GENETICS OF TRIATOMINE BUGS (FAMILY REDUVIIDAE) IN RELATION TO THEIR ROLE AS VECTORS OF CHAGAS' DISEASE, TRYPANOSOMAL INFECTION OF THE AMERICAS

by

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"The aim of life is self-development. To realize one's nature perfectly - that is what each of us is here for".

Oscar Wilde

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ABSTRACT

Trypanosomiasis caused by Trypanosoma cruzi and transmitted by triatomine bugs is widespread in South and Central America, human infections, chronic or acute, being known as Chagas' disease. Since blood forms of the trypanosome in man and animals are commonly too few to be found in blood films, conclusive evidence of infection is still by xenodiagnosis. This requires vector bugs to be engorged on a suspected infected vertebrate and the bug faeces examined a month or so later by which time the trypanosomes should have greatly multiplied in the insect gut. It is known that there is variation between triatomine species and between individuals within species in ability to develop T. cruzi infections. The present research investigated the genetic basis of this variable susceptibility in one species, Rhodnius prolixus, by selection for susceptible and refractory bug populations and revealed evidence of polygenic control of susceptibility to infection with T. cruzi: this contrasts with the major gene mechanisms reported for other pathogens in other insect vectors. Male bugs showed significantly higher levels of infection than females and may be more efficient for xenodiagnosis.

The radioresistance of triatomine bugs may be related to their chromosome morphology. Male R. prolixus given a sub-sterilizing irradiation dose were less sterile than their progeny, this delay resulting from the diffuse structure of the centromeres of triatomine chromosomes. These results are discussed in relation to possible control of triatomines by genetic manipulation.

Investigation of spermatogenesis by autoradiographic techniques in male R. prolixus revealed that starvation induced diapause inhibits

spermiogenesis. A blood-borne factor produced when the diapausing bug is fed may directly affect the rate of meiosis.

The karyotype is described for several triatomine species. A method was developed for distinguishing between individual mitotic chromosomes within the complements of *Triatoma infestans* and *R. prolixus*, the chromosome markers being valuable for further genetic studies of triatomine bugs.

GENERAL INTRODUCTION

Chagas' disease is a serious clinical condition and constitutes one of the most widespread and dangerous human diseases in many South and some Central American countries. This disease is virtually [illegible], nor is there an effective prophylactic drug; it can give rise to severe cardiac abnormalities and mega conditions of the alimentary canal and other organs with death from heart failure and [illegible]. It has been estimated by [illegible] expert committees (W.H.O., [illegible]) that [illegible] people may be exposed to the risk of infection and that at least 7 million people were actually [illegible] Chagas' disease. The majority of these cases occurred in Brazil, where it was estimated in 1960 that [illegible] had a billion cases of Chagas' disease (Roberts, 1970). Nine other countries in South America have also reported large numbers of [illegible] (Dias, 1961; [illegible], 1962); Argentina, 1.5 million (Romana, 1963); Bolivia, 60 thousand (Diaz, 1956); Chile, 75 thousand (Apt, 1971). Although the cases have been confined [illegible] South American countries, although the disease is endemic in all of them. Human cases are not normally found beyond northern Mexico [illegible] and Argentina [illegible] (Romana, 1963).

The disease was first described by the Brazilian scientist, Carlos Chagas, who found epimastigote forms of a flagellate in the gut of a triatomine bug, [illegible] in Minas Gerais, Brazil. These bugs proved to be infective to laboratory animals and Chagas [illegible] (Chagas, 1909). Chagas subsequently described the life cycle of T. [illegible] in its [illegible] and [illegible], and the processes of the disease in man (Chagas, 1909, 1911). Chagas was also the first to describe [illegible] of a reservoir host for T. cruzi in wild animals, the armadillo

Dasyprocta [illegible] ([illegible], 1912), and the first to show that triatomine [illegible] showed that the trypanosome completed its development in the hind-gut [illegible] thought that the trypanosomes developed in the salivary glands of the [illegible] hypothesis and demonstrated that *T. cruzi* was indeed transmitted in the bug faeces.

[illegible] cycle [illegible] infected bug faeces first invade cells of the reticulo-endothelial [illegible] enter the bloodstream and spread to various parts of the body where they invade cells of different organs and again multiply. Different [illegible], but muscle (including heart muscle), [illegible] and reproductive organs are all favoured sites. On entering a cell the body of [illegible] and its flagellum [illegible]. [illegible] transformed into trypomastigotes via an intermediate epimastigote stage. After 5 days the host cell becomes distended, its cytoplasm having been consumed by the parasites, forming a pseudocyst which then [illegible]. [illegible] do not divide in the blood and the numbers present vary with the phase of infection; in man they are only numerous during the acute phase of the disease and after 4 weeks may be too few to be detected in the [illegible]. [illegible], blood forms are so few as to be seldom or never seen in routine blood [illegible]

in the tissues and be released into the blood intermittently.

Dias (loc. cit.) and more recently Brack (1968) have described the life cycle of the trypanosome in the bug, where its entire development takes place in the gut. [illegible] lasts 6-7 days in young insects and 10-11 days in adults. A few hours after an infecting blood-meal, short trypomastigotes without a free flagellum occur in the gut of the bug and after 14-20 hours are replaced by amastigotes with a short internal flagellum which later protrudes, the resultant [illegible]. These then multiply by binary fission and after about 24 hours divide and give rise to small [illegible] and epimastigotes. [illegible] blood-meal epimastigotes appear in the rectum where they attach themselves to the epithelium and become sphaeromastigotes with the flagellum [illegible]. These develop into short trypomastigotes which elongate and by the seventh to eighth day slender metacyclic trypanosomes are produced which do not divide and are passed with the faeces of the bug.

Arising from the paucity of trypanosomes in the peripheral blood of the chronically infected vertebrate host - human or other animal (the avian hosts which triatomines do feed on as well are insusceptible to T. cruzi infection) - and this substantial multiplication of the trypanosomal organism within the bug is the basis of the xenodiagnostic [illegible] this, bugs [illegible] or animals and kept for several weeks until a patent rectal infection is detectable, or not. This is a special aspect of vector function [illegible] in which vector-host processes [illegible]. It is examined genetically in this thesis as the xenodiagnostic test, despite several

[illegible] still provides the critical parasitological evidence of infection in [illegible] animal.

Barretto (1968) lists 86 species of American Triatominae, 38 of which have been found to be [illegible] T. [illegible]. Triatomines have a much wider distribution from 42°N in the United States to 43°S in Argentina (Romaña, 1931). This difference has been attributed [illegible] by variation in the habits of the bug which in endemic areas have become adapted to living in human dwellings and it is these domiciliated rather than sylvatic species which are responsible for most of the transmission of Chagas' disease to man. Romaña (loc. cit.) states that the sylvatic species produce very few cases of the disease and that of the domestic species Triatoma infestans and Rhodnius prolixus have the widest distribution and are the most important vectors. The principal vectors of Chagas' disease in South America are shown in Table 1.

Table 1. Principal vectors of Chagas' disease in South American countries with high incidence of Chagas' disease

Country	Species	Reference
Chile	Triatoma infestans	Neiva and Lent (1943)
Argentina	[illegible]	Abalos and Wygodzinsky (1951)
Colombia and Ecuador	Rhodnius prolixus T. dimidiata	Dias (1952)
Venezuela	[illegible]	Dias (1952)
Brazil	Panstrongylus megistus T. infestans [illegible] [illegible]	Barretto (1968)

Because of their importance as vectors of Chagas' disease, triatomine bugs have been the subject of a great deal of research work in relation to their distribution, vector potential and control, and one species in particular (Rhodnius prolixus), has long been established as laboratory colonies and used for basic researches on insect physiology besides studies bearing on its vector function. However, very little research effort has been applied to the genetics and cytogenetics of triatomine bugs, despite their medical importance, notwithstanding that for other medically important insects genetic research has expanded in recent years because of the possibility of controlling vector and pest populations by genetic manipulation, either at chromosomal or gene level - methods which have the great advantage over conventional pesticide usage of not polluting the environment. The lack of genetic research in triatomine bugs can be related directly to the biology of these insects; genetic experiments inevitably require that successive generations of a given species be reared which, if the work is to be completed within a reasonable time-span, means that organisms with short generation times are preferred.

In the case of insect vectors of disease, the Diptera have become common laboratory tools for genetic research, not only because many of them are pests and vectors, but also because most of them have short generation times and many require relatively simple techniques for colonization. Thus, large amounts of useful information have been gained in relatively short periods of time about, for example, mosquito genetics, and with relatively small budgets.

Triatomine bugs, on the other hand, do not make ideal laboratory tools for genetic research. Their main disadvantage lies in their long generation time (5-6 months is the minimum period from egg to adult,

while some species occupy considerably longer times than this) which makes only two generations per year possible at most. Also, because they are obligatorily haematophagous, these bugs are expensive to keep in large numbers - they need to be fed and take long blood-meals on live hosts (usually, guinea-pigs, chicks, hamsters) at frequent intervals. Every stage (and in females even each egg batch) requires a blood-meal. This is a labour-intensive process and correspondingly expensive in man-power and vertebrate hosts.

The cultures established at the L.S.H. and T.M. provided a selection of triatomids essential for the present work, many of them (e.g. Triatoma infestans and Rhodnius prolixus) having been kept for many years and bred well adapted to laboratory conditions. The following species were used in the present study, some of which were long-standing laboratory cultures and others built up more recently from bugs supplied from various sources in South America:-

Triatoma rubrofasciata (De Geer, 1773). This species was collected in Belize, British Honduras, by Mr. A.M. Minter in 1957, and since established at L.S.H. and T.M.

T. infestans (Klug, 1834). This species was received from Professor E. Escomel (Lima) and established at L.S.H. and T.M. in 1956 but its precise origin is unknown. It was subsequently mixed with T. infestans received from Argentina, Brazil and Venezuela in 1956.

T. lecticularia (Stål, 1859). This species was collected in Belize, British Honduras, by Mr. A.M. Minter in 1957, and since established at L.S.H. and T.M.

T. sanguisuga (LeConte, 1855). This species was brought to the L.S.H. and T.M. in 1956 by Professor F.A. Dodge from a colony in

Alaquez, Colombia.

T. tibio-maculata (Pinto, [illegible]). This species was collected in Bahia, Brazil by Dr. M.A. Miller and colonized at L.S.U. and T.M. in 1973.

T. [illegible] (Mayr, 1863). This species was donated by Cambridge University, Department of Zoology and introduced at L.S.U. and T.M. in 1972. Its origin is not known.

T. perrensis Viereck, 1903. The parasite wasps and T.O. insect was supplied by Cambridge University in 1971 and reintroduced from L.S.U. and T.M. in the Spring. This species collected in Mexico and is more common from Mexico.

T. sierrensis Girault, 1913. This species was collected in Bahia, Brazil, by Dr. M.A. Miller and introduced at L.S.U. and T.M. in 1973.

Trichogramma minutum Riley, 1879. This species was collected in Bahia, Brazil by Dr. M.A. Miller and introduced at L.S.U. and T.M. in 1971.

Trichogramma semblidis Oatd, 1900. This species was collected in Bahia, Brazil by Dr. M.A. Miller and colonized at L.S.U. and T.M. in 1972.

T. pretiosum Riley, 1879. The colony of T. pretiosum was established at the L.S.U. and T.M. in 1973 by Lcl. A. Almeda using eggs given to him by Professor H. Knipp who obtained them from Venezuela at approximately between 1972-1973. This original colony was subsequently mixed with T. pretiosum also by Professor H. Knipp who obtained this from Caracas, Venezuela.

Trichogramma pretiosum-minutum (Riverso and Nagarkatti, 1964).

A T. pretiosum Riley, 1879. This hybrid was made by Dr. A. Nagarkatti-Sivakumar in Bhavanagar, Gujarat and Raleigh, Carolina with the parental cultures in L.S.U. and T.M. in 1973.

Rearing methods

Bugs were reared by a combination of the methods described by Buxton (1930) for [illegible] small numbers of Triatominae, and by Gardiner and Maddrell (1972) for [illegible] bugs of R. prolixus. Each were kept in [illegible] at 26±1°C and approximately 70% R.H., and given blood-meals [illegible] lop-eared rabbits. Prior to feeding, larvae and adults were placed in [illegible] tubes (1" x 3/4", [illegible] with nylon 'tights'. The open ends were sealed with [illegible] tape. Cages containing bugs were placed on [illegible] and [illegible] on to a [illegible] and, after feeding, the bugs were allowed to [illegible] for a large amount of urine produced after a meal. The [illegible] bugs were [illegible] and [illegible] and a [illegible] strip of [illegible]. To [illegible] bugs, we used the bag method. The bags on the jars were sealed by clamping a section of nylon 'tights' over the mouth with the pulled [illegible] plugged with filter [illegible]. For safety in handling, all the insects were anaesthetized with CO_2 for transfer from the storage jars to the feeding cages the day before feeding, since it was found that anaesthesia immediately before feeding interfered with the feeding response of the bugs.

Eggs, collected [illegible] from [illegible] rabbits, were placed in 2" x 1½" flat-bottomed tubes, and the first instar larvae which hatched were placed for feeding in perspex containers with a gauze covered end, identical to those designed by Gardiner and Maddrell (1972) for this purpose and not in the Geigy-cages used for [illegible]. After the first [illegible] feed, bugs were moved to fifteen jars, until the next feed was due. First instar larvae were fed one

days to second instar larvae, were fed two weeks later as second instar larvae. [illegible] fed. [illegible] took about three [illegible] to moult [illegible] to fourth instar larvae, and [illegible] from these first moults moult [illegible] fifth instar larvae. The final moult to adult occurred after 3 or 4 weeks and adults were fed a week after emergence. [illegible] intervals and after three feeds they were disposed of to make way for more breeding stock. Only [illegible] large numbers of bugs could be reared without adverse effect on the [illegible] work to be planned well in advance.

[illegible] different larval stages to avoid [illegible] and eggs to hatch [illegible]. As already mentioned, [illegible] species, the [illegible] species particularly [illegible] which most of the present study was carried out. Other species, such as Panstrongylus megistus, could take as long as 9 months or more to complete the life-cycle, R. prolixus having a relatively short life-[illegible] of about 3 months. For species other than R. prolixus, material was supplied at appropriate stages and condition by other workers in the department from the colonies which they were particularly concerned with rearing for other researches.

[illegible] of its relatively short life-cycle, the present research was concentrated on R. prolixus. Four selected problems, deemed feasible in three years, were chosen for this study as likely to be of particular interest to problems of triatomine systematics, [illegible] to [illegible].

[illegible]. [illegible] researches are presented in the thesis in four parts, as briefly indicated below.

PART [illegible] – [illegible] of the [illegible]

The cytotaxonomy of insects is now a well established technique, and can provide great insight into the relationships of different [illegible] on the basis of chromosomal differences. The cytotaxonomy of triatomine bugs has, [illegible] these bugs do not possess the giant polytene chromosomes so useful to [illegible] karyotypes of the triatomine species and hybrid species available and developing methods of producing chromosome markers for this group of [illegible] staining [illegible].

PART TWO – The regulation of [illegible]

Hormones have been shown to control many aspects of growth and differentiation in insect development, but the control factors involved in development of the testis, and the [illegible] in the fully developed testis, remain subject to debate. Most work carried out on the development of insect testes has concentrated on the growth [illegible]. The present experiments examined the effects of starvation and feeding on the intra-meiotic events of spermatogenesis in R. prolixus fifth instar larvae and adults.

[illegible]

[illegible] vary,

in bug susceptibility to the parasite infection affecting the outcome. The present experiment was designed to study whether the susceptibility of R. prolixus to T. cruzi is genetically determined by following a selection programme to breed refractory and susceptible populations of bugs. Should susceptibility be related to genotype, then it should be possible to breed a highly susceptible strain to improve the sensitivity of the complement test.

PART FOUR - The inheritance of radiation induced semi-sterility in Rhodnius prolixus:

Insects, in recent years, as the control of insect vectors and pests by the release of artificially sterilized males and the successful developed lethal mutations in the past few decades. It has been shown that, like the lepidoptera, triatomine bugs are very resistant to sterilization by irradiation, such that doses required for complete sterility of males result in them being unable to compete with normal male bugs in mating. The present experiments examined the effect of sub-sterilizing doses of irradiation on the fertility of irradiated males, and successive generations of their offspring, and related the changes in fertility to chromosomal damage found in spermatocytes of experimental males. The release of semi-sterile Lepidoptera has been used as a means of controlling this radio-resistant group. The purpose of this aspect of the thesis research on triatomines was to assess the fertility of both R. prolixus treated with sub-sterilizing doses of irradiation and to relate these effects to chromosomal changes; the feasibility of controlling this vector species by releasing semi-sterile males could then be properly assessed.

PART ONE

CYTOTAXONOMY OF THE TRIATOMINAE

INTRODUCTION:

Insects of the sub-family Triatominae (Family Reduviidae, Sub-Order Heteroptera, Order Hemiptera) are widely distributed in the [illegible] species (Usinger et al., [illegible]). [illegible] also involved experimental hybridization of different species to determine the nature of their relationships from their hybrid fertility [illegible]. [illegible] Brodie and Ryckman (1967) examined the antigenic relationships of 18 populations of bugs comprising five Triatoma species from both North and South America using [illegible] techniques.

Differences in chromosome morphology between species have provided taxonomists in recent years with additional criteria to aid classification. [illegible] including insects. [illegible] Compared with other disease vectors however, the cytotaxonomy of the [illegible] autosomes and sex-chromosomes for several species of triatomine.

The Triatominae have large numbers of relatively small and almost

indistinguishable autosomes, with usually 20 Autosomes plus an XY system in the male, although some species have multiple X-chromosome systems which may be used as specific markers (Wenzel, 1968). The only detailed study of the cytotaxonomy of these pests is given by Ueshima (loc. cit.) who describes details of meiotic chromosome behaviour for 20 species and 1 species hybrid from studies of testis squashes. These studies showed, unfortunately, that the meiotic chromosomes of triatomine spermatocyte nuclei do not provide the taxonomist with a useful cytogenetic tool, because of the similarity of the small chromosomes. A further limitation was that relative arm length of chromosomes, which can be a useful diagnostic feature in [illegible] species, has no systematic value in hemipterous insects which have [illegible] centromeres.

Polytene chromosomes, in which minute differences in morphology can be detected, have facilitated the construction of detailed maps of Dipteran chromosomes so that sibling species may be distinguished. Such studies of polytene chromosomes in mosquitoes (Kitzmiller et al., 1967) black-flies (Dunbar, 1966) and Tsetse-flies (Southern and Pell, 1973) have brought great precision to studies of systematic relationships within these vector species. Polytene chromosomes have, however, been recognized from any tissue of triatomine bugs at any developmental stage so that, again, this tool, so valuable in these several vector insects is not available for bug taxonomists.

Cytogenetic studies of [illegible] have, however, been handicapped by the nature of their chromosome morphology, and this was also the case for other animal groups, notably for mammals and particularly for human cytogeneticists faced with large numbers of very similar sized chromosomes in the human cell (for a recent review see

Ford, 1973). However, in recent years, the study of mammalian cytogenetics has been revolutionized, Caspersson et al. (1968, 1969a and b) showing that by using fluorescent dyes they could distinguish brighter and darker zones in metaphase chromosomes of man when the preparations were examined under U.V. light on a fluorescence microscope. Later, with the dye quinacrine mustard (Q.M.), Caspersson et al. (1970a, b and c) were able to differentiate every chromosome of the human karyotope. The precise nature of these Q-bands, however, remained controversial; it was assumed initially that the alkylating agent, mustard, bound preferentially to regions of DNA rich in guanine and that the fluorochrome quinacrine attached to the mustard would fluoresce most strongly in these regions (Caspersson, 1968). However, it has since been shown that Q.M. has an affinity for adenine-thymine rich DNA regions and not guanine-cytosine regions, which makes the original hypothesis untenable (Weisblum and DeHaseth, 1972). Despite the controversy which has surrounded the mechanism of chromosome fluorescence, its usefulness for delineating different chromosomes was an important advance, although the technique required complex and expensive equipment and the fluorescence of the preparations faded with time. These problems were circumvented by a technique that could also be produced in human chromosomes using Giemsa stain following a pre-treatment incubation in a saline solution (sodium citrate) (Drets and Shaw, 1971; Dutrillaux et al., 1971). Using this Giemsa (G-band) technique higher resolution of bands could be obtained if the preparation was also given a brief pre-treatment in trypsin (Seabright, 1971). As with the Q-bands, the cytochemistry of G-band production remains a matter for debate; it was thought originally that G-bands reflected a denaturation-reassociation process of repetitive DNA (Sumner et al., 1971) but when the bands were

produced by an enzyme which did not act by denaturation (Seabright, 1972) this theory was rejected. Kato and Moriwaki (1972) found that bands could be induced in mammalian chromosomes by a variety of chemicals but could suggest no common mode of action. Sanchez and Yunis (1974) have recently suggested that the basic organization of DNA in eukaryotes and, in particular, its repetitive nature are responsible for band production but an ultrastructural study of G-bands (Burkholder, 1975) has shed little light on their nature. Whatever their origin, G-bands have improved the study of mammalian cytogenetics and since the chromosomes of triatomine bugs presented problems akin to mammalian chromosomes (i.e. large numbers of small, indistinguishable chromosomes) it was decided as part of the present work to attempt to apply G-banding techniques to mitotic cells of triatomine material. Although little work has been published related to banding of insect chromosomes, several workers have reported successful application of the C-banding technique to insect material. The C-banding technique involves pre-treatment of cells in an alkaline or acid solution followed by S.S.C. incubation and Giemsa staining, which stains a single band of hetero-chromatin in the centromeric region. Nau (1971) first showed that C-bands could be produced with insect material using Drosophila melanogaster, and Gallimore et al. (1975) differentially stained the supernumerary B-chromosomes of the grasshopper Myrmeleotettix maculatus, using the same technique. Newton et al. (1974) were able to distinguish the X and Y-chromosomes of Melanoplus femurrubrum in larval brain cells by C-banding and Breta and Stoll (1974) demonstrated C-bands in mitotic and meiotic chromosomes of the cricket Gryllus argentinus. However, C-banding provides only a limited system of chromosomal markers and it was hoped that by applying the G-banding technique to triatomine chromosomes,

a more detailed account of these could be consulted.

In addition, the karyotypes of 13 species of triatomine and one species hybrid were examined at early meiosis. Meiotic meiosis, hitherto unreported in triatomine bugs, was investigated in one species, R. prolixus.

I now turn to describe the techniques used, for conventional orcein staining of chromosomes and those developed on the principles of G-banding for human chromosomes, for triatomine species and the results obtained regarding the karyotypes for the following triatomine material:- T. infestans, T. sordida, T. rubrovaria, T. phyllosoma, T. pseudomaculata, T. megista, T. brasiliensis, P. megistus, R. prolixus, and T. dimidiata x T. sordida hybrid.

Methods

(a) Male Material

Cytogenetic techniques. Testes were dissected from 2nd adult males under Ringer's saline (9 g NaCl, 0.42 g KCl, 0.25 g $CaCl_2$ per litre), fixed in 3:1 absolute ethanol:acetic acid and stored at 4°C in labelled screw vials. Squash preparations were made by teasing out fixed follicles out on clean microscope slides in lacto-propionic-orcein using a small brass rod with a flat end. The orcein stain was made by dissolving 2 g synthetic orcein (Gurr, High Wycombe, England) with 50 ml 45% propionic acid and 50 ml 45% lactic acid for 24 hours and then filtering. Preparations were squashed by finger pressure under 22 x 22 mm coverslips and sealed with rubber solution to prevent evaporation. After examination and photography, coverslips were removed by freezing in liquid nitrogen and prizing off with a razor

Plate. The preparations were then dehydrated into absolute ethanol and mounted in Depex as permanent mounts. Photographs of the resultant chromosome configurations were taken with Recordak 35 mm micro-file film (Kodak, London, England) developed in FF contrast developer (Ilford, Essex, England) and fixed in Amfix (May and Baker, Dagenham, England).

Results. Diagnostic material has been obtained for all the species studied. The polytene permeabilist nucleus has a heteropycnotic body representing the association, X and Y chromosomes.

The autosomes do not stain as deeply as the sex-chromosomes in early prophase but at diakinesis they have become heteropycnotic, and the autosomes can be seen to be joined by a single terminal chiasma in each bivalent.

Tatera leucogaster, T. robertsi, T. cafricola, T. inclusa-mariquensis and T. cordilla. The 5 species in this group all have 36 autosomes +XY and morphologically the autosomes of all 3 species are very similar, each having 1 larger sized bivalent which is distinguishable from the other pairs.

Plate 1.1 shows a spermatocyte of Gerbillurus from T. leucogaster, at prophase I the autosomes condense as ring bivalents while the sex-chromosomes associate, and at this stage become negatively heteropycnotic relative to the autosomes and remain so throughout the rest of meiosis; Plate 1.2 shows a metaphase I cell from T. robertsi. The sex-chromosomes are often seen like so in Plate 1.2 at the centre of a ring of autosomes and this arrangement is also illustrated by Plate 1.3 with a metaphase I cell from T. inclusa. The karyotypes of the species T. pseudo-maculata and T. cordilla will

be dealt with later in the section related to the cytogenetics of the hybrids produced by crossing these two species.

Triatoma tibio-maculata, T. phyllosoma, T. protracta, T. lenti and T. vitticeps. These 5 species of the genus Triatoma all have multiple non-chiasmate X_nY systems in the male, 4 of them with X_1X_2Y and T. vitticeps with an $X_1X_2X_3Y$ system. The course of meiosis in these species is the same as for the 3 species described above. The 20 autosomes arrange themselves as 10 bivalents on the metaphase plate and the sex-chromosomes are usually situated peripherally. Plates 1.5, 1.6 and 1.7 show metaphase I cells from T. tibio-maculata, T. protracta and T. sordida and Plate 1.8 shows a cell in diakinesis from T. lenti. At second metaphase in this group of species the sex-chromosomes always lie in the centre of the ring of autosomes and assume the characteristic 'heart and dot' pairing which is illustrated for T. phyllosoma in Plate 1.9. At second anaphase the Y-chromosome goes to one pole and the multiple X-chromosomes go to the other pole. T. vitticeps is of special interest being the only species examined with three X-chromosomes. Plate 1.10 shows a metaphase I cell of this species, and it can be seen that the Y-chromosome is much larger than all the X-chromosomes being about the size of the autosomes.

Panstrongylus megistus. This species is characterized by having only 18 autosomes which form 9 bivalents at metaphase I. The sex-chromosomes (X_1X_2Y) lie at the periphery of the spindle at metaphase I (Plate 1.11) and divide precociously on the first division. Plate 1.11 shows an anaphase I division. At metaphase II (Plate 1.12) the sex-chromosomes again lie on the periphery of a ring of autosomes.

Rhodnius neglectus and R. prolixus. The two species studied of the genus Rhodnius both have 20 autosomes plus an XY sex-system. Their meiotic chromosomes are much smaller than those found in the genera

Plate I.1. [illegible] spermatocyte of [illegible]. 2n = 20 + XY.
Note sex-chromosomes isopycnotic with autosomes. Ten
bivalents each with a single terminal chiasma. X 2500.

Plate I.2. Metaphase I spermatocyte of [illegible] maculata. 2n = 20 + XY.
Note negative heteropycnosis of sex-chromosomes which lie
at centre of ring of autosomal bivalents. X 2500.

Plate I.D. Metaphase- I [illegible] T. [illegible], 2n = 20 + XY. X [illegible].

Plate I.E. Metaphase II spermatocyte of T. infestans. Note sex pseudo-bivalent (sb) [illegible] ring of autosomes. [illegible].

Plate 1.5. Metaphase I spermatocyte of T. tiblo-maculata, 2n = 20 + X_1X_2Y. X 2500.

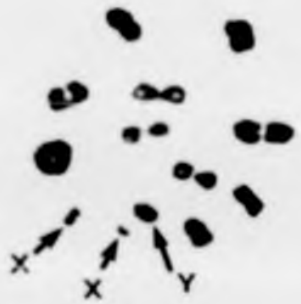


Plate 1.6. Metaphase I spermatocyte of T. phyllosoma, 2n = 20 + X_1X_2Y. X 2500.

Plate 1.7. Early metaphase I spermatocyte of T. protracta, 2n = 20 + X_1X_2Y. X 2500.

Plate 1.8. Diakinesis spermatocyte of T. lenti 2n = 20 + X_1X_2Y. Note very small X_2. X 2500.

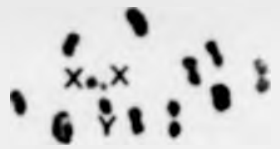


Plate 1a). Metaphase II spermatocyte of T. [illegible]. 2n = 20 + X_1X_2Y.
Note sex-chromosomes at centre of ring of autosomes and their characteristic 'touch and go' pairing. X 2500.

Plate 1b). Metaphase I spermatocyte of T. vitticeps. 2n = 20 + $X_1X_2Y_1Y_2$.
Note large Y-chromosome similar in size to autosomes. X 2500.

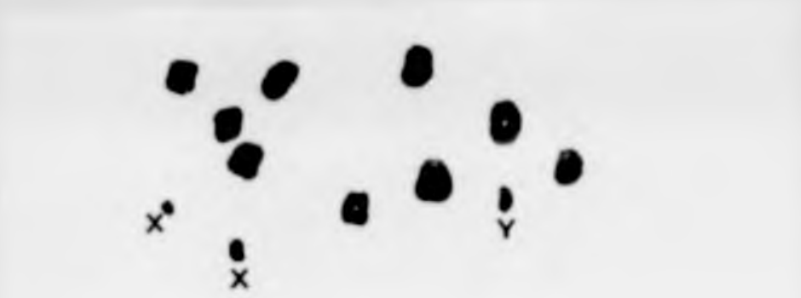


Plate 1.11. Metaphase I spermatocyte of P. megistus, 2n = 18 + X_1X_2Y. Note only 9 bivalents. X 2500.

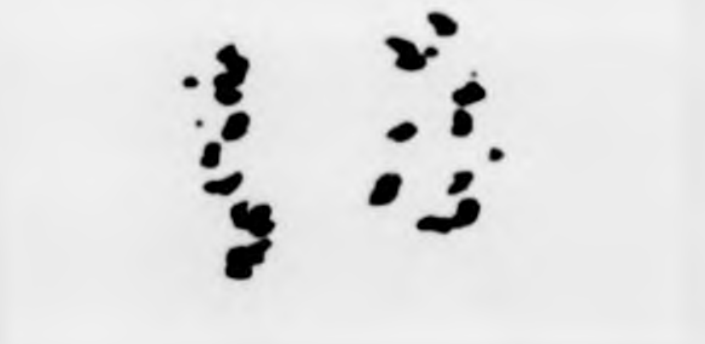


Plate 1.12. Anaphase I spermatocyte in P. megistus. X 2500.

Plate 1.13. Metaphase I spermatocyte of P. megistus. X 2500.

Plate 1.14. Metaphase I spermatocyte of R. neglectus, 2n = 20 + XY. Note small size of chromosomes compared to Triatoma species. X 2500.

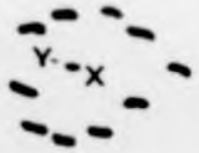


Plate 1.15. Metaphase II spermatocyte of R. neglectus. X 2500

Triatoma and Panstrongylus as seen in Plate 1.14 which shows a metaphase I cell of R. neglectus with the X and Y-chromosomes at the periphery of the spindle. Plate 1.15 shows a metaphase II cell of this species with the XY pseudo-bivalent at the centre of a ring of autosomes. The karyotypes of these 2 Rhodnius species are barely distinguishable in meiotic preparations except that the chromosomes of R. pallescens are slightly larger (see later and Plate 2.8, Part Two).

(b) Female Meiosis

Cytogenetic technique. The method of Perry and Jones (1971) developed for the study of grasshopper oocyte nuclei was used to study female meiosis in triatomine bugs. Eggs were placed in 3:1 absolute ethanol:acetic acid fixative, their caps removed with forceps and fixed for 5-10 minutes. The contents of the eggs were then squeezed out of the case with forceps, placed in a drop of lacto-propionic-orcein on a siliconized coverslip ('Repelcote', Hopkin and Williams, Essex, England) and allowed to stain for one hour. The coverslips were then inverted on to clean slides, squashed and examined for the egg nucleus.

Results. Because of the large numbers of cells in division in a testis these organs have always been favoured for the study of meiosis. The female gonad, consisting of ovarioles filled in insects is surrounded by large amounts of yolk and therefore presents considerable technical difficulties in dissecting ovaries to squash preparations. Perry and Jones (loc. cit.), however, developed an oocyte squash technique which proved to be suitable for application to triatomine eggs. Since a large colony of R. prolixus was being maintained for the present work the opportunity existed to examine large numbers of eggs of this species. It is usual for the first meiotic division of the ovum to occur at the time of ovulation following the

Plate 1.16. Metaphase I oocyte nucleus from an egg of R. prolixus. Cut-out karyotype shows 2n = 20 + XX. X 2500.

entry of the sperm into the plasma of the egg and for fertilisation of the ovum nucleus to take place just before the egg is laid (Wigglesworth, 1965). The female reproductive organs of R. prolixus consist of 2 ovaries each made up of several ovarioles of the telotrophic type in which the nurse cells are confined to the apex of each ovariole and are connected to the developing oocytes by long [illegible] (Wigglesworth, 1965; Davey, 1961). Ovulation occurs when the leading oocyte of each ovariole is ripe and the eggs pass one after the other into the lateral, on their way to the common, oviduct. Examination of large numbers of eggs from the reproductive tracts of female R. prolixus at all stages of development up to the substantial yolk laden egg at oviposition revealed the oocyte nuclei, but this may have been due in part to the technical difficulties of locating the small nuclei in the mass of yolk in the eggs. The 3 oocyte nuclei which were found at first metaphase were all located in the lateral [illegible]. One of these female metaphase I nuclei is shown in Plate 1.16 which shows that the female has 10 bivalents of very similar size with 2 X-chromosomes.

(c) Q-Banding of Mitotic Chromosomes

Published photographs of mitotic chromosomes of triatomine bugs have shown groups of very small [illegible] chromosomes already indistinguishable (White, 1954a and b; Smith, 1966). Wigglesworth (1967) examined mitotic cells in the fat body, epidermis and muscle of R. prolixus larvae and his results also showed minute mitotic metaphase chromosomes. Examination of these tissues in the course of the present work confirmed these observations - mitoses in these somatic tissues appeared to be similar to those found in gonadic cells (Part Two, Plate 2.1). However, studies by Mellanby (1936a and b) of the embryonic

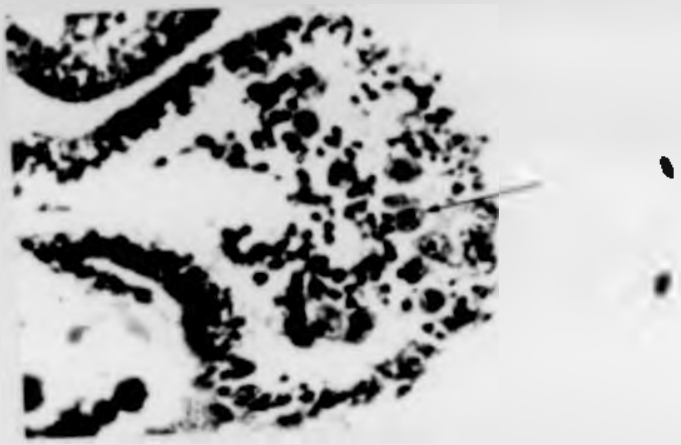


Plate 1.17. [illegible] of embryo of T. [illegible] showing large [illegible] metaphase [illegible]. X 800.

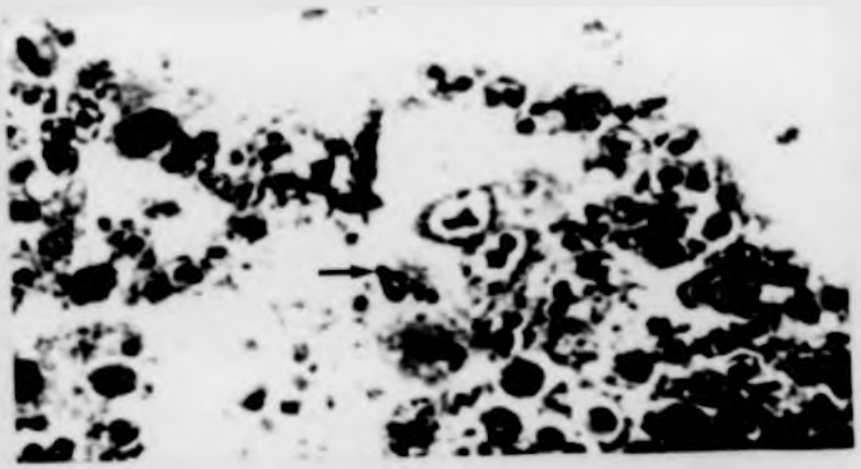


Plate 1.18. L.S. of embryo of T. infestans showing large mitotic metaphase chromosomes of neuroblasts. X 800.

development of R. prolixus and [illegible] large, conspicuous cells present in the embryo at the time of head and thoracic segmentation. Mellanby (1936a) found that these large cells were neuroblasts responsible for the formation of the ventral nerve cord; they appeared at day 5 of embryonic development. Sections of 5-7 day-old eggs were, therefore, studied [illegible] eggs initially) and large neuroblasts resembling those reported by Mellanby (loc. cit.) for R. prolixus were found. Batches of eggs were prepared using the method described by Anderson (1960) for tsetse eggs, and were stained with haematoxylin and eosin. Plates 1.17 and 1.18 show sections through T. infestans eggs and demonstrate the neuroblast cells.

Cytogenetic technique for G-banding mitotic chromosomes. Squash preparations of 5-7 day-old T. infestans eggs confirmed that the chromosomes of these neuroblasts were indeed larger than those found elsewhere in embryonic tissues. Having located these cells with large chromosomes which seemed suitable for G-banding the main technical problem was to produce a suspension of these cells. It is an essential pre-requisite for the G-banding technique to have a [illegible], produces a preparation with individual cells randomly dispersed over the slide and showing good metaphase spreads. Cytogeneticists working with human material routinely employ a technique developed by [illegible] to produce [illegible].

Five to seven day-old embryos were dissected from the triatomine eggs in L15 medium (Leibovitz, 1963) and placed in hypotonic (0.8% sodium citrate for 8 minutes. The embryonic cells were then dispersed in 0.25% trypsin (Difco Trypsin, 1:250, Difco Laboratories, Detroit, Michigan) made up in 0.85%

versene (0.1 g EDTA, 4.0 g NaCl, 0.1 g KCl, 0.58 g Na_2HPO_4, 0.1 g KH_2PO_4 and 0.1 g glucose in 500 ml distilled water), the resulting cell suspension washed in insect saline and centrifuged at 1000 r.p.m. for 5 minutes (Mistral 6L centrifuge head 8.5 cm). The supernatant was discarded and the pellet flicked to form a thin layer up the walls of the centrifuge tube which was rapidly fixed by adding drops of freshly made 3:1 absolute ethanol:acetic acid fixative on the cells. After standing for 5 minutes the cells were centrifuged at the same speed, re-suspended in fresh fixative and, after standing for a further 10 minutes, were centrifuged and finally suspended in a few drops of fixative. Air-drying procedures were those by the method of Evans et al. (1964), the suspension being dropped on to acid-cleaned slides and blown dry when the interference rings appeared. The preparations were kept for one week in dust-proof boxes before further treatment, since attempts to produce banded preparations immediately after fixation were unsuccessful. The G-banding technique employed was essentially that described by Sumner et al. (1971); slides were incubated for one hour in 2 x SSC (0.3M sodium chloride, 0.03M trisodium citrate) at 60°C, rinsed in absolute ethanol and stained in Giemsa (1 in 5 Gurr's Improved R66 stain, Gurr, London, England) at pH 6.8 for 3 minutes. Fresh buffer was prepared by diluting 1.5 ml of 0.1M citric acid to 50 ml with distilled water, and adjusting the pH to 6.8 with 0.2M disodium phosphate. After staining, the preparations were rapidly rinsed by running distilled water into the Coplin jars and finally removing to be air-dried and mounted in Eupiral.

The results of using this technique with neuroblasts of 2 species of Locusta, L. migratoria and L. succincta are shown in Plates 1.17 and 1.18

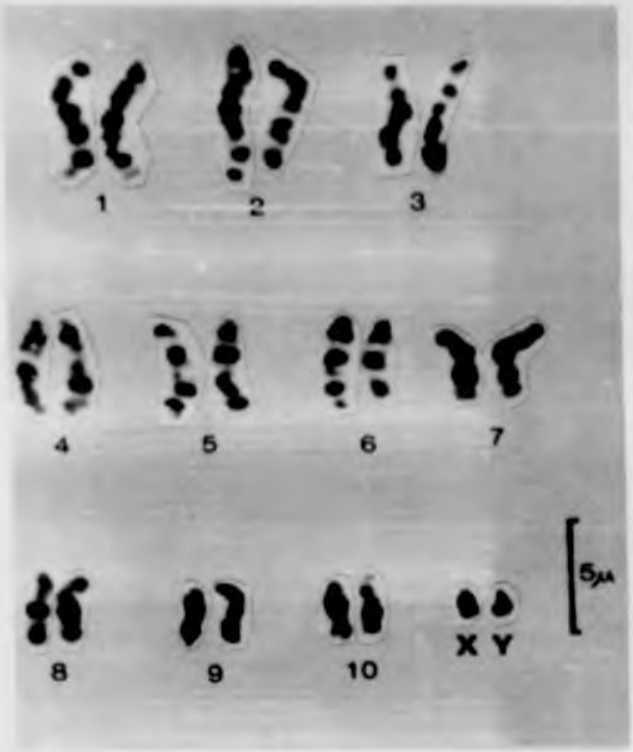


Plate 1.19. Cut-out karyotype of *P. prolixus* from neuroblast of male embryo G-banded by A.S.G. technique.

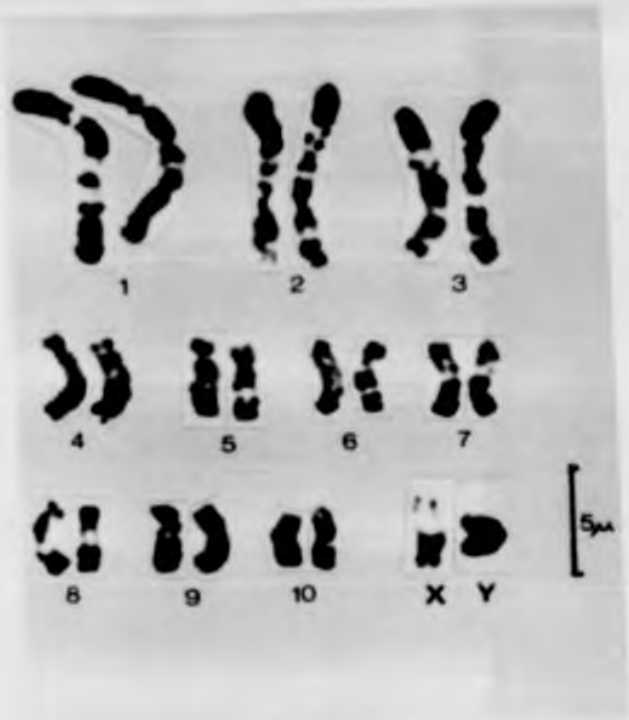


Plate I.20. Cut-out of karyotype of T. infestans from neuroblast of male embryo G-banded by A.S.G. technique.

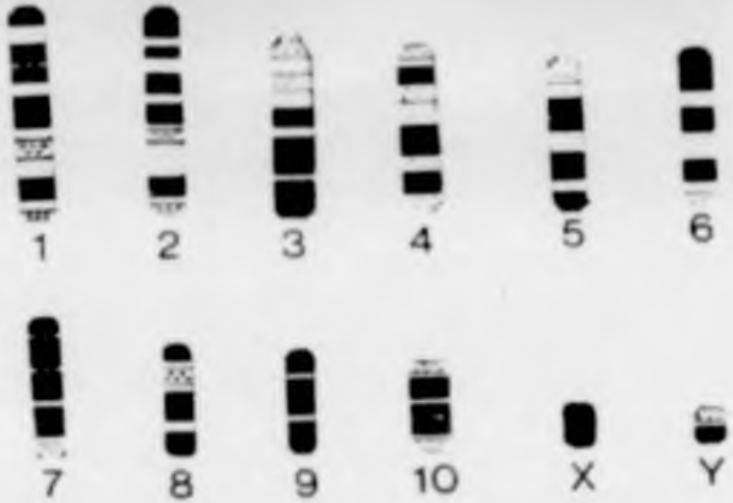


Plate I.21. Idiogram of mitotic chromosome complement of R. prolixus.

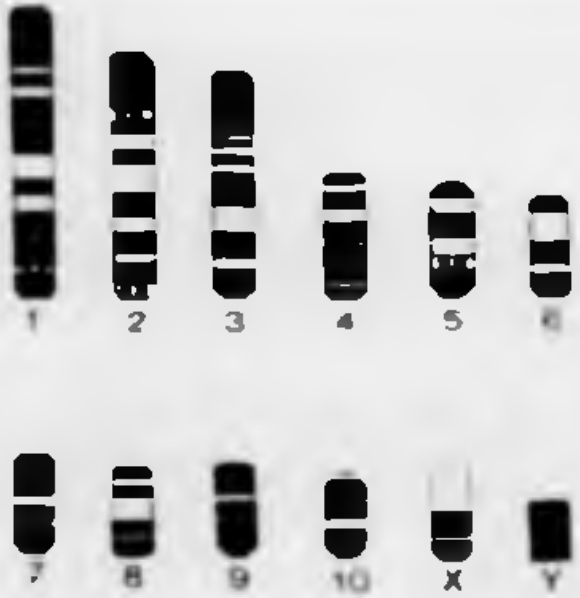


Plate I-15. Idiogram of mitotic chromosome complement of *T. intestinus*.

It is clear from a study of these photographs that very definite distinctions can be made between chromosome pairs within complements and that G-bands provide excellent markers of particular chromosomes. Comparison of the 7 cut-out karyotypes shown a great deal of inter-specific difference which is exhibited also [illegible] (Figures 1.71 and 1.72) provided [illegible] comparisons are examined. Construction of these idiograms revealed sufficient distinguishing features for the recognition of each pair of homologues within each species and although the bands are not as detailed as those found in the large Dipteran [illegible] they are sufficient, it is hoped, to advance in triatomine cytogenetics which has so far been limited to the study of male meiotic chromosomes conventionally prepared and stained with orcein as illustrated by the numerous photographs (Plates 1.1 - 1.15) of meiotic karyotypes in the early sections of this study.

(d) Hybrid species meiosis

Dr. A. Perlowagora-Szumlewicz has been studying the fertility of F_1 hybrids formed by crossing various species of triatomine bugs with a view to the production of sterile males which could perhaps be used in a programme designed to control triatomine populations by releasing [illegible]. She found that the cross T. pseudo-maculata ♀ X T. sordida ♂ and its reciprocal were both semi-fertile and the F_1 males from these crosses were always completely sterile, while the F_1 females had very low fertility. Dr. [illegible] found that the F_1 male sterility was cytogenetic in origin and she sent F_1 and parental males of both species to London for cytogenetic analysis, the results of which are presented here.

Plate 1.23. Metaphase I spermatocyte of T. pseudo-maculata, 2n = 20 + XY. X 2500.

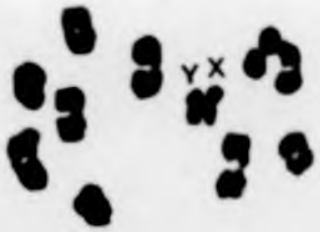


Plate 1.24. Metaphase I spermatocyte of T. sordida, 2n = 20 + XY. X 2500.

Plate 1.25. Metaphase I spermatocyte of T. pseudo-maculata ♀ X T. sordida ♂ F_1 hybrid. Note chain of III and univalents. X 2500.

Plate 1.25. Metaphase I spermatocyte of T. sordida ♀ X T. pseudo-maculata ♂ F_1 hybrid. Note chain of VII and ring of V associations plus univalents. X 2500.

Plate 1a(i). Metaphase I spermatocyte of T. candida ♂ x T. pseudo-maculata ♂ F_1 hybrid. Note chain of IV and ring of IV associations. X 2500.

Plate 1a(ii). Metaphase I spermatocyte of T. candida ♂ x T. pseudo-maculata ♂ F_1 hybrid. Note chain of IV association and 10 univalents. X 2500.

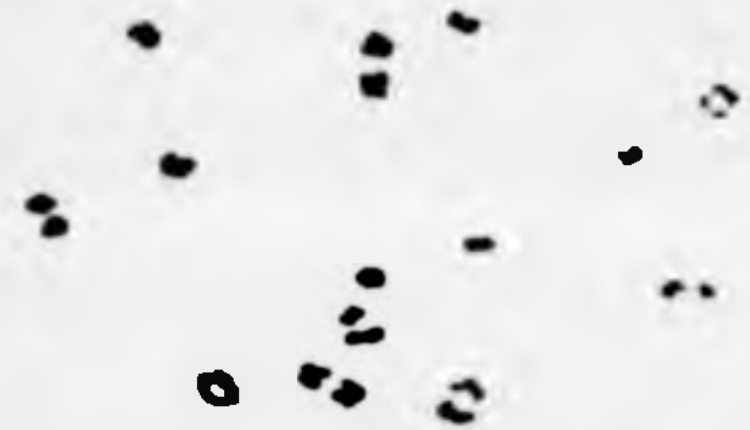


Plate I, 20. Diakinesis spermatocyte of *T. [illegible]* ♀ X *T. [illegible]* ♂ F_1 hybrid. [illegible] 6 bivalents and 8 univalents. X 2500.

Parental strains

(i) T. pseudo-maculata. The karyotype of this species shows no differences at male meiosis from that of T. maculata. Plate 1.23 shows a metaphase I cell from T. pseudo-maculata with 20 autosomes plus an Xf system.

(ii) T. sordida. This species also has 20 autosomes and an XY system although the chromosomes of this species are all somewhat larger than those of T. pseudo-maculata (Plate 1.24).

F_1 hybrids

Meiosis in the F_1 hybrid males formed by crossing these 2 species was characterized by the formation of multiple associations and univalents. Multiple associations assumed a variety of forms in the hybrid males, the most common being chains which varied in size from III (Plate 1.25) to chains of VII (Plate 1.26). Although chains were the most frequently seen in multivalent associations, ring multivalents were also found in these hybrid males and they too varied in number of elements forming them (Plates 1.26 and 1.27). These multivalent associations most probably resulted from interchanges between the 2 parental chromosome sets at meiotic prophase in the hybrid males. As expected in a hybrid between 2 apparently unrelated species, univalent formation was often of a very high order (Plates 1.28 and 1.29), even though the parental types had the same diploid number and it is clear that synapsis failed to occur in many bivalents which might have been considered to be morphological homologous.

DISCUSSION

Karyotypes

The basic chromosome complement or karyotype of a species has proved useful to taxonomists in defining evolutionary relationships

between species within groups naturally defined by morphological and ecological characteristics. Because chromosomes are subject to breakage which may result in the production of inversions and translocations, the karyotype is subject to variation, so that differences between related species which have come about by the accumulation of small changes over long periods may be used to study the evolution of a group. Karyotype evolution may also take the form of changes in the number and/or the morphology of the chromosomes. It is evident from previous studies of the Triatominae that changes have taken place in the number of chromosomes and chromosome mechanisms. The present study of 13 species of bug, all from South or Central America has revealed a variation in diploid numbers from 21 to 24; but only one species showed sex-linked inheritance, all showing 22 autosomes and XY. P. geniculatus with 24, however, showed 22 autosomes and X0 in males as in most triatomine bug and he concluded that the primitive number was 20+ XY for the sub-family. Darlington (1937) suggested that changes in the basic number of chromosomes could occur by means of reciprocal translocations of unequal chromosome sections, producing an increase or decrease in the number. Patterson and Stone (1952) in a study of

changes in the group had come about mainly by a reduction in number of chromosomes by centric fusions, inversions and translocations, thus confirming Darlington's (1937) theory. Such precise studies of chromosome behaviour are fairly easily carried out in the polytene chromosomes of the Diptera which provided large numbers of markers. In the absence of such markers, and with further comparative studies are carried out, it remains difficult to say what constitutes the archetypal karyotype of the Triatominae, but since the condition in P. megistus is unique,

Ueshima (1966) is probably correct in assuming that 20 is the basic autosomal complement of the group.

Much greater variation was found in the numbers of sex-chromosomes in the Triatominae studied. It is generally assumed that the XY system represents the primitive condition for sex-chromosomes (John and Lewis, 1965) and the present study has shown 7 species of triatomine with [illegible] (T. sordida, R. prolixus and R. neglectus). Six species were found to have multiple sex-[illegible], 5 of which were of the type [illegible] (T. [illegible], T. [illegible], T. [illegible], T. protracta and P. megistus) and one, T. vitticeps, which conformed to $X_1X_2X_3Y$. These X-chromosomes behaved in a regular fashion at meiosis, dividing equationally at the first division and segregating from the Y in a group at the second division. Manna (1958, 1962) has shown that compound X-chromosome systems are typical of the Heteroptera and it is generally thought that the extra X-chromosomes have arisen by fragmentation of an archetypal single X and not by alteration of the autosomes. This hypothesis is strengthened by the demonstration that chromosomal fragments induced by irradiation in species with diffuse [illegible] a demonstration which has been confirmed with irradiated R. prolixus in the present work (Part Four), further suggesting that the multiple XnY system of the Triatominae may have arisen by fragmentation of [illegible]. [illegible] number of 20 reinforces the view that X-fragmentation is responsible for the XnY system, otherwise a reduction in the number of autosomes would [illegible] ed.

[illegible] species were available for study in the present work, the karyotypes of which had not previously been described:- T. lenti, [illegible] and T. pseudomaculata. T. lenti had a 20+ X_1X_2Y complement while the two T. maculata sub-species were 20+ [illegible]. Since T. [illegible] by Pinto [illegible] is clearly published on karyotype grounds.

Ueshima (1966) concluded from his study of male meiosis in Triatominae that South American species of the genus were of two [illegible], those with an [illegible] and those with X_1X_2Y systems, but the present study has demonstrated three South American species (T. lenti, T. [illegible], T. phyllosoma) all of which had X_1X_2Y systems. [illegible] are not confined to species in North America as Ueshima (loc. cit.) has suggested.

Female Meiosis.

Female meiosis was studied only in a single species, R. neglectus. [illegible] the maturation divisions were seen, and these were found in eggs which were about to be [illegible]. It is concluded that the first maturation division takes place either at or just before the anterior limit of the common oviduct and sperm enter the micropyle at this point as they pass into the [illegible]. The fully [illegible] may indeed precede the formation of the first meiotic division, but as most [illegible] the ovary of the male genus Rhodnius only the second meiotic division (Wigglesworth, 1965).

Pratt and Davey (1972a) state that work done in their laboratory by Case (1967) showed that the first reduction division of the oocyte nucleus took place in larval R. prolixus, the age of the larvae not

being specified, but it is clear from the present work that Case (1970) was mistaken and that meiosis, as expected, occurs in adult females only.

Banding of triatomine chromosomes

The present work has demonstrated that G-banding techniques, as developed in recent years for mammalian mitotic chromosomes may be valuably applicable to furthering knowledge about insect cytogenetics. The results obtained with R. prolixus and T. infestans demonstrate that good G-banding can be produced [illegible]. A crucial factor in their induction is apparently the initial step of forming a cell suspension. [illegible] cytogeneticists are experienced in preparing cell suspensions in work on blood cultures but insect cytogenetic studies have been restricted to squash techniques which are adequate for the production of C-bands but are not adequate for G-banding which requires separation of cells on the slide and complete spreading of the chromosomes. This was achieved in the present study by using the enzyme trypsin with versene to disperse the cells of triatomine embryonic tissue, the resulting cell suspension being fixed, air-dried and banded using the A.S.G. technique of Sumner et al. (1971). [illegible] of mitotic chromosomes within a species, and between species as briefly published by Mandlin (1974). In the present context this is of particular importance to the further study of triatomine bugs, since [illegible], chromosomes have been of limited use as taxonomic [illegible]. The polytene chromosomes of the Diptera have, of course, provided numerous genetic markers for that group and fostered sophisticated genetic experiments in relation to the control of Diptera in vectors of disease. It is hoped

that the G-banding technique for triatomine chromosomes described here will provide chromosomal markers for further studies of genetical aspects of problems in the systematics, biology and control of triatomine bugs, [illegible] America, possibly for other Hemipteran insects, and perhaps genetical investigations in insects generally.

Hybrid meiosis

Meiosis in male F_1 hybrids from the cross T. [illegible]-maculata X T. sordida was characterized by multiple associations and univalent formation, the multiples commonly taking the form of chains of varying size [illegible]. The production of [illegible] multiples suggests that interchanges were produced in the hybrids between [illegible] prophase. The chains produced are similar in morphology to those demonstrated (see later, Part Four) in R. prolixus as a result of interchange formation following irradiation damage. It is difficult to see how such interchanges could persist in holokinetic bugs with low chiasma frequency and linear orientation of chromosomes. The 'convergent' orientation of chain multiples which can result in genetically balanced gametes was not observed in these hybrids and the maintenance of ring multiples involving 4 or more chromosomes would have required regular pairing and crossing over which would be difficult given the low chiasma frequency of these bugs. It is not surprising, given the high incidence of univalent and multiple formation, that these F_1 males were found to be infertile and that no F_2 generation could be bred (Panzera-Baumelwicz and Correia, 1972).

The only other cytogenetic study of bug hybrids is by Yoshima (1966) who examined the cross [illegible] and found

that the F$_1$ males typically formed univalents at meiosis. But he did not observe any multiple associations. Further studies of hybrids of such species as could be induced to copulate successfully would probably yield information useful to taxonomists on the basis of the pairing affinities of chromosomes of different species.

PART TWO

THE REGULATION OF SPERMATOGENESIS IN MALE *RHODNIUS PROLIXUS*

INTRODUCTION

Although it is now well established that hormones play a major role in the regulation of insect growth, the factors responsible for the initiation and control of spermatogenesis in the developing insect remain the subject of debate. Wigglesworth (1940), from his intensive work with R. prolixus, concluded that there were two major hormonal regulators of growth and differentiation; firstly a moulting hormone [illegible]. Wigglesworth (loc. cit.) showed [illegible] R. prolixus were given a blood-meal, the resultant abdominal stretching provided a nervous stimulus for neurosecretory cells in the brain to discharge their secretion along their axons to the corpus cardiacum where it was [illegible] into the blood as the brain hormone. This brain hormone activated the thoracic gland which in turn secreted the moulting hormone which led directly to the renewal of growth in many organs and tissues of the body, particularly epidermal/cuticular changes associated with the impending ecdysis. The inhibitory hormone secreted by the corpus allatum became operative a few days after the growth and moulting process had already been initiated by the moulting hormone, and for the successive larval stages, prevented the development of imaginal characters. When the final fifth instar R. prolixus larvae moult, they undergo a striking metamorphosis and turn into adults with wings and a totally different pattern in their cuticle. Wigglesworth (1936) [illegible], could be modified by implanting the corpus allatum of earlier stage larvae of R. prolixus into fifth instar larvae; instead of moulting to become adults, the fifth instar larvae moulted to become sixth instar larvae known as supernumerary larvae. Later, Wigglesworth (1968)

suggested that the capacity to form the adult morphology was latent in the larva but was suppressed by the larval genome, but became 'switched on' in the absence of juvenile hormone so that the normal adult form resulted at the final moult.

In holometabolous insects, one instar, the pupa is normally formed between the larva and the adult and metamorphosis starts with pupal formation. The moulting hormone of Holometabola was shown to be similar to the substance found in Hemimetabola. It is neither larval-specific, including moulting in Hemimetabola and puparium formation in Holometabola [illegible]. It is generally responsible for initiating changes throughout the insect body in those tissues which are involved in growth and differentiation [illegible]. The juvenile hormone in Holometabola was shown to be produced in small amounts in the last larval stage, which led to the formation of the pupa, while in the pupa no juvenile hormone was required, allowing the adult to develop.

The life cycle of many insects is interrupted periodically by a phase of arrested development during which metabolism proceeds at a reduced rate, and this arrest of growth is called diapause which is essentially an adaptation to various adverse conditions. Diapause may be divided into two types, firstly, true diapause which occurs independently of changes in the environment and secondly, periods of quiescence produced by adverse environmental conditions such as cold or desiccation. Many factors may be responsible for the induction of true diapause but the most important single factor is said to be day length (de Wilde, [illegible]). Hormones have been implicated in the control of both types of diapause. For example Agrawal (1964) found larval _T. molitor_. Other species of adult blood-meals instead of one large meal, failed to grow because the moulting hormone was not

secreted indicating that growth or moulting could take place in diapausing bugs only if the requisite hormone was given. In a series of experiments with diapausing Lepidoptera, Williams (1942, 1946, 1947 and 1948) showed that the stimulus to diapause in overwintering pupae was the absence of growth promoting factors, the production of which was directed by the brain. Williams (loc. cit.) restored growth to diapausing pupae of the giant silkworm (Platysamia cecropia) by chilling, which stimulated the brain to secrete a hormone which in turn activated the prothoracic gland which caused renewed growth by secreting ecdysone.

Spermatogenesis may be considered as a part of the process of insect growth and differentiation and like all other growth processes may be arrested periodically. Using the silkworm moth Williams (1947) showed that pupal diapause could result in the inhibition of spermatogenesis and, since virtually all aspects of insect metamorphosis were known to involve hormones, Schmidt and Williams (1953) investigated the hormonal control of spermatogenesis using in vitro culture techniques. In the Holometabola all the primary spermatocytes are formed by the end of the larval stage but meiosis and spermiogenesis occur in the pupae. Schmidt and Williams (loc. cit.) found that germinal cysts removed from the testes of diapausing larvae would not develop in their culture medium but, when they added blood from metamorphosing silkworms, meiosis began within 24 hours followed by spermiogenesis. Analysis of silkworm larvae haemolymph at different times during metamorphosis revealed a fluctuation in the content of a non species-specific substance, the "macromolecular factor" (MF), which Schmidt and Williams (loc. cit.) suggested was the hormone necessary for sperm maturation. Butenandt and Karlson (1954) found that injection of ecdysone into diapausing

moth pupae rapidly stimulated spermatogenesis *in vivo* but when ecdysone was added to *in vitro* cultured testes it had no effect (Karlson, 1956). This paradox was resolved by Kambysellis and Williams (1971a and b) who cultured intact diapausing pupal testes of the silkworm *Hyalophora cecropia* and showed that both ecdysone and the "macromolecular factor" were essential for *in vitro* spermatogenesis to proceed. However, removal of the germinal cysts from the testis walls prior to culture showed that spermatogenesis would proceed in the absence of ecdysone and it was concluded that this hormone served only to alter the permeability of the testis wall to the passage of MF which was directly responsible for the induction of spermatogenesis.

The regulation of spermatogenesis in Hemimetabola is different from the Holometabolous process, for in the former some mature sperm may form in the larval stages and spermatogenesis may continue during the adult life. In the [illegible], which is hemimetabolous, meiotic division starts in the fifth instar larva and proceeds, at a slower rate, in the adult. Economopoulos and Gordon (1971) showed that testes taken from fourth instar larvae of the milkweed bug and transplanted into mature male or female adults underwent extensive spermatogenesis and concluded that moulting was not essential for testis differentiation. Since it was known that a high titre of ecdysone was normally found in fifth instar larvae [illegible], Economopoulos and Gordon (1971) suggested that spermatocyte differentiation in the fifth instar was stimulated by some change in the blood composition related to the 'metabolic' chemical changes which followed the moult to the fifth instar, and was not caused by the direct action of ecdysone on the spermatocytes.

This hypothesis was very similar to that proposed by Kambysellis and Williams (1971) for the regulation of spermatogenesis in holometabolous insects.

The juvenile hormone has also been implicated as a regulatory factor in spermatogenesis. Senhal (1968) implanted corpora allata into pupae of the holometabolous lepidopteran Galleria mellonella and found that testis development was inhibited. Takeuchi (1969) found that spermiogenesis did not occur in testes transplanted from third instar larvae of the silkworm Bombyx mori to pupae with intact corpora allata but did occur in about half of the pupae which had their corpora allata removed, and he concluded that the juvenile hormone had an inhibitory influence on spermatogenesis in the silkworm. In the hemimetabolous cockroach Blattella germanica the testis consists of spermatogonia until the fourth instar when differentiation into primary spermatocytes occurs followed by meiosis and later spermiogenesis which continues in the fifth instar and is completed when metamorphosis to the adult occurs (Amerson and Hays, [illegible]). [illegible] found that removal of corpora allata from fifth instar larvae of the cockroach Leucophaea maderae stimulated spermatogenesis in the 'adultoid' insect produced at the next moult and suggested that juvenile hormone normally inhibited sperm differentiation. Economopoulos and Gordon (1971) treated fourth and fifth instar Oncopeltus fasciatus with synthetic juvenile hormone but could find no changes in the process of spermatogenesis in this insect and, contrary to other workers, claimed that juvenile hormone did not play an inhibitory role in spermatogenesis of hemimetabolous insects.

The hemimetabolous haematophagous bug Rhodnius prolixus has five larval stages, in each of which it takes a single large blood-meal and

then proceeds to moult and grow [illegible]. Wigglesworth (1934) showed that starved R. prolixus larvae would enter a state of diapause which was broken by feeding which led to the release of ecdysone and the renewal of growth. Preliminary investigations for the present work had shown that testis differentiation in R. prolixus was similar to that of the milkweed bug (Economopoulos and Gordon, 1971) in that meiotic divisions started in the fifth instar larva. However, R. prolixus, being haematophagous, has a completely different feeding pattern from the plant-sucking bugs and Schreiber et al. (1968) have suggested that meiosis in triatomine bugs was initiated by the blood-meal, but did not present any experimental evidence to [illegible]. Recently Dumser and Davey (1974) have investigated the role played by the juvenile hormone in controlling spermatogenesis in R. prolixus and found that they could produce a dose-response related inhibition of the number of spermatid cysts produced by the bug after topical application of juvenile hormone analogue to fifth instar larval males.

The present work was designed to investigate the influence of diapause and blood-meal on spermatogenesis in R. prolixus and in particular to study their influence on intra-meiotic events.

MATERIALS AND METHODS

Materials

All experiments were carried out on R. prolixus from the L.S.H. and T.M. colony, details of which have already been given.

Method

Bugs were kept in incubators at 25°C (except where specified below) and approximately [illegible] R.H., in 2" x 1" glass tubes and fed on rabbit blood.

1. Preliminary experiments

The process of spermatogenesis in normal, untreated, bugs was first examined by making, at daily intervals, testis squash preparations from larval R. prolixus selected at random from groups of bugs of the same age. This was done for fed and unfed fourth and fifth instar larvae.

The testes were dissected out in insect saline and fixed in 3:1 absolute ethanol:acetic acid and stored at 4°C. Squash preparations were made by tapping out testes on to clean slides in a drop of lacto-propionic-orcein, and squashing them under a coverslip, which was then ringed with rubber solution prior to microscopic examination.

2. Timing of meiosis by autoradiography

Groups of male fifth instar larvae and adults, both fed and starved, were injected with an aqueous solution of thymidine-6-^{3}H (specific activity 5000 μCi/mM, Radiochemical Centre, Amersham, England) using a finely drawn glass pipette attached to a micrometer syringe calibrated to deliver known volumes to the nearest microlitre. Each bug was given approximately 1 μCi ^{3}H-thymidine in 4 μl distilled water injected, via the thin cuticle at the base of the metathoracic legs, into the haemolymph, the puncture being sealed with adhesive wax. For each group of experimental bugs injected with ^{3}H-thymidine, a control group was injected with an equal volume of water.

Insects were randomly chosen from each group and sacrificed at daily or 12 hourly intervals, testes were dissected out under saline, fixed in 3:1 absolute ethanol:acetic acid and stored at 4°C. Following hydrolysis in 5N HCl for 1 hour at room temperature, testes were stained by the Feulgen reaction and squash preparations of whole testes made in a drop of 45% acetic acid on acid-cleaned slides. Preparatory to

applying the photographic emulsion, coverslips were removed with a razor blade after the preparations had been frozen in liquid nitrogen, and the slides then plunged into absolute ethanol, transferred via 85%, 70%, 55%, 40%, 15% and 10% ethanol to distilled water and allowed to air dry overnight in a dust-free atmosphere.

Autoradiographs were prepared by dipping the slides into a Coplin jar containing [illegible] Nuclear Research Emulsion (Ilford Ltd., Essex, England) diluted 1:1 with distilled water, the emulsion being allowed to dry, and the slides then being placed in light-tight boxes for exposure for 7 weeks at -20°C. The autoradiographs were developed in Kodak D19 developer (Kodak, London, England) for 4 minutes and fixed in Kodak Amfix for two minutes. After rinsing and drying, autoradiographs were permanently mounted in Depsral.

3. RNA synthesis during meiosis

A group of fifth instar male P. prolixus which had moulted from the fourth instar 21 days previously was divided into two groups, one of which was given a blood meal and the other left unfed. Eight days after this feed testes were removed from the larvae by vivisection under insect saline and placed in solid watchglasses containing pre-warmed sterile insect saline with uridine-5,6-^{3}H (specific activity 53,000 μCi/mM, Radiochemical Centre, Amersham, England) at a concentration of 100 μCi per 0.1 ml saline (Henderson, 1964). Testes from bugs, in groups of five, were incubated at 27.5°C for ½ hour and 1 hour while control groups were incubated in insect saline. Following incubation, testes were washed in two changes of insect saline and fixed in 3:1 absolute ethanol:acetic acid and stored at 4°C until preparations were made.

Squash preparations for each group were made on clean slides in

45% acetic acid after passing through the alcohol series to distilled water. All except two preparations from each group which were discarded. These two slides from each experimental group were incubated for 1 hour at 37°C in a solution of Chemicase (B.D.H., Poole, England) at a concentration of 0.1 mg/ml in phosphate buffer pH 6.5 (Markenthaler, 1964), rinsed in distilled water and air-dried.

All slides were dipped in L4 nuclear emulsion, exposed for 7 days at -20°C, developed, fixed and rinsed in distilled water for 15 minutes. The slides were then soaked in acetate buffer pH 3.6 and stained for one minute in a 0.1% solution of Toluidine blue made up in the same acetate buffer. Following a rinse in two changes of distilled water, the preparations were air-dried and mounted in Euparal, ready for examination by light microscopy for evidence of RNA synthetic activity.

RESULTS

1. Preliminary investigations of spermatogenesis in R. prolixus

The structure and cytology of the testis of triatomine bugs have been described in detail by Barth (1956a and b) using T. infestans, and this is outlined below for R. prolixus, supported by observations made for the present work on testes from fourth and fifth instar larvae and adult males of R. prolixus, to serve as a foundation for the more elaborate studies which were planned. In the adult, each testis is made up of seven follicles, two of which are much larger than the rest, and each follicle has a germarium at its apex consisting of primordial germ cells. The spermatogonia produced by the germ cells pass down the follicle, divide repeatedly and become covered by a mantle of somatic cells to form a cyst, mature cysts containing up

to 256 spermatogonia (Barth, 1956b). The spermatogonia then become spermatocytes which undergo meiosis.

The successive stages of normal spermatogenesis are illustrated by the photomicrographs (Plates 2.1 - 2.14) of testis squashes of adult male R. prolixus. Plate 2.1 shows a gonial cell at mitotic metaphase which eventually leads to the formation of spermatocytes which undergo meiosis starting with prophase leptotene (Plate 2.2). This in turn is followed by zygotene (Plate 2.3) and pachytene (Plate 2.4). Prophase in Heteropteran meiosis is characterised by a 'diffuse stage', following pachytene, during which the autosomes lose their affinity for nuclear stains but the sex-chromosomes (X,Y) remain heteropycnotic; this stage is shown in Plate 2.5. The 'diffuse stage' is followed by a normal diplotene (Plate 2.6), diakinesis (Plate 2.7) and the first spermatocyte division (metaphase 2.8) at which point the sex-chromosomes are metaphasic and the autosomes have become polarised. The first metaphase is followed by anaphase and telophase I (Plate 2.9) in which the sex-chromosomes lie at the centre of a ring of autosomes, and the second meiotic division follows immediately with metaphase II (Plate 2.10), anaphase II (Plate 2.11) and telophase II. There then follows the process of spermiogenesis by which the rounded primary spermatids (Plate 2.12) are converted into elongated secondary spermatids (Plate 2.13) and finally into mature filamentous spermatozoa (Plate 2.14).

Using this knowledge of the stages of spermatogenesis, a preliminary comparison was made of the testes of fed and unfed fourth, and fed and unfed fifth larval instars, and of male adults allowed to moult through from fed fifth larval bugs. Methods for squash preparation and staining with orcein were as given above (Part One).

Plate 2.1. Gonial mitosis from R. prolixus testis at metaphase. X 2500

Plate 2.2. Leptotene nucleus from R. prolixus testis. X 2500.

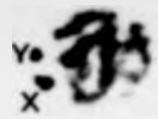


Plate 3.3. Zygotene nucleus from *T. spatiosum* testis. Note distinct heteropycnotic X and Y. X 2500.

Plate 3.4. Pachytene nucleus from *T. spatiosum* testis. X and Y still heteropycnotic. X 2500.

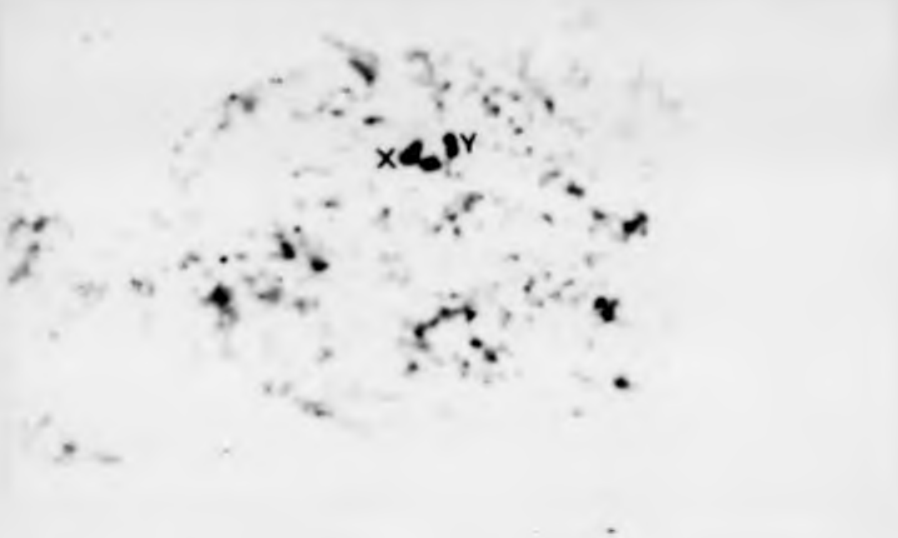


Plate 2.5. 'Diffuse stage' of meiotic prophase from R. prolixus testis. Note euchromatic, diffuse autosomes. X 2500.

Plate 2.6. Diplotene spermatocyte from R. prolixus testis. Autosomes now more deeply stained. X 2500

Plate 3.3. Spermatocyte of Blattella from G. portalis testis. X 2500.

Plate 3.4. Metaphase I spermatocyte from G. portalis testis. Autosomes are more deeply stained than sex-chromosomes. X 2500.

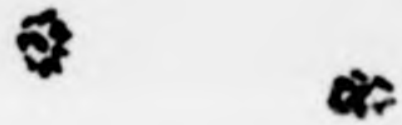


Plate 2.9. Telophase I from R. prolixus testis. Note sex-chromosomes surrounded by a ring of autosomes. X 2500.

Plate 2.10. Metaphase II from R. prolixus testis. Note X and Y in centre of a ring of more deeply staining autosomes. X 2500.

Plate 2.11. Anaphase II from R. prolixus testis. X 2500.

Plate 2.12. Rounded primary spermatids from R. prolixus testis. X 2500.

Plate 2.13. Primary and secondary spermatids from R. prolixus testis. X 2500.

Plate 2.14. Mature spermatozoa from R. prolixus testis. X 2500.

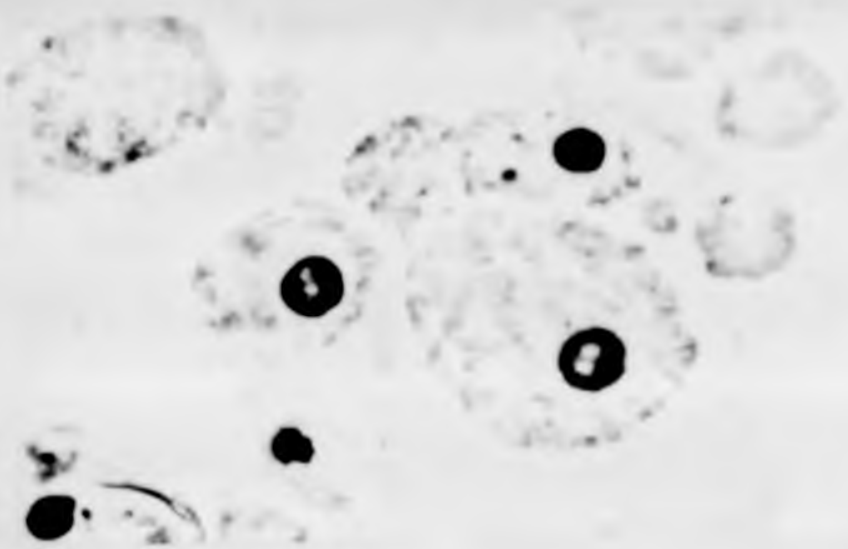


Plate 2.15. 'Aborted' spermatids from R. prolixus male in diapause.
X 2500.

Unfed fourth instar larvae. A large group of fourth instar larvae which had all moulted to this instar on the same day were sampled daily, commencing 14 days after the moult and continuing for 30 days, no blood-meal being given. Two insects were sampled on each day and testis squashes prepared from each one. These testes were all very small on dissection and the squash preparations showed that spermatogenesis had not been initiated in these unfed bugs, not even gonial divisions, as late as 44 days after the previous ecdysis.

Fed fourth instar larvae. A group of fourth instar larvae, all of the same age, were fed on rabbit blood on the same day and sampled at daily intervals for 7 days. At day 3 the first gonial mitoses were detected in squash preparations and these continued up to day 7 with no more advanced stages being seen up to that time. The bugs moulted about the 12th day.

Fed fifth instar larvae. A large group of these larvae was fed at fourth instar on the same day and those bugs which moulted to the fifth instar, 12 days later, were kept unfed until, at 28 days after the moult (experimental day 0), the testes were dissected and squash preparations made from 5 of them. This was repeated with 5 more of the bugs on every other day up to experimental day 40. On microscopic examination, the follicles of these diapausing larvae were found to be populated largely by two cell types, firstly diffuse stage spermatocytes (Plate 2.5) and secondly what appeared to be 'aborted' primary spermatids. Very few cells in the 1st or 2nd meiotic divisions were seen in these preparations compared with normal testes from fed adults. Secondary spermatids and mature spermatozoa were completely absent from all these larval testes. The 'aborted' primary spermatids were of particular interest and their numbers appeared to increase

proportionately as the [illegible] lengthened. Plate 2.15 shows 'aborted' spermatids photographed in a squash from the testis of a diapausing fifth instar larva. These spermatids were more densely stained than normal (see Plate 2.12) and appeared to undergo a process of degeneration during which their volume increased greatly [illegible]. These [illegible] degenerating passively or being actively disposed of by the insects.

[illegible]. A large group of male larvae was fed on fourth instar [illegible] on the same day and allowed to moult into fifth instar larvae. These were next fed 28 days after the moult (experimental day 1). After this meal 2 bugs were selected from the group and their [illegible] testes, and 2 bugs were dealt with in this way daily for a further 24 days, on into the adult stage into which they moulted in about 18 days. Examination of these squashes revealed great differences between these testes and those of the unfed diapausing fifth larval bugs. In the fed bugs 1st and 2nd meiotic divisions were abundant by 3 days after the blood-meal. This wave of meiosis was followed on day 10 by the first signs of spermiogenesis and on day 13 the first mature spermatozoa were seen.

These bugs moulted to the adult stage about day 18 after the blood-meal and examination of the adult testes continued for 6 more days during which time they were still actively undergoing meiotic [illegible]. [illegible] found in unfed fifth instar larvae were not seen in fed fifth instar larvae after the first 3 days following the blood-meal.

Having completed these preliminary investigations of spermatogenesis in *R. prolixus* the following conclusions were drawn:-

1. Testis differentiation commences in fourth instar males following a blood-meal.
2. Spermatocyte production does not start until the [illegible] instar.
3. [illegible] instar larvae and the primary spermatids produced develop no further and 'abort' during diapause.
4. Following a blood-meal, fifth instar larval spermatocytes rapidly undergo meiosis and the process of spermiogenesis is inaugurated. Some 'aborted' primary spermatids are evident only for about 3 days after the blood-meal and these are, presumably, those which form during the pre-feed period, in this case 28 days.

2. Timing of meiosis

Because of the apparent importance of the blood-meal to the process of spermatogenesis, autoradiographic experiments were carried out to time the process in both fed and unfed bugs to determine if the blood-meal affected the rate of intra-meiotic events. The regulation of the meiotic process, whilst of scientific interest, must also be considered of importance in relation to the control of this vector species. The inhibition of spermatogenesis may be a means of producing sterile males for population control purposes.

The principle involved in these experiments is well established. It involves simply recording the most advanced cell stage which is labelled at different time intervals after a 'pulse' label of ^{3}H-thymidine is given, the first labelled cells to reach a particular

meiotic stage being those which incoporated the thymidine at the end of the preceding DNA replication period or S-phase (Monesi, 1962; Muckenthaler, 1964; Taylor, 1965; Callen and Taylor, 1968; Moens, 1970; Coggins and Gall, 1972). Selman and Kafatos (1974) have recently shown that nearly all ^{3}H-thymidine is incorporated into DNA when injected into insects and after a single injection is rapidly cleared from the haemolymph, resulting in a maximum 'pulse' of 4 hours. The sampling interval between injection and examining insects on the first occasion and subsequently in the present experiments, was a minimum of 12 hours which in view of the work of Selman and Kafatos (loc. cit.) must be given an error margin of 4 hours. In scoring labelled slides, comparison was made throughout with control autoradiographs from bugs injected with water.

Fed fifth instar larvae

(a) At 25°C. Bugs used in this experiment were fed simultaneously as fourth instar larvae and again as fifth instar larvae 28 days after the fourth instar feed. The bugs were then injected 7 days after the blood-meal (experimental day 0). The rate of progress of cells through the process of meiosis for fed fifth instar larvae kept at 25°C is shown in Fig. 2.1 in which each line represents the stage(s) found labelled within a single insect. It may be seen from this figure that meiosis was completed in 12 days at this temperature, the longest stage being the 'diffuse stage' which lasted about 4 days.

(b) At 27,5°C. Bugs for this experiment were of the same age and received the same treatment as fed fifth instar larvae kept at 25°C. The results of this experiment are given in Fig. 2.2 which shows that at this higher temperature meiosis was completed in 11½ days.

It is clear from a comparison of Figs. 2.1 and 2.2 that increasing

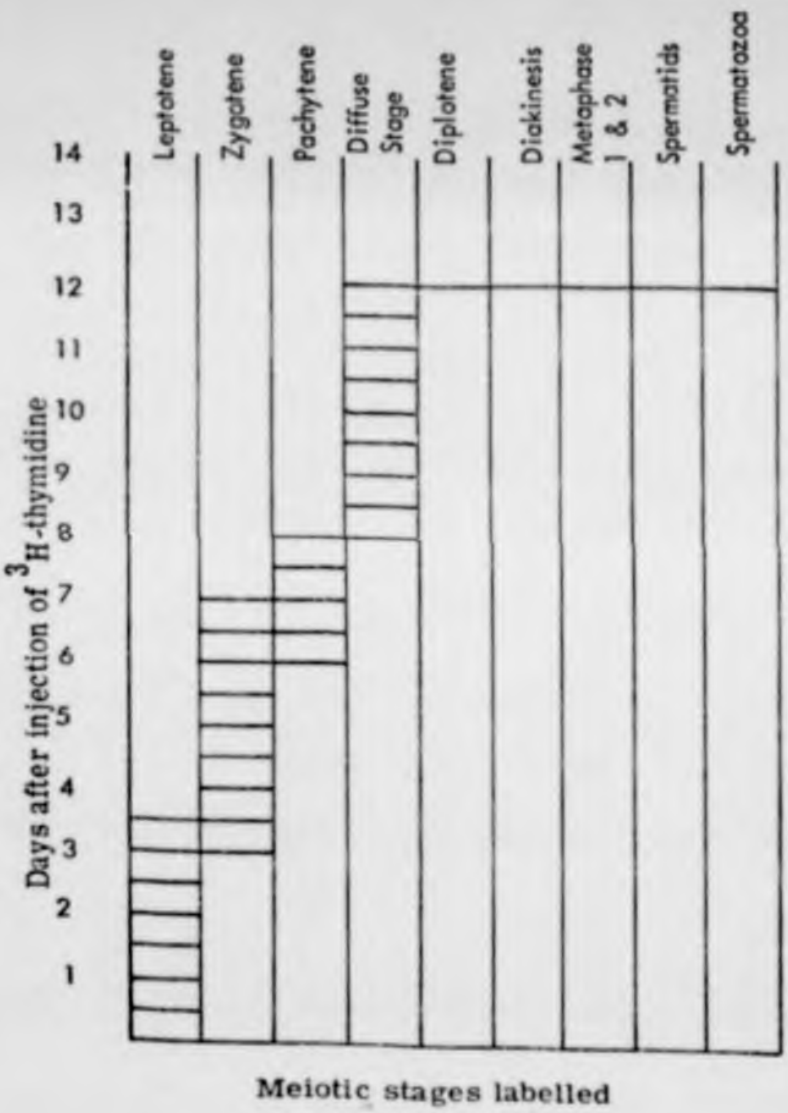
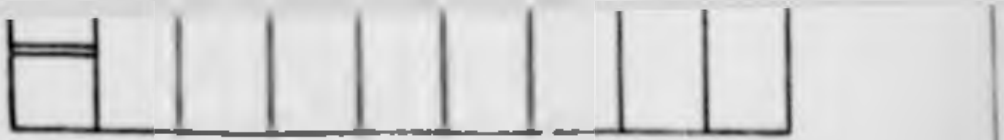


Figure 2.1. Timing of meiosis in Rhodnius prolixus fed fifth instar larvae at 25°C. Each line represents the stages labelled within a single insect.



Meiotic stages labelled

Figure 2.2. Timing of meiosis in Rhodnius prolixus fed fifth instar larvae at 27.5°C. Each line represents the stages labelled within a single insect.

Days after injection of ^{3}H-thymidine

2 3 4 5 6 7 8 9 10 11 12

Leptotene

Zygotene

Pachytene

Diffuse Stage

Diplotene

Diakinesis

Metaphase 1 & 2

Spermatids

Spermatozoa

environmental temperature shortens the time spermatocytes take to pass from early prophase to mature spermatozoa. As at 25°C, so for 27.5°C, spermatocytes on leaving the 'diffuse stage' passed through diplotene, diakinesis, both meiotic divisions and spermiogenesis in less than 24 hours. Fig. 2.2 shows that the first labelled metaphase II cells were seen on day 10 but the first labelled spermatozoa were not seen until day 10½ which suggests that spermiogenesis can occupy about 12 hours at this temperature.

Unfed fifth instar larvae

The bugs used in this experiment were all fed as fourth instar larvae on the same day, allowed to moult to fifth instar larvae but these were not fed. Experimental day 0, when the ^{3}H-thymidine was injected, was 35 days after the fourth instar blood meal was given, and approximately 21 days after the moult to the fifth instar. This long period of starvation ensured a state of diapause (Wigglesworth, 1934).

The results of this experiment are given in Fig. 2.3 which shows that, in diapausing fifth instar larvae, meiosis takes approximately twice as long as in fed fifth instar larvae at the same temperature. Meiosis took as long as 20½ days in the unfed fifth instar larvae compared with only 12 days in fed larvae. Healthy spermatids and mature spermatozoa were not found in the testes of unfed bugs and the results show that the first labelled 'aborted' spermatids appeared on day 21. Whilst the prophase stages of leptotene, zygotene and pachytene occupied approximately the same time periods in fed and diapausing larvae, the 'diffuse stage' lasted from day 11 to day 20 in unfed bugs (a period of 9 days) while in fed bugs it occupied only 4 days.

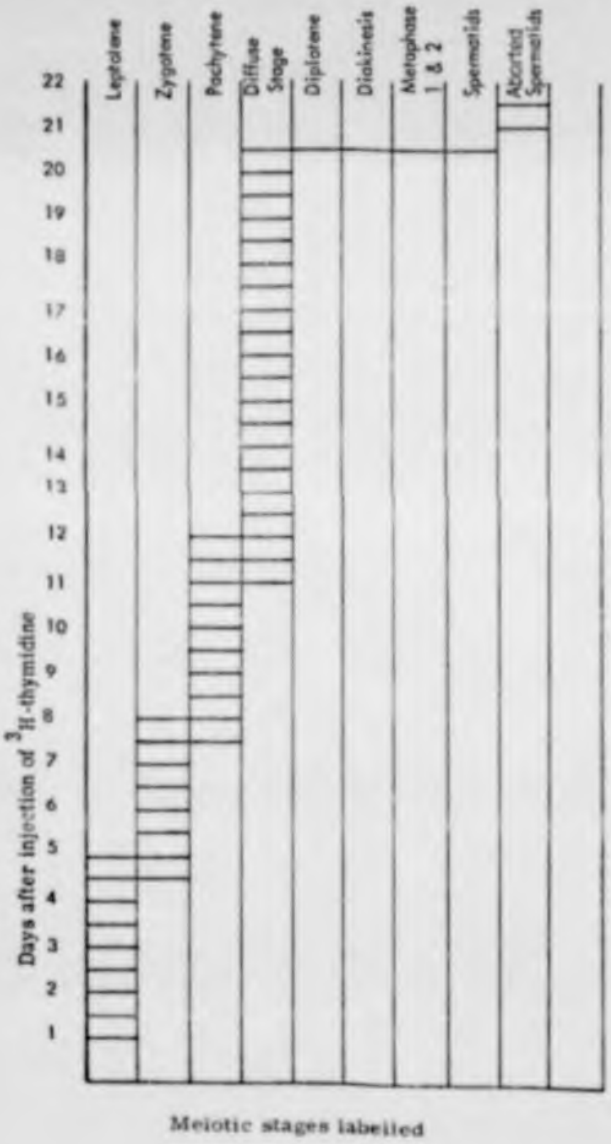


Figure 2.3. Timing of meiosis in Rhodnius prolixus unfed fifth instar larvae at 25°C. Each line represents the stages labelled within a single insect.

Fed adults

Adult males, which, as fifth instar larvae had been fed on the same day, were fed again as adults 40 days later, and then injected with ^{3}H-thymidine 7 days after this adult blood-meal (experimental day 0). The results (Fig. 2.4) show that the successive stages occupied approximately the same time periods in fed adults as they did in fed fifth instar larvae (Fig. 2.1). The process of meiosis was completed in 12½ days in fed adults. Some of the labelled stages in this experiment are shown in photomicrographs or Plates 2.16 - 2.21. Plate 2.16 shows a labelled zygotene cell from a fed adult and the other labelled stages shown are pachytene (Plate 2.17), 'diffuse stage' (Plate 2.18), metaphase I (Plate 2.19), metaphase II (Plate 2.20) and Plate 2.21 shows labelled spermatozoa.

Unfed adults

The males used in this experiment were fed as fifth instar larvae on the same day as in the above experiment with fed adults, but they were not given a blood-meal after moulting to adults. They were injected with ^{3}H-thymidine 47 days after feeding as fifth instar larvae (experimental day 0) and approximately 35 days after they had moulted to adults.

The results of this experiment are given in Fig. 2.5 which shows that meiosis occupied 16-17 days in these diapausing adult males as opposed to 12½ days for fed adult males. Some labelled spermatozoa as well as labelled 'aborted' spermatids were found in the unfed male adults, suggesting that spermiogenesis is not completely inhibited in diapausing adults. Nevertheless, the fact that no labelled metaphase divisions or spermatids were seen in these preparations (Fig. 2.5) suggests that few cells were passing through division or

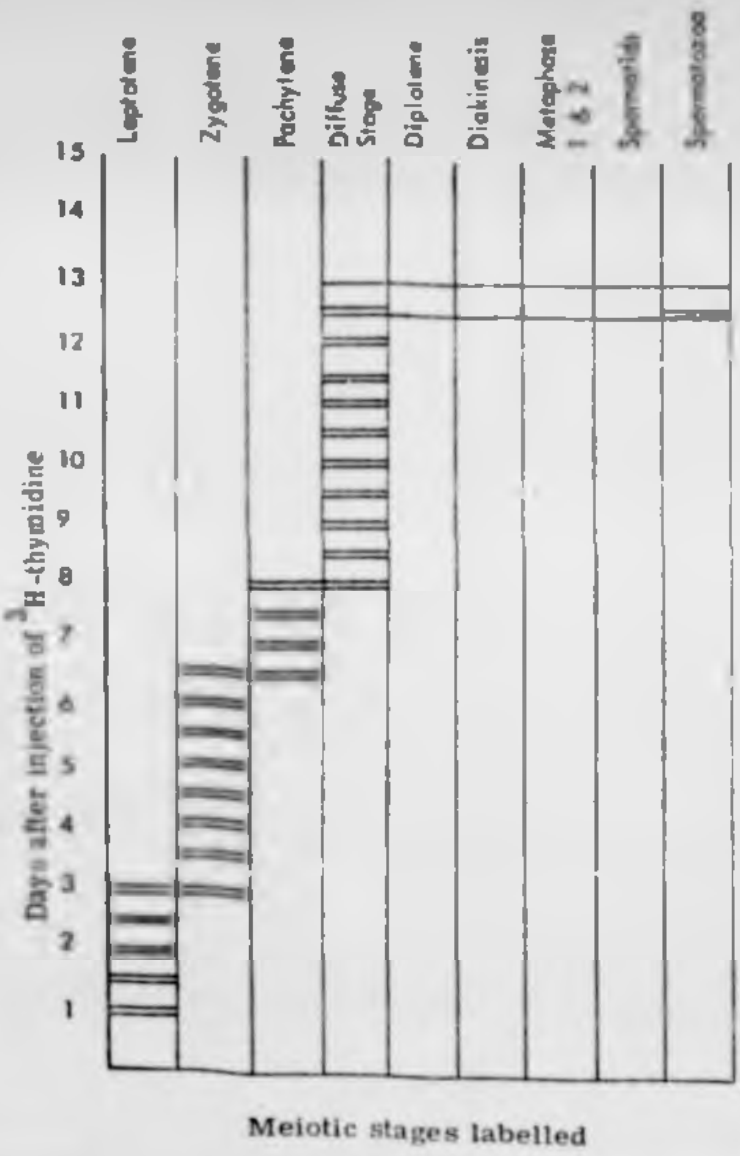


Figure 2.4. Timing of meiosis in Rhodnius prolixus fed adults at 25°C. Each line represents the stages labelled within a single insect.

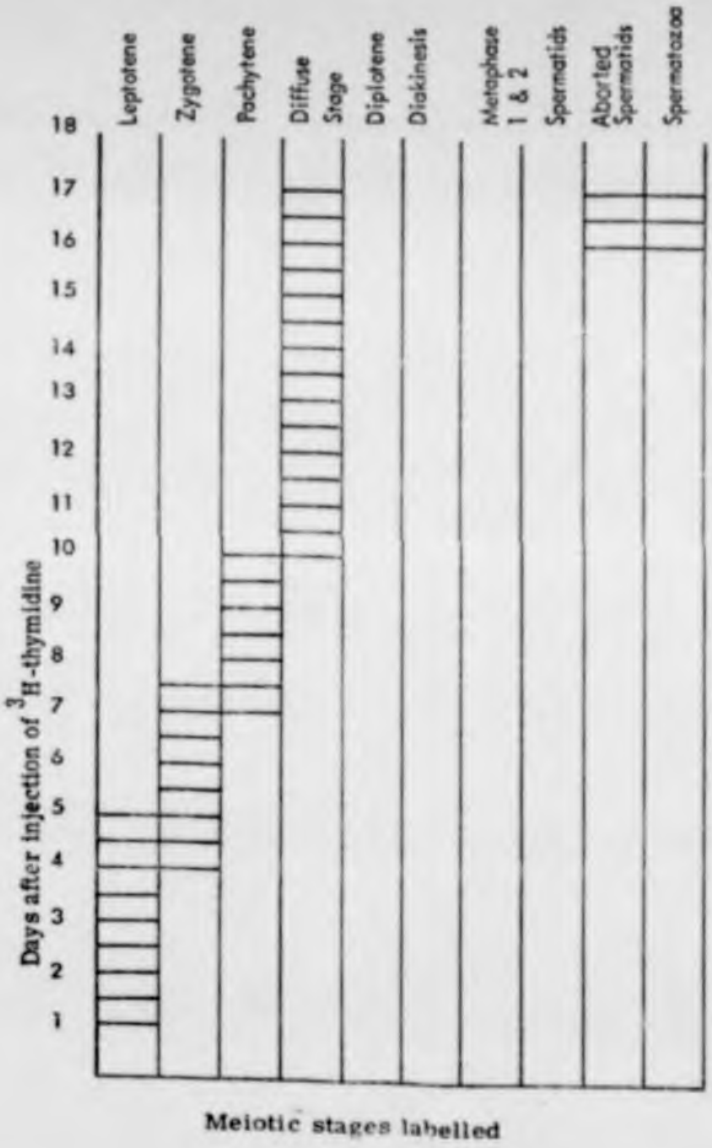


Figure 2.5. Timing of meiosis in Rhodnius prolixus unfed adults at 25°C. Each line represents the stages labelled within a single insect.

Plate 2.16. Autoradiograph from adult H. brevicaule testis labelled with ^{3}H-thymidine showing zygotene nucleus. X 2500.

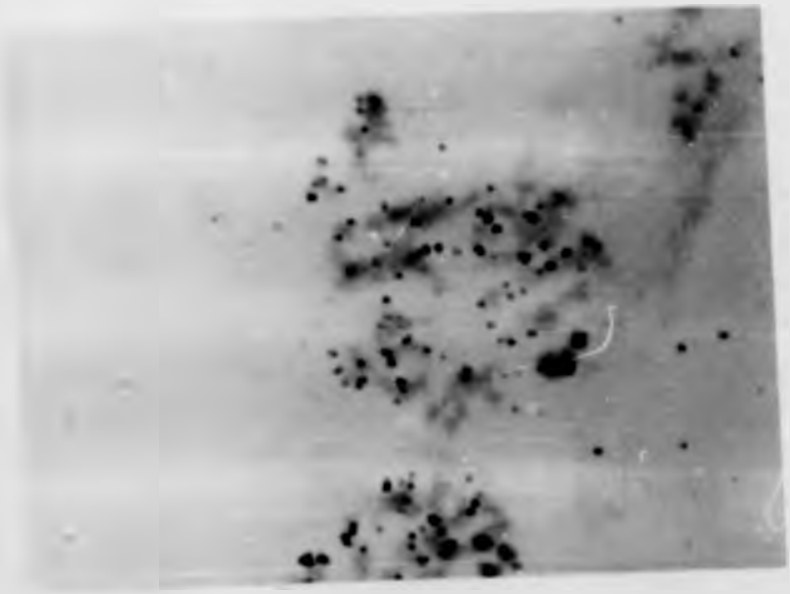


Plate 2.17. Autoradiograph from adult H. prolixus testis labelled with ^{3}H-thymidine showing labelled pachytene nucleus. X 2500.

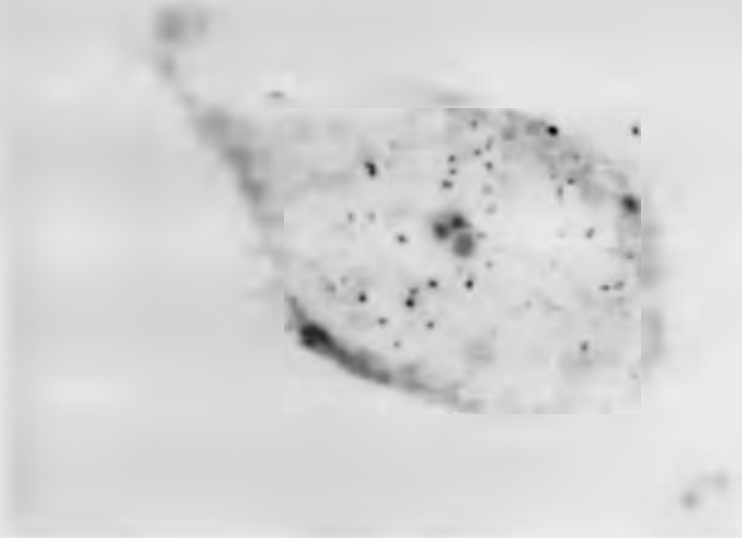


Plate 2.18. Autoradiograph from adult R. prolixus testis labelled with ^{3}H-thymidine showing labelled 'diffuse stage' nucleus. X 2500.

Plate 2.19. Autoradiograph from adult R. prolixus testis labelled with ^{3}H-thymidine showing labelled metaphase I nucleus. X 2500.

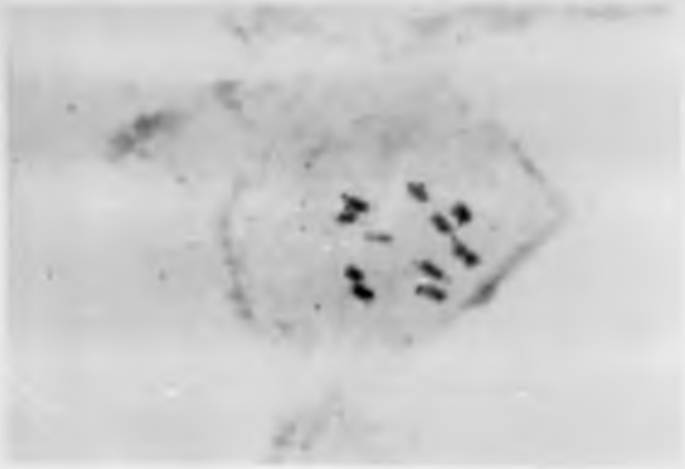


Plate 2.20. Autoradiograph from adult R. prolixus testis labelled with ^{3}H-thymidine showing labelled metaphase II nucleus. X 2500.

Plate 2.21. Autoradiograph from adult R. prolixus testis labelled with ^{3}H-thymidine showing labelled spermatozoa. X 2500.

spermiogenesis and that inhibition of these processes was severe in these diapausing adults.

As in the unfed fifth instar larvae, the 'diffuse stage' was [illegible] days longer than expected also occurred.

In summary, these autoradiographic studies show that the rate of meiosis in *R. prolixus*, judged by the first appearance of successive labelled stages, varies according to the nutritional status of the bug. Thus, at the same temperature, fed fifth instar larval spermatocytes took about 12 days to pass through meiosis, while in unfed larvae the process occupied about 21 days, this difference being accounted for mainly by variation in the amount of time cells spent in the 'diffuse stage'. The rate of meiosis appeared to be very similar in fed adults and fed fifth instarlarvae, both groups taking about 12 days. However, cells of diapausing larvae took 5 days longer to complete meiosis than those of diapausing adult cells, which may be related to the degree of starvation of the two groups.

3. RNA synthesis during spermatogenesis

Because of the difference found between fed and unfed bugs in the time taken to pass through the different spermatogenic stages, it was decided to compare the process of RNA synthesis in fed and unfed insects in case if differences could be detected in cell synthesis related to the nutritional status of the bugs.

The incorporation of ^{3}H-uridine into the nuclei of spermatocytes of fifth instar larvae was examined by grain counts made on the nuclei of unextracted squash preparations made from the testes of fed and unfed fifth instar larvae incubated for ½ and 1 hour in the

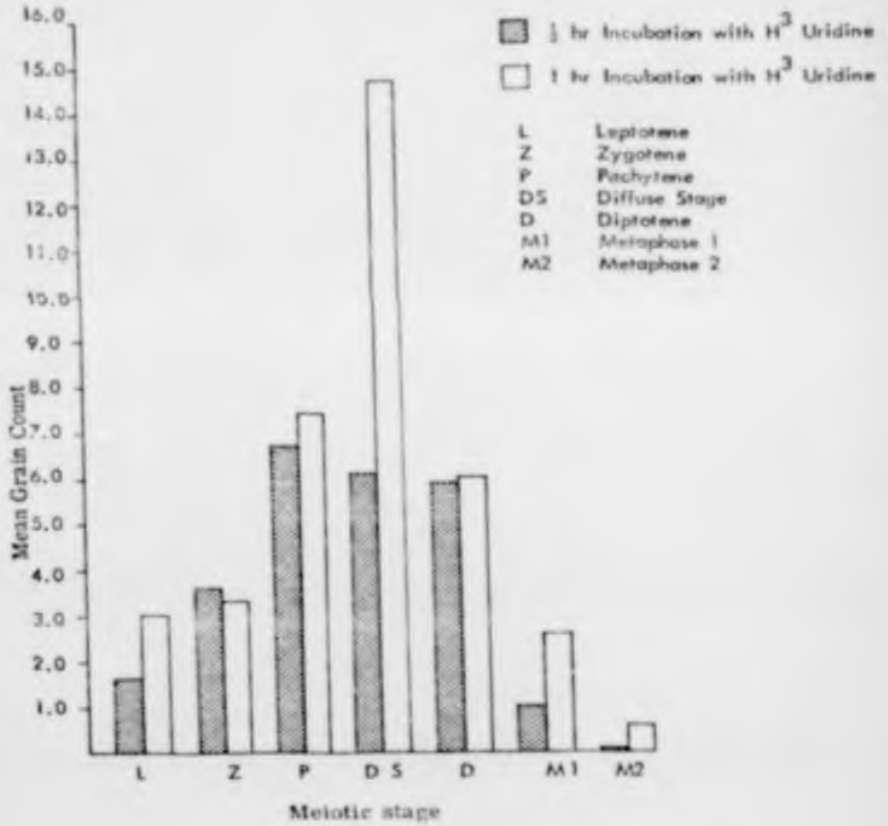


Figure 2.6. RNA synthesis in Rhodnius prolixus fed fifth instar larval testis.

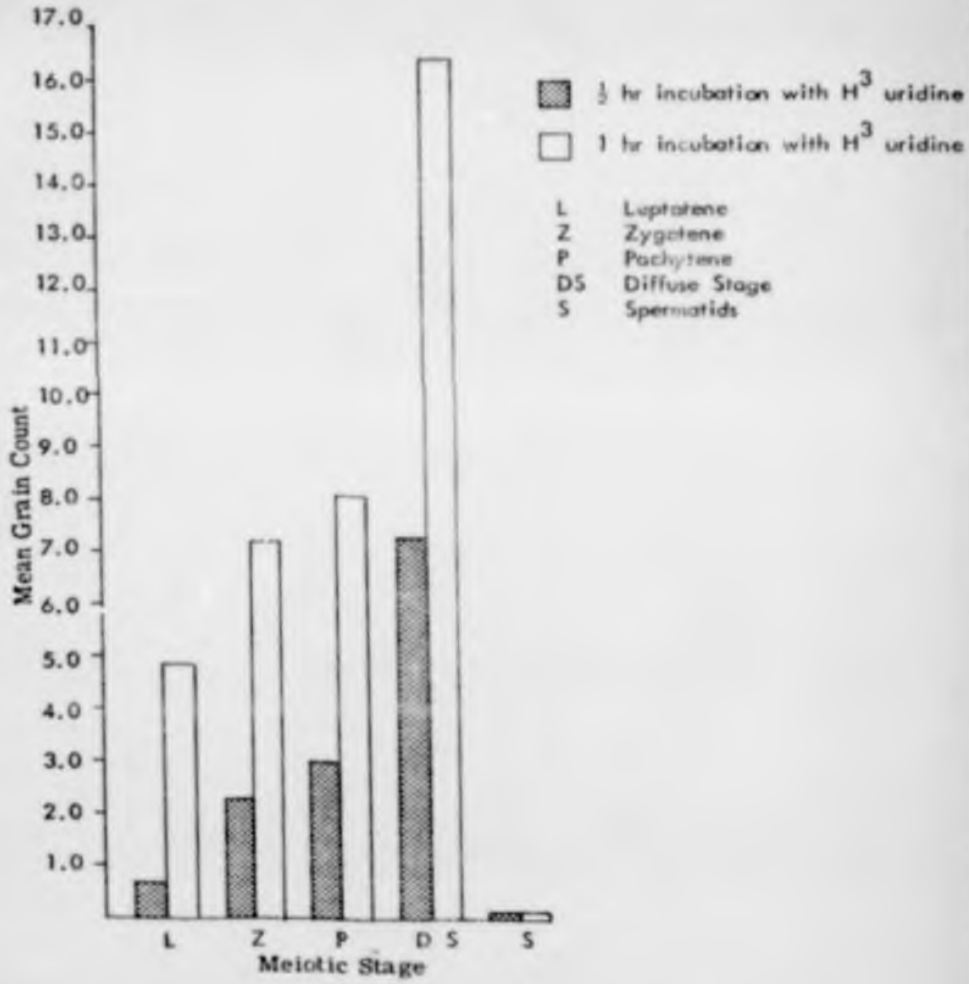


Figure 2.7. RNA synthesis in Rhodnius prolixus unfed fifth instar larval testis.

labelled precursor. The slides extracted with RNA-ase showed no evidence of any labelling and did not differ from untreated control slides, which confirmed that the labelled slides were indicating synthesis of RNA.

Grain counts were carried out over 10 cells of each meiotic stage from each preparation, both control and labelled, and the mean cell count was calculated by taking the mean count for 10 labelled cells of any one stage and subtracting the mean score of 10 cells of the same stage in the control slides. In practice, the counts from the control slides were very low, often scoring zero.

The results of this experiment are shown in Fig. 2.6 for fed fifth instar larvae and Fig. 2.7 for unfed fifth instar larvae.

These figures show that the labelling patterns were very similar for both the ½ and the 1 hour incubation periods. Throughout meiotic prophase spermatocyte nuclei were actively synthesising RNA in both fed and unfed larvae. In fed and in diapausing larvae the 'diffuse stage' nuclei were found to be the most heavily labelled and by inference the most actively synthetic. High levels of nuclear labelling continued up to diplotene in the spermatocytes of fed larvae and the level of labelling fell off rapidly in cells engaged in the meiotic divisions. There was no evidence of any RNA synthesis during spermiogenesis in these fed bugs.

In the testes of diapausing larvae [illegible] diplotene, diakinesis or metaphase stages were not found and, as in the studies of ^{3}H-thymidine incorporation, there appeared to be no spermiogenesis taking place in these testes and the 'aborted' spermatids showed no evidence of synthesising RNA.

Plate 2.22 shows a 'diffuse stage' spermatocyte labelled with

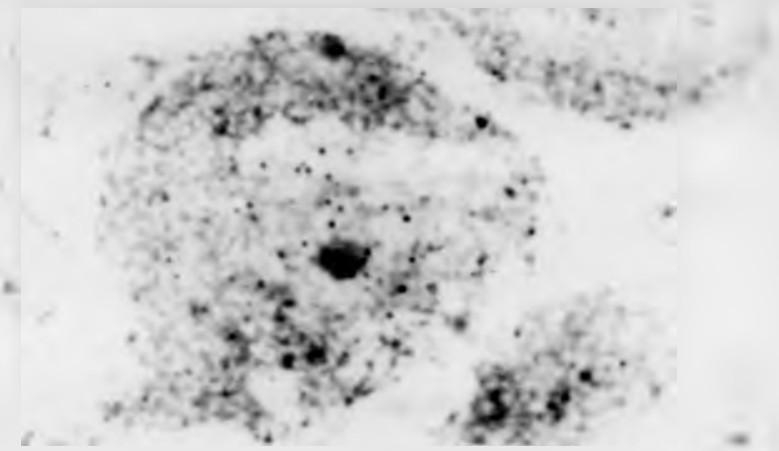


Plate 5.10. Autoradiograph from dispersed testis tubule of *R. sanguinus* testis labelled with ^{3}H-uridine showing labelled 'mature stage' nucleus. X 2500.

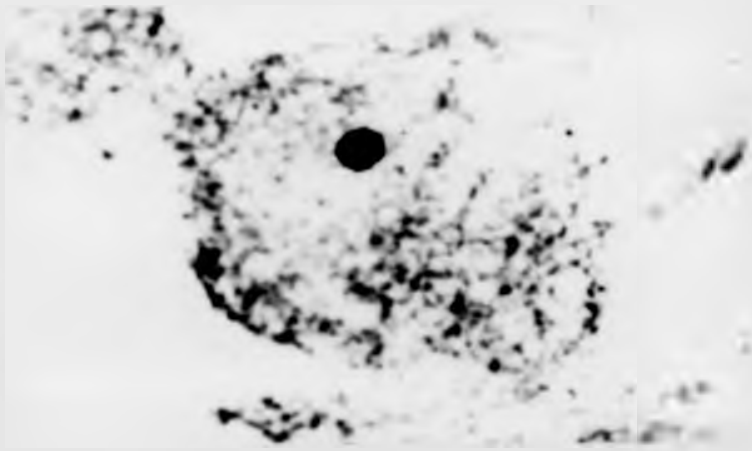


Plate 5.11. Autoradiograph from dispersed testis tubule of *R. sanguinus* testis labelled with ^{3}H-uridine showing unlabelled 'aborted' spermatid. X 2500.

^{3}H-uridine from the testis of a diapausing larva after 1 hour incubation in the precursor and the high level of label indicating synthetic activity in readily observable. Plate 7.3 shows an 'aborted' spermatid from the same bug which indicates that there was no RNA synthesis taking place in these nuclei. These results suggest that the elimination of 'aborted' spermatids may be a passive process in the diapausing bug involving little or no nuclear transcription of RNA.

DISCUSSION:

A preliminary examination of squash preparations taken from testes of fourth and fifth instar male larvae killed sequentially revealed that mature flagellated spermatozoa were first produced in fifth instar larvae after a blood-meal. In unfed fifth instar larvae, spermatocytes passed through meiosis and rounded primary spermatids were produced, but spermiogenesis, the process by which spermatids are converted into flagellated spermatozoa (Wilson, 1925), did not take place and instead primary spermatids became densely stained and apparently 'aborted'. Spermiogenesis was rapidly initiated in diapausing fifth instar larvae given a blood-meal so that prior to the moult to the adult form, R. prolixus males were found to have a supply of mature spermatozoa. These preliminary investigations were in close agreement with the results of Dumser and Davey (1974) who studied sections of R. prolixus testes and who further suggested that 'aborted' spermatids which they found in diapausing bugs were removed by autolysis.

The critical factors affecting production of mature spermatozoa in R. prolixus appeared to be age and nutritional status; age being

critical since testis differentiation would not take place in fourth instar larvae, and the blood-meal being critical since spermatogenesis would proceed only as far as the production of primary spermatids in diapausing fifth instar larvae. This critical effect of age is similar to that demonstrated by Economopoulos and Gordon (1971) in the milkweed bug testis which they attributed to chemical change in the insect associated with age. The critical sensitivity of testis development to bug age suggests the influence of a genetic mechanism in R. prolixus switching on the process of differentiation in a manner analogous to that proposed by Wigglesworth (1968) for other adult structures in [illegible] suggested that the juvenile hormone is responsible for the inhibition of spermatogenesis in R. prolixus [illegible] since secretion of juvenile hormone is prevented in fifth instar R. prolixus [illegible], however, the present work has shown that diapausing fifth instar larvae will not produce mature spermatozoa and therefore some signal or product associated with the blood-meal is necessary for spermatogenesis to be completed. The present experiments do not reveal the nature of such a signal; it may be that the situation in R. prolixus parallels that in Holometabola in which a blood-borne macromolecular factor (MF) is apparently required to break the diapause inhibition of spermatogenesis in silkworm pupae (Kambysellis and Williams, 1971a and b).

Although the precise nature of the blood-meal stimulus to spermatogenesis was not determined, the effect of the blood-meal on the rate of spermatogenesis has been investigated by meiotic timing techniques using ^{3}H-thymidine label. Spermatocytes of fed fifth instar larvae kept at 25°C passed through meiosis in approximately 12 days while at [illegible]

between temperature and rate of meiosis is well documented for other insects (Craig-Cameron and Jones, 1970) and was not of primary interest in the present experiments except to note that all comparative studies were carried out at 25°C. A further experiment showed that meiosis from leptotene to primary spermatid stage took approximately 21 days in diapausing fifth instar larvae, 9 days longer than in fed larvae, a difference which was largely accounted for by the time taken for spermatocytes in the unfed bugs to pass through the 'diffuse stage' of meiosis. Similar results were recorded for fed and diapausing adult males in which the 'diffuse stage' was extended from 4 days in fed bugs to 7 days in unfed bugs. Although the same period of diapause was imposed on both fifth instar and adult bugs (35 days), diapausing larvae took 5 days longer to complete meiosis than did diapausing adults. Fifth instar larval R. prolixus [illegible] about 250 mg which is more than twice the amount taken by fourth instar larvae (Buxton, 1930) so that adult bugs may have derived greater food reserves from the fifth instar than those derived from their fourth instar after a similar period of starvation. This may account for the difference in rate of meiosis between starved fifth instar larvae and starved adult males.

Dumser and Davey (1974) found that application of juvenile hormone analogue to R. prolixus produced a dose-related inhibition of meiosis which they assessed by the number of spermatid cysts present in sections of testis. They found that the size of the 'spermatocyte containing' part of the testis remained constant and concluded that the effect of the juvenile hormone had been on the rate of gonial mitoses and not on the meiotic process itself. From these experiments Dumser and Davey (loc. cit.) proposed a kinetic hypothesis for the

regulation of spermatogenesis in R. prolixus, which assumed an endogenous level of production in the absence of endocrine activity which would be increased by ecdysone following the blood meal, to produce a high rate of cell division and differentiation which could be returned to the endogenous rate by the secretion of the inhibitory juvenile hormone. However, this hypothesis was based on the premise [illegible] of meiotic prophase was a constant and that only the rate of gonial input varied. The present experiments have shown that meiotic prophase is not of fixed duration in spermatogenesis and may vary in length considerably depending on the nutritional status of the bug, the flexibility lying in the amount of time spent [illegible] 'diffuse stage'. The 'diffuse stage' of meiosis is characteristic of the suborder Heteroptera (Order Hemiptera) (Lewis and Southwood, 1958) and is associated in R. prolixus with a great increase in nuclear volume (Schreiber et al., [illegible] of the condensation they have gone through up to pachytene and lose their affinity for nuclear stains. The ability of spermatocytes in this 'diffuse stage' to respond to changes in the internal environment of the bug is of considerable interest since, in many cases meiosis, Special Features [illegible] have been demonstrated in nuclear behaviour at the [illegible] stage of meiosis. For example, [illegible] meiosis is typified by lateral 'lampbrush' loops which extend from the chromosomes during pachytene and a prolonged diplotene, but prior to first metaphase the lateral loops are withdrawn and compact chromosomes are [illegible]. Evidence for 'lampbrush' chromosomes are widespread throughout the animal kingdom in meiotic cells of both sexes (Callan, 1963). For example, Gall (1956) and Bl has demonstrated 'lampbrush' chromosomes in the oocyte nuclei

of grasshoppers, locusts and cockroaches and there is evidence that chromosomes pass through a 'lampbrush' stage during male meiotic prophase in [illegible]. Callan (1967) has suggested that the pattern of chromosome organisation in eukaryotes consists of a series of repeated DNA base sequences, each made up of a 'master' followed by 'slaves' and that extension of the lateral loops during 'lampbrush' formation results from the progressive matching of 'slaves' against the 'master' copy thus correcting any errors in the 'slaves' which are then passed on into the lateral loops. All [illegible] pachytene in meiotic cells, [illegible] this stage follows the process of genetic recombination in meiosis and, following correction, the 'slaves' are free to transcribe RNA if required. There is evidence that RNA synthesis takes place in the lateral loops of 'lampbrush' chromosomes of urodeles (Gall and Callan, 1962) and [illegible] the 'threads' loops of the Y-chromosomes of Drosophila spermatocytes are associated with intense RNA synthesis.

Although 'lampbrush' chromosomes have not been demonstrated in Rhizomicrobium, the 'filiform stage' of male meiosis, which is an extended pachytene/diplotene phase, may represent a system of chromosome [illegible] 'lampbrush' formation and serving similar functions. The incorporation of [illegible] into testes of R. prolixus demonstrated in the present work has shown that 'filiform stage' spermatocytes do not [illegible] although synthesising DNA. The [illegible] of meiotic prolongation has been shown to be [illegible] in other insects (Nicklas, 1956; Rees and Peacock, 1961) and Rees (1961) has suggested that [illegible]

produced during meiosis may carry information related to the control of subsequent steps of spermatogenesis. This hypothesis was confirmed in part by Hess (1965) who showed that *Drosophila* males which lacked a single Y-chromosome 'lampbrush' loop were sterile; this sterility resulted from the arrest of spermiogenesis at different stages depending on which [illegible] was missing, suggesting that loop products were released at first metaphase specifically to control spermiogenesis.

The present experiments have shown that spermiogenesis is inhibited during diapause in both larval and adult *R. prolixus* and ^{3}H-thymidine labelling has shown that the blood-meal has a remarkable accelerative effect, [illegible] on 'diffuse stage' spermatocytes. The results suggest that a blood-borne factor is released following the blood-meal and it may be that a 'macromolecular factor' similar to [illegible] is involved. Pratt and Davey (1972a, b and c) have shown that egg production in *R. prolixus* females may be affected by many factors, including hormones and starvation, which may be seen as an adaptation to the haematophagous way of life, eggs being produced in a cyclical fashion each feed resulting in a cycle of egg production, the number of eggs depending on the size of the meal ingested. It could be postulated that the production of sperm in the male is similarly governed by the nutritional status of the bug. If this were [illegible], it could further be postulated that a blood-borne factor released after a blood-meal interacts directly with the uncondensed 'diffuse stage' spermatocyte chromosomes and that the RNA synthetic activity demonstrated in these nuclei was in part associated with control of the subsequent stages of spermatogenesis and, in particular, spermiogenesis, in a way analogous to the 'lampbrush' control of meiosis demonstrated for *Drosophila hydei* (Hess, 1968).

It is known that insect hormones can act directly on chromosomes, for example Clever and Karlson (1960) induced puffs in the giant chromosomes of Chironomus tentans larvae with ecdysone and it has been shown that the hormone acts directly on the nucleus to induce RNA and in turn enzyme synthesis (Karlson, 1961, 1963 and 1967). Considering this information from other organisms, it is reasonable to propose that a hormonal stimulus (possibly a 'macromolecular factor') acts as a switch to turn on m-RNA synthesis in the 'diffuse' chromosomes of the spermatocytes of diapausing R. prolixus, thus providing the biochemical stimulus for spermiogenesis to proceed after the blood-meal. However, since it has been shown in the present work that diapausing unfed adult males that at least a few cells pass from the 'diffuse stage' and through spermiogenesis, it may be that the control substance is quantitative in its effect, depending on the degree of starvation of the bug.

PART THREE

INHERITANCE OF SUSCEPTIBILITY TO *TRYPANOSOMA CRUZI* INFECTION OF *RHODNIUS PROLIXUS*

INTRODUCTION

The clinical diagnosis of Chagas' disease is difficult in the chronic phase, the persons may harbour trypanosomes all their lives yet may be symptomless and acting as carriers (Marsden et al., 1969). Even in acute cases the number of trypanosomes in the blood may be too few to be detected by direct methods and other means must be employed such as immunodiagnosis. The various immunological tests for Chagas' disease have recently been reviewed (Goble, 1970) and the oldest of these, the complement fixation test, first used by Guerreiro and Machado (1913) only 4 years after the discovery of *T. cruzi*, is still the most widely used serological test.

Immunological tests rely on sophisticated techniques and yield 'false positive' diagnoses; the simplest and least expensive way of diagnosing chronic Chagas' disease is by the method of xenodiagnosis which does not produce 'false positives' and of all the diagnostic tests available is the most sensitive (Brumpt, 1950). Xenodiagnosis was first described by Brumpt (1914) as a method for using the natural vector of a disease to diagnose an infection in a host animal. The procedure is simple, usually 6 fifth instar larvae from a clean laboratory colony are allowed to feed on the patient and the bugs are then examined some time later by either pulling out the gut and examining the contents or by compressing the abdomen causing involuntary excretion and then examining the faeces (Dias, 1934b). Maekelt (1964) suggested an improvement to the technique which involved homogenizing the test bugs in saline, filtering, centrifuging and examining the sediment for trypanosomes. Comparing this method with the squash technique on the same group of patients, Maekelt (loc. cit.) found a significant improvement in the sensitivity of the test. However, the squash technique,

whereby the gut contents are simply pulled out on to a slide, diluted with saline and examined for trypanosomes, is still the most widely used method (Siqueira, 1968). The main objection to xenodiagnosis is its inefficiency in detecting very low levels of parasites; for example, Pifano (1954) using laboratory animals with known chronic infections, found that Rhodnius prolixus detected 44% of the positive cases in guinea-pigs and 77% in dogs. Moreover, within the groups of bugs tested on each positive animal, only 50% were individually positive. Romana and Romana (1957) in an experiment on 433 patients showing clinical symptoms of Chagas' disease found that only 30% were positive by xenodiagnosis while 98% were positive by complement fixation test. De Freitas (1961) using fifth instar Triatoma infestans obtained only 20% positive results on patients with chronic Chagas' symptoms and Marsden et al. (1969) found that 16 out of [illegible] Rhodnius prolixus fed on patients with a patent parasitaemia failed to develop flagellates. Clearly, estimates of the efficiency of xenodiagnosis vary widely and the present work was designed to investigate the extent to which the insect genotype affects this variation and if so, whether the efficiency of the insects as diagnostic tools could be improved.

Many factors may influence the development of a T. cruzi infection in a bug after the infecting blood-meal at which the vector ingests the parasite. A bug which will support the full development of the parasite is said to be 'susceptible' and in the same way the term 'refractory' describes individuals or populations in which the parasite will not develop (Macdonald, 1967). 'Intensity of infection' is used to describe the numbers of parasites which the vector will support. Whether or not a bug is susceptible, and the intensity of this infection, both affect the vector efficiency, which will influence the choice of

bugs for xenodiagnosis. Factor efficiency may be affected by both bug and parasite characteristics, environmental factors and interactions between these (see Fig. 3.1). The most important environmental factors which influence the physiology of insects are humidity and temperature (Wigglesworth, 1965). Buxton (1932) showed that adult R. prolixus possess considerable powers of regulating the proportion of water in their bodies and that only prolonged desiccation (42 days at less than 60% R.H. and 23°C) would cause these regulatory processes to break down. Desiccation results eventually in body injury and fall in metabolic rate and it is therefore important to ensure humid conditions in keeping bugs experimentally (Buxton, 1931). Estimates of optimal conditions of humidity vary, Ryckman and Ryckman (1966) recommend [illegible] for Triatominae while Zeledon et al. [illegible] 23°C. Temperature affects many aspects of the bug-trypanosome relationship since, as the external temperature rises, so the metabolic rate of insects rises. The relationship between temperature and the rate of biological processes is described by an S-shaped logistic curve (Davidson, 1942, 1944) which represents the speed of development of many insects over most of the temperature range which will support development. The rate of development of a bug from a first instar larva to the adult is, of course, a function of metabolic rate and in turn temperature, given that appropriate blood-meals are taken between moults. (Triatomine bugs have 5 larval instars in each of which they take one or more large blood-meals and then moult to the next instar). Brumpt (1912a) found metacyclic trypanosomes in the bug Eratyrus mucronatus at varying time intervals following an infecting blood-meal, depending on the temperature at which the bugs were kept and Phillips (1960b) found that the interval between an infecting meal and evidence

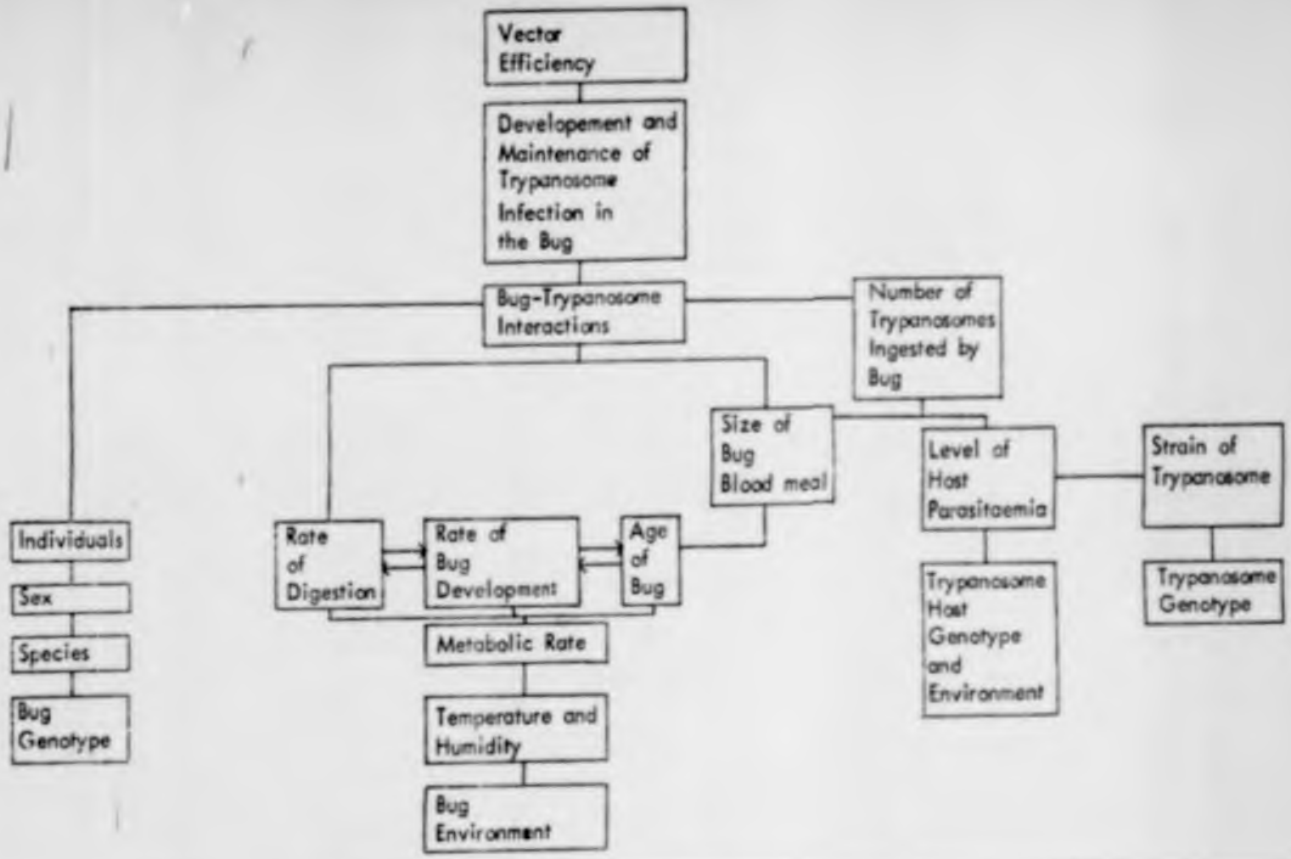


Figure 3.1. Factors affecting vector efficiency

of trypanosomes appearing in the bug faeces decreased progressively as the temperature increased in bugs of all larval stages. Wood (1954) noted that adult Triatoma protracta caught during the Summer in California had more trypanosomes in their faeces than bugs caught in mid-Winter and also showed that increasing the temperature increased the number of trypanosomes in the faeces of experimentally infected bugs.

Since temperature affects the rate of development of a bug, the age of a bug may be regarded as a function of temperature and several studies have examined the effect of age on the [illegible] relationship. Brumpt (1913) showed that he could infect bugs at all ages experimentally, and in a survey of wild caught R. megistus Torres (1915) noted that the proportion of infected bugs increased with age, which he attributed to the fact that older bugs had taken in more blood and would have had more opportunities for reinfection. By counting the number of trypanosomes in faecal droplets, Wood (1954) found that adult T. protracta excreted more trypanosomes than larvae over a 6 hour period. Phillips and Bertram (1967) compared the percentages of bugs of each larval instar of R. prolixus which were positive for metacyclic trypanosomes in their faeces after an infecting blood-meal and found that the proportion declined with each larval instar, so that 91% of first instar larvae were infected while only 7% of fifth instar larvae were positive, although adults showed an increase to 88% positive. In explaining these results several factors must be noted; firstly, bugs take increasingly larger blood-meals at successive larval stages ranging from about 6 mg in first instar R. prolixus to about 280 mg in fifth instar larvae and falling to about 170 mg in the adult (Buxton, 1930); secondly, rate of digestion of the blood-meal increases as the bug ages.

the fifth instar larva digesting its meal 20 times faster than the first instar larva (Buxton loc. cit.). Thus, if bugs are fed experimentally on an animal with a known parasitaemia, the more blood a bug digests the more parasites it will ingest. Phillips and Bertram (1967) estimated that first instar larvae ingested approximately 40,000 trypanosomes while fifth instar ingested over a million trypanosomes when fed on rats with known parasitaemia and yet the percentage remaining infected decreased with age. Wood (1954) also noted that bugs which received fewer parasites with their infecting meal showed as many parasites in their faeces as those ingesting large numbers of trypanosomes. [illegible] that the rate of infection was higher in later stages of experimentally infected *R. neglectus*, but their data shows that this trend was not consistent between replicate experiments and that in one experiment second instar larvae showed the highest infection rate and fifth instar larvae the lowest.

From these results it appears that neither the size of the blood-meal nor the number of trypanosomes ingested determines whether or not a bug will maintain an infection, or the intensity of that infection. Phillips and Bertram (1967) suggested that the increased rate of digestion of older bugs was harmful to the development of ingested trypanosomes and Dias (1934a) also related parasite survival with the rate of haemolysis of the blood-meal, which he also suggested was influenced by the development of intestinal bacterial symbionts. It has long been known that triatomine bugs have bacterial symbionts in their guts (Wigglesworth, 1936) which in the case of *R. prolixus* was named by Brecher and Wigglesworth (1944) as *Actinomyces rhodnii*. However, the function of these symbionts is not as Dias (1934a) thought, to aid

digestion or haemolysis, but rather to provide missing nutritional factors which may be absent from the host's blood. Several studies have been carried out to determine specifically which factors the bacteria provide, with some emphasis on their role in synthesizing vitamin B factors although this is still a subject of recurring debate and further experiments (Goodchild, 1955; Baines, 1956; Harington, 1960; Lake and Friend, 1968; Auden, 1974). Phillips and Bertram (1967) found that the rate of reduction in the proportion of bugs infected at each larval stage was not linear and concluded that while the rate of digestion was probably the most important single factor influencing the development of trypanosomes in the gut of the bug, a complex of physiological processes was probably involved.

It is clear from the literature that variation exists both in the intensity of an infection of individual bugs and in the proportion of susceptible individuals in a population. This variation may be due to environmental factors affecting the bug and the parasite, but may also be affected by the bug genotype.

The possibility that the species of a bug may affect the vector-trypanosome relationship has been investigated by several workers. Dias (1940a), using a single Brazilian strain of T. cruzi, obtained infection rates of 80-90% with Brazilian vectors of the disease (T. infestans, P. megistus and T. sordida) but was only able to infect 56% of a group of R. prolixus from Venezuela with this strain of trypanosome. Dias (1940b) tested the same colony of R. prolixus with a Venezuelan strain of T. cruzi and obtained the same infection rate (56%) but when P.megistus and T. infestans from Brazil were tested with the same Venezuelan strain of T. cruzi they showed a much reduced infection rate (40% and 36% respectively). Similarly, Floch and Lajudie

(1945) using local strains of T. cruzi in French Guiana found that 70% of R. pictipes and 85% of R. prolixus developed infections while only 31% of T. rubrofasciata could be infected, the degree of infection varying with their importance as vectors. Torres, Redondo and Vieto (1957) found that T. dimidiata, the natural vector of T. cruzi in Costa Rica, with an infection rate of 76% was less susceptible than 4 exotic species T. Phyllosoma (from Mexico) 83%, T. infestans (from Chile) 86%, R. prolixus (from Salvador) [illegible] and R. pallescens (from Panama) 94%. Phillips and Bertram (1967) fed adult bugs of 4 species on rats with high levels of parasitaemia and found R. prolixus to be less susceptible (with infection rates 64-98%) than the other species T. infestans, T. protracta and T. sordida (infection rates of 97-100%). Little et al. (1968), using Mexican strains of T. cruzi found T. barberi, a species of triatomine common in Mexico, to be more susceptible than T. infestans from Chile and concluded that inter-specific differences in bug physiology produced this variation between species. Rabinovich et al. (1973) fed [illegible] species of triatomine larvae on 3 patients with chronic Chagas' disease in Argentina and T. infestans larvae gave a much higher infection rate (81%) than other species, for example T. guasayana (9%). The highest infection rate of T. infestans being attributed to the fact that this is the most common vector in Argentina and therefore the strains of trypanosomes and the bug were co-adapted. It may be concluded from this work that variation in infection rates are related in part to the species of the bug which is in turn determined by its genotype.

The infection rates cited above demonstrate that there exists variation between individuals within a species which may be expressed in two ways, firstly, as a 'susceptibility rate' (the proportion of

susceptible individuals in a population expressed as a percentage) and secondly, as variation in 'intensity of infection' (the number of trypanosomes which an individual bug will support). Phillips and Bertram (1967) showed that individual bugs of 4 species were unable to support an infection despite heavy and repeated intakes of large numbers of parasites from host rats. This refractoriness they thought might have a genetic basis and by selecting refractory parents from a group of R. prolixus with a susceptibility rate of 81% and inbreeding them, they produced offspring with a susceptibility rate of only 57% which suggested that susceptibility may be genetically controlled.

Few attempts have been made to quantify differences in the numbers of parasites supported by individual bugs. [illegible] counted the number of metacyclic and non-metacyclic trypanosomes in adult T. protracta experimentally infected with T. cruzi to determine the effect of temperature on the rate of trypanosome development in the insect host. Wood (1960) constructed an infectivity index which compared the proportions of metacyclic to non-metacyclic trypanosomes per faecal droplet. Phillips and Bertram (1967) assigned an arbitrary numerical value to individual bugs using a scale from 1 to 6 by estimating the numbers of trypanosomes in the first faecal droplet released by an adult bug after feeding. Using this scale they observed differences in individuals of a group but not between the parental and F_1 generations of R. prolixus as mentioned above. Although they did detect slight differences between species using this technique.

Wood (1960) noted a difference in intensity of infection between sexes of adult T. protracta: he counted 1954 metacyclic trypanosomes per male (mean of 9 males) and 1821 per female (mean of 6 females). Phillips and Bertram (1967), however, found no significant difference

between sexes in any of the bug species they used, although they did note that females showed a slightly higher susceptibility rate (82%) than male *R. prolixus* (64%) infected with the same strain of *T. cruzi*. Likewise, Almeida et al. (1978) showed no selection of more heavily and adult *R. neglectus* and found no difference in susceptibility rate between sexes.

It appears that only Phillips and Bertram (1967) have compared the ability of different colonies of the same species of bug to transmit *T. cruzi*: an inbred laboratory culture of *R. prolixus* and a recently isolated field stock, showed no significant difference in susceptibility rates or in intensity of infection between the 2 populations studied. However, when they compared an inbred laboratory stock of *T. infestans* with field isolates of the same species, they found that the 3 wild stocks had an overall susceptibility rate of 100% while the laboratory stock had a slightly reduced rate of 98%. They also scored a small quantitative difference in intensity of infection of individuals between the 3 wild stocks but again this was not significant. It is not clear from this evidence if intensity of infection differs in individual bugs in a systematic fashion which could be related to genetic control, but it appears that such variation exists and may be due in part to the bug genotype.

Variation in the behaviour of different strains of *T. cruzi* was first recognised by Brumpt (1913c) who noted that the virulence of two strains differed. Characteristics other than virulence (as measured by mortality) which have been reported to differ among strains are morphology, aspects of early growth, tissue affinity, course of parasitaemia, drug susceptibility, response to temperature, prepatent period and elaboration of various antigens (Hoare, 1970). Variation in strain characteristics

has been shown to affect the bug-trypanosome relationship. Phillips and Bertram (1967) noted a slight variation between strains in ability to infect bugs and this difference was constant for 4 species of bug. Sherlock and Almeida (1973) fed 10 species of triatomine bugs on dog, armadillo and mouse infected with 3 different strains of T. cruzi: on dog, T. infestans and R. neglectus showed the highest susceptibility rates; on armadillo, T. infestans and T. brasiliensis had the highest rates, while on mouse, R. prolixus and T. brasiliensis had the highest rates. Deane (1964) showed that when North American species of triatomine bug were infected with South American strains of T. cruzi a much lower density of metacyclic trypanosomes was found in the rectum than when the same species of bug were infected with a North American strain of T. cruzi and vice-versa. Ny Kraus (loc. it.) concluded that strains of T. cruzi in nature are adapted to populations of triatomine bug from the same geographical regions.

Various vertebrates have been shown to differ in their susceptibility to T. cruzi. For example, it has long been known that birds are not susceptible (Brumpt, 1914) and the guinea-pig is known to have poor susceptibility in the laboratory (Phillips, 1958), although it is said to be a natural host of the parasite (Deane, 1960). It has also been shown that the resistance to infection increases with the age of the vertebrate host (Regendanz, 1930; Dias, 1934a; Kolodny, 1939a, b; Culbertson and Kessler, 1942) and the severe forms of Chagas' disease in man are commonly encountered in infants. The strain of laboratory host is also said to influence its susceptibility suggesting genotype involvement: Filizzi et al. (1960) showed that albinos were increasingly susceptible the more homozygous they became. The sex of the host has also been examined for its effect on susceptibility and

Mauschka (1947) demonstrated that male mice were more susceptible than females of the same stock to 3 strains of T. cruzi. There is also evidence to suggest that environmental temperature affects the course of infection in the host; Kolodny (1939c, 1940) correlated variations in intensity of infection in experimental rats with seasonal temperature changes and Mauschka (1949) noted a temperature-dependent seasonal rhythm in the blood-trypanosome level of infected mice over a 27 month period. It has also been suggested that differences in behaviour within strains can be produced in the vertebrate host by altering the size of the inoculum given to the host animal. Phillips (1960a) investigated this by quantifying the parasitaemia of infected animals and found that the intensity of infection of 2 strains of T. cruzi measured in terms of parasite density in the blood and of virulence, varied with the number of parasites initially inoculated. Failure to inoculate sufficient parasites resulted in numerically attenuated infections with these 2 strains but in a third strain examined, the number of parasites inoculated proved unimportant. Marsden (1967) and Marsden and Hagstrom (1968), in experiments with mice and dogs, found that the pathogenicity of the 'Peru' strain of T. cruzi was related to the dose of infecting organisms and furthermore, that the route of inoculation could influence the course of the disease. Mahelbwala and Ormerod (1973) found great similarities between infections in mice both in intensity and mortality produced by trypanosome doses ranging over 4 orders of magnitude. Phillips (1960a) concluded that, given a sufficient number of parasites is inoculated to produce the maximum pathogenicity of which a strain is capable, the trypanosome will behave in a stable fashion and Marsden (1967) found that mice given more than 1000 trypanosomes always died. It seems, therefore,

that the importance of the inoculating dose is still a matter for debate. Although it is reasonable to envisage that size and site of inoculum may have some effect on the course of an infection. It has also been suggested that the virulence of strains of T. cruzi could be lost over long periods of passage in laboratory animals (Gaillard, 1952) but Mauschke (1949) found that passaging two strains of T. cruzi in mice for over 2 years increased their virulence. Phillips (1960a) quantitatively assessed a 'B.H.' strain infection of T. cruzi and found that it sustained its virulence unchanged for 2½ years in routine mouse passage.

From the work reviewed above it appears that several factors may affect the bug-parasite relationship and therefore, in order to study specifically the effect of bug genotype, factors other than this must be held constant if possible, including those affecting the bug (species, population, age, sex and external environment) and the trypanosome (strain, vertebrate host, host characteristics and inoculum).

The present work describes experiments designed to breed susceptible and refractory populations of R. prolixus. A pure breeding susceptible population would have great value in xenodiagnosis.

MATERIALS AND METHODS

Materials

One strain of colonized Rhodnius prolixus, 2 strains of Trypanosoma cruzi and T.C. strain were as vertebrate hosts.

R. prolixus. A single species of triatomine, R. prolixus (Stal, 1859), was used in this experiment and was chosen because of its relatively short life-cycle (egg to adult taking about 6 months at 28° C (Gardiner

and Maddrell, 1972)) and because it requires only one blood-meal between moults (Wigglesworth, 1934). The L.S.H. and T.M. colony of R. prolixus is now a highly adapted laboratory strain, an indication of its fitness is that in 1930 the fertility of the colony was estimated to be about 82% (Buxton, 1930) and is now about [illegible]% (see Part Four).

[illegible]. Two strains of T. cruzi were used in this experiment and were chosen for their well-documented, and differing characteristics.

1. Peru strain. A strain of high [illegible] isolated in 1963 by Dr. F. Naquira in Arequipa, Peru. It was given that year to Dr. F. Goble (Nussenzweig and Goble, 1966) who [illegible] gave it to Dr. P.D. Marsden of L.S.H. and T.M. (Marsden, 1967). The strain was subsequently passaged over 160 times in mice but at passage 155 it was stabilated by standard methods of cryopreservation as reference code LUMP 691 (for details of this procedure see Lumsden et al., 1973). This stabilate was then used for the present experiment, and is herein referred to as the Peru strain. This strain has a pre-patent period of 8-14 days in mice and is lethal at 25-30 days in inocula containing more than 100 organisms (Mahmoud and Ormerod, 1973).

2. Strain 7. A [illegible] stabilate of T. cruzi. Isolated in 1965 from an acute case of Chagas' disease in man in Sao Filipe, Brazil, by Dr. P.D. Marsden and brought to the L.S.H. and T.M. in London in 1966. The strain was then passaged in mice 37 times, stabilated as LUMP 518 and 519, passaged once more in mouse and stabilated as reference code LUMP 692. This stabilate was used in this work. Strain 7 is not lethal to mice and they may recover from inocula of over ½ million trypanosomes. The pre-patent period in mice is 8 days and after 30 days parasitaemia declines and the mice recover

(Ketteridge, personal communication).

Experimental host. Six to eight week old male mice of the random bred T.O. (Theiler's Original) strain weighing [illegible] g were used throughout and maintained in one room at 24°C.

Methods

The protocol adopted for this experiment to ensure the synchrony of infected mice with bugs of the correct age is shown in Fig. [illegible] and was adhered to over successive generations of bugs.

Treatment of mice. Stabilates of both strains of T. cruzi were inoculated into mice and the infection followed by examination of tail blood one week after inoculation and thereafter daily. The procedures followed in handling infected material are detailed in [illegible] et al., 1973). Mice with a parasitaemia of 1-5 trypanosomes per field (estimated by scoring 100 fields, X 45) were bled from the heart. 1 ml of infected blood was diluted with [illegible] balanced salt solution (Oxoid, England) and 0.1 ml of this diluted blood was injected intra-peritoneally (i.p.) into [illegible] experimental mice. This standardized system, although subject to variation in the numbers of trypanosomes inoculated, did produce very regular patterns of infection in the experimental mice as observed by tail bleeding. Mice infected with the Peru strain regularly developed parasitaemia of 1-5 trypanosomes per field [illegible] fields, X 450) by examination of tail blood 24 days post-inoculation. Mice infected with Strain 7 developed a parasitaemia of approximately 1 trypanosome per field 21 days after inoculation by the same method of examination. These results were consistent throughout the experiment. Although a review of previous work suggested that the number of trypanosomes ingested by a bug was not a critical factor in determining the level of infection developed by the bug, this

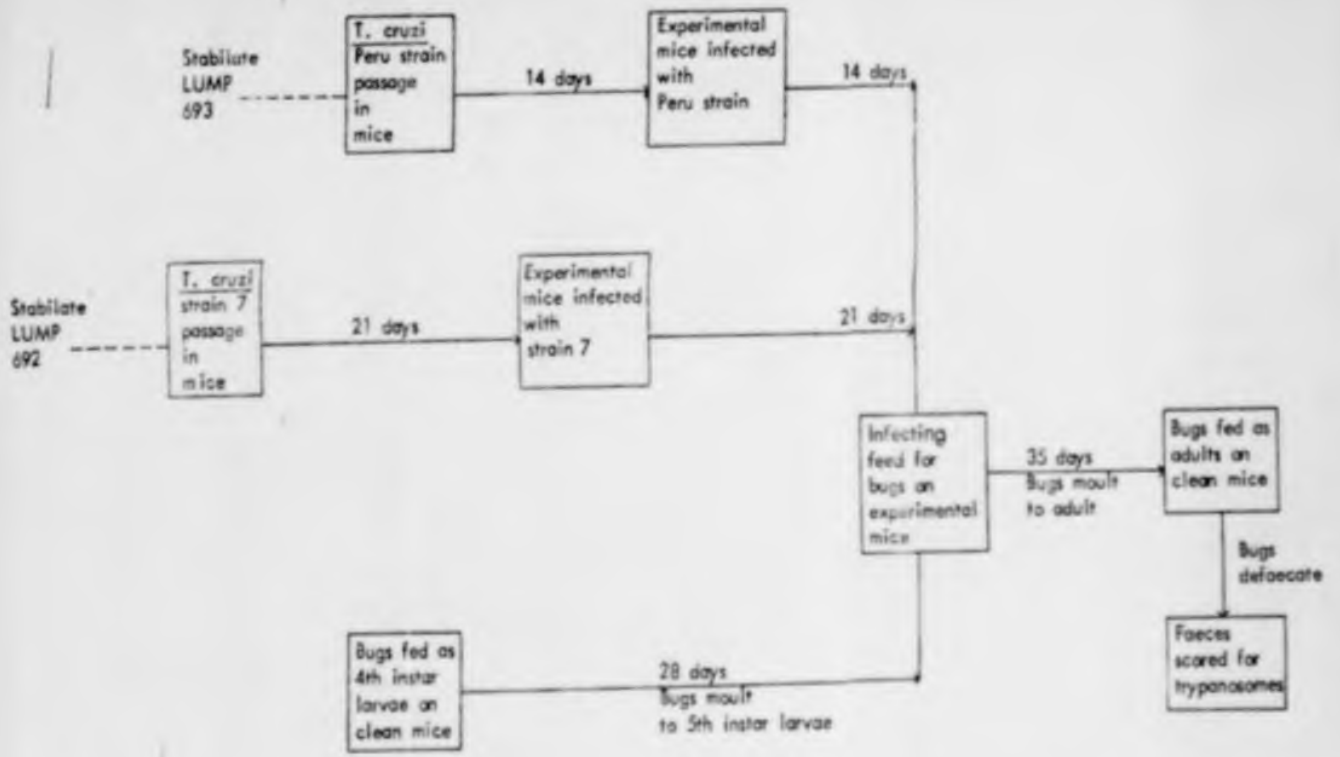


Figure 3.2. Protocol for mouse and bug infections

standardized method of mouse inoculation was an attempt to ensure that bugs were ingesting approximately the same numbers of trypanosomes in terms of orders of magnitude. With such large numbers of mice involved in this experiment, it would have been difficult to ensure that each mouse had exactly the same level of parasitaemia.

Treatment of bugs. R. prolixus were kept in an incubator at a constant temperature of 28°C and R.H. of approximately 70% and contained in 2" x 1" flat-bottomend glass tubes, sealed with fine mesh nylon gauze held in place by adhesive tape. Since the care and feeding of larval stages may influence the susceptibility to disease of adult insects (Burtt, 1946), great care was taken to provide optimal and identical rearing conditions for the larval stages of experimental bugs. Larvae were reared in tubes with only a few larvae per tube to preclude competition when feeding, and the tubes of larvae were maintained in incubators under the same conditions of temperature and humidity described above. Larval blood-meals were given on uninfected mice in the same way as described for infecting feeds. To ensure that the experimental bugs were all of the same age when given their infecting feed, they were all fed simultaneously as fourth instar larvae, allowed to moult to fifth instar larvae and, as fifths were given the infecting blood-meal exactly 28 days after this fourth instar blood-meal. Fifth instar larvae were chosen for the infecting feed because they take more blood than any other stage of R. prolixus; a feed of 200-300 mg blood is usual for fifth instar larvae (Wigglesworth, 1934) which guaranteed an adequate intake of trypanosomes.

Infecting feed. For the infecting blood-meal the fifth instar larvae were placed in pairs in tubes, filter paper having been placed in the tubes to assist their stance while feeding and to absorb their faeces. The parasitaemia of each infected mouse was first estimated by

standardized method of mouse inoculation was an attempt to ensure that bugs were ingesting approximately the same numbers of trypanosomes in terms of orders of magnitude. With such large numbers of mice involved in this experiment, it would have been difficult to ensure that each mouse had exactly the same level of parasitaemia.

Treatment of bugs. R. prolixus were kept in an incubator at a constant temperature of 28°C and R.H. of approximately 75% and contained in 2" x 1" flat-bottomed glass tubes, sealed with fine mesh nylon gauze held in place by adhesive tape. Since the care and feeding of larval stages may influence the susceptibility to disease of adult insects (Hurst, 1938), great care was taken to provide optimal and constant rearing conditions for the larval stages of experimental bugs. Larvae were reared in tubes with only a few larvae per tube to preclude competition when feeding, and the tubes of larvae were maintained in incubators under the same conditions of temperature and humidity described above. Larval bloodmeals were given on anesthetized mice in the same way as described for infecting feeds. To ensure that the experimental bugs were all of the same age when given their infecting feed, they were all fed simultaneously as fourth instar larvae, allowed to moult to fifth instar larvae and, as fifths were given the infecting bloodmeal exactly 28 days after this fourth instar bloodmeal. Fifth instar larvae were chosen for the infecting feed because they take more blood than any other stage of R. prolixus: a feed of 200-300 mg blood is usual for fifth instar larvae (Wigglesworth, 1934) which guaranteed an adequate intake of trypanosomes.

Infecting feed. For the infecting bloodmeal the fifth instar larvae were placed in pairs in tubes, filter paper resting bases placed in the tubes to assist their stance while feeding and to absorb their faeces. The parasitaemia of each infected mouse was first estimated by

examination of tail blood on the day of feeding and mice were selected for the feeds using the criteria described above. Mice used for feeding bugs were anaesthetised with 0.2 ml of a 20% solution of 'Nembutal' (Pentobarbitone sodium B. vet. C.P., administered i.p. and, after sedation, they were placed on top of the tubes containing the bugs so that each mouse covered 2 tubes and thereby fed 4 fifth instar larvae. The tubes containing the bugs were placed upright randomly in a polystyrene box prior to the feed, the lid being placed on the box after the mice had been laid on the tubes, to provide insulation. Since R. prolixus is attracted to its host from short range mainly by the warm air diffusing from it (Wigglesworth and Gillett, 1934), insulating the mice in this way prevented the fall in body temperature normally associated with this anaesthetic. After the bugs had fed, they were returned in their tubes to the incubator at 28°C. The mice were killed with ether, before they recovered from the anaesthetic, and incinerated.

One week after the infecting feed, and prior to the moult to the adult, the bugs were placed individually in 15mm glass tubes which prevented any unplanned matings. Following the moult the adult bugs were placed in clean tubes which, instead of filter paper, contained a strip of nylon mesh which enabled the bugs to climb up the tubes to feed on a clean mouse when offered in due course, but would not absorb any of the faeces which would be required for examination for trypanosomes. At this stage also, bugs were sexed and a code number was written on the side of each tube for identification.

Measurement of bug infections

1. Time of examination. Mackerl (1964) examined bugs for xenodiagnosis 40-60 days after the infecting feed and Freitas (1950)

examined bugs 30, 60 and 90 days after feeding and found that the group of bugs examined at 60 days had a significantly higher infection rate than the other 2 groups. Patterson and Miles (1973) found that the intensity of infection in the rectum of R. prolixus fed on rhesus monkeys infected with T. cruzi did not remain constant and was highest 25 days after the blood-meal. In the present experiment all adult bugs were examined exactly 35 days after their infecting feed as fifth instar larvae (Patterson and Miles, loc. cit. have shown that metamorphosis and ecdysis of fifth instar larvae to adults have no effect on the intensity of infection in the gut of the bugs.

2. Method of examination of bugs. Since the bugs were required to mate to produce progeny for other genetic studies, routine dissection of rectal contents not squeezing the abdomen could be employed for faecal examination for trypanosomes, and therefore bugs were fed on uninfected mice and were allowed to defaecate naturally during and after this 'clean feed'. For this 'clean feed' the same apparatus was used as for the infecting feed. The anaesthetised, clean mice were placed on top of the bug tubes, randomly arranged in a polystyrene box, so that each mouse fed 2 bugs. But, for this meal, [illegible] ml Hanks solution was injected to the bottom of each bug tube using sterile graduated syringes, and the bug faeces, as they were excreted, dropped from the nylon mesh in the tube into the Hanks solution in which dessication of the trypanosomes was prevented. Any faecal material remaining on the nylon mesh strip was mixed with the Hanks solution before sampling. Wigglesworth (1931) showed that R. prolixus voids the residue of its previous meal immediately after feeding, then a few minutes later excretes a cloudy, watery fluid and for the next 3-4 hours passes clear, colourless, urine at intervals, although the

rate of excretion declines rapidly 1-2 hours after feeding. Since the faeces of many bugs often had to be separately examined on the same day, it was not possible to control strictly the time interval between feeding a bug and examination of faeces, but when numbers were large on the same day, the bug tubes were grouped randomly and groups fed at conveniently spaced intervals, so keeping to a minimum variation in the time between feeding, excretion and examination of the faeces. Using this system the faeces of most bugs were produced and available for microscopic examination between one and two hours after feeding. This seemed to be a reasonable and practicable procedure. The clean mice were killed immediately after use, unless they were being kept for further bugs, to exclude the possibility of cross-infection.

3. Scoring of faecal infections. The number of parasites excreted by each bug was estimated by sampling the Hanks solution after it had been well mixed with the faeces. Sampling was carried out using calibrated Pasteur micropipettes (measured volume 0.. microl), a clean pipette being used for each bug's faeces. A micropipette was pushed through the gauze covering the top of a tube containing a bug and its faeces in Hanks solution, and allowed to fill by capillary action. Three .. of samples were then expelled on to clean microscope slides using a rubber bulb. No. 1 coverslips (.. x .. mm, Chance Propper Ltd., England) were placed on the droplets and the slides were marked with code numbers corresponding to those on the tubes containing the bugs.

Scale of examination.

It would perhaps have been more accurate to have used a haemocytometer to estimate the numbers of trypanosomes in the samples but

this would have involved too much time per insect, making the assessment of infection in a large number of bugs impossible. Moreover, haemacytometers have an inherent error related to mathematical considerations of the patterns in which organisms are expected to occur in the squares of the apparatus (Dacie and Lewis, 1963). The method chosen was to count 100 fields (X 450, using phase contrast microscopy) of each sample, working in a systematic way across the [illegible] examined except for bugs of the F_3 generation infected with [illegible] of the error variation between [illegible] of trypanosomes in [illegible] a 'score'. It must be noted that all forms of trypanosome recognised in each sample were counted; previous workers have sometimes distinguished between the transmissible, infective metacyclic forms and other forms (amastigotes and epimastigotes, Mahalbeala and Ormerod, 1973) which are sometimes found in the faecal test, since the production of trypanosomes of whatever stage of development is evidence that the vector is supporting the development of the parasite, the counting of all forms, rather than time-consuming differentiation of different forms, seemed to be both a practicable and adequate measure of vector efficiency. Moreover, for the purposes of xenodiagnosis, the evidence of trypanosomes in the faeces, of whatever developmental stages, is proof that the original donor patient, or animal, is infected.

If no parasites were seen in a faecal sample, the bug was kept and given a second 'clean feed' 14 days later when, if it proved negative again, a sample of the diluted faeces was injected i.p. into a clean mouse. The tail blood of the mouse was examined for trypanosomes

14 and 21 days later. If the bug showed no evidence of parasites on all three tests it was assumed to be refractory to infection, and assigned a score of zero. If a bug was negative at the first test but proved positive at a later test it was assigned an arbitrary score of one, since the second defaecated sample or, the final assay (the recipient mouse parasitaemia) could not be quantitatively related to the first defaecated sample scores of other bugs in the experiment.

Methods of selection of bugs for breeding

The parental generations of R. prolixus were selected randomly from the stock colony as fourth instar larvae and, after feeding and moulting to the fifth instar, 2 groups of bugs each consisting of 50 male larvae and 50 female larvae were given an infecting blood-meal on mice as described above. These 2 groups of bugs were known as 'Peru' and 'Strain Y' according to the strain of T. cruzi imparted. Both groups were allowed to moult to adult, then fed on clean mice, and their faeces allowed to develop above. Bugs which did not readily engorge at an infecting meal were rejected not only in these parental groups but also in subsequent generations.

Selection of bugs as parents for the next generation was made on the basis of individual faecal scores for trypanosomes. Selected males from each of the parental groups were mated with several females from the same group and 3 blood-meals given to each pair at 14 day intervals. The eggs laid by each female over the 3 feeds were pooled, each group of eggs representing an F_1 family. On emergence, the F_1 first instar larvae were fed on clean mice and reared to the fifth instar when they were given an infecting feed and subsequently scored, when adults on the basis of faecal infection. Single pair reciprocal assortative matings were made from these F_1 adults using individual

scores as the basis for selection. This process of infecting feed, followed by scoring, selection, and assortative mating, was repeated to produce an F_2 generation from both 'Peru' and 'Strain 7' bugs. However, only the 'Strain 7' group was reared to the F_3 generation, the 'Peru' experiment being discontinued at the F_2 generation.

Methods of data analysis

1. Analysis by class

For a major gene interpretation, infected bugs fall into 2 obvious discrete classes, (a) refractory - bugs with no trypanosomes in their faeces, and (b) susceptible - bugs with trypanosomes in their faeces. It is not essential in analysing such data to give a numerical definition of the individuals within the classes defined; it is sufficient that the classes are clearly distinct producing a discontinuous distribution so that analysis is based simply on the number of individuals in each class. The present experiment examined the [illegible] structure of 2 populations of bugs, Strain [illegible] and Peru, through generations of selection in order to examine any changes in proportion of refractory to susceptible bugs which would allow for a Mendelian interpretation of the control of susceptibility.

2. Analysis by intensity of infection.

Analysis of continuous variation presents different problems, but each observation is important and reflects the expression of the character. The number of trypanosomes excreted by a bug cannot be continuous [illegible], the fraction of trypanosomes do not exist and it is therefore essential to treat the scores as if such minor discontinuities did not exist, that is as 'quasi continuous' variation [illegible]. In describing and analysing intensity of infection it is therefore necessary to replace frequencies by means

and variances. For the application of most statistical methods to a set of data, its frequency distribution should be normal, but the dispersal pattern of trypanosomes is not normal since they tend to be clumped or aggregated in space. This distribution, which is described as 'contagious' (Southwood, 1966) is non-random and in order to normalize it, the data must be transformed; that is, the actual numbers must be replaced by a function whose distribution is such that it normalizes the data. The appropriate transformation for trypanosome population estimates has been arrived at empirically and it has been shown that a logarithmic transformation is most suitable (Lumsden et al., 1971). Accordingly, in assessing the intensity of infection of bugs in the present work all scores were transformed logarithmically, and, in order to overcome difficulties with zero counts, a constant was added to the original scores so that all scores were transformed to a log (n + 1) scale (where n represents the score) before analysis.

A quantitative analysis was carried out on the transformed scores of the bugs in the present selection experiment to allow for the possible polygenic control of susceptibility.

RESULTS

Numbers of bugs selected

Peru. Of the 100 fifth instar larval bugs initially infected with Peru strain T. cruzi, 81 fed fully, moulted successfully and were scored as adults, 41 of which were male and 40 were female. Eleven of these 81 bugs had a zero score. Twenty contacted on all tests of their faeces, and of these 11 refractory bugs (7 males and 4 females) survived and were used for mating. Four arrays of

half-sib families were established, being 4 males each mated to 4 females. Some matings were unsuccessful and only 10 of the 16 matings remained. In addition, 1 further matings were established using randomly chosen parents, giving rise to 12 F_1 families in all (see Table 3.1).

These F_1 families were reared, scored as adults and 19 full-sib matings were selected from among 8 of the F_1 families. Thirteen of these matings were selected for high score and 6 for low scores. The offspring of these full-sib assortative matings were reared to adults, infected and scored. The Peru experiment was not taken further than this F_2 generation.

Strain T. From a large population of 170 first instar *S. mansoni* larvae infected with Strain 7 *S. mansoni*, 77 (41 males and 36 females) were reared successfully to the adult stage and scored. Of 77 bugs, 8 had scores of zero and were found to be refractory on all tests of their faeces. Six 14 males, 2 individuals of these 8 survived and were used for mating. The design of these matings was similar to that for the Peru bugs consisting of 4 males each of which was mated to 4 females (see Table 3.1). Eleven of these 12 matings produced viable F_1 families which were reared, infected and scored as adults. On the basis of these F_1 scores, 16 full-sib assortative matings were established together with 2 unrelated crosses from among 9 of the 11 F_1 families. These 16 F_2 families were reared and scored as adults and from 9 of these families further selections were made and 17 full-sib matings set up. These 17 selected pairs were divided into high and low lines. Four pairs were assortatively mated for high score and from the same F_2 families 4 pairs with low scores were mated to provide reverse selection controls. Seven pairs were mated assortatively for low score and 4 reverse selections were also

established from these low line families, but only 2 of these control matings were fertile.

The progeny of these 17 full-sib F_2 matings were reared, infected and scored as F_3 adults. Those F_3 individuals were scored twice, 2 faecal samples being examined from each bug to increase the precision of the F_3 statistics.

Analysis by class

Peru. The parental matings made from the Peru population are shown in Table 3.1 in which bug scores are presented untransformed. F_1 families were coded according to the number given to their female parent. F_2 families 73 and 61 were produced from crosses in which both the parents were refractory (i.e. score zero). Family 73 was bred to produce F_2 families 17 and 18 using only refractory parents while family 61 was similarly bred to produce F_2 family 16. The results of this breeding experiment over 2 generations of selection are shown in Table 3.2 where results are expressed in terms of the proportions of refractory to susceptible bugs in the families of each generation raised.

Strain 7. The 4 groups of parental matings selected from the base population of R. prolixus infected with T. cruzi Strain 7 are shown in Table 3.3.

F_1 families 93 and 94 were produced from parents with zero scores. F_1 family 93 was bred successfully to F_2 family 2 using only refractory parents, and family 94 was bred to F_2 family 14 using parents with high scores in selecting for susceptibility. The results of selection and breeding for susceptible and refractory bugs are shown in Table 3.4. Only those F_1 and F_2 families which provided parents for subsequent generations are included in Table 3.4.

Table 3.1. Twelve matings from base population R. prolixus infected with Peru strain T. cruzi showing code numbers and scores (untransformed).

Array 1			Array 2			Array 3			Array 4			Random Mating					
Sex	Bug No.	Score*	Sex	Bug No.	Score	Sex	Bug No.	Score	Sex	Bug No.	Score	Sex	Bug No.	Score	Sex	Bug No.	Score
♂	37	11	♂	52	0	♂	1	0	♂	40	4	♂	25	4	♂	66	3
♀	21	0	♀	73	0	♀	61	0	♀	20	0	♀	43	4	♀	63	1
♀	48	3	♀	55	3	♀	58	5	♀	56	4						
♀	6	3	♀	2	9												

* No. of trypanosomes/100 fields

Table 4.2. Proportions of reflective bugs in the F_1 and F_2 generations of *P. vanii* Peru strain infected *R. prolixus*. Scores are shown untransformed.

F_1						F_2						Direction of selection
Family number	Parental score ♂	Parental score ♀	T	n	% with zero score	Family number	Parental score ♂	Parental score ♀	T	n	% with zero score	
43	4	4	15	[illegible]	0	[illegible]	4	24	18	[illegible]	5.6	S
						[illegible]	3	10	8	[illegible]	[illegible]	S
						3	2	5	15	1	6.7	[illegible]
63	3	[illegible]	28	4	14.3	4	20	5	15	2	13.3	[illegible]
55	0	3	36	1	2.8	6	67	29	15	[illegible]	6.7	[illegible]
						7	25	24	13	1	7.7	[illegible]
						8	96	27	12	[illegible]	8.3	[illegible]
56	4	4	24	2	8.3	9	6	23	19	2	10.5	S
						10	6	3	19	1	5.3	S
2	0	9	37	1	2.7	11	19	20	20	[illegible]	0	S
						12	15	25	11	[illegible]	0	S
						13	74	24	19	2	10.5	S
						18	141	11	20	3	15.0	S
						Total			204	15	7.3	

.....Continued/

Table 3.2. (Continued)

F_1						F_2						Direction of selection
Family number	Parental score ♂	Parental score ♀	T	n	% with zero score	Family number	Parental score ♂	Parental score ♀	T	n	% with zero	
61	0	0	43	5	11.6	16	0	[illegible]	14	1	7.1	R
73	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] 18	[illegible] 0	[illegible] 0	[illegible] 16	[illegible] 3	[illegible] 18.8	[illegible] R
6	11	3	9	2	22.2	19	0	0	16	2	12.5	R
21 x 48	[illegible] 11	[illegible] 3	[illegible] 23	[illegible] 1	5.7 4.4	20	0	0	16	2	12.5	[illegible]
2 x 55	0 0	9 3	37 36	1 [illegible]	2.7 2.8	21	0	0	20	0	0	R
						Total			99	11	11.1	

T = Total No. bugs scored

n = No. of refractory bugs (with zero score)

R = Selection for refractoriness

[illegible] = Selection for susceptibility

Table 4.3. [illegible] T. cruzi, showing code numbers and scores (untransformed)

Array 1			Array 2			Array 3			Array 4		
Sex	Bug No.	Score*	Sex	Bug No.	Score	Sex	Bug No.	Score	Sex	Bug No.	Score
♂	51	0	♂	42	0	♂	17		♂	15	0
♀	50	3	♀	93	0	♀	94	0	♀	70	7
♀	69	30	♀	73	7	♀	58	9	♀	76	14
						♀	46	17	♀	66	28
						♀	55	61			

* No. of trypanosomes/100 fields

Table 3.4. Proportions of refractory eggs in the F_1, F_2 and F_3 generations of *T. crassiceps* strain T infected *M. musculus*. Scores are given untransformed.

F_1						F_2						F_3						
Family number	Parental score ♂	♀	T	n	% with zero score	Family number	Parental score ♂	♀	T	n	% with zero score	Family number	Parental score ♂	♀	T	n	% with zero score	Direction of selection
93	0	0	22	5	22.7	20	0	0	17	2	11.8	1 2	138 0	10 [illegible]	23 24	6 5	26.1 20.8	Rs R
70	0	7	22	10	45.5	11	0	[illegible]	19	7	36.8	4 5	[illegible] 0	[illegible] [illegible]	31 13	1 0	3.2 0	R R
73	1	7	20	11	55.0	13	0	0	21	3	14.3	7	0	0	37	2	5.4	R
69	0	30	22	2	9.1	15	0	0	20	2	10.0	8 9	86 0	42 9	13 18	1 1	7.7 5.6	Rs R
73	1	7	20	11	55.0	14	0	0	21	3	14.3	10 11	[illegible] 0	[illegible] 0	24 30	10 14	41.7 46.7	R R
												Total			213	40	18.7	

.....Continued

Table 3.4. (Continued)

F_1						F_2						F_3						
Family number	Parental score ♂	♀	T	n	% with zero score	Family number	Parental score ♂	♀	T	n	% with zero score	Family	Parental score ♂	♀	T	n	% with zero score	Direction of selection
50	0	3	36	3	8.3	2	156	33	15	0	0	12 13	38 2	14 6	35 8	2 0	5.7 0	S Sr
94	0	0	36	0	0	5	118	103	21	1	4.8	14 15	114 4	20 0	33 24	0 1	0 4.2	S Sr
70	0	7	22	10	45.5	8	184	[illegible]	17	1	5.9	16	370	30	27	0	0	S
46	1	17	34	3	8.8							17	2	0	24	0	0	Sr
58	1	9	26	1	3.8	3	286	256	26	0	0	18 19	355 6	88 4	24 10	1 1	4.2 [illegible]	S Sr
												Total			[illegible]	5	2.7	

T = Total No. bugs scored

n = No. of refractory bugs (with zero score)

Rs = Reverse selection from refractory

Sr = Reverse selection from susceptible

Differences between parental populations

The proportions of refractory bugs in the 2 base populations were compared by a χ^2 test, the results of which are shown in Table 3.5.

Table 3.5. χ^2 test for difference in proportions of refractory bugs between 2 base populations of R. prolixus infected with T. cruzi Peru strain and Strain 7.

Bug population					
Peru	81	11	13.6		
Strain 7	77	8	10.4		

Symbols in calculations

N.S. = Probability is non-significant

\+ = Probability 0.05 - 0.10

* = Probability 0.01 - 0.05

** = Probability 0.001 - 0.01

*** = Probability <0.001

P = Probability

The results show that there was no significant difference in the proportion of refractory bugs between the 2 base populations of R. prolixus infected with the 2 different strains of T. cruzi.

Differences between selection lines

The effect of selection for susceptibility and refractoriness on the proportions of the 2 types after successive generations of selection

was compared for both groups of bugs infected with different strains of T. cruzi:

(a) Peru. The proportions of refractory bugs in 19 F_2 families of 2 selection lines, were compared by a χ^2 test (Table 3.6.).

Table 3.6. χ^2 test for differences in proportion of refractory bugs between 2 selection lines from 19 F_2 families of R. prolixus infected with Peru strain T. cruzi.

Selection line	T	n	%	χ^2	P
Susceptible families	204	15	7.3	0.77	N.S.
Refractory families	99	11	11.1		

The results show that selection for refractoriness and susceptibility over 2 generations of assortative mating did not significantly alter the proportions of the 2 classes of bug in the F_2 generation of Peru strain infected R. prolixus.

(b) Strain 7. The proportion of refractory bugs in 9 F_2 families infected with T. cruzi Strain 7 were compared by a χ^2 test (Table 3.7.).

Table 3.7. χ^2 test for differences in proportions of refractory bugs between 9 F_2 families from 2 selection lines of R. prolixus infected with T. cruzi Strain 7.

Selection line	T	n	%	χ^2	P
Susceptible families	79	2	2.5	18.7	<0.001***
Refractory families	98	17	17.4		

The results show that for R. prolixus infected with T. cruzi Strain 7, after 2 generations of assortative mating there was a highly significant difference in the proportions of refractory bugs between families selected for susceptibility and those selected for refractoriness.

A similar analysis was carried out on the proportions of refractory bugs found in F_3 families infected with T. cruzi Strain 7 (Table 3.8).

Table 3.8. χ^2 test for differences in proportions of refractory bugs between 17 F_3 families of two selection lines of R. prolixus infected with T. cruzi Strain 7.

Selection line	T	n	%	χ^2	P
Susceptible families	185	5	2.7	19.2	<0.001***
Refractory families	213	40	18.7		

The results show that the highly significant differences detected in generation F_2 were reproduced in generation F_3 in which highly significant differences in the proportions of refractory bugs between selection lines were found. These significant differences can only have resulted as a consequence of the selection pressure applied to the original population.

Difference between families within selection lines

The proportions of refractory to susceptible bugs within families are shown in Tables 3.2 and 3.4 together with the direction of selection applied to each family. The proportions of refractory bugs should have

increased in those families deliberately selected for refractoriness, and a test for linear trends in proportion was applied to selected families to detect any consistent change over generations. Three F_2 families from the Peru group, numbers 17, 18 and 19 were chosen since they were bred from parents and grandparents which were phenotypically refractory. The results of this analysis are shown in Table 3.9. These analyses were carried out following the method of Armitage (1955).

Table 3.9. Test for linear trend in increase of proportions of refractory bugs in 3 families of *L. longipalpis* selected for refractoriness to *T. cruzi* over several generations, using P, F_1 and F_2 data

F_2 family number	$\hat{b}$	S.E.	χ^2 1df	P
17	0.0090	0.0371	0.06	N.S.
18	0.0117	.0377	0.10	N.S.
19	0.0027	0.0409	0.004	N.S.

$\hat{b}$ - Slope of regression line

S.E. - Standard error $\hat{b}$

The results show that there was no significant trend towards an increase in the proportions of refractory bugs in these 3 families over 3 generations. Despite the inbreeding of refractory parents in family 16 which was bred from refractory parents and grandparents, in fact,

Table 4-15. Tests for linear trends in proportions of refractory *R. prolixus* in 9 selection lines selected for refractoriness to *T. cruzi* Strain 7 using P, F_1, F_2 and F_3 data.

F_3 Family No.	b	S.E.	χ^2, 3df	P
1	0.0321	0.0228	1.9846	N.S.
2	0.0099	0.0200	0.2475	N.S.
4	0.0011	0.0230	0.0019	N.S.
5	0.0275	0.0294	0.8759	N.S.
7	-0.0125	0.0209	0.3523	N.S.
8	-0.0056	0.0229	0.0587	N.S.
9	-0.0103	0.0210	0.2376	N.S.
10	0.0563	0.0244	5.3126	N.S.
11	0.0636	0.0233	7.4348	0.05 - 0.1
Pooled	-0.0068	0.0137	0.2393	N.S.

b = slope of regression line

S.E. = standard error b

showed a decreased proportion of refractory bugs, having only 7% while its parents, which were both refractory, came from an F_1 family (No. 61) with 11% refractory bugs.

Similar analyses were carried out using data from the P, F_1, F_2 and F_3 generation families infected with T. cruzi Strain 7, which were selected for refractory bugs. The results of these analyses are shown in Table 3.10. The results show that there were no highly significant trends towards an increase in the proportions of refractory bugs in any of these families from the parental to the F_3 generation. The most surprising result in this connection is family 1 which had parents, grandparents and great-grandparents all phenotypically refractory and yet showed no trend towards an increasing proportion of refractory members, having 20% refractory members compared with its parental family proportion of 11% and its grand-parental family with 22% refractory. Furthermore, pooling the data from all these families failed to show any significant linear trend towards increasing proportions of refractory bugs; indeed the pooled data produced a negative slope indicating a slight overall decrease in the proportions of refractory bugs.

A similar analysis was carried out using those families infected with T. cruzi Strain 7 which had been selected for susceptibility (Table 3.11).

Table 3.11. Test for linear trend in proportion of refractory bugs in 8 F_3 families selected for susceptibility to infection with T. cruzi Strain 7 using P, F_1, F_2 and F_3 data.

F_3 family numbers	$\hat{\beta}$	S.E.$\hat{\beta}$	χ^2	P
12 - 19 pooled	[illegible]	0.0092	9.76	[illegible]

The results show that in these 8 families there was a significant linear trend downwards in the proportion of refractory bugs from the parental generation to the F_3 ($b = -0.21$) which indicates that selection for susceptibility in these families had indeed steadily increased the proportion of susceptible bugs.

It is difficult to give an explanation for the apparently conflicting results presented above. For example, after 2 generations of assortative mating, the Peru strain F_2 family with the greatest proportion of refractory bugs (F_2 family 18) did not have a significantly greater proportion of refractory members (18.8%) than the original parental generation (14.6%) [illegible] χ^2 [illegible], P [illegible], [illegible] F_3 in the families selected for susceptibility, there was no significant overall increase in the proportions of susceptible bugs (84.6% to 90.7%, χ^2 [illegible].6, P = N.S.).

The F_3 families bred for refractoriness to T. cruzi strain 7 had a mean proportion of 18.7% refractory members which was not significantly different from the parental generation level of 14.6% (χ^2 = 1.6[illegible], P [illegible]. Moreover, the F_3 families with the greatest proportions of refractory bugs (>40%) were F_2 families 10 and 11 which were bred initially from F_1 family 75, the parents of which were not refractory (see Table 3.6).

The F_3 families selected for susceptibility did however show a significant increase in the proportion of susceptible members compared with the base population (from 85.6% to 97.3%, χ^2 = 6.6, P = 0.05 > [illegible].47) and this increase was fairly consistent over all families. The results have not, however, demonstrated any radical changes in the proportions of either susceptible or refractory bugs as a result of selection which, assuming a major gene controlling factor, would have been expected after 3 generations of selection and sib-mating.

Quantitative analysis

An alternative genetic analysis was attempted using the scores of individual bugs (i.e. intensity of infection) exposed in the selection experiments described above. Instead of assigning each bug to a class (i.e. positive or negative for T. cruzi) on the basis of its score, the actual score (number of trypanosomes/[illegible] found in each bug was transformed and then used as raw data in computing first and second degree statistics for families and selection lines. The transformed data are presented as family and generation means in Tables 3.12 - 3.28. These data are based on scores from a total of 1725 R. prolixus adults. The family sizes, numbers, variances and untransformed scores of selected parents have already been given in Tables 3.1 - 3.4 together with their selection lines. In the present analysis, selection for entomophthorous implies low score, and selection for susceptibility implies selection for high score.

Differences between strains of T. cruzi

The mean scores presented in Tables 3.12 - 3.28 show that intensity of infection was strikingly different in the bugs infected with Peru strain from the bugs infected with Strain 7 T. cruzi.

Differences between the 2 parental generations were compared by analysis of variance of scores of bugs infected with Peru and Strain 7, analyses of male and female scores being carried out separately as well as together, and the results are shown in Tables 3.29, 3.30 and 3.31.

Table 3.12. Transformed data for Peru parental generation R. prolixus

Sex	n	$\bar{x}$	S.E.	S.D.
♂	41	0.4797	0.0408	0.2609
♀	40	0.5005	0.0428	0.2706
♂ + ♀	81	0.4900	0.0294	0.2643

Key to annotation for data Tables 3.12 - 3.28

n = Number of bugs tested

$\bar{x}$ = Mean score

S.E. = Standard error of mean

S.D. = Standard deviation

Table 3.13. Transformed data for Peru F_1 generation sale *R. prolixus*

Family number	n	$\bar{x}$	S.E.	S.D.
21	20	0.6021	0.0778	0.3478
48	10	0.4970	0.0710	0.2246
6	5	0.7360	0.2556	0.5716
73	15	0.8915	0.1133	0.4389
55	17	1.1400	0.1281	0.5281
2	23	1.1525	0.1200	0.5752
61	15	0.9130	0.1664	0.6443
58	5	1.0673	0.2648	0.5922
20	8	0.7456	0.1045	0.2954
56	12	0.6966	0.0704	0.2439
43	3	0.5927	0.0642	0.1112
63	15	0.5488	0.1125	0.4358

Table 3.14. Transformed data for Peru F_1 generation female *G. prolixus*

Family number	n	$\bar{x}$	Var.	S.D.
21	15	0.6119	0.1036	0.4012
48	13	0.5841	0.1035	0.3731
6	4	0.6851	0.3230	0.6460
73	18	0.4493	0.0777	0.3298
55	19	0.8620	0.1054	0.4595
2	14	1.0804	0.0983	0.3679
61	27	0.6396	0.0825	0.4288
58	3	0.6514	0.1901	0.3292
20	9	0.8412	0.1731	0.5193
56	12	0.5054	0.1161	0.4022
43	12	0.6899	0.1015	0.3517
63	11	0.5663	0.1212	0.4369

Table 3.15. Transformed data for Peru F_1 generation families (male and female) of R. prolixus

Family number	n	$\bar{x}$	S.E.	S.D.
21	35	0.6063	0.0619	0.3659
48	23	0.5462	0.0654	0.3138
6	9	0.7134	0.1887	0.5662
73	33	0.6503	0.0763	0.4382
55	36	0.9933	0.0843	0.5058
2	37	1.1253	0.0826	0.5024
61	42	0.7372	0.0811	0.5253
58	8	0.9114	0.1863	0.5269
20	17	0.7962	0.1016	0.4189
56	24	0.6010	0.0693	0.3400
43	15	0.6705	0.0819	0.3172
63	28	0.5569	0.0809	0.4282

Table 3.16. Transformed data for Peru F_1 generation male *P. prolixus*

Family number	n	$\bar{x}$	S.E.	S.D.
1	19	0.7169	0.1321	0.4178
2	4	0.7107	0.2747	0.5493
3	9	0.7584	0.1803	[illegible]
4	3	0.9619	0.1772	0.4688
5	7	0.8219	0.2158	0.5710
7	8	0.7556	0.1712	0.4841
8	5	1.1554	0.2319	0.5185
9	19	0.6616	0.0758	0.2734
10	50	0.7816	0.0524	0.1657
11	13	1.1488	0.0828	0.2986
12	5	0.7482	0.1095	0.3284
13	11	1.0614	0.1637	0.5429
13	11	0.8848	0.1069	0.3547
14	7	1.0323	0.2414	0.6388
17	6	0.6609	0.2150	0.4808
18	8	0.3106	0.1261	0.3782
19	6	0.6032	0.1648	0.4038
20	4	0.5295	0.0778	0.2334
21	11	1.0217	0.1711	0.5675

Table 3.17. Transformed data for Peru F_2 generation female *R. prolixus*

Family number	n	$\bar{x}$	S.E.	S.D.
1	8	0.2634	0.0376	0.1064
2	4	0.8919	0.2259	0.4517
3	6	0.7564	0.1391	0.3407
4	8	0.4921	0.1510	0.4270
6	8	0.8917	0.0560	0.1583
7	5	0.9426	0.2708	0.6054
8	7	0.6460	0.1458	0.3856
9	6	0.3465	0.1360	0.3331
10	9	0.6724	0.1562	0.4686
11	7	0.8205	0.1387	0.3669
12	4	0.6276	0.1468	0.2936
13	8	0.7773	0.1779	0.5033
15	9	0.2928	0.1006	0.3019
16	7	0.9775	0.2384	0.6306
17	12	0.4998	0.1338	0.4633
18	7	0.5649	0.1707	0.4516
19	10	0.6808	0.1594	0.5042
20	7	0.4959	0.2053	0.5432
21	9	0.6748	0.1083	0.3249

Table 3.18. Transformed data for Peru F_2 families (male and female) of *R. prolixus*

Family number	n	$\bar{Y}$	S.E.	S.D.	Parental family number
1	18	[illegible]	0.0916	0.3884	43
2	8	0.8013	0.1681	0.4756	43
3	15	0.7576	0.1179	0.4567	43
4	15	0.7113	0.1276	0.4942	63
6	15	0.8591	0.1012	0.3918	55
7	13	0.8275	0.1436	0.5176	55
8	12	0.8583	0.1436	0.4977	55
9	19	0.5621	0.0737	0.3214	56
10	19	0.7299	0.0776	0.3383	56
11	20	1.0339	0.0789	0.3530	[illegible]
12	13	0.7111	0.0863	0.3112	[illegible]
13	19	0.9418	[illegible]	[illegible]	[illegible]
15	20	0.6184	0.0990	0.4426	[illegible]
16	14	1.0049	0.1632	0.6105	61
17	17	0.5472	0.1114	0.4595	73
18	16	0.4218	0.1045	0.4181	73
19	16	0.6517	[illegible]	0.4565	[illegible]
20	16	0.5148	0.0960	[illegible]	[illegible]
21	20	0.8656	0.1108	0.4953	55

Parental family number - F_1 family from which parents of F_2 families were selected.

Table [illegible]: Transformed data for Strain 7 parental generation

[illegible]

Sex	[illegible]	[illegible]	S.E.	S.D.
♂	[illegible]	0.8314	0.0981	0.6282
♀	36	[illegible]	[illegible]	[illegible]
♂ + ♀	77	0.9560	[illegible]	0.5934

Table 3.20. Transformed data for Strain 7 F_1 generation male *P. proximus*

Family number	n	$\bar{x}$	S.E.	S.D.
50	20	1.3559	0.1849	0.8270
69	11	1.4781	0.2005	0.6651
93	14	1.1160	0.2281	0.8533
73	12	0.5382	0.1561	0.5407
94	21	1.4630	0.1035	0.4745
58	17	1.5263	0.1750	0.7214
46	16	1.2555	0.1748	0.6991
55	4	1.5588	0.3173	0.6347
70	7	1.4313	0.3007	0.7954
76	3	0.9830	0.6112	1.0587
66	13	1.4672	0.1441	0.5197

Table 3.21- Transformed data for Strain 7 F_1 generation female S. cerealicum

Family number	n	$\bar{x}$	S.E.	S.D.
50	16	0.9944	0.1666	0.6665
69	11	0.6919	0.1617	0.5363
93	8	0.9106	0.2249	0.6362
73	8	0.0376	0.0376	0.1064
94	15	[illegible]	0.1384	0.5361
58	9	[illegible]	0.0571	0.1713
46	18	1.1226	0.1562	0.6626
55	6	1.2872	0.3209	0.7860
70	[illegible]	[illegible]	0.2063	0.7990
76	8	0.8255	0.1722	0.4870
66	[illegible]	1.1404	0.1832	0.5812

Table 3.12. Transformed data for Strain 7 F_2 families (male and female) of *P. prolixus*

Family number	n	$\bar{x}$	S.E.	S.D.
5[illegible]	36	1.1952	[illegible]	[illegible]
69	22	1.0850	0.1522	0.7138
93	22	1.0413	0.1646	0.7719
73	20	0.3379	0.1088	0.4866
94	36	1.3500	0.0853	0.5118
58	26	1.5414	0.1148	0.5856
46	34	1.1851	0.1154	0.6729
55	10	1.3958	0.2230	0.7051
70	22	0.7961	0.1911	0.8965
76	11	0.8685	0.1896	0.6290
66	21	1.3427	0.1160	0.5315

Table 3.23. Transformed data for Strain 7 F_2 generation male *B. prolixus*

Family number	n	$\bar{x}$	S.E.	S.D.	Parental family number
1	7	1.1440	0.2387	0.6315	50
2	7	0.6468	0.1696	0.4486	50
3	13	1.5463	0.1483	0.5347	58
4	8	1.5444	0.2875	0.8132	58
5	15	1.2261	0.1356	0.5251	94
6	5	1.2393	0.1269	0.2839	94
7	10	1.3600	0.1604	0.5072	46 x 70
8	9	1.7106	0.2282	0.6847	70 x 46
9	12	1.3895	0.1328	0.4601	93
10	13	0.9369	0.1582	0.5705	93
11	10	0.9527	0.2517	0.7960	70
12	14	1.4922	0.0970	0.3629	73
13	10	0.8785	0.1915	0.6056	73
14	6	0.4762	0.2493	0.6106	73
15	12	1.1378	0.1839	0.6371	69
16	6	1.3303	0.1644	0.4026	76

Table 3.24. Transformed data for Strain 7 F_2 generation female *R. prolixus*

Family number	n	$\bar{x}$	S.E.	S.D.	Parental family number
1	10	0.6583	0.1672	0.5286	50
2	8	0.9028	0.0965	0.2729	50
3	13	1.3250	0.1914	0.6903	58
4	13	1.1154	0.1462	0.5270	58
5	6	0.9140	0.2380	0.5829	94
6	8	1.0489	0.1735	0.4908	94
7	7	0.9758	0.1378	0.3646	46 x 70
8	8	1.1017	0.1684	0.4764	70 x 46
9	8	0.6904	0.1751	0.4953	93
10	4	0.5909	0.2291	0.4581	93
11	9	0.5203	0.2504	0.7511	70
12	14	0.6913	0.0870	0.3255	73
13	11	0.5619	0.1818	0.6030	73
14	5	0.0602	0.0602	0.1346	73
15	8	0.9375	0.1864	0.5272	69
16	4	0.6694	0.2268	0.4535	76

Table 3.24. Transformed data for Strain 7 F_2 generation female *H. prolixus*

Family number	n	$\bar{x}$	S.E.	S.D.	Parental family number
1	10	0.6583	0.1672	0.5286	50
2	8	0.9028	0.0965	0.2729	50
3	13	1.3250	0.1914	0.6903	58
4	13	1.1154	0.1462	0.5270	58
5	6	0.9140	0.2380	0.5829	94
6	8	1.0489	0.1735	0.4908	94
7	7	0.9758	0.1378	0.3646	46 x 70
8	8	1.1017	0.1684	0.4764	70 x 46
9	8	0.6904	0.1751	0.4953	93
10	4	0.5909	0.2291	0.4581	93
11	9	0.5203	0.2504	0.7511	70
12	14	0.6913	0.0870	0.3255	73
13	11	0.5619	0.1818	0.6030	73
14	5	0.0602	0.0602	0.1346	73
15	8	0.9375	0.1864	0.5272	69
16	4	0.6694	0.2268	0.4535	76

Table 3.7a. Transformed data for [illegible] F_2 families (male and female) of *K. prolixum*

Family number	n	$\bar{x}$	S.E.	S.D.	Parental family number
1	17	0.8583	0.1470	0.6062	50
2	15	0.7833	0.0969	0.3755	[illegible]
3	26	1.4357	[illegible]	0.6154	58
4	21	1.2789	0.1454	0.6661	58
5	21	1.1389	0.1193	0.5466	94
6	13	1.1221	0.1166	0.4203	94
7	17	1.2018	0.1170	0.4823	46 x 70
8	17	1.4240	0.1594	0.6571	70 x 46
9	20	1.1099	0.1297	0.5880	93
10	17	0.8555	0.1342	0.5534	93
11	19	0.7478	0.1802	0.7853	70
12	28	1.0918	0.1001	0.5299	73
13	21	0.7127	0.1333	0.6108	73
14	11	0.2871	0.1480	0.4908	73
15	20	1.0577	0.1318	0.5895	69
16	10	1.0659	0.1658	0.5245	76

Table 3.26. Transformed data for Strain 7 F_1 male *M. prolixus*, based on the mean of two observations for each bug

Family number	n	$\bar{x}$	s.d.	s.e.	Selection lines	Parental family number
1	12	0.3274	0.0905	0.3133	2	10
2	16	0.6970	0.0689	0.2757	3	14
4	20	0.6999	0.0806	0.3603	1	11
5	8	1.1599	0.3114	0.6228	4	13
7	10	1.1603	0.0903	0.3833	1	17
8	5	0.9796	0.2333	0.6558	1	19
9	13	0.6849	0.1792	0.4478	1	15
11	11	0.6827	0.2045	0.6884	1	16
12	15	0.5841	0.1660	0.5988	3	18
13	17	1.4104	0.0993	0.4095	4	2
14	3	1.6855	0.0000	0.0449	4	1
16	20	1.5172	0.0954	0.4196	4	5
17	12	1.4884	0.1583	0.5428	4	9
18	19	1.6806	0.1058	0.4090	4	8
19	16	1.3956	0.1111	0.4483	4	6
20	16	1.4370	0.1078	0.4149	4	3
21	6	1.2918	0.2611	0.6459	3	4

Key to selection lines for Strain 7 families:-

1 = selection for low score

2 = reverse selection from low-scoring families

3 = reverse selection from high-scoring families

4 = selection for high score

Table 3.27. Transformed data for Strain 7 F_3 female [illegible], based on the mean of two observations for each bug

Family number	n	[illegible]	[illegible]	[illegible]	Selection line	Parental family
1	11	0.3662	0.1131	0.1752	[illegible]	[illegible]
2	18	0.4097	0.0834	0.3536	[illegible]	[illegible]
4	11	0.4466	0.0850	0.2820	[illegible]	[illegible]
6	9	0.7438	0.1382	0.4146	[illegible]	[illegible]
7	19	0.6814	0.1083	0.4721	[illegible]	[illegible]
8	8	0.9904	0.1499	0.4241	[illegible]	[illegible]
9	6	0.7326	0.0718	0.1758	[illegible]	[illegible]
10	13	0.3644	0.1178	0.4967	[illegible]	[illegible]
11	15	0.4347	0.1508	0.5841	[illegible]	[illegible]
12	18	1.2190	0.1366	0.5795	[illegible]	[illegible]
13	5	1.3052	0.2360	0.5278	[illegible]	[illegible]
14	9	1.0102	0.1432	0.4295	[illegible]	[illegible]
15	12	[illegible]	0.1455	0.5041	[illegible]	[illegible]
16	12	0.9960	0.1659	0.5746	[illegible]	[illegible]
17	8	1.0322	0.0704	0.1991	[illegible]	[illegible]
18	8	1.2303	[illegible]	0.5405	[illegible]	[illegible]
19	4	1.4082	0.2134	[illegible]	[illegible]	[illegible]

Table 3.28. Transformed data for Strain 7 F_3 families (male and female) of *R. prolixus*, based on the mean of two observations for each bug

Family number	n	$\bar{x}$	S.E.	S.D.	Selection line	Parental family number
1	41	0.3459	0.0702	0.3368	7	10
2	35	0.5449	0.0595	0.3467	6	10
3	31	0.6100	0.0632	0.3520	7	12
4	18	0.8719	0.1391	0.5015	7	11
5	47	0.4161	0.0805	0.4896	6	12
6	17	0.9862	0.1382	0.4083	7	10
7	49	0.7008	0.0880	0.3732	7	10
8	34	0.5103	0.1216	0.5959	7	11
9	30	0.5095	0.1070	0.5862	6	11
10	35	1.3119	0.0856	0.5062	5	7
11	9	1.4478	0.1575	0.4455	4	8
12	11	1.3789	0.0778	0.4467	5	9
13	24	1.2379	0.1188	0.5803	5	8
14	27	1.3763	0.1138	0.5915	4	9
15	28	1.2745	0.0851	0.4168	5	8
16	28	1.3948	0.0947	0.4640	4	9
17	51	1.3383	0.1721	0.5441	5	9

Table 3.10. Analysis of variance of male scores of parental generations of R. prolixus infected with T. [illegible] of 2 strains, Strain 7 and Peru.

Item	df	MS	VR	P
Between strains	1	2.5362	[illegible].6433	[illegible]**
Within strains	80	0.2630		

df = Degrees of freedom

MS = Mean square

VR = Variance ratio

Table 3.11. Analysis of variance of female scores of parental generations of R. prolixus infected with T. [illegible] of 2 strains, Strain 7 and Peru.

Item	df	MS	VR	P
Between strains	1	6.7611	26.3283	<0.001***
Within strains	74	0.2568		

Table 3.11. Analysis of variance of male and female scores of parental generations of R. prolixus infected with T. [illegible] of 2 strains, Strain 7 and Peru.

Item	df	MS	VR	P
Between strains	1	8.5730	32.6839	<0.001***
Within strains	156	0.2623		

These 3 analyses show that in the parental generation there were highly significant differences in intensity of infection between bugs infected with the 2 difference strains of trypanosome, when compared to variation between bugs within strains and that these differences were common to both sexes.

A similar analysis was carried out on the data from F_1 bugs infected with different strains of trypanosome (Table 3.32.).

Table 3.32. Analysis of variance of male and female scores of F_1 generations of *R. prolixus* infected with *T. cruzi* of 2 strains. Strain 7 and Peru.

Item	df	MS	VR	P
Between strains	1	20.4891	12.1979	[illegible]***
Between bug families (within strains)	21	1.6811	5.3267	<0.[illegible]1***
Between individual bugs (within families)	[illegible]	0.3156		

This analysis shows that there were highly significant differences in intensity of infection between F_1 bugs infected with the 2 strains of *T. cruzi*, even when this variation was tested against the considerable differences between F_1 families. Comparison of further generations was not made as such analyses would not be strictly valid because of the different amounts of selection pressure which were applied to subsequent generations of the 2 groups of bugs. However, it is clear that differences between *T. cruzi* strains were very large and these are reflected in the

mean scores for the parental generations, shown in Tables 3.12 and 3.19: Peru mean = 0.49, S.E. = 0.031; Strain 7 mean = 0.96, S.E. = 0.07. It may be concluded that bugs infected with Strain 7 *T. cruzi* excreted much greater numbers of trypanosomes (bearing in mind that these scores have been transformed logarithmically) than those infected with the Peru strain. This result is even more remarkable when it is recalled that bugs feeding on mice infected with Peru strain were ingesting more parasites than the bugs feeding on mice infected with Strain 7, the parasitaemia of the Strain 7 mice being <1 trypanosome per field of tail blood at the time of feeding, while the Peru mice had 1-9 trypanosomes per field. These differences suggest strongly that the intensity of infection of an infected bug is markedly influenced by the genotype of the trypanosome ingested. The significant difference demonstrated between bugs of the parental generations is important since these bugs were selected at random from the same colony, and prior to selection may be assumed to have been genetically homogeneous, suggesting that differences between parental groups are a consequence of genotypic differences between strains of trypanosome. However, the highly significant differences between F_1 families within *T. cruzi* strains when tested against the variation between individual bugs within families further suggests that the bug genotype (determined by family differences), as well as trypanosome genotype, is involved in determining the intensity of infection in *R. prolixus*.

Differences between sexes and families

The variation in intensity of infection between sexes of *R. prolixus* was analysed for both Strain 7 and Peru infections in bugs of all generations.

1. Parental generation.

Difference between male and female scores for bugs of the parental generation were compared by analysis of variance, the results of which are shown in Tables 3.33 and 3.34.

Table 3.33. Analysis of variance of male and female scores for parental generation R. prolixus infected with T. cruzi Peru strain.

Item	df	MS	VR	P
Between sexes	1	0.0088	—	N.S.
Within sexes	78	0.0706		

Table 3.34. Analysis of variance of male and female scores for parental generation R. prolixus infected with T. cruzi strain 7.

Item	df	MS	VR	P
Between sexes	1	1.3610	4.0183	0.05-0.01*
Within sexes	75	0.3387		

These results show that in the parental generation there were no significant differences between male and female scores for bugs infected with Peru strain while there were significant differences between sexes for bugs infected with Strain 7 T. cruzi when compared to the variation between individuals within each sex.

2. F_1 generation

(a) Peru strain. The mean male score in F_1 generation for male bugs infected with T. cruzi Peru strain was greater than the mean female score ($\bar{x}$ males = 0.83, S.E. $\bar{x}$ = 0.20; $\bar{x}$ females = 0.68, S.E. $\bar{x}$ = 0.21).

These differences in mean score were examined using analysis of variance, the results of which are shown in Table 3.35.

Table 3.35. Analysis of variance of male and female scores for F_1 generation [illegible] infected with T. cruzi Peru strain

Item	df	MS	F	P
[illegible]	1	0.7453	[illegible]	0.05-0.1*
Increment due to families	[illegible]	[illegible]	3.781	[illegible]***
Residual	283	0.1937		

The results show that there were slightly significant differences between sexes when compared to the variation between F_1 families. The increment of variation due to families was highly significant compared to the variation between individuals within families. This variation between sex-families was partitioned and a further analysis carried out, the results of which are shown in Table 3.36.

Table 3.36. Analysis of variance of male and female scores for F_1 generation *R. prolixus* infected with *T. cruzi* Peru strain.

Item	df	ME	VR	P
Between sexes	1	0.7453	[illegible]	[illegible]*
Between families	11	0.9697	5.0052	<0.001***
Sex X families	11	0.2120	1.0944	N.S.
Residual	283	0.1937		

This analysis shows that variation between sexes was significant when tested against the error item. The variation between families was highly significant when tested against the error and there was no significant interaction between sex and family, indicating that the difference between sexes was constant for all the F_1 families infected with Peru strain *T. cruzi*.

(b) Strain 7. The mean score of F_1 males was greater than that of females infected with Strain 7 ($\bar{x}$ males = 1.31, S.E. $\bar{x}$ = [illegible].44; $\bar{x}$ females = [illegible].94, S.E. $\bar{x}$ = 0.31).

This difference was again examined by analysis of variance, the results of which are shown in Tables 3.37 and 3.38.

Table 3.37. Analysis of variance of male and female scores for F_1 generation *R. prolixus* infected with *T. cruzi* Strain 7.

Item	df	ME	VR	P
Between sexes	1	6.3751	4.7612	0.01-0.05*
Increment due to families	20	1.3390	3.1714	<0.001***
Residual	238	0.4222		

Table 3.36. Analysis of variance of male and female scores for F_1 generation *R. prolixus* infected with *T. cruzi* Strain 7, partitioning the variation due to families.

Item	df	MS	VR	P
Between sexes	1	6.3751	15.0994	<0.001***
Between families	10	2.1975	5.2047	<0.001***
Sex X families	10	0.4340	1.0279	N.S.
Residual	238	0.4222		

The results in Table 3.37 show that the differences between sexes were significant when tested against the variation due to families, but when this increment due to families was partitioned (Table 3.38) the variation between sexes was highly significant when tested against the residual variation. Table 3.38 also shows that there was no significant interaction between sex and family when this item was tested against the error, indicating that differences between sexes were consistent for all families of this generation. As with the *T. cruzi* Peru strain infected bugs, differences between families in intensity of infection were highly significant when tested against the error for F_1 bugs infected with *T. cruzi* Strain 7.

3. F_2 generation

(a) Peru strain. The mean score of F_2 males infected with Peru strain *T. cruzi* was greater than the mean score for females infected with the same strain ($\bar{x}$ males = 0.81, S.E. $\bar{x}$ = 0.19; $\bar{x}$ females = 0.63, S.E. $\bar{x}$ = 0.14). These differences were again examined by analysis of variance, the results of which are shown in Tables 3.39 and 3.40.

Table 3.39. Analysis of variance of male and female scores for F_2 generation *A. prolixus* infected with *T. cruzi*, Peru strain.

Item	df	MS	VR	P
Between sexes	1	1.7408	4.6423	[illegible]*
Increment due to families	36	[illegible]	2.0470	[illegible]**
Residual	267	0.1832		

Table 3.40. Analysis of variance of male and female scores for F_2 generation *A. prolixus* infected with *T. cruzi*, Peru strain, partitioning the variation due to families.

Item	df	MS	VR	P
Between sexes	1	1.7408	[illegible]	[illegible]**
Between families	18	[illegible].4870	[illegible]	[illegible]***
Sex X families	18	[illegible].2419	1.3203	N.S.
Residual	267	0.1832		

The results in Table 3.39 show that the differences between sexes were significant when tested against the variation due to families and on partitioning this last item (Table 3.40) the differences between sexes were shown to be highly significant when tested against the error variation. The variation between F_2 families was highly significant when tested against the error variation (Table 3.40) but there was no

significant interaction between sex of bug and family, again showing that sex differences were constant over families.

(b) Strain 7. The mean scores for the two sexes of F_1 bugs again differed with this strain of trypanosome; the males having the higher mean score as in previous generations ($\bar{x}$ males = [illegible], S.E. [illegible]; $\bar{x}$ females = [illegible], S.E. $\bar{x}$ = [illegible]), and this difference was tested by analysis of variance, the results of which are shown in Tables 3.41 and 3.42.

Table 3.41. Analysis of variance of male and female scores for F_1 generation *Rh. prolixus* infected with *T. cruzi* Strain 7.

Item	df	MS	VR	P
Between sexes	1	9.8183	11.3665	<0.001***
Increment due to families	30	0.8638	2.8770	<0.001***
Residual	261	0.3002		

The difference between sexes was highly significant even when tested against the large increment of variation due to families, but this increment was again partitioned to test for any sex X family interaction.

Table 5.4j. Analysis of variance of male and female scores for F_2 generation *R. prolixus* infected with *T. cruzi* Strain 7, partitioning the variation due to families.

Item	df	MS	VR	P
Between sexes	1	9.8183	32.7016	<0.[illegible]1***
Between families	15	[illegible]	[illegible]	<0.001***
Sex X families	15	0.2775	[illegible]	N.S.
Residual	961	0.3002		

Differences between F_2 families were highly significant in both analyses but the sex X families interaction was not significant, demonstrating once more that differences between sexes were constant for families of different pedigree. The highly significant differences between families which are a constant feature of the analyses of intensity of infection shown above strongly suggest that bug genotype is involved in regulating [illegible] growth in the gut. These family differences were demonstrated for both trypanosome strains used and were independent of sex differences. [illegible] significant sex X family interaction terms were found on analysis of data. Differences in variance between families must reflect the similarity of within-family scores which can only have resulted from the genetic resemblance of siblings.

4. F_3 generation

Only bugs infected with Strain 7 *T. cruzi* were bred to the F_3 generation. An analysis of variance of male and female scores was

carried out, the results of which are shown in Table 3.43.

Table 3.43. Analysis of variance of male and female scores for F_3 generation *H. prolixus* of 2 selection lines from 17 families infected with *T. cruzi* Strain 7, each score based on the mean of 2 observations.

Item	df	MS	VR	P
1. Between sexes	1	10.9109	30.4859	<0.001***
2. Sex x Lines	1	8.0911	22.6141	<0.001***
3. Sex X families (within lines)	15	0.2732	-	N.S.
4. Residual	374	0.3613		
3.+ 4. Pooled	389	0.3579		

The results show that differences between sexes were highly significant when tested against items 3 and 4 pooled. There was no significant sex x family interaction, which would indicate that the significant differences between sexes were constant for different families. The sex X lines interaction (item 2) was highly significant when tested against the error variation indicating that differences between sexes was affected by the direction of selection. A survey of the mean values for the F_3 generation shows that while the mean male score ($\bar{x}$ = 1.11) was greater than the female mean ($\bar{x}$ = 0.78) for all families, the difference between sexes was greater in families

numbered 12 - 19 of the high selection lines ($\bar{x}$ males - $\bar{x}$ females = 0.13) than between families numbered 1 - 11 of the low selection lines ($\bar{x}$ males - $\bar{x}$ females = 0.04). It may be that this difference is an effect of scaling, such that the logarithmic transformation did not completely normalize the data or, alternatively, it could be that control of susceptibility is determined by a sex-linked gene or genes.

Since throughout the experiment the results have shown that male scores for trypanosomes in their faeces were greater than female scores, several possible causes, other than bug or trypanosome genotype were tested. First, the explanation for higher levels of male infections may have been simply that fifth instar males (the stage at which the bugs, male and female, were given their infecting meal throughout these experiments) were taking larger infecting blood-meals than female larvae. This possibility was investigated by weighing 2 groups of fifth instar larvae of different sex before and after a blood-meal, and comparing the weights of the blood-meals. The results of this experiment are shown in Table 3.44.

Table 3.44. Analysis of blood-meal weights for 20 male and 20 female fifth instar *R. prolixus* fed on mice. Weights in grams.

		Mean weight of unfed bugs	Mean weight of fed bugs	Mean weight of blood-meal
Males	$\bar{x}$	[illegible]	0.1815	[illegible]
	S.E. $\bar{x}$	0.0009	[illegible]	[illegible]
Females	$\bar{x}$	0.0257	[illegible]	[illegible]
	S.E. $\bar{x}$	0.0011	0.0067	[illegible]
	t	1.1959	1.8398	1.4929
	P	N.S.	[illegible]	[illegible]

$\bar{x}$ - Mean S.E. - Standard error

The results of the t-tests show that there was no significant difference between the amount of blood taken by male and female fifth instar larvae, and it may be inferred that experimental bugs of different sex took approximately the same size infecting blood-meals.

Secondly the relationship between sex and trypanosome score was further investigated by comparing the rate of defaecation of male and female adult R. prolixus. For this comparison male and female adults (which, like the experimentally infected bugs, had not had a previous meal as adults) were individually fed to repletion and then each allowed to excrete for one hour, when the weight of faeces collected in the tubes was calculated by difference in weight. The results of this experiment are shown in Table 3.45.

Table 3.45. Analysis of blood-meal and excreta weights for 10 male and 10 female R. prolixus adults. Weights in grams.

	Mean weight of unfed bugs	Mean weight of fed bugs	Mean weight of blood-meal	Mean weight of excreta after 1 hr.	% Blood-meal excreted in 1 hr.
Males $\bar{x}$	0.0509	[illegible]	[illegible]	[illegible]	[illegible]
S.E. $\bar{x}$	0.0019	0.0046	0.0031	0.0035	
Females $\bar{x}$	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
S.E. $\bar{x}$	0.0033	0.0075	0.0046	0.0054	
t	3.8252	6.7063	7.9208	3.3056	
P	<0.001***	<0.001***	<0.001***	0.01-0.001**	
χ^2					16.2082
P					[illegible]***

$\bar{x}$ = Mean

S.E. $\bar{x}$ = Standard error of mean

Weight excreta = weight of excreta collected in 1 hr. post feeding

$$\% \text{ blood-meal excreted} = \frac{\text{weight excreta}}{\text{weight blood-meal}} \times 100$$

The results of the t-tests between sexes show that adult females took significantly larger blood-meals than males and also excreted significantly larger [illegible] of faeces than [illegible] after feeding. The χ^2 test for proportions showed that females also excreted a greater proportion of their blood-meal within one hour of feeding than did the adult males. [illegible], it would be expected that, at an infecting blood-meal, fifth instar males and females would ingest approximately the same [illegible] of trypanosomes and, when subsequently given a clean feed, the females would excrete a greater number of trypanosomes within a limited period. However, the results of the present work have demonstrated the contrary; males excreted significantly more trypanosomes than adult female R. prolixus throughout the experiment. It may be concluded that differences in intensity of infection between sexes are independent of variations in blood-meal size (and thereby numbers of trypanosomes ingested) and of differences in rates of excretion, since females took larger blood-meals and yet excreted significantly fewer trypanosomes. These sex differences appear to be independent of environmental variables and must be attributed to genetically determined differences between the two sexes of R. prolixus.

Difference between selection lines

In analysing the effects of selection for intensity of infection in successive generations of R. prolixus, only data for bugs infected with [illegible] Strain 7 (Tables 4.19 - 4.28) have been used since this strain infected bugs were only bred to the F_2 generation and could not provide sufficient information for a reasonable assessment of the changes brought about by selection. The bugs of the F_1 generation infected with Strain 7 were each scored twice by repeated sampling of

The results of the t-tests between sexes show that adult females took significantly larger blood-meals than males and also excreted significantly larger amounts of faeces than males within one hour after feeding. The χ^2 test for proportions showed that females also excreted a greater proportion of their blood-meal within one hour of feeding than did the adult males. Given these results, it would be expected that, if an infecting blood-meal, [illegible] approximately the same numbers of trypanosomes and, when subsequently given a clean feed, the females would excrete a greater number of trypanosomes within a limited period. However, the results of the present work have demonstrated that [illegible] more trypanosomes than adult female *R. prolixus* throughout the experiment. It may be concluded that differences in intensity of infection between sexes are independent of variations in blood-meal size (and thereby numbers of trypanosomes ingested) and of differences in rates of excretion, since females took larger blood-meals and yet excreted significantly fewer trypanosomes. These sex differences appear to be independent of environmental variables and must be attributed to genetically determined differences between the two sexes of *R. prolixus*.

Difference between selection lines

In analysing the effects of selection for intensity of infection in successive generations of *R. prolixus*, only data for bugs infected with *T. cruzi* Strain 7 (Table 3.19 - 3.28) have been used since Peat strain infected bugs were only bred to the F_1 generation and could not provide sufficient information for a reasonable assessment of the changes brought about by selection. The bugs of the F_2 generation infected with Strain 7 were each scored twice by repeated sampling of

their diluted faeces and analyses for this generation have been based on the mean of these 2 observations. The effects of selection pressure on mean family scores over successive generations of bugs infected with T. cruzi Strain 7 are illustrated in Figs. 3.3 - 3.8. In these diagrams the mean family scores have been plotted for each generation of selection and assortative sib-mating, the pedigree of each family being traced by the lines connecting the means. The numbers beside the F_3 means correspond to the F_3 family code numbers in Tables 3.26 - 3.28. Figs. 3.3 and 3.4 show the changes in mean family score for both sexes, Fig. 3.3 showing results of selection for low scores and Fig. 3.4 showing results of selection for bugs with high intensity of infection. Comparison of these 2 figures shows that family means did change in response to selection pressure for both high and low scores. However, since significant differences between sexes have already been demonstrated, it is more meaningful to plot response to selection of family means with sexes treated separately and these changes are illustrated in Figs. 3.5 - 3.8. These diagrams show that both sexes of Strain 7 infected bugs responded to selection pressures for high and low scores but they also reflect the differences between sexes demonstrated statistically above. For example, a comparison of F_3 family 19 males in Fig. 3.7 with the mean values for females of the same family in Fig. 3.8 shows that the males responded to selection pressure, but the females of the same family did not. However, a closer inspection of the results in Tables 3.26 and 3.27 shows that the mean male value was based on scores from 6 males while the female value was based on only 4 females. In fact, family 19 was one of the smallest F_3 families and therefore too much emphasis cannot be placed on this particular family, but it serves to emphasise that the mean values shown in

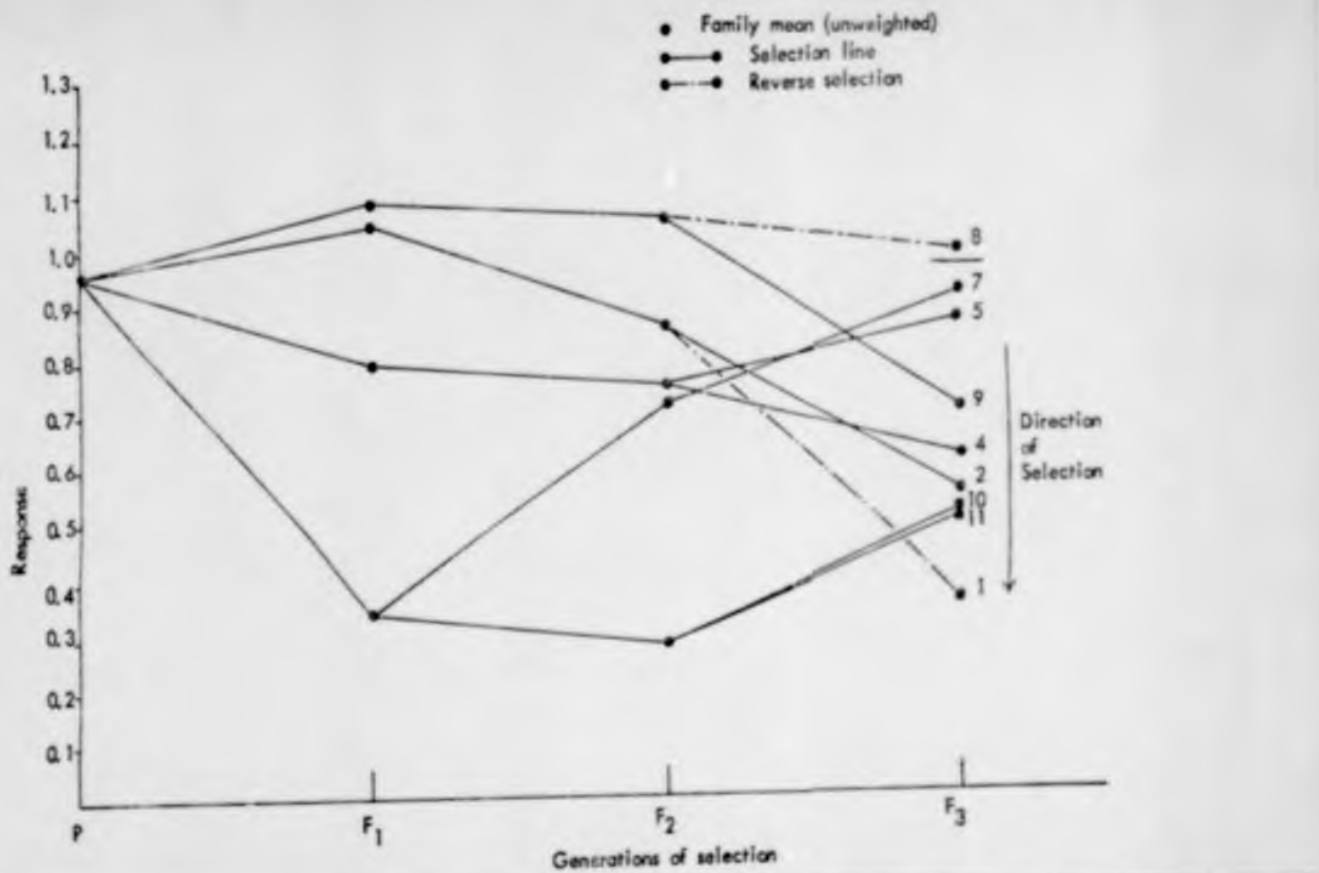


Figure 3.3. Changes in family means in response to selection for low levels of infection in *R. prolixus* infected with *T. cruzi* Strain 7.

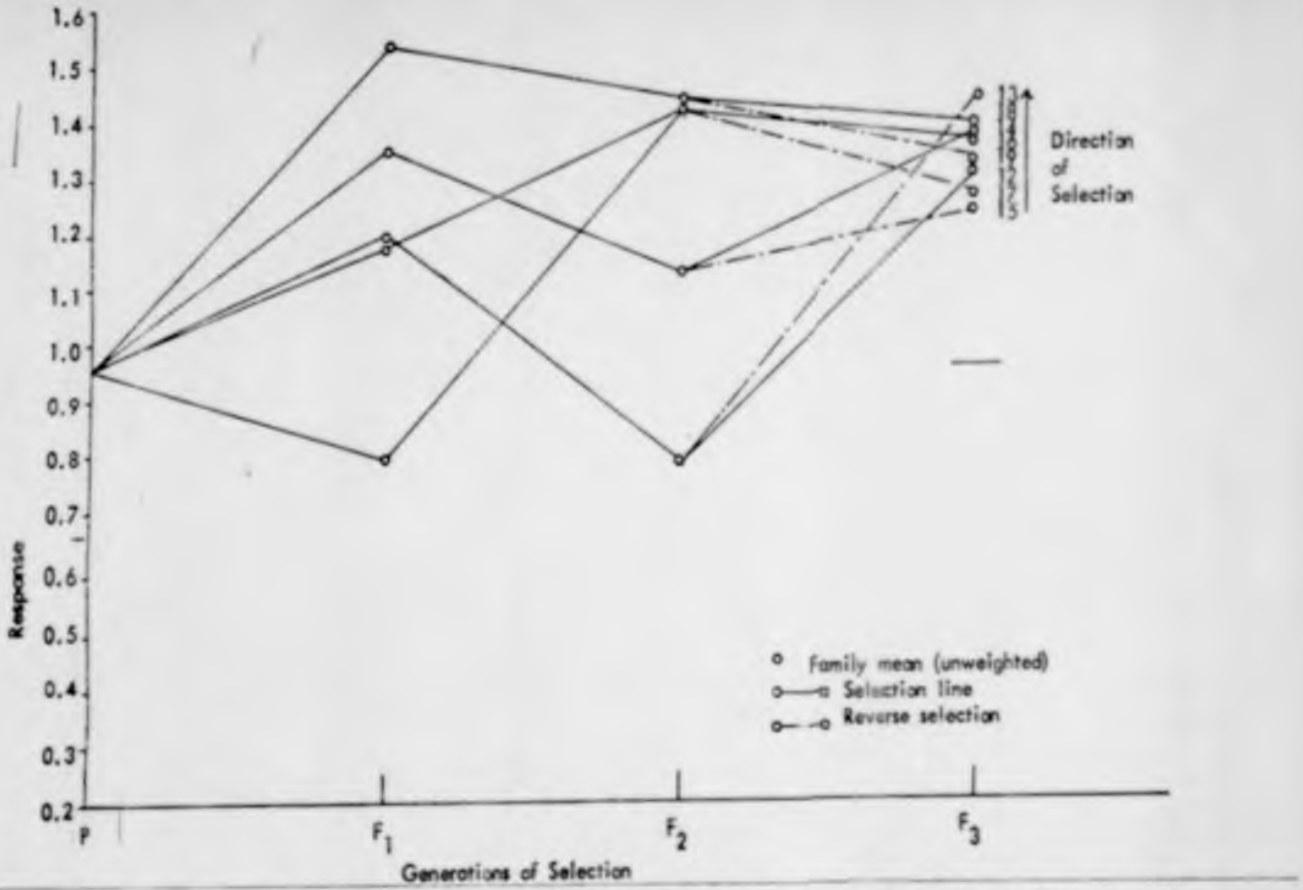


Figure 3.4. Changes in family means in response to selection for high levels of intensity of infection in R. prolixus infected with T. cruzi.

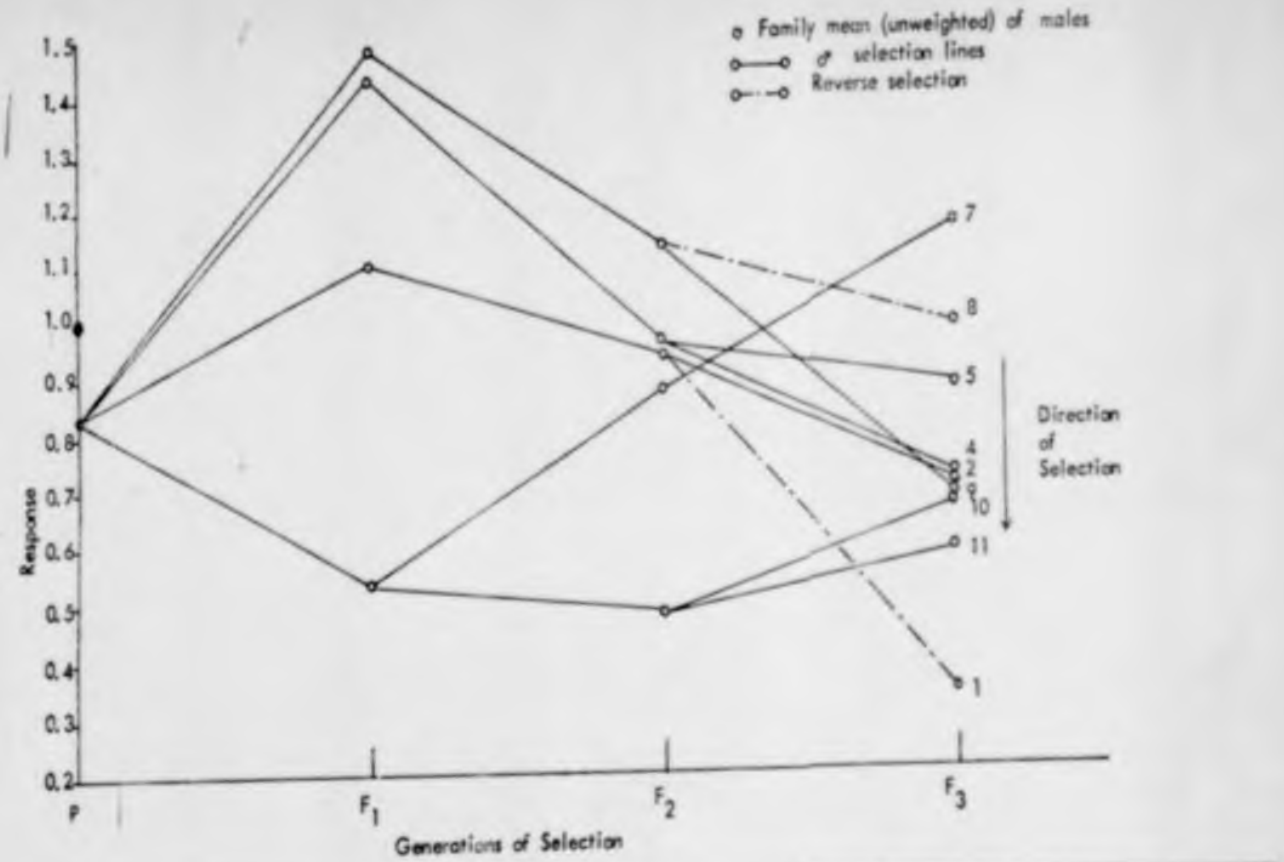


Figure 3.5. Changes in mean male scores in response to selection for low levels of intensity of infection in R. prolixus infected with T. cruzi Strain 7.

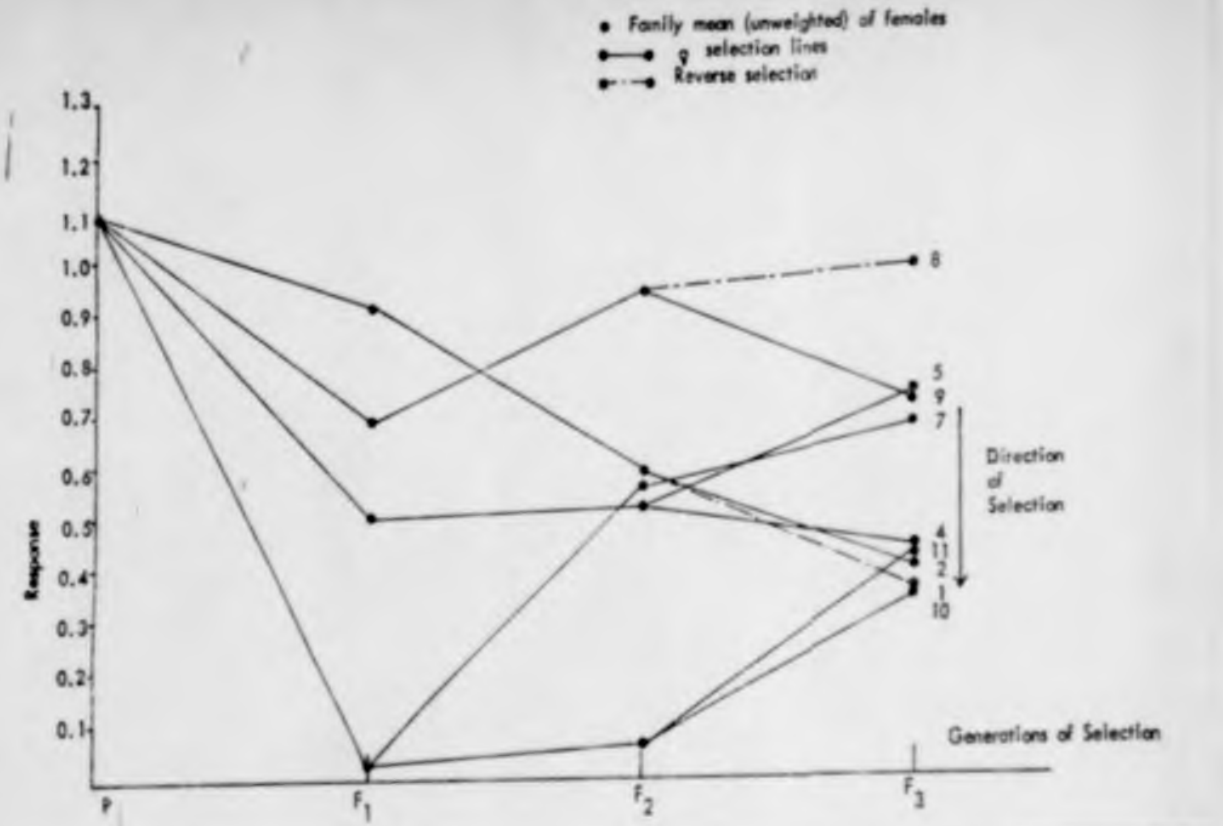


Figure 3.6. Changes in mean female scores in response to selection for low levels of intensity of infection in R. prolixus infected with T. cruzi Strain 7.

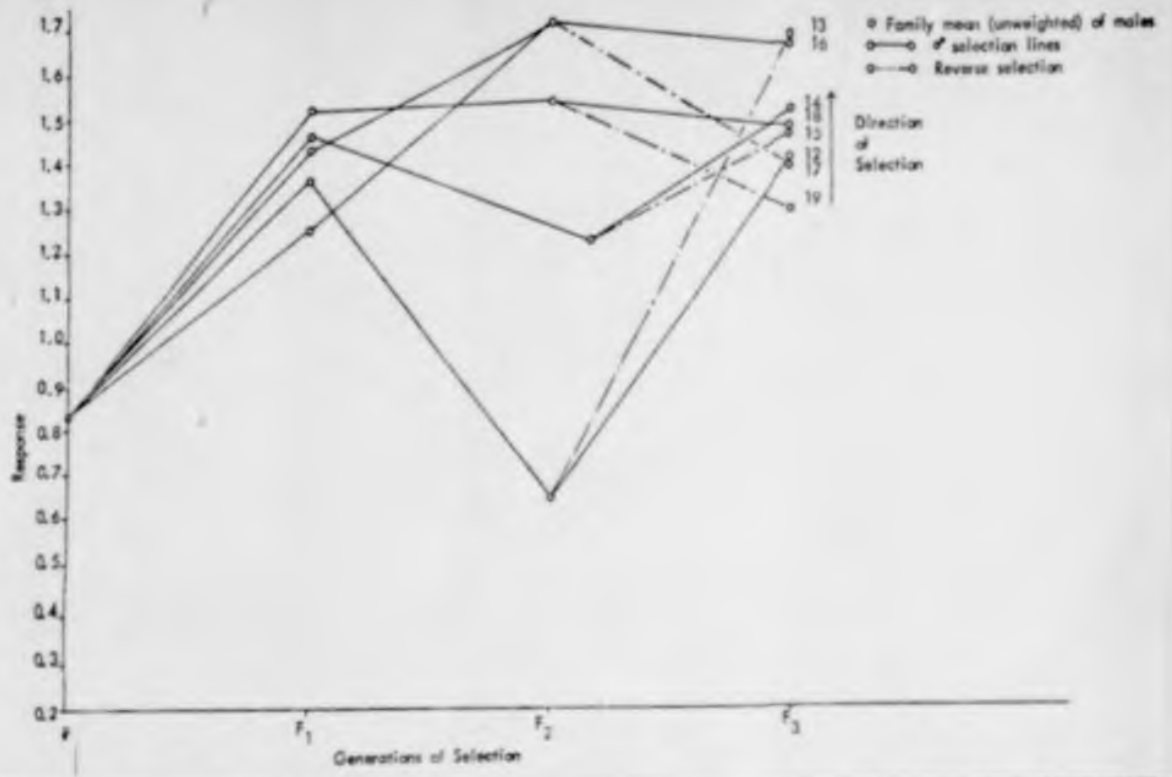


Figure 3.7. Changes in mean male scores in response to selection for high levels of intensity of infection in R. prolixus infected with T. cruzi Strain 7.

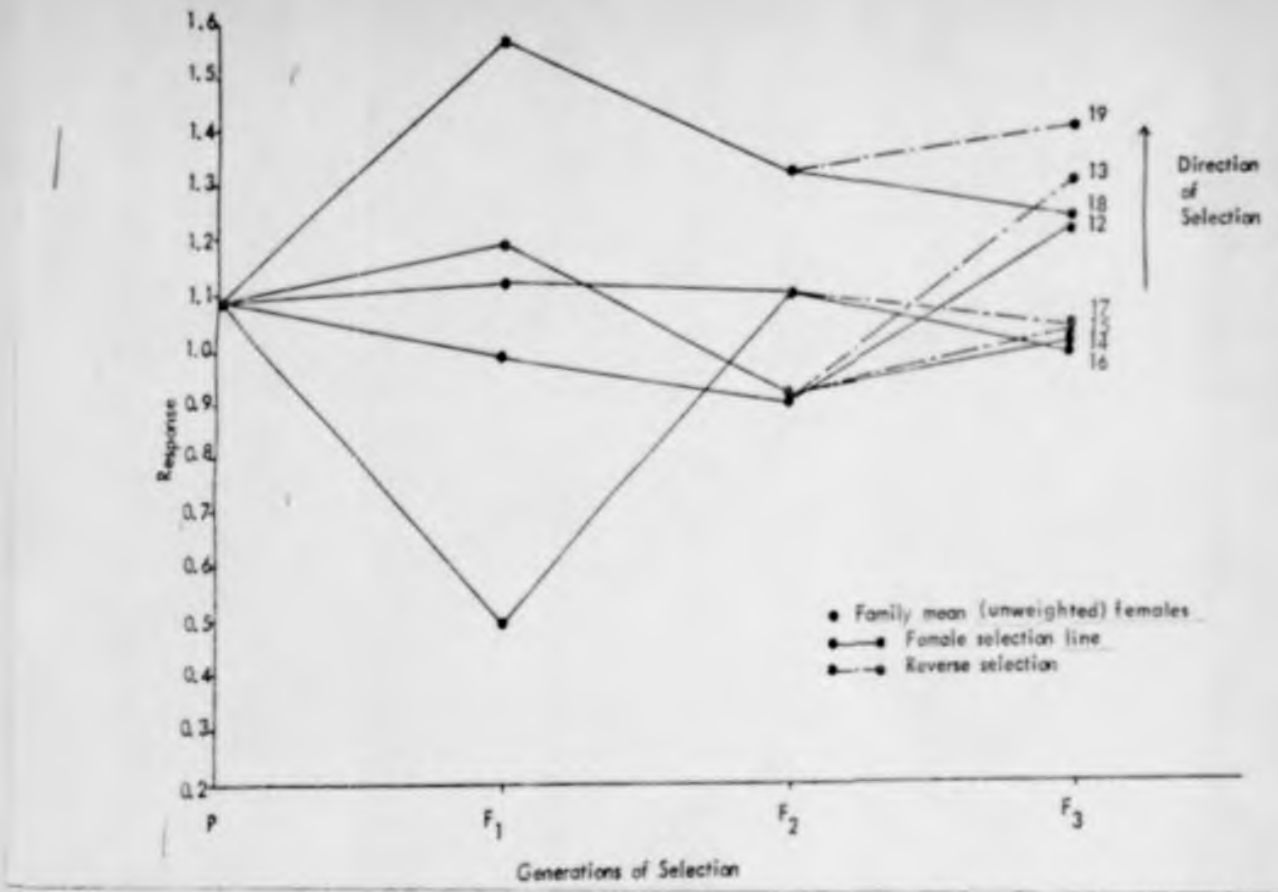


Figure 3.8. Changes in mean female scores in response to selection for high levels of intensity of infection in *H. prolixus* infected with *T. cruzi* Strain 7.

Figs. 3.1 - 3.8 do not convey a very meaningful impression of the response of family means to selection; firstly the means are based on differing numbers of individuals but are not weighted to compensate for this, and secondly, these diagrams give no impression of the degree of selection applied to each family.

However, differences between F_3 families infected with *T. circe* Strain 7 have been quantified, by using analysis of variance, which gives a meaningful comparison because sums of squares are calculated in relation to the number of observations in each family. The differences between males of F_3 families selected for high and low intensity of infection were examined by an analysis of variance, the results of which are shown in Table 3.46.

Table 3.46. Analysis of variance of male scores of 16 F_3 generation families infected with *T. circe* Strain 7 from 2 selection lines. Each score based on the mean of 2 observations.

Item	df	MS	VR	P
Between selection lines	1	30.5385	58.0911	[illegible]***
Between families (within lines)	15	[illegible]	[illegible]	0.01-0.001**
Between individuals (within families)	[illegible]	0.20[illegible]		

The results of this analysis show that there were highly significant differences in intensity of infection between males selected for high and low levels of intensity of infection when tested against the variation between families within selection lines. The variation between families

was also significant when tested against the variation between individuals within families.

A similar analysis of variance was carried out on females of the F_3 generation, the results of which are shown in Table 3.47.

Table 3.47. Analysis of variance of female scores of 17 F_3 families infected with *T. cruzi* strain 7 from 2 selection lines. Each score based on the mean of 2 observations.

Item	df	MS	VR	P
1. Between selection lines	1	15.2808	[illegible]	[illegible]***
2. Between families (within lines)	15	0.3438	[illegible]	[illegible]
3. Between individuals (within families)	168	0.2157		
Between selection lines	1	15.2808	[illegible]	[illegible]***
Pooled SS for 2 and 3	184	0.2262		

This analysis of female scores shows that there were highly significant differences in intensity of infection between bugs selected for high and low scores when compared to the variation between families within selection lines. The variation between families within lines was slightly significant when compared to the variation between individuals within families, and these 2 items were pooled to test further the differences between selection lines, and again the differences between

selection lines were shown to be highly significant. It may be concluded from these analyses that, as a result of selection by assortative sib-mating from a single parental population, 2 groups of families were produced in the F_3 generation, one of which contained bugs with high levels of intensity of infection, the other bugs with low levels, and that differences between these 2 groups were highly significant. Thus the differences between the 2 groups of F_3 families illustrated in Figs. 3.3 - 3.6 represent real differences and both sexes of bug infected with T. cruzi Strain 7. A further comparison was made between the F_3 family with the highest mean score from the families of the low selection lines (family 7, Fig. 3.3) - ignoring reverse selected families - and the family with the lowest mean score from families selected for high scores (family 12, Fig. 3.4). The results of this analysis are shown in Table 3.4b.

Table 3.4b. Analysis of variance of F_3 families 7 and 12 from 2 selection lines for high and low score.

Item	df	MS	VR	P
Between families	1	2.8430	11.4776	0.01-0.001**
Within families	70	0.2477		

The results show that there was a highly significant difference between these 2 families when tested against the variation between individuals within families which is reflected in the means: family 7 $\bar{x}$ = 0.9144, S.E. = 0.0878; family 12 $\bar{x}$ = 2.5417, S.E. = [illegible]. This

analysis provides further evidence of the efficacy of the two-way selection programme which produced 2 clearly separated groups of families by the F_3 generation.

Reverse selection.

Attempts were made to reverse the selection process from some F_2 families and these families are indicated by broken lines in Figs. 3.1 - 3.8. Owing to sterilities, only 7 reverse selection matings were successful from the low selection lines (F_3 families 1 and 8), and comparisons between these families and the other 7 families of the same selection group have not been made since insufficient reverse selected control data was available for a meaningful analysis. However, from each F_2 family of the high selection group, 2 matings were established, firstly a mating selected for high score and secondly a mating reverse selected for low score. A pair comparison can be made of these 7 groups, each of 4 families, and an analysis of variance was carried out using this data, the results of which are shown in Tables 3.49 and 3.50 for males and females respectively.

Table 3.49. Analysis of variance of male scores for F_3 families 12, 14, 16 and 18 of high selection lines and families correspondingly reverse selected for low score, 13, 15, 17 and 19.

Item	df	MS	VR	P
Between lines	1	0.2154	[illegible]	[illegible]
Between families (within lines)	6	[illegible].1508	—	N.S.
Between individuals (within families)	101	[illegible].1894		

Table [illegible]. Analysis of variance of tonic scores for F_3 families 13, 14, 16 and 18 of high selection lines and families correspondingly reverse selected for low score, 13, 15, 17 and 19.

Item	df	MS	VR	P
Between lines	1	0.0	-	N.S

These analyses show that there were no significant differences between bugs of either sex of the high selection lines and those families reverse selected for low score for [illegible]. However, it must be noted that these families were inbred as a result of sib-mating to F_3 and therefore the reverse selected and control families would be genetically similar, [illegible] the similarity of response in the 2 groups. But before any conclusive interpretation could be based on these results, further generations of reverse selection would have had to be carried out.

Having demonstrated in analyses in Tables [illegible] and 3.48 that selection for high and low scoring bugs was successful by the F_3 generation, it is necessary, to gain an overall insight into these results, to relate the amount of selection pressure applied to the response of the selected families.

Measurement of response to selection

i. Theoretical considerations. The change in a quantity that brought about by selection is called the response, but when more than one

generation of selection has been made, the measurement of the response is not simple. Inspection of Figs. 3.3 - 3.8 shows that the generation means of the families infected with Strain 7 did not progress in a regular fashion but fluctuated erratically. This is a phenomenon common to most selection programmes and Falconer (1967) attributes it to two causes: firstly, sampling variation which is determined by the number of individuals measured and, secondly, environmental variation. In the present experiment the sampling variation was large, variable numbers of bugs being raised in each family, and environmental variation may also have affected the results, for although temperature and humidity were easily regulated, other factors outlined in Fig. 3.1 may have affected the results, if only in a minor way. Where there is variation in generation means, Falconer (loc. cit.) states that the best measure of the average response per generation is obtained by fitting a regression line to the means, thereby assuming that the true response has been constant over the selection programme. The use of a two-way selection procedure in the present experiment allowed for a more accurate measurement of the response to selection to be made than would have been obtained from a comparison with an unselected control population, since environmental changes equally affected both selection lines. The response to selection measured from the divergence of the two regression lines will be about twice that of the lines separately, each line acting as a control for the other.

The response (R) to selection is defined as the difference in mean phenotypic value between the offspring of the selected parents and the whole of the parental generation. The measure of the selection

applied is the average superiority of selected parents and is called the selection differential (S) which is defined as the mean phenotypic value of selected parents expressed as a deviation from the population mean before selection (Falconer, loc. cit.). The regression of offspring score on parents is thus R/S and therefore $R = b_{op}S$ where b_{op} is the regression (b) of offspring on mid-parent value (op). Having carried out a selection programme, the equation of response given above can be used to estimate the heritability of a character from the results of the selection experiment. It can be shown that $b_{op} = h^2$ (where h^2 is the heritability) thus $h^2 = R/S$, this ratio being defined as the realized heritability (Falconer, loc. cit.) and providing a measure of the effectiveness of selection. Realized heritability may be measured by plotting response against selection differential and, to obtain a measure of this differential that is relevant to families of different size, the selection differential must be weighted according to the numbers of progeny scored. The effective selection differentials, appropriately weighted, may then be summed over successive generations to give the total selection applied up to any given generation and this cumulated selection differential may then be plotted against the response. A regression line is then fitted to the points and the slope of this line measures the average value of h^2, the realized heritability (Falconer, loc. cit.).

2. Estimates of heritability. To determine the realized heritability of susceptibility to infection from the present work, cumulated selection differentials and response statistics, as defined above, were calculated for families infected with *E. coli* strain T, the results of which are shown in Tables 3.51 and 3.52 for families selected for low scores and high scores respectively (reverse selections were excluded). Weighted regression analyses were carried out using the R and S values given in these

Table 3.51. Cumulated selection differentials (C.S.), response (R) and weightings(WT) for families selected for low score, infected with T. cruzi Strain 7.

Generation	Family number	WT	C.S.	R
F_1	93	22	-0.9560	0.0853
F_2	10	17	-1.7615	-0.1005
F_3	2	24	-2.5670	-0.4111
F_1	70	22	-0.5045	-0.1599
F_2	11	19	-1.0090	-0.2082
F_3	4	31	-2.5225	-0.3460
F_3	5	13	-2.5225	-0.0841
F_1	73	20	-0.3540	-0.6181
F_2	13	21	-0.7080	-0.2433
F_3	7	37	-1.0620	-0.0416
F_1	69	22	-0.2103	0.1290
F_2	15	20	-0.4206	0.1017
F_3	9	18	-1.1266	-0.2552
F_1	73	20	-0.3540	-0.6181
F_2	14	11	-0.7080	-0.6689
F_3	10	24	-1.0620	-0.4457
F_3	11	30	-1.0620	-0.4465

WT - Number of individuals scored per family

Mean score of parental generation = 2.7080

Table 3.42. Cumulated selection differentials (S.D.) response (R) and weightings (WT) for families selected for high score, infected with T. crassi Strain 7

Generation	Family number	WT	S.D.	R
F_1	50	36	-0.6550	0.2392
F_2	3	15	1.1469	-0.1677
F_3	12	35	2.2295	0.3558
F_1	94	35	-0.8055	0.3940
F_2	5	21	1.4942	0.1803
F_3	14	33	2.2196	0.4229
F_1	70	22	-0.5045	-0.1559
F_2	8	17	1.1757	0.4680
F_3	16	27	2.2501	0.4203
F_1	58	36	-0.5950	0.2854
F_2	3	26	0.8454	0.4797
F_3	18	24	2.2366	0.4388

WT = Number of individuals scored per family

Mean score of parental generation = $\bar{y}_0$=7.43

Tables and the results have been expressed graphically in Figs. 3.9 and 3.10, in which the best fitting lines have been drawn in accordance with the regression analyses. These weighted regression analyses were computed according to the following equations:-

SS (sum of squares) of x = $\Sigma (wx - w\bar{x})^2$

$SSy = \Sigma (wy - w\bar{y})^2$

(Sum of products) SP (xy) = $\Sigma (wx - w\bar{x})(wy - w\bar{y})$

Regression SS = $SP(xy)^2/SSx$

Total SS = SSy

Where w = weight

x = [illegible]

y = R

n = no. pairs of observations

The regression lines were tested by analysis of variance, the results of which are shown in Tables 3.53 and 3.54.

Table 3.53. Analysis of variance of regression lines for families selected for low levels of intensity of infection with *T. cruzi* Strain 7.

Item	df	MS	VR	P
Regression	1	[illegible]	[illegible]	[illegible]***
Remainder	369	1.3992		

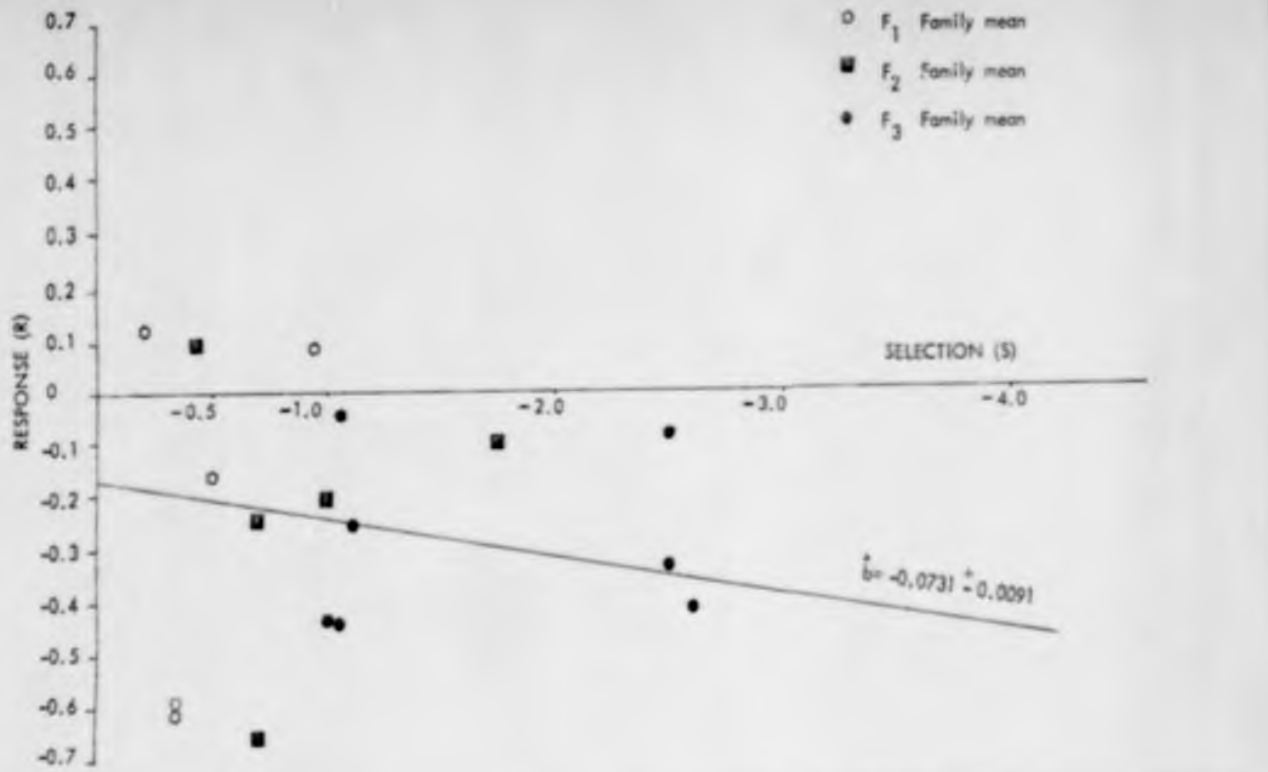


Figure 3.9. Response (R) to selection (S) for low levels of intensity of infection in R. prolixus infected with T. cruzi Strain 7.

R = Mean family score minus mean score of parental generation.

S = Cumulated selection differential (weighted).

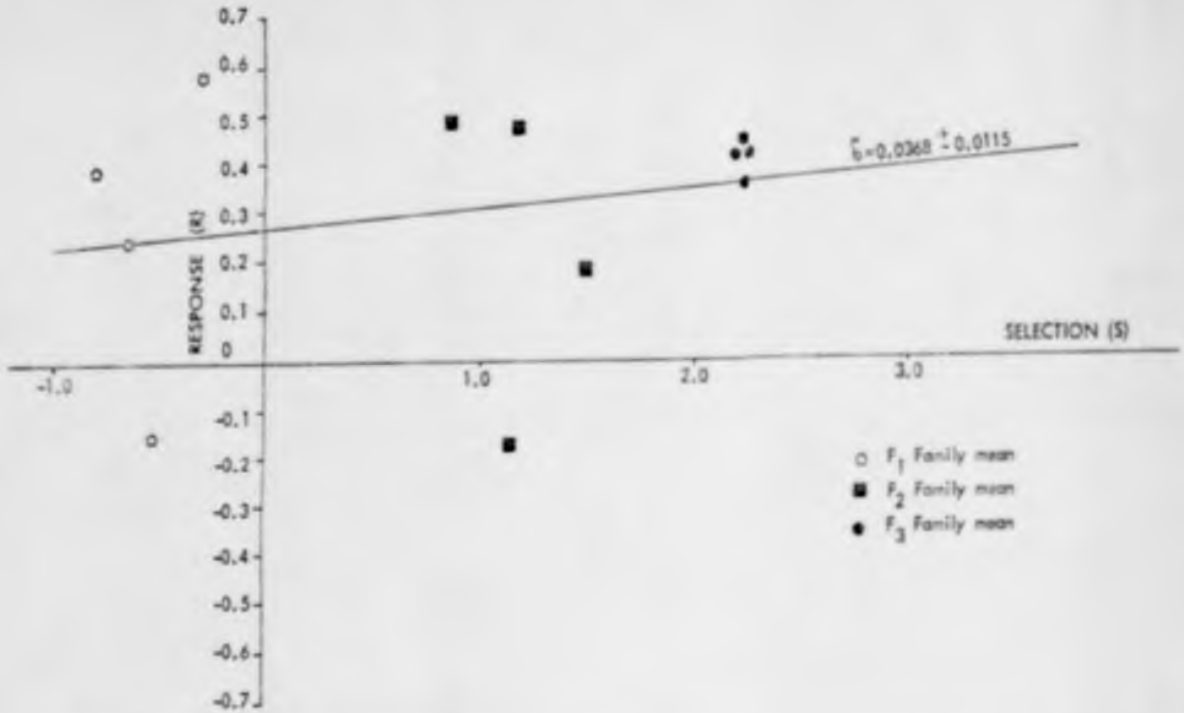


Figure 3.10. Response (R) to selection (S) for high levels of intensity of infection in *R. prolixus* infected with *T. cruzi* Strain 7.

Table 3.54. Analysis of variance of regression items for families selected for high levels of intensity of infection with *T. cruzi* Strain 7.

Item	df	MS	VR	P
Regression	1	427.44	147.7735	.1***
Remainder	316	2.8929		

The results of these analyses show that the regression clearly accounts for practically all of the variance in both analyses, the remainder (non-linear) item being insignificant in both cases. These analyses show conclusively that response changed systematically with the selection pressure applied in both upward and downward directions.

The regression line for downward selection (Fig. 3.40) had a slope (b) of - .7811 ± .0[illegible] and, since this regression was highly significant, it may be concluded that the realized heritability for bugs selected for reduced levels of intensity of infection was .39 ± .0[illegible]. The regression line for upward selection (Fig. 3.41) had a slope ($\hat{b}$) of .4388 ± 0.0145 and, since this regression was also highly significant, it may be concluded that the realized heritability for *T. cruzi* selected for high levels of intensity of infection was .39 ± .01. From these two estimates, it was concluded that the mean realized heritability for this character was 0.39.

Reverse selection

Although an analysis of variance of mean F_3 family scores (Tables 3.43 and 3.44) failed to show any differences for reverse selected families, that analysis did not allow for the amount of selection

Table 3.55. Cumulated selection differentials (C.S.), response (R) and weightings (WT) for families reverse selected from high scoring lines infected with T. cruzi Strain 7.

Generation	Family number	WT	C.S.	R
F_1	50	36	-0.6550	0.2392
F_2	2	15	1.1469	-0.1677
F_3	13	8	0.8520	0.4918
F_1	94	36	-0.8055	0.3940
F_2	5	21	1.4842	0.1803
F_3	15	24	0.8777	0.2819
F_1	46	34	-0.1779	0.2291
F_2	8	17	1.5647	0.4680
F_3	17	24	0.8572	0.3185
F_1	58	26	-0.3055	0.5845
F_2	3	26	0.8454	0.4797
F_3	19	10	0.6645	0.3823

pressure applied to the 4 groups of families. The combined selection differentials and response values have been calculated for F_3 families 13, 15, 17 and 19, and are set out in Table 3.57 together with the appropriate weightings. The resulting weighted regression analysis showed a slope (b) of - 0.1181 ± 0.0535 and the regression lines were tested by analysis of variance, the results of which are shown in Table 3.56.

Table 3.56. Analysis of variance of regression items for families reverse selected from high lines.

Item	df	MS	VR	P
Regression	1	2.9495	-	N.S.
Remainder	275	3.3875		

The results show that the regression item was not significant indicating that reverse selection from F_2 families did not produce the systematic increase in family mean demonstrated above for F_3 families 13, 15, 16 and 18 and that the relationship of families 13, 15, 17 and 19 in response to selection was not linear. This suggests that reverse selection was effective in disrupting the linear response to selection for increasing levels of intensity of infection.

4. Asymmetry of response to selection. The results illustrated in Figs. 3.9 and 3.10 were here put together for comparative purposes in Fig. 3.11 which illustrates the inequality of response to selection in two directions achieved in the present experiment: selection for low

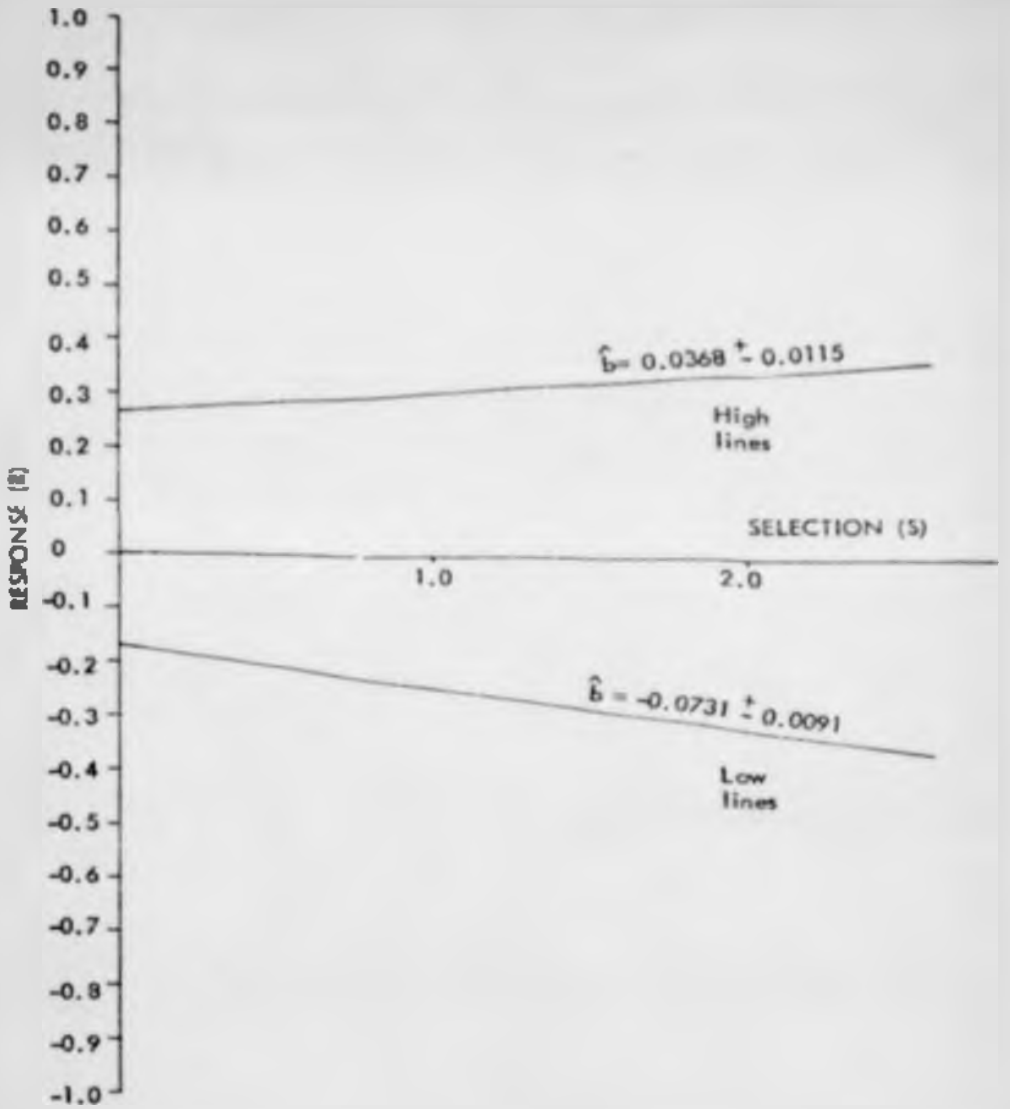


Figure 3.11. Asymmetry of response (R) to selection (S) for high and low levels of intensity of infection in *P. prolixus* infected with *T. cruzi* strain 7.

levels of intensity of infection was approximately twice as effective as selection for high levels. This asymmetry of response to selection has been found by different workers in selection experiments with other organisms and its possible causes will be discussed further.

DISCUSSION

The purpose of these genetic investigations on triatomine bugs was to determine what genetic mechanisms might be controlling their susceptibility to infection with the trypanosome, T. cruzi, for which they are natural vectors to man and other vertebrates in South (and parts of Central) America. This study was undertaken only with R. prolixus (infected with 2 strains of T. cruzi) as this species had the shortest generation time (approximately 5-6 months) of the species kept at the L.S.H. and T.M. Moreover, this species was available in colony in large numbers and, of importance, some earlier work by others had touched on the possibility of genetic inheritance of susceptibility in this species, an important vector in Venezuela and other adjacent territories. Since bugs, and not least, R. prolixus, are frequently used routinely for xenodiagnosis to assist clinical diagnosis of infection in man, and also in experimental or "wild" infections in other vertebrates, evidence for a genetic mechanism in bugs controlling infection rates in groups, and levels of faecal infection in individual bugs could possibly contribute to improved efficiency of xenodiagnosis by selective breeding of highly susceptible bug populations to be used for the purpose. It might also aid interpretation of infections in wild populations of bugs, although many other factors would also be important in nature. Admittedly, infection rates for T. cruzi, whether natural in wild bugs or experimentally derived, as for example in xenodiagnosis, are high for virtually all species adequately documented,

had this is, at the moment, a particular reference to the genetical. Evidence, as cited later, for Mendelian mechanisms of transmission in other insects in their vector functions in filariasis or malaria also justified the genetical study being carried out with *R. prolixus* and *T. cruzi*.

The results reported in the foregoing pages show that detection and interpretation of the genetic control of bug susceptibility was by no means simple and on the basis of percentage infection rates the data obtained did not support a Mendelian monogenic mechanism. On the other hand, analysis of first and second degree statistics derived from quantitative assessment of faecal infections in parental bugs and their progeny, elicited evidence of polygenic control of infection with *T. cruzi* in the form of response to two-way selection over 3 generations for populations with increased or reduced ability to support infections, as a process of discovering the answer. It is now necessary to consider the selection procedures adopted and to follow that with discussion of the more important findings on which the conclusions drawn are based.

After an infecting blood-meal as fifth instar larvae, *R. prolixus* adults were given a clean blood-meal and the numbers of trypanosomes excreted estimated by scoring standardly diluted samples of their faeces in terms of the number of trypanosomes per [illegible] fields. Two parental groups of bugs infected in this way with different strains of *T. cruzi* (Peru strain and Strain 7) were scored and selected parents from both groups were mated. The progeny from [illegible] were reared, given an infecting feed as fifth instars, and scored when moulted to F_1 adults; selected bugs were then [illegible]. This process was repeated with the F_2 generations of bugs infected with both strains of trypanosome, and with the F_3 generation of bugs infected with *T. cruzi* Strain 7.

Analysis by class

Bugs were classed as susceptible to T. cruzi infections if they showed any sign of faecal infection following an infecting blood-meal on mice, or as refractory if no trypanosomes of any type could be detected either in the faeces or in recipient clean mice injected with samples of the faeces. In the present experiments, 11.6% of bugs tested were refractory to infection with T. cruzi Peru strain, while 10.4% of bugs infected with Strain 7 were refractory. Alternatively the populations may be considered as having susceptibility rates of 86.4% and 89.6% respectively, which are very close to the figure of 88.4% obtained by Phillips and Bertram (1967) based on the mean of 3 groups of R. prolixus infected with different strains of T. cruzi.

Attempts to increase the proportion of refractory bugs within families by sib-mating refractory or low scoring parents failed in both experimental groups. For example, after two generations of selection the proportion of refractory bugs in F_2 family 18 of the Peru strain group was 16.0%, a figure not significantly different from the proportion of refractory bugs in the parental generation of 11.6% (corrected χ^2 = 0.006, P = N.S.) and yet this family had a greater proportion of refractory members than any other F_2 family selected for refractoriness. Similarly, selection for refractoriness from bugs infected with T. cruzi Strain 7 produced F_3 families with a mean proportion of 18.7% refractory which was not significantly different from the parental generation figure of 10.4% (χ^2 = 1.4, P = N.S.). Statistical tests to detect any trend towards an increase in the proportion of refractory bugs as a result of selection were negative whether bugs were infected with Peru strain or Strain 7 of T. cruzi. Nevertheless, F_3 families 11 and 13, which contained the

greatest proportion of refractory bugs, were descended from susceptible parental stock.

Selection for increasing proportions of susceptible bugs infected with Peru strain *T. cruzi* gave similar discouraging results, there being no significant effect by the F_2 generation and, moreover, there was no significant difference in proportions of susceptible bugs between the two groups of F_2 families selected for susceptibility and refractoriness. However, families selected for susceptibility to *T. cruzi* infection over 3 generations did show significant increases in the proportions of susceptible bugs within families. Selected F_3 families had a mean proportion of 97.3% susceptible members which was significantly greater than the 89.6% in the parental generation ($t' = 4.6$, $0 < P < 0.05$). A test for linear trends in proportions confirmed that over the 3 generations there was a systematic increase in the proportions of susceptible bugs in these selected families.

The results of these analyses are unfavourable. Selection for refractory bugs (as defined in the present work) was clearly unsuccessful. Families and sexes (Tab. 10.1.) were pooled for only comparable data increased the proportions of refractory bugs in the F_3 to 45.5% from the parental mean of 43.6% by crossing refractory *T. cruzi*. A survey of the F_1 data in the present experiments shows that high proportions of refractory bugs were obtained in some families of the Strain 7 infected group; for example, F_1 family 70 had 45.5% refractory bugs (10 out of 22) but subsequent selection and assortative mating from this family resulted in F_3 families 4 and 5 with 3.2% and 0% refractory members respectively. The results of breeding from F_1 family 73 of the Strain 7 group are interesting, since selection for refractoriness from this family, which was 5.3% refractory, produced

3 F_3 families F_1 [illegible] and 11 with 5.4%, 41.[illegible]% of refractory members respectively. The parents of F_1 family 73 were both susceptible which makes a Mendelian interpretation of the data rather difficult. If susceptibility were controlled by a single Mendelian recessive factor then both the parents of F_1 family 73 would have to be homozygous and could not, therefore, have produced refractory offspring. Alternatively, if refractoriness were the homozygous state then the susceptible parents of F_1 family 73 must be assumed to have been heterozygous and should have produced a 3:1 ratio of susceptible to refractory progeny. [illegible] parents should then have all been refractory as should the F_2 offspring. This, clearly, was not the case. However, selection for susceptibility did produce significant changes in the proportion of the 2 classes in families infected with Strain 7 trypanosomes which suggests that susceptibility is, at least in part, genetically controlled, but these results give no indication as to the nature of the control. The fact that selection for susceptibility was successful while selection for refractoriness was not, [illegible] refractoriness were controlled by a recessive allele (s) with a dominant allele [illegible] for susceptibility, then the failure to select for refractoriness may be explained as a failure to recognise homozygous (ss) bugs phenotypically using the test [illegible] adopted for the present experiment. This would produce results which do not fit expected Mendelian proportions. Alternatively, it might be that the significant differences in proportions of refractory bugs between F_3 families of the Strain 7 group selected for refractoriness and susceptibility (χ^2 = 11.[illegible], P < 0.[illegible]***) were simply a consequence of changes in the intensity of infection brought about in the 2 groups.

Suppose that the intensity of infection of a bug was reduced to a low level by selection, then the correct phenotypic classification of this bug as refractory, or susceptible, would have been difficult because the method of checking microscopically negative faeces by inoculation into a clean mouse could overlook a very low infection if the immune response of the recipient mouse eliminated the infecting parasites.

In conclusion, a major gene interpretation of the data presented here, that is that susceptibility to *T. cruzi* infection in *R. prolixus* is controlled by alleles at a single locus, cannot be supported, but neither has such an interpretation been completely excluded by the present work. Further generations of selection and inbreeding, had this been practicable, might have clarified the situation, but this could be doubted as, in a similar selection experiment for *T. lariaxi*, Macdonald (1962a) produced a susceptible, true-breeding line of the mosquito *Anopheles gambiae*, in the F_2 generation using four-fold matings, and was able to demonstrate that the gene controlling susceptibility to the filarial worm, *Dirofilaria*, in the mosquito was a Mendelian recessive factor. Moreover, Macdonald (loc. cit.) was able to increase the proportion of susceptible mosquitoes from 27.7% in the parental generation to 85.7% in the F_2. The present work has failed to demonstrate such dramatic changes in the proportions of either susceptible or refractory bugs as a result of selection and inbreeding, and while this does not rule out a major gene control factor, such an hypothesis has not been substantiated.

I turn now to the conclusions drawn by quantitative analysis dealing with levels of infection of trypanosomes in the faecal excrement of individual *R. prolixus*, i.e. patterns of trypanosome per standard samples of bug faeces.

Quantitative analysis

The intensity of infection of each infected bug was assessed by counting the number of trypanosomes in 100 fields (X 450) of a 20 µl sample of faeces of a standard dilution. The resulting scores were transformed to log (n + 1) to normalize the data for statistical analysis. Changes in family means and variances following selection and sib-mating were analysed to determine whether intensity of infection was a heritable quantitative character. These analyses were carried out on the scores per 100 fields obtained from the same bugs which were analysed by class as positive or negative. The third analysis was designed to detect differences in intensity of infection between bugs infected with the 2 strains of trypanosomes. Although the experiment was not strictly replicated, as that is not possible. (I mean, to test the same group of bugs with both strains of trypanosomes, the bugs used in the parental generation were randomly chosen from the same colony and may be assumed to be genetically homogeneous.) In this parental generation, significant differences were found in intensity of infection between bugs infected with the 2 different strains; bugs given an infecting feed on T. cruzi Strain 7 had a mean score approximately twice that of bugs fed with the more virulent Peru strain. This result is most surprising since the bugs ingesting the Peru strain (a virulent, lethal strain for mice) were fed on mice with higher levels of parasitaemia than those infected with Strain 7 T. cruzi (a strain of low parasitaemia from which mice could survive). It can be assumed that both groups of bug took approximately the same size blood-meal. Therefore, Peru strain infected bugs must have ingested more trypanosomes, yet they excreted fewer than the Strain 7 group of bugs, an indication that the virulent trypanosomes of the Peru strain, possibly as in mice, had a

lower potential for producing a correspondingly heavy infection in the vector bug.

There are no comparable quantitative studies of the nature covered in the present work. In the literature, on the intensity of infection of different strains of trypanosome in the bug. True, Rydzewski (1965) did report a quantitative difference in the density of trypanosomes in the faeces of triatomines infected with different [illegible] strains; also the effect of trypanosome strain on susceptibility rates (i.e. proportions of infected bugs in [illegible] triatomine [illegible] and Bertram (1967) and [illegible] and Nicolle (1911). But these studies were not designed to follow the inheritance of quantitative changes in intensity of infection within bugs. Further analyses of F_2 families of the present series again showed highly significant differences in intensity of infection in individual bugs between groups infected with the 2 different strains of trypanosome. Analyses of further generations were not carried out since the effects of selection may have affected variation between strains disproportionately. However, it may be concluded from the results from F_1 families, and from the parental generations that intensity of infection in R. prolixus is affected in part by the genotype of the strain of trypanosome which the bug ingests.

A particularly interesting finding was obtained by analyses of variance of F_1 and F_2 families infected with the 2 strains of trypanosome in that all analyses revealed significant differences in intensity of infection of male and female bugs when tested against the considerable variation between families. Highly significant differences ($P < 0.001$) were also found between sexes in F_2 families of the Nicole I group. These differences between sexes were constant for the several families of both

lower potential for producing a correspondingly heavy infection in the vector bug.

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group tested since no significant sex X family interactions were detected in the analyses. It was thought with [illegible] reference to these sex differences, that they may have been attributable to differences in the volume of blood taken at an infecting feed at their fifth instar blood-meal or to differences between sexes in rate of excretion when tested as flukes. [illegible] differences in blood-meal size between male and female fifth instar R. prolixus larvae; differences in numbers of trypanosomes ingested could not [illegible] faeces collected within 1 hour [illegible] shown that adult females [illegible] excreted a greater mass of faeces and excreted a greater proportion of their blood-meal within 1 hour of feeding. A reasonable explanation would be, that [illegible] but the results showed this [illegible] being significantly greater than female scores throughout the selection experiment. The conclusion [illegible] individual bugs is influenced by the sex of the bug ingesting trypanosomes regardless of the strain of T. [illegible]. [illegible] determined character in bugs, these results provide good evidence for the involvement of bug genotype in determining the numbers of trypanosomes which develop in a bug's gut after an infecting feed.

The analysis of F_3 families infected with T. [illegible] strain 7 showed a further interesting result in which there was a significant sex X selection line interaction when tested against the error [illegible] differences between sexes were greater in families selected for high score than in those selected for low score. This result may have been produced by sex-linkage of genes controlling susceptibility or, alternatively, may

have been the result of a failure in scaling by which the log transformation did not fully remove the positive skewness evident in the frequency distribution of the raw data. The precise nature of the genetic control of sex-differences in intensity of infection could not be determined from the present work. It may be that the control of susceptibility is sex-linked but the significant sex-differences may have been produced by sex-linked genes. Phillips and Nelson (1967) found no significant difference in infection rate between sexes of H. protixus experimentally infected from mice and Arneida et al (1973) failed to detect a difference in susceptibility rate between sexes of R. [illegible]. However, neither of these studies was based on precise quantitative estimates of the intensity of infection and are not strictly comparable with the present work.

A constant feature of the analyses was the significant differences between families. These between-family differences were detected in the analysis of trypanosome strain differences indicating that family differences were to be found in both groups of bugs infected with different strains. In the analyses of sex differences, the between-family item was found to be highly significant in all the analyses of variance of bug scores for the F_1 and F_2 generations and for both strains of trypanosome used. Furthermore, tests for sex x family interaction effects proved negative, indicating that family differences were independent of the significant sex differences. These significant between-family differences are of great genetic importance for they signify resemblance between related individual bugs for a quantitative character (intensity of infection) since significant differences between several families must reflect the similarity of individuals in any one family. The resemblance between relatives is

one of the basic characteristics displayed by metric characters (Falconer, 1967) and these differences in variance are a strong indication that intensity of infection in H. prolixus is polygenically controlled.

The analysis of F_3 females infected with T. cruzi Strain 7 (Table 3.47) showed only barely significant differences between families (P = 0.05–0.10) and was in contrast to the analysis of male F_3 scores of the same families which showed highly significant differences between families (Table 3.46; [illegible]). This difference may be explained by the fact that the item tested in Table 3.47 was variation between female families within selection lines and it is to be expected that after selection and inbreeding to F_3, variation between families within a selection line would be reduced. This result may be taken as an indication of the effectiveness of the selection procedure for females which may have been reaching the limits of their selection potential, at which point it would be expected that family means of a particular selection line would be [illegible].

The degree of resemblance between relatives is a property of a polygenic character which can be quantified by experimental means or by analysing the results of a selection programme. The degree of resemblance between relatives provides an estimate of the amount of additive variance, and it is the proportionate amount of additive variance which determines the breeding value or heritability of a polygenic character. The heritability has great predictive value to breeders and is a most important property of a polygenic character, the greater the proportion of the heritable variation which is additive and [illegible], the greater will be the effectiveness of selection. The heritability of intensity of infection was estimated, in the

present work, from the ratio of response (R) to selection differential (S) from a two-way selection programme applied to *H. prolixus* infected with *T. cruzi* Strain 7. Two lines were derived from the parental population of bugs selected for high and low levels of intensity of infection over 3 generations. In the F_1 generation, highly significant differences were found between bugs of the 2 selection lines of both sexes ($P < 0.001$***). Furthermore, when the F_2 family with the lowest mean score from the high line was compared with the family with the highest mean score from the low line, differences between these 2 families were highly significant ($P < 0.01$**) when compared with the variation between bugs within families. Thus, selection from the parental generation of randomly selected bugs produced in the F_3 two discrete populations with significantly different levels, on a logarithmic scale, of intensity of infection. The realised heritability for the character was determined from the regression of response on selection differential for both selection lines. The regression lines for both lines were highly significant ($P < 0.001$***) so that the slopes of the lines provided sound estimates of the heritability of this character. The response to selection measured from the divergence of regression lines, taken as twice that of the line separation, produced an estimate of 0.67 for the heritability of intensity of infection in *R. prolixus* under the experimental conditions of this work.

Reverse selection from F_2 families infected with *T. cruzi* strain 7 did not, however, produce F_3 families with mean scores significantly different from the families selected throughout the high scores. This result is, perhaps, not surprising after only one generation of reverse selection. However, when the regression of response on selection differential for the reverse selected high lines was carried out, the result

was a negative slope (b = 0,1181) and the [illegible] not significant, indicating that there was no longer a linear upward trend in mean score after reverse selection. Taking these two findings together, it may be concluded that reverse selection had a small effect on family scores but this effect was not large enough to produce significant differences when compared with the variation within families.

When the regressions of response on selection differential for high and low lines were further considered it was clear that the response to selection pressure was not equal in both directions (Fig. 4.11). This asymmetry of response is a common feature of two-way selection programmes with animals and has been attributed [illegible] to two main causes, inbreeding depression and directional dominance. It is known that inbreeding can lead to a reduction in the mean phenotypic value of a quantitative character which is connected with fitness - such as reproductive capacity (Falconer, [illegible]). Characters with low heritability are often related to fitness; for example, the amount of white spotting in Friesian cattle has a very high heritability of 75% (Briquet and Lush, 1947) while conception rate (which is fitness related) in the same cattle has a very low heritability, of [illegible] (Robertson, 1967). The low heritability found in the present work for intensity of infection (5,5%) suggests that this character may be related to fitness and therefore subject to inbreeding depression. Characters which show inbreeding depression will respond more rapidly to downward selection than to upward selection and, since the asymmetry of response demonstrated for H. polygyrus showed downward selection to be twice as effective as upward selection, this may further indicate that intensity of infection is related to fitness. Alternatively, it could be argued that the genes controlling intensity of infection are merely linked to

those of another character more directly concerned with fitness or, it may be, that in selecting for trypanosome density in bug faeces an unconscious selection of the fittest individuals was taking place. It is important to consider, therefore, what direct effect density of a trypanosome infection in the gut of a bug could have on the fitness of a bug. Moore (1972) has suggested that mammalian trypanosomes have evolved from the monoxenic trypanosomatid parasitic flagellates of non-blood-sucking insects, and that bugs infected with trypanosomes acquired the blood-sucking habit, defaecated at the site of the bite and the trypanosomes of their gut entered the mammal where eventually they established themselves as haemoflagellates, the bugs being reduced to intermediate host status. Whether this hypothesis is true or not, it is known that the relationship between *T. cruzi* and the triatomines as vectors is extremely complex and, if the trypanosomes were more solely exploiters of bugs, it is to be expected that bugs would have evolved genotypes specifically adapted to cope with the bug-parasite relationship, and that such genes could be related to the fitness of the bug.

Falconer (1967) also states that if the genes that increase a metric character are dominant over their alleles responsible for a reduction in its value, then inbreeding will result in a reduction in the population mean for the character, i.e. a change in the direction of the more recessive alleles. Thus, it would be expected that a character which showed inbreeding depression would respond more rapidly to downward selection, in which case the results of the present work suggest intensity of infection may be related to fitness in *T. protozoa* and also that the genes for increasing susceptibility could be dominant to those for refractoriness and decreasing intensity of infection.

Knowledge of genetic mechanisms controlling the susceptibility of insect vectors to infection is limited to mosquitoes and their malarial and filarial parasites. Briefly, Huff (1929) was the first to suggest that susceptibility of a vector was genetically controlled from his study of susceptibility of Culex pipiens to Plasmodium cathemerium. Further experiments by others were subsequently done with various mosquito vector-malaria associations but the mode of inheritance remained obscure until recently when Kilama and Craig (1969) demonstrated that susceptibility of Aedes aegypti to infection with Plasmodium gallinaceum [and malarial was controlled by a pair of

The genetic control of susceptibility of the mosquito Aedes aegypti to infection with the filarial nematode Dirofilaria immitis was first investigated by Rouband et al. (1936) who found variation in the susceptibility rate of strains of mosquito from different geographic regions. [illegible] demonstrated that susceptibility of the mosquito Aedes aegypti, to filarial infection with Dirofilaria immitis was controlled by a sex-linked recessive gene. Recently Zielke (1973) and Mahaney et al. [illegible] extended this, showing that the susceptibility of Ae. aegypti to another filarial worm, Dirofilaria immitis, is also controlled by a single sex-linked recessive gene.

It is notable that this work on susceptibility of mosquitoes to parasites which they transmit has so far revealed only major gene control mechanisms. There have been attempts to quantify mosquito susceptibility in terms of infection levels of individual insects but these have not produced clear-cut results; for example, [illegible] were unable to produce parent-offspring regressions for oocyst counts. However, it is interesting that selection experiments with mosquito filarial systems have often failed to produce completely refractory or completely susceptible

stocks of mosquitoes even after many generations of inbreeding. For example, Macdonald (1962a) selected a strain of Ae. aegypti susceptible to infection with [illegible] for 15 generations at which point the susceptibility rate was still only 84.8%. [illegible] (1974) attributed the variation in susceptibility rate of selected populations of Ae. aegypti infected with L. longipalpis to the fact that the male genotype cannot be determined. It may be that intensity of infection in mosquitoes is controlled, in part, polygenically and this could influence the interpretation of data concerned, at present, only with dividing populations into - classes, refractory and susceptible.

The present work has demonstrated that intensity of infection with L. braziliensis in H. tropicus is a quantitative character with low heritability and that this character is sex-linked, or sex-limited. It is further suggested that genes for increasing intensity of infection are dominant to those for decreasing intensity. This experiment has failed to demonstrate that susceptibility to infection with L. braziliensis in H. tropicus is controlled by major genes, but further generations of selection would be necessary to clarify this point. Mather and Jinks (1971), in their work on biometrical genetics, suggest that with the possible exception of antigenic traits, all the characters of an organism are subject to both continuous and discontinuous variation as a result of major gene and polygenic differences. It is, therefore, unwise to rule out the possibility that a major gene mechanism may be involved in the susceptibility of H. tropicus to L. braziliensis infection, as well as the concentration polygenic control of intensity of infection.

Finally, the question of xenodiagnosis must be mentioned. This experiment has shown that it is possible to increase the intensity of infection in H. tropicus by selection. For the purposes of xenodiagnosis

it would be desirable to breed and maintain in standard laboratory culture, bugs which, on feeding on patients with very low [illegible] infection, would be so highly susceptible to multiplication of the few trypanosomes ingested that they would be detected readily within a month or so in the bug faeces. Since it has been shown that the heritability of this character is low, the best method of producing such a population of bugs would be by using family rather than individual selection (Falconer, 1967). The present work indicates that, having established an inbred line of bugs with high levels of intensity of infection, only male bugs should be used for xenodiagnosis (usually, late pre-adult larvae are used, but it is a simple matter to use fifth instar bugs - Chagas, 1912 - before using them on patients). The prospects are that, using males, the sensitivity of this diagnostic test would be considerably improved, and with the added benefit of using fewer bugs per patient, by no means a negligible matter from the point of view of the psychological stress some patients feel with this technique, and the logistic problems of maintaining adequate supplies of bugs.

PART FOUR

THE INHERITANCE OF RADIATION INDUCED SEMI-STERILITY IN *RHODNIUS PROLIXUS*

INTRODUCTION

In recent years several genetic methods have been proposed for the control of insect pests, both of agricultural and public health importance. The subject was recently reviewed by Davidson (1973). Because of its demonstrated efficacy, the sterile male technique has become the most widely known and studied of these genetic control methods. The sterile male technique, first advocated as a practical reality by Knipling (1955), involves sterilizing males by irradiation or chemosterilization and releasing them, usually in vast numbers, into the wild population to mate with and inseminate normal wild females with resultant sterility of their eggs. It was found that the dose of mutagen required to induce the maximal chromosomal damage in the sperm of irradiated male insects was of critical importance, the aim being to give a dose which would not affect male competitiveness but would ensure that translocations [illegible] which at fertilization would result in embryonic mortality. Investigations, first made by Muller (1927), have been shown by a series of complex experiments with the parthenogenetic wasp Habrobracon juglandis to result from chromosomal breakage rather than from point mutations (Smith and von Borstel, 1972). Knipling's mathematical models of the population dynamics of sterile male release [illegible], [illegible] were put on trial in the field and the first successful results were achieved in Curacao when the screwworm fly Cochliomyia hominivorax was eradicated from the island by releasing sterilized males (Baumhover et al., 1955). Following this success, the sterile-male technique was extended to the mainland of the United States and the screwworm fly was eliminated first from the state of Florida (Knipling, 1959). Again after this success, the method was extended to the States bordering on Mexico,

where a barrier was set up along the border. This biological barrier has involved releasing one thousand million sterile flies per year along the Mexico-Texas border (Bushland, 1971). Recently this control programme has apparently suffered a setback, a recrudescence of large numbers of "strikes" by screw-worm, particularly in Southern U.S. territory contiguous with Mexico being reported in Texas (1973), and attributed to the reduced fitness of the factory-bred flies in competition with the wild-type. However, following the initial success of the screw-worm fly control programme, the sterile male technique has been tried with varied success against pests, especially those in which the female does not mate more than once, thus preventing the normal populations to eradicate since re-invasion by neighbouring populations is precluded; for example, the melon fly [illegible] has been eliminated from the island of Rota in the Pacific Ocean by sterile male release (Steiner et al., 1965).

The success of sterile male control programmes stimulated interest in alternative forms of genetic manipulation and, in particular, in the release of partially sterile males or fully fertile males carrying inherited characters which would affect not only the first but also subsequent generations of the species. Several methods have been proposed for the production of insects with inherited sterility factors including chromosomal translocations, recessive lethal mutations, conditional lethal mutations, and meiotic drive which would produce sex ratio distortions. The idea of using translocations to control insect populations was proposed initially by Serebrovsky (1940) and later by Curtis (1968). A chromosomal translocation arises when two non-homologous chromosomes in the same cell are broken and the fragments are interchanged. [redacted] The process of interchange production is illustrated in Fig. 4.1 which shows that when meiosis occurs in the heterozygote formed by the union of an egg with a sperm carrying a translocation, a cross configuration must be formed at

[illegible] in which the chromosomes may pair. If the cruciform is formed in each of the arms of such a cross, the bivalents form a ring at first metaphase (Fig. 4.2a) and it is the way in which this metaphase ring disjoins (Fig. 4.2b) which determines whether viable gametes are produced. Balanced gametes only result from the so-called "alternate" disjunction when alternate centromeres of the metaphase ring go to the same pole at anaphase which involves a twisting of the ring multiple. If adjacent centromeres proceed to the same pole then the resultant gametes will be genetically unbalanced because of duplication and deficiencies of chromosomes (Fig. 4.2b). Of the balanced gametes produced by alternate orientation, half are wild-type and half carry the translocation and can pass it on to the next generation in which a heterozygote would again produce balanced and unbalanced gametes. Clearly, the mode of orientation of the ring multiple is critical in determining the fertility of translocation heterozygotes. Although 6 possible genetic types of gamete may be produced by a ring multiple (Fig. 4.2c) they are not in practice produced with equal frequency and it has been shown that a single translocation will usually result in producing a heterozygote with 50% fertility i.e. producing a 1:1 ratio of balanced to unbalanced gametes (Curtis, 1968). This apparent anomaly is a result of the non-random orientation of ring multiples; John and Lewis (1965) state that the expectation is 1:1 alternate to adjacent orientation in organisms with a single centromere if random behaviour of the two co-orientated adjacent centromeres is assumed, hence the 50% sterility of the translocation heterozygote.

Serebrovsky (1940) and Curtis (1968) further proposed that if translocation heterozygotes were inbred, insects homozygous for the translocation could be produced which on mating with the wild-type would again yield semi-sterile offspring; they further suggested that this

translocation homozygote would be fully fertile since it would be genetically balanced. This hypothesis has been substantiated for some insects; for example, Laven et al. (1971) produced fully fertile Culex pipiens homozygous for an autosomal translocation. Unfortunately, translocation homozygotes may suffer from reduced fitness in competition with the wild-type insect population (Curtis et al., 1977). Bearing this loss of fitness in mind, and also considering the very low yield of viable translocation homozygotes which is normally achieved by irradiation treatment of normal stock insects, McDonald and Kai (1971) suggested that translocation heterozygotes be released in control programmes. This method could also be used for species which cannot be mass produced, when wild caught insects could be partially sterilized thereby introducing translocations into the population (Laven et al., 1971). It has also been suggested (Curtis and Robinson, 1971; Whitten, 1971) that multiple translocations would be more desirable for pest control programmes than single interchanges as they would yield a higher level of sterility. A disadvantage of controlling insect populations by releasing semi-sterile males could be that, instead of eradicating a pest, the population would be simply replaced by the translocation - carrying population. This could, however, prove advantageous if the translocation stock was also carrying desirable genetic linkages. Curtis (1968), for example, has suggested that in this way a wild-type population could be replaced by a translocation stock carrying genes for susceptibility to insecticides or refractoriness to parasites.

Holokinetic insects. Schrader (1936) first noted that different forms of centromere were to be found in chromosomes of different species; firstly, a sharply localised type and secondly, a diffuse type. The diffuse type of centromere was later called holokinetic (Hughes, 1952).

Holokinetic (or holocentric) chromosomes show no primary constriction and the spindle fibres appear to be attached along the entire length of the chromosomes. Organisms with holokinetic chromosomes are sporadically distributed throughout the animal and plant kingdoms. In the animal kingdom, holokinetic species are limited to the arthropods and are most common amongst the insects (John and Lewis, 1965), and holokinetic chromosomes are known to be typical of the Hemiptera (Hughes-Schrader and Schrader, 1961). It is important to appreciate that a diffuse centromere imparts certain characteristics to a chromosome. For example, at mitotic anaphase holokinetic chromosomes proceed to the poles with their long axes perpendicular to the long axis of the spindle (Schrader, 1953). It was on these behavioural characteristics that holokinetic chromosomes were recognized in many organisms and, subsequently, experimental proof of their structure was obtained. Hughes-Schrader and Ris (1941), were able to show that X-ray induced fragments of coccid chromosomes, behaved normally at mitosis, each fragment, regardless of its size, dividing and passing to the poles at anaphase through many cell divisions. The peculiarities of the holokinetic system later proved to be useful in insect control programmes involving partially sterile males. It was observed by Proverbs (1962) that male codling moths (Laspeyresia pomonella) given a sub-sterilizing dose of radiation and then mated to normal females resulted in reduced numbers of F_1 progeny, the majority of which were males with very low fertility. This delayed effect of irradiation in Lepidoptera was confirmed by other workers including North and Holt (1968a) for cabbage loopers (Trichoplusia ni) and Walker and Quintana (1968) for the sugar-cane borer (Diatraea saccharalis), the level of sterility being greater for both species in F_1 progeny than in the

treated male parents. The use of sub-sterilizing doses of radiation in these experiments had not been accidental since it was already known that the Lepidoptera were highly resistant to induced reduction in fertility by irradiation; for example, 30–40 K rad had been required to completely sterilize codling moths in an experiment carried out by Proverbs and Newton (1963). Such high doses produced [illegible] damage and rendered the males less competitive so that male cabbage loopers given high doses of gamma-rays failed to transfer any sperm when mating with normal females (North and Holt, 1968b). The explanation for this high degree of radio-resistance encountered in Lepidoptera, and for the phenomenon of delayed sterility of males given sub-sterilizing doses was provided by Bauer (1967) working with [illegible]. He compared the observed rate of viable translocations in this butterfly, after irradiation, with the expected rate for a monokinetic species and, from this, proposed that, in a holokinetic organism, lethality due to a single translocation could not occur at all or only exceptionally for the following reason: Following a break in two non-homologous chromosomes in a monokinetic animal there are two possible results; firstly, the proximal broken ends can unite to produce a dicentric chromosome while the distal ends join to form a fragment or, secondly, a symmetrical reciprocal translocation can be produced as shown in Fig. 4.1. Normally, dicentric chromosome production will lead to gamete duplication-deficiencies due to bridge formation at anaphase and to the loss of the acentric fragments, but Bauer (loc. cit.) proposed that in holokinetic species dicentric chromosomes could not be formed. Indeed, his experimental results confirmed this hypothesis for he obtained a much greater proportion of viable translocations than would be expected with a monokinetic organism. In fact, Bauer obtained 20%

zygote lethality in this experiment which was much higher than he expected from his hypothesis and he attributed these deaths to gene mutation or extra-nuclear radiation effects, maintaining that they could not have been caused by small chromosome fragments.

North and Holt (1968b) confirmed Bauer's hypothesis when they found, by microscopical examination of large numbers of translocations in the spermatocytes of F_1 male cabbage loopers whose male parents had been given sub-sterilizing doses of radiation, some translocations, which they suggested, were the result of 'dicentric' chromosomes behaving as reciprocal translocations in this holokinetic species. North and Holt (loc. cit.) provided further cytogenetic explanation for the sterility of F_1 males being greater than that of their irradiated parents, by showing that intercalary reciprocal translocations could result in the formation of bridge-and-fragment tetrads, depending on the mode of orientation of the multiples at metaphase, so that when F_1 sperm were used in fertilization they could be lethal to the embryo, the degree of F_1 sterility depending on the number of interchanges induced by the radiation.

In addition to the production of reciprocal translocations, diffuse centromeres also ensure that chromosomal fragments which would normally be lost in monocentric organisms, are mitotically stable in holokinetic species. LaChance and Degrugillier (1963) working with the holokinetic milkweed bug, Oncopeltus fasciatus, showed that X-ray induced fragments were not only mitotically stable but were also meiotically stable and could be transmitted through 3 generations of outcrosses to normal females. Furthermore, LaChance et al. (1971) demonstrated that a single chromosomal fragment could lower the fertility of O. fasciatus appreciably, the degree of sterility depending on the size of the

fragment and its pairing behaviour so that 3 fragments produced almost total sterility while random segregation of a single fragment produced 50% sterility. [illegible] Sharp's (1917) hypothesis that chromosomal fragments were not responsible for embryonic deaths was shown to be incorrect. Knipling (1970) devised mathematical models to estimate the effect of the release of partially sterile Lepidoptera on wild populations and concluded that in mass releases, such males would be more effective in suppressing pest populations than completely sterile males. It is clear from all this work with Lepidoptera that centromere structure can play an important part in determining the radiosensitivity of an insect and in deciding the correct approach to controlling a wild population.

The control of Triatominae (Hemiptera, Reduviidae) is of great interest because of their importance as vectors of Chagas' disease and some work has been carried out to investigate ways of sterilizing these pests. Gomez-Nunez et al. (1962, 1964) and Baldwin and Shaver (1963) have studied the effect of irradiation on Rhodnius prolixus and have produced dose-response curves with respect to fertility. Gomez-Nunez et al. (1964) using Y-irradiation and Baldwin and Shaver (1963) using X-rays found that 20 K rad were required to sterilize completely adult R. prolixus, this dose being comparable to that required to sterilize Lepidoptera (North and Holt, 1968b). Gomez-Nunez et al. (1964) also studied the effect of irradiation on longevity and competitiveness and found that at 5 K rad irradiated males were as competitive as normal males and produced sterile sperm, while their longevity was not reduced significantly. Baldwin and Chant (1971) showed that by irradiating R. prolixus in an atmosphere of nitrogen the competitiveness of males given sterilizing doses of X-rays was improved over controls irradiated in air. Sterility studies have also been carried out with triatomine

bugs using alternative methods to irradiation. Chu... [illegible] (1972) investigated the effect of the chemosterilant metepa on R. prolixus; Triatominae have, however, not received the attention that other insect vectors of disease have, in this whole context, particularly in terms of the genetic and cytogenetic mechanisms involved in irradiative sterilization.

Since the Triatominae are [illegible], they are of special interest cytogenetically because, as with other [illegible] they have been assumed to be holokinetic (Wenrich, 1945). Mack (1971) in an ultrastructural study of R. prolixus found that the mitotic chromosomes had a diffuse centromere but found that the kinetochore was apparently absent from meiotic chromosomes. Comings and Okada (1972) in an ultrastructural study of Oncopeltus fasciatus also showed that kinetochore plates were present along most of the length of mitotic chromosomes while, in the meiotic chromosomes, they could find no kinetochore plates but, found spindle microtubules inserted along the length of the chromosomes which were thought to be responsible for chromosomal movements during meiosis. They suggest that in holokinetic species, suppression of kinetochore activity is essential during meiosis to facilitate chiasma terminalization.

The present study is concerned with the inheritance of radiation-induced partial sterility in R. prolixus. [illegible] (Mack, 1971) have indicated that R. prolixus has holokinetic chromosomes, it could be that induced partial sterility in males would be preferable to completely sterile males as a genetic mechanism for control by sterile male release, as suggested by Gomez-Nunez et al. (1964) and Baldwin and Chant (1970), especially as these workers also showed that small, sterilizing doses of radiation seriously impairs the sexual fitness of R. prolixus males.

MATERIALS AND METHODS

Materials

R. prolixus randomly selected from the colony kept at L.S.H. and T.M. were used for this experiment. Details of the history of this colony have already been given.

Methods

All bugs in this experiment were contained in 2" x 1½" glass flat-bottomed tubes covered with fine mesh nylon gauze held in place by adhesive tape. The tubes were kept in [illegible] at a constant temperature of 27°C and approximately 75% R.H. Bugs were fed throughout on lop-eared rabbits.

The radiation source was a ^{60}Co gamma cell which gave a dose rate of 2.5 K rad per minute, the facilities being kindly provided by the Department of Physics, Middlesex Hospital, London.

To determine the radiation dose required to produce semi-sterile males, a dose-response curve for fertility was constructed from a preliminary experiment by irradiating adult males 4 days after moulting to adults, in groups of 6 with doses ranging from 0 to 20 K rad in 2 K rad stages. Each male was then separately paired [illegible] with a normal female of the same age and seven days later the paired bugs were fed for the first time. They were subsequently fed twice more at 14 day intervals. The eggs laid after each of the 3 blood-meals were collected and scored for hatchability. Simultaneously, an untreated control group of bugs were similarly paired and reared and their fertility measured by scoring their eggs for hatch. Both males and females of these series had been fed on rabbit as fifth instars on the same day and had been sexed and separated at this non-mating larval stage so that mating was precisely timed to begin when the paired sexes were set up later in the adult stage, as above.

Since the protocol for the proposed experiment demanded that males be killed for cytogenetic examination, a control experiment was first run to determine the effect on female fertility of removing the male at different time intervals after mating. For this purpose, [illegible] groups of bugs, each consisting of 7 pairs of males and females, were fed [illegible] days after moulting to adults and then fed twice more at [illegible]-day intervals, the males being removed from the first group 7 days after the first blood-meal and from the second group [illegible] days after the second blood-meal. The fertility of the [illegible] groups was then compared for the [illegible] blood-meals.

For the main experiment in which the effect of parental irradiation on subsequent generations was investigated genetically and cytogenetically, bugs were fed on the same day in fifth larval instar, sexed then and isolated one per tube. Four days after moulting to adults, 4 males were irradiated, placed together in a [illegible] petri dish with 6 K rad X-rays. The males were then immediately paired with the normal females which had been isolated at the fifth instar, and which were of the same age and nutritional state. [illegible] pairs were fed 5 days post-irradiation and subsequently given 3 further meals at [illegible]-day intervals. Mated [illegible] females were kept in a controlled fashion, each feed resulting in a cycle of egg production and oviposition, the numbers of eggs produced being dependent on the size of the blood-meal (Buxton, 1930; Friend et al., 1961). The eggs produced from each of the 4 blood-meals given to the females in this experiment were collected and scored for hatchability. The F_1 larvae hatching from these 4 batches of eggs were labelled and kept as a separate batch throughout the experiment and, on reaching the fifth instar, they were sexed and placed individually in separate labelled tubes. On moulting to adults, one normal male, pair or female of the same age was placed

in the tube with each F_1 adult according to sex. Eighty-six of these backcross type matings were made, 51 of which were of the form F_1 ♂ X normal ♀ and 35 were F_1 ♀ X normal ♂. Each of these pairs was given 3 blood-meals at 14 day intervals and the eggs laid were collected and scored for hatchability. [illegible] blood-meal all F_1 males were removed from the tubes, their testes dissected out in saline and stored in 3:1 absolute alcohol:acetic acid fixative at 4°C.

For the next generation of matings, parents were chosen only from those F_1 X normal matings which were of low fertility; the [illegible] sexed and isolated at fifth instar to avoid inwanted matings. [illegible] 16 such families were chosen and bugs from these families were reared to adults and [illegible] at approximately the same age.

Twenty-eight of these 'F_1' X normal matings were made from among the 16 selected F_1 families ('F_1' is used in this experiment merely to denote bugs reared from F_1 X normal outcrosses and does not imply genetic [illegible]), 14 of which were of the type 'F_1' ♂ X normal ♀ and 2 of which were 'F_2' ♀ X normal ♂. These 28 pairs were each given 3 blood-meals at 14 day intervals and [illegible] eggs [illegible] were saved for hatchability. [illegible] Males were removed, their testes dissected out and stored in fixative. Although only 16 F_1 families were chosen for further breeding, all viable 'F_1' bugs from all the F_1 X normal [illegible] were reared to the adult stage and although not mated, testes were dissected from the males for cytogenetic examination. The progeny of these 'F_1' bugs descended from the first egg batch laid by the parental generation were reared to adult, testes dissected from the males and stored in fixative. As

before. No assessment was made of the fertility of these F_1 males.

Lacto-acetic-orcein squash preparations were made from the fixed testes and a postmeiotic second male of any cytological abnormalities. These cytological examinations were made prior to the analysis of fertility data for each generation and were therefore unbiased by fertility estimates.

RESULTS

Fertility studies

The fertility data for males given increasing doses of X-rays are shown in Table 4.1 together with the control fertility calculated from 10 untreated pairings. All fertility results in this experiment have been calculated as corrected fertility (or sterility), this being computed using Abbott's correction (Abbott, 1925) such that:

$$\text{Corrected fertility} = \frac{\text{Experimental fertility}}{\text{Control fertility}} \times 100$$

A dose-response curve was constructed from the data given in Table 4.1 and this curve is shown in Fig. 4.5 in which, for comparative purposes, dose-response curves constructed from the data of Sobels et al. (1964) and Baldwin and Shaver (1963) have also been drawn. It is noticeable that the curve constructed from the data of Sobels et al. (1964) has a very similar slope to that produced in the present work (labelled L.M.), while the curve constructed from Baldwin and Shaver's (1963) data has a slightly different form, although the same species was used in all experiments. These differences may be due to the use of different radiation sources with different dosimetries. Sobels et al. (1964) used X-rays at a dose-rate of 0.675 rad per minute. Baldwin and Shaver (1963) used X-rays at a dose-rate of 1 K rad per

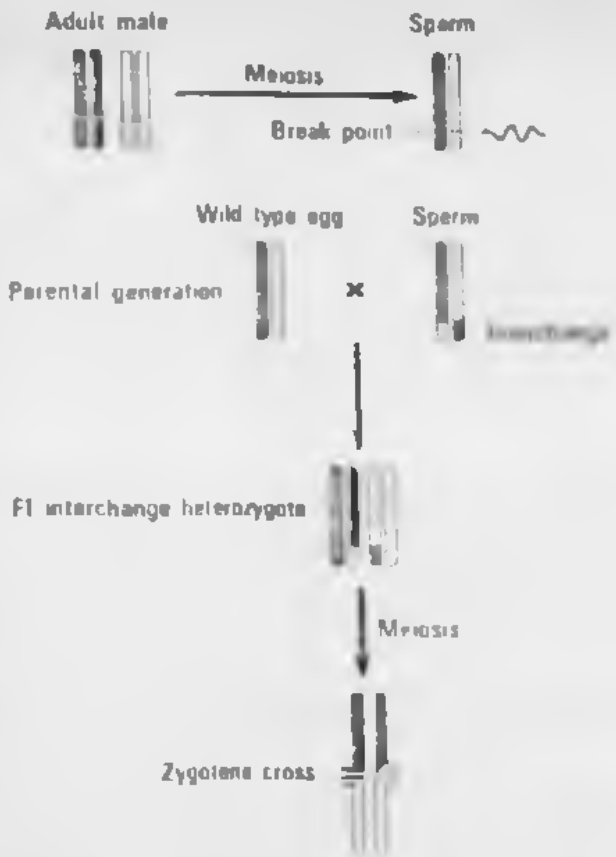

Figure 4.1. Production of interchange heterozygotes in [illegible] in holokinetic insects.

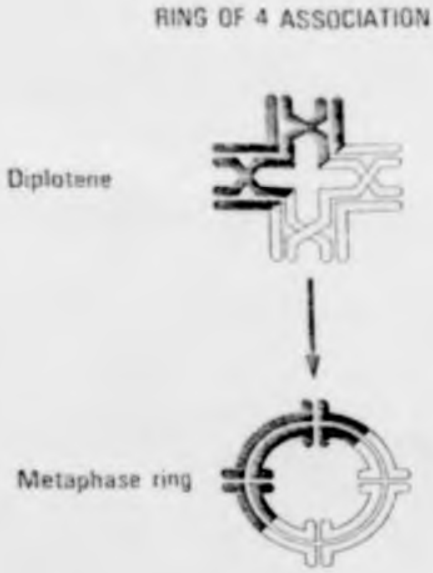

Figure 4.2 (a). Formation of ring of 4 association in interchange heterozygotes.

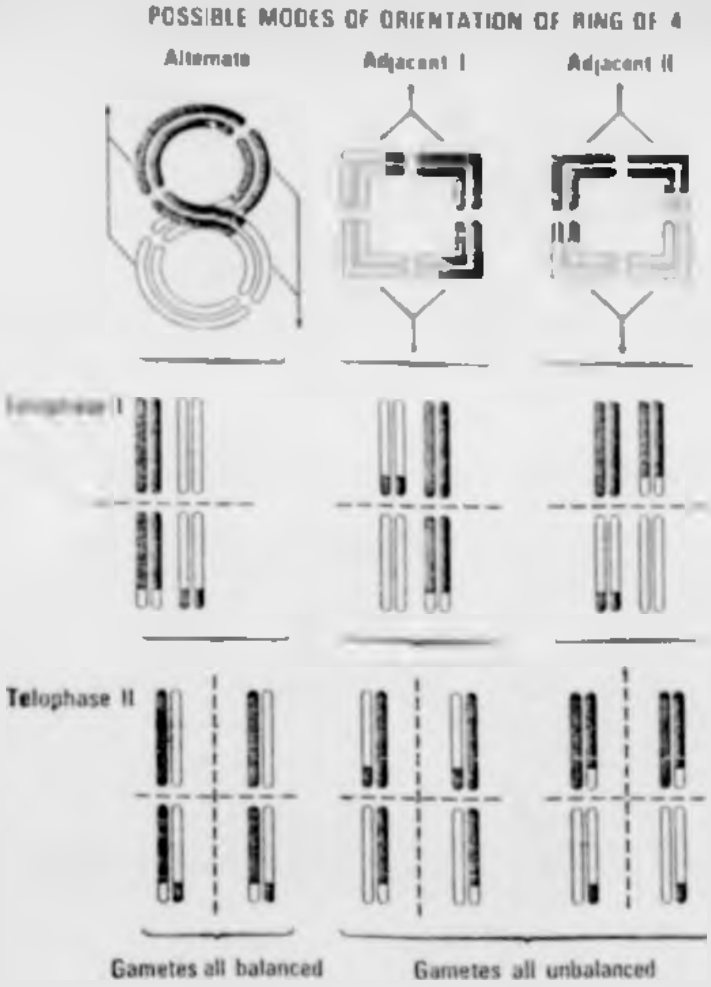

Figure 4.2 (b). Possible modes of orientation of a ring of 4 association.

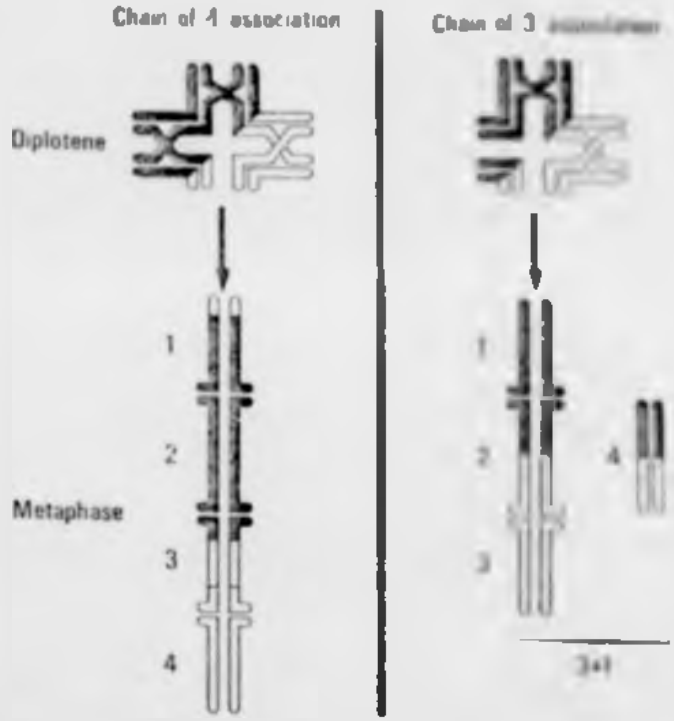

Figure 8.3 (a). Formation of chain associations by interchange heterozygotes.

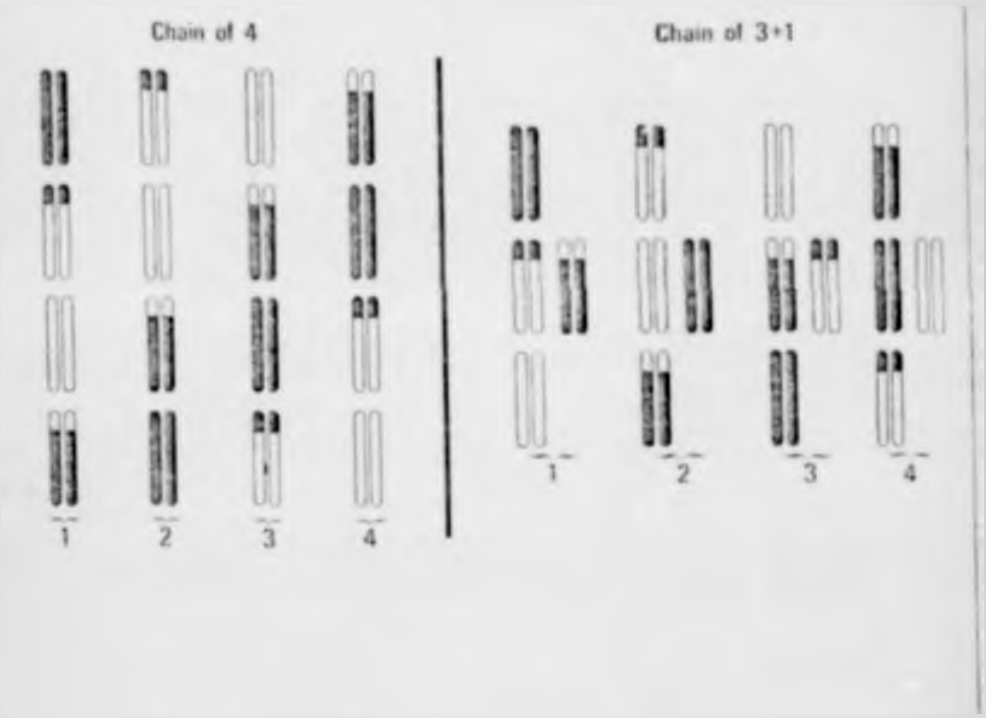

Figure 5.3 (b). Possible associations of chain multivalents, depending on positions of chiasmata.

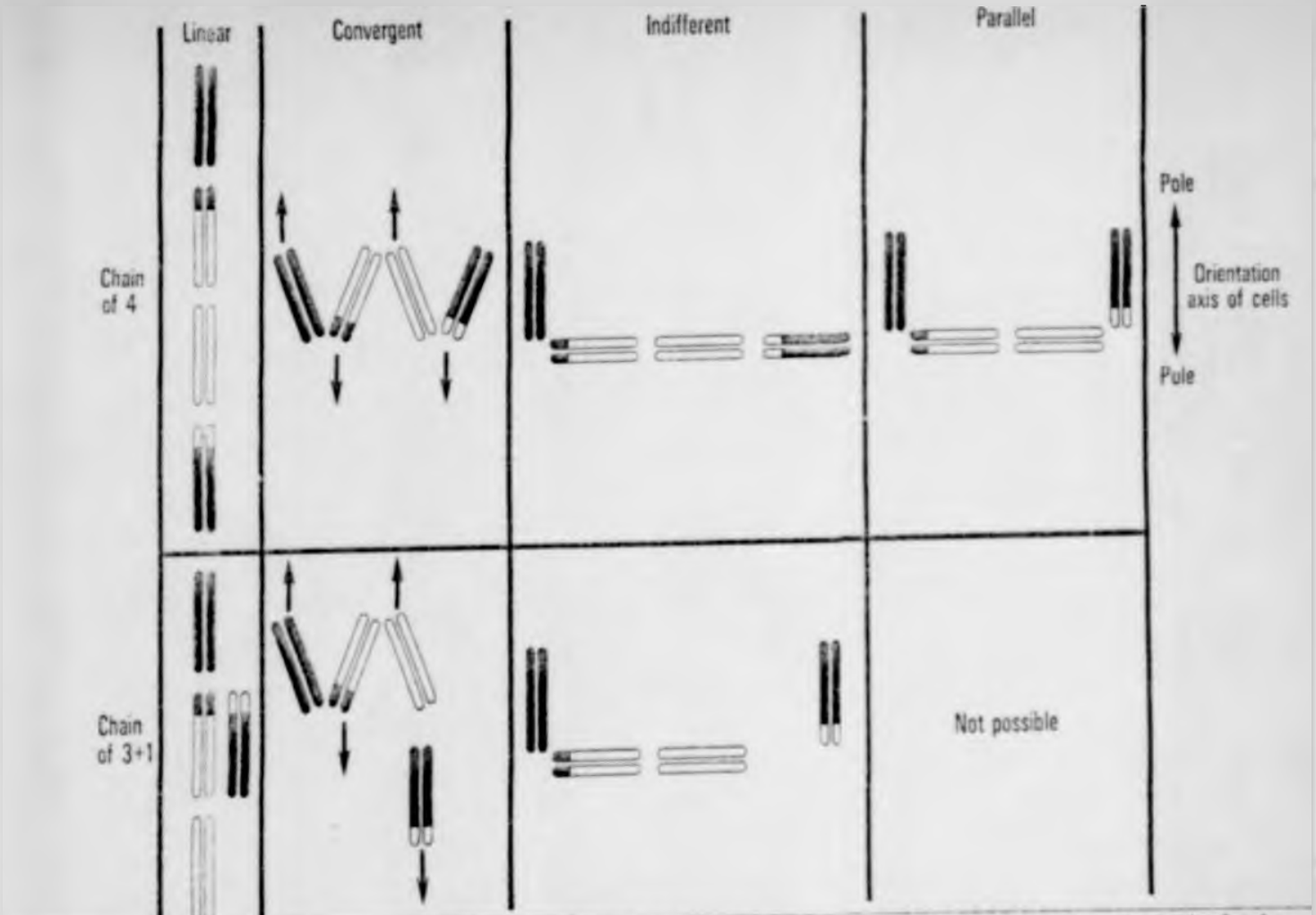

Figure 4.4. Orientation of chain multiples, considering a single association. Only convergent orientation leads to the production of balanced gametes.

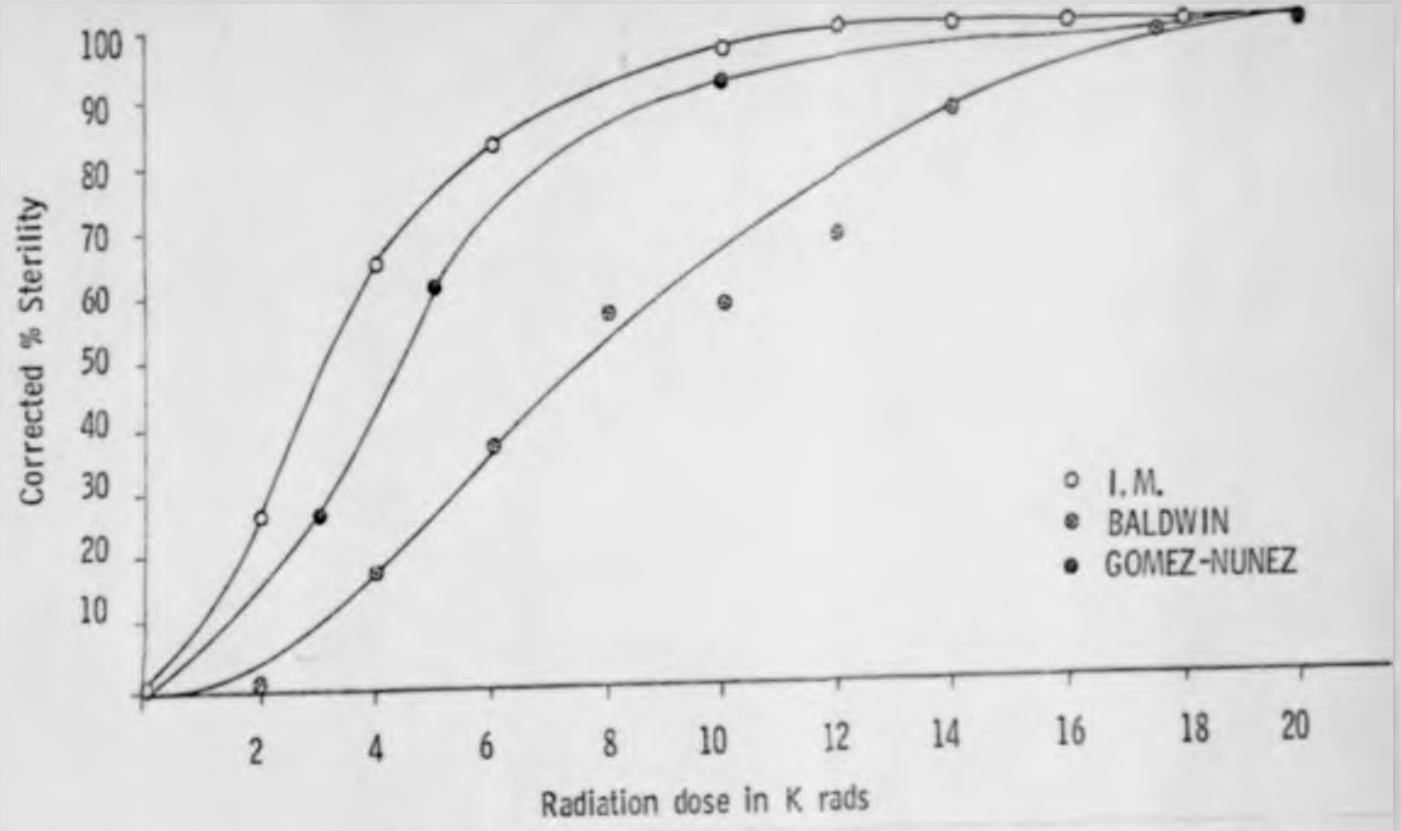

Figure 4.5. Comparison of dose-response curves for B. proliinx compiled from the data of Gomez-Nunez et al. (1964), Baldwin and [illegible] (1963) and from the present work (I.M.).

minute. These qualitative and quantitative differences in radiation applied, probably account for the much smaller dosage required to produce complete sterility in the present experiment (12 K rad) than that needed by Baldwin and [illegible] to produce complete sterility (20 K rad).

From this dose-response data, it was decided that 8 K rad should be used as the experimental dosage since this dose produced a high level of sterility (83.16%) and, also, Gomez-Nunez et al. (1964) had shown that such a dose did not appreciably affect the mating vigour of male R. prolixus. At this point, the cytogenetic effects of such a dose were unknown so that this dosage was chosen, with particular respect to chromosomal damage, essentially arbitrarily.

The preliminary check on time effects on ejaculation and insemination efficiency of normal paired bugs was made as follows: Removing normal males from normal females at 7 days after the first meal (Group 1) or, leaving pairs together for a further 14 days and removing them 7 days after their second blood-meal (Group 2). Only 2 lots of 7 pairs of R. prolixus reared on Virginia adults. The results of this experiment are shown in Table 4.2. A χ^2 test for proportions, applied to the 2 sets of results, showed that there was no significant difference in fertility between the 2 groups (χ^2 = 1.24, P = N.S.), so that removal of the male as early as 7 days after pairing clearly had no effect on hatchability of eggs laid after subsequent blood-meals by the female over a period of 35 days. The data from these two groups were pooled to give a figure of 83.16% for the fertility of 14 pairs of bugs and this was used as the control value for Abbott corrections for subsequent experiments which involved the removal of males after mating.

Table 5.1. Dose-response fertility data for H. prolixus males irradiated with gamma rays and mated to normal females. Data pooled for eggs laid after each [illegible] blood-meals.

Dose in K rad.	Number of pairs	Number of eggs laid	Number of eggs hatched	Corrected % sterility
0	4	198	[illegible]	[illegible]
2	4	189	130	26.56
4	4	81	[illegible]	[illegible]
6	4	146	23	83.16
8	4	113	[illegible]	100.00
10	4	247	7	96.97
12	4	166	[illegible]	[illegible]
14	4	62	[illegible]	[illegible]
16	4	102	[illegible]	[illegible]
18	4	109	0	100.00
20	4	40	[illegible]	100.00

Table 4.2. The effect on fertility of removing male *R. prolixus* from females at different times after mating. The numbers of eggs laid are the totals for 3 blood-meals given at 14 day intervals.

Group	No. pairs	No. eggs laid	No. eggs hatched	% fertility	$\bar{x}$	s
1	7	507	[illegible]	[illegible]	[illegible]	[illegible]
2	7	516	[illegible]	[illegible]		
Total	14	[illegible]	[illegible]	[illegible]		

Group 1 - males removed 7 days after first blood-meal (= 7 days paired)

Group 2 - males removed 7 days after second blood-meal (= 21 days paired)

Having decided on a dosage of 6 K rad, the parental generation of 40 male *R. prolixus* were irradiated and each paired with a normal female. The hatchability of the eggs laid by these 40 females after 4 blood-meals are shown in Table 4.3, showing that with a dose of 6 K rad the mean fertility of the 39 surviving pairs was [illegible] and 1 female bug died during the course of this experiment and the data from these bugs are not included in the calculations of fertility. Two pairs had zero fertility but it is important to note that 28 of the 30 surviving pairs were semi-sterile to a greater or lesser extent, their corrected fertility ranging from [illegible]. The mean fertility of 23.9% differs from that given for the dose-response curve in which bugs given 6 K rad had a mean fertility of 14.8%, but the latter figure was based on only 4 pairs of bugs.

Table 4.3. Fertility data for the parental generation of 40 virgin male *R. prolixus* irradiated with 6 K rad and mated with normal virgin females. The data for eggs laid after 4 blood-meals have been pooled. Control fertility = 91.88%

Pair number	Number of eggs laid		Number of eggs hatched	Corrected % fertility
1	62		17	29.84
2	65		23	36.84
3	47		11	25.47
4	29		7	26.27
5	58		18	33.78
6	71		7	10.73
7	0		0	0
8		Male died		—
9	80		14	19.04
10	61		11	19.63
11	56		4	7.78
12		Male died		—
13	59		14	25.83
14	38		7	20.05
15		Male died		—
16	22		2	9.89
17		Male died		—
18	77		14	19.79
19	51		6	12.80
20	14		0	0
21		Male died		—
22		Male died		—
23		Female died		—
24	19		5	22.91
25	14		3	15.55
26		Male died		—
27	38		4	11.46
28	62		33	57.93
29	43		14	35.44
30	37		8	23.53
31	32		6	20.41
32	60		14	25.40
33		Male died		—
34	66		12	19.79
35	45		7	16.93
36	49		7	15.55
37	101		25	26.94
38	31		8	28.09
39		Male died		—
40	35		14	43.54
Total	1420		313	23.66

eggs laid after each of the 4 parental blood-meals were kept separately, and, on hatching, ten larvae were reared to F_1 adults as 4 separate batches derived from egg batches 1, 2, 3 or 4. All of the offspring from these 28 partially fertile pairings were fed but not all of them were successfully reared to adults. Of the 5[illegible] first instar larvae which hatched, only 147 were reared to adults (47.1%). The majority of the 165 bugs which were not reared successfully died during the early stages of development as first or second instar larvae. Of the 147 bugs reared to adults 80 were male and 67 female, and a χ^2 test showed that there was no significant departure from an expected 1:1 sex ratio in these F_1 bugs (χ^2 = 3. , P = N.S.).

Only 86 of the 147 F_1 adults were used in further matings. Selection of these 86 bugs was made to include bugs from families with few surviving progeny and to limit the number of bugs used from large F_1 families in which survival to F_1 adult had been good; thus the choice of parents was not random. These 86 selected adults were each mated with normal mates and given 3 blood-meals at 14 day intervals. The hatchability data of the eggs laid by these 86 F_1 pairs were pooled for all eggs laid by each pair over 3 blood-meals (Tables 4.4 - 4.7 - each of these 4 Tables relating to crosses of normal adult X F_1 adults from the egg batches 1 to 4 laid by the parental female X irradiated parent male cross).

The results show that 2 of the 86 F_1 X normal matings were fully fertile (No. 2_1 from the first egg batch and No. 11 from the third egg batch) and that both of these were F_1 ♀ X normal ♂ crosses. Forty three of the F_1 X normal crosses were completely sterile and 41 were semi-sterile. The semi-sterile crosses showed a wide range of fertility from 1.0% (No. 11_3 egg batch No. 3) to 74.2% (No. 2_4 egg batch No. 4).

Table 4.4. Fertility data for 14 crosses of F_1 X normal *R. prolixus*. the F_1 adults being derived from egg batch 1 of normal female parent X irradiated male parent. Data pooled for eggs laid after 3 blood-meals by F_1 females.

Control fertility = 91.88%

Bug number		F_1 sex	Number of eggs laid	Number of eggs hatched	Corrected % fertility
1	1	♂	31	0	0
	2	♀	94	51	59.05
2	1	♀	132	114	94.00
	2	♀	12[illegible]	0	0
4	1	♂	85	13	16.65
	2	♀	143	5	[illegible]
	3	♂	86	3	3.80
9	1	♀	124	3	2.63
19	1	♂	74	24	35.30
29	1	♂	79	25	34.44
	3	♂	63	2	[illegible]
30	1	♂	76	45	[illegible]
	2	♂	61	5	8.92
34	1	♀	62	2	3.51
Total			[illegible]	[illegible]	[illegible]

Table 5.1. Fertility data per [illegible] ages of F_1 X normal *S. mediterranea*, the F_1 adults being derived from egg batch 2 of normal female parent X irradiated male parent. Data pooled for eggs laid after 3 blood-meals by F_1 females.

Control fertility 91.88%

Reg number F_1	F_1 sex	Number of eggs laid	Number of eggs hatched	Corrected % fertility
1	♀	48	1	2.27
	♀	82	1	1.33
	♀	39	0	0
	♀	81	0	0
2	♂	59	11	20.29
	♂	72	43	71.05
	♂	87	0	0
	♂	33	0	0
5	♀	165	0	0
5	♀	27	0	0
	♀	100	9	9.80
6	♀	20	0	[illegible]
	♀	95	4	4.58
7	♀	39	0	0
	♀	[illegible]	0	0
	♀	84	23	[illegible]
14	♀	61	0	0
	♀	38	0	0
	♀	66	1	1.65
	♀	71	1	[illegible]
19	♀	25	0	0
28	♀	4	0	0
	♀	51	3	6.40
32	♀	56	1	1.94
	♀	110	0	0
34	♀	12	0	0
37	♀	66	3	4.95
	♀	22	0	0
	♀	67	35	45.48
	♀	30	0	0
	♀	70	1	1.55
	♀	135	69	55.63
	♀	117	4	3.72
38	♀	113	11	[illegible]
	♀	45	3	7.62
	♀	115	38	35.96
	♀	70	1	1.55
40	♀	50	0	0
	♀	40	0	0
Total		[illegible]	[illegible]	11.39

Table 4.6. Fertility data for [illegible] of F_1 X normal *C. pallidus*. The F_1 adults being derived from egg batch 3 of normal female parent X irradiated male parent. Data pooled for eggs laid after 3 blood-meals by F_1 females. Control fertility = 91.88%.

Egg batch ♀	F_1 ♂	F_1 sex	Number of eggs laid	Number of eggs hatched	Corrected % fertility
2	2	♀	179	0	[illegible]
5	1	♀	147	3	[illegible]
	4	♂	64	0	[illegible]
10	1	♂	120	0	[illegible]
13	1	♂	49	0	[illegible]
	2	♂	138	[illegible]	[illegible]
18	1	♂	71	[illegible]	[illegible]
28	1	♂	71	[illegible]	[illegible]
	2	♂	120	[illegible]	[illegible]
	3	♂	81	[illegible]	[illegible]
	4	♂	94	[illegible]	[illegible]
	5	♂	134	[illegible]	[illegible]
	6	♂	37	[illegible]	[illegible]
	7	♂	32	[illegible]	[illegible]
34	2	♂	[illegible]	[illegible]	[illegible]
	3	♂	69	[illegible]	[illegible]
36	2	♂	47	[illegible]	[illegible]
	3	♂	12	[illegible]	[illegible]
37	1	♂	95	[illegible]	[illegible]
	2	♂	104	[illegible]	[illegible]
[illegible]			[illegible]	[illegible]	[illegible]

Table 3.7. Fertility data for 13 crosses of F_1 X normal R. prolixus, the F_1 adults being derived from egg batch 4 of normal female parent X irradiated male parent. Data pooled for eggs laid after 3 clear females. Control fertility = 91.88%.

Bug number P	F_1	F_1 sex	Number of eggs laid	Number of eggs hatched	Corrected % fertility
2	1	♂	46	0	0
	2	♂	70	0	0
3	1	♀	139	0	0
	2	♀	26	0	0
13	1	♂	91	13	15.55
	3	♀	142	0	0
25	1	♀	102	0	0
29	2	♀	9	0	0
37	1	♂	40	0	0
	3	♀	116	0	0
40	1	♂	51	0	0
	2	♀	89	1	1.22
	3	♂	86	21	26.58
[illegible]			[illegible]	[illegible]	[illegible]

Table 4.8. F_1 X normal *R. prolixus* fertility data pooled from 4 parental egg batches (see Tables 4.4 - 4.7) representing 22 parental crosses. Control fertility - 91.88%.

Parental family No.	Number of F_1 pairs in sibship	Number of eggs laid	Number of eggs hatched	Corrected % fertility
1	6	375	53	15.38
2	6	[illegible]	172	23.46
3	3	330	0	0
4	5	[illegible]	[illegible]	7.40
5	3	211	3	1.55
6	4	124	10	8.78
7	5	256	25	10.63
10	1	120	0	0
13	4	[illegible]	[illegible]	35.76
14	4	236	2	0.92
18	1	71	0	0
19	3	99	24	26.38
25	1	102	0	0
28	4	[illegible]	24	3.96
29	5	151	27	19.46
30	3	137	50	39.72
32	2	166	1	0.66
34	4	[illegible]	3	0.93
36	3	59	0	0
37	11	862	106	13.38
38	8	343	55	17.45
40	5	326	35	11.68
Total for 22 sibships	[illegible]	6520	757	12.64

The data from these four groups presented in Tables 4.4 - 4.7 have been pooled and are presented in Table 4.8 as data from 22 families.

The results show that the mean corrected fertility for the 86 F_1 X normal crosses was 12.6% ± 0.04. Five of the 22 F_1 sibships were completely sterile and the mean fertility of the other 17 sibships ranged from 1.4% to 70.7%. These figures may be compared with the parental fertility of 73.9% (range 1.8% to 91.7%) which indicates that the F_1 progeny were much less fertile than their irradiated parents. However, a straightforward comparison of these two values is not meaningful since the F_1 matings were derived from several different parents, but it is clear from a study of the range of fertilities for the 2 generations that the F_1 families were less fertile overall. A family by family comparison (on fertility data from Tables 4.3 and 4.8) showed that in only 3 of the 22 families was the F_1 fertility greater than that of the corresponding parental family. The 3 exceptions, families F_1 9 and 8 (see paragraph 4) fertilities but χ^2 tests of the data showed that these differences were not significant (for parental fertility χ^2 = 0.80, 1.96 and 1.41 respectively, P = N.S.).

A comparison of the fertility of F_1 matings established from each of the 4 parental egg batches revealed that the fertility of these 4 groups differed. The mean fertility of the 4 groups (see in Tables 4.4 - 4.7) were 23.0%, 11.3%, 7.1% and 5.1% respectively, at least the F_1 bugs from the first parental egg batch were more fertile than those of the later batches. A simple statistical comparison of these 4 proportions would not be meaningful since not all F_1 sibships were represented in each of the 4 parental egg batches. A comparison was therefore made between F_1 families within sibships for the 4 batches

the data from these four groups presented in Tables 4.4 – 4.7 have been pooled and are presented in Table 4.8 as data from 22 families.

The results show that the mean corrected fertility for the 80 F_1 mated crosses was 11.6% ± 1.9%. Five of the 22 F_1 matings were completely sterile and the mean fertility of the other 17 sibships ranged from 1.6% to 57.7%. These figures may be compared with the parental fertility of 13.7% (range 7.8% to 67.0%) which indicates that the F_1 progeny were much less fertile than their irradiated parents. However, a straight-forward comparison of these 2 mean values is not meaningful since the F_1 matings were derived from several different parents, but it is clear from a study of the range of fertilities for the 2 generations that the F_1 families were less fertile overall. A family by family comparison of the fertility data from Tables 4.3 and 4.8 shows that in only 3 of the 22 families was the F_1 fertility greater than that of the corresponding parental family. The 3 exceptions, families 13, 19 and 30 had increased F_1 fertilities but χ^2 tests of the data showed that these increases were not significant over parental fertility (χ^2 = 1.81, 1.46 and 1.41 respectively, P = N.S.).

A comparison of the fertility of F_1 matings established from each of the 4 parental egg batches revealed that the fertility of these 4 groups differed. The mean fertility of the 4 groups shown in Tables 4.4 – 4.7 were 25.8%, 11.5%, [illegible] respectively, so that the F_1 bugs from the first parental egg batch were more fertile than those of the later batches. A simple statistical comparison of these 4 proportions would not be meaningful since not all F_1 sibships were represented in each of the 4 parental egg batches. A comparison was therefore made between F_1 families within sibships for the 4 batches

Table 4.9. X^2 tests for differences in fertility between 62 F_1 families of R. prolixus grouped into 11 sibships and produced from 4 egg batches laid by the parental generation.

F_1 sibship number	Parental egg batch								df	X^2	P
	1		2		3		4				
	L	H	L	H	L	H	L	H			
1	125	51	250	2					1	75.8	<0.001***
2	252	114	251	58	179	0	116	0	3	108.9	<0.001***
4	314	21	127	9					1	0.02	N.S.
9	124	3	132	22					1	12.2	<0.001***
14			236	2	187	125			1	116.9	<0.001***
19	74	24	25	0					1	7.6	0.01-0.001**
28			91	3	569	21			1	0.02	N.S.
29	142	27					9	0	1	1.69	N.S.
34	62	2	12	0	159	0			2	5.4	0.1-0.05
37			507	105	199	1	156	0	2	65.7	<0.001***
40			90	0			226	22	1	8.5	<0.001***
T o t a l	1093	242	1886	201	1293	147	507	22			

L = No. of eggs laid

H = No. of eggs hatched

df = Degrees of freedom

P = Probability

and the results of these analyses are shown in Table 4.9. Only those 11 F_2 sibships which were each represented for more than one parental batch could be compared and the results show that there were significant differences in fertility between families for 7 of these 11 sibships. These analyses do not reveal whether there was any uniformity or trend in these differences between egg batches, therefore a test for linear trend in proportions was carried out on the column totals given in Table 4.9, the results of which are shown in Table 4.10.

Table [illegible]. χ^2 test for linear trend in fertility for [illegible] F_2 families from 4 parental egg batches.

	Parental egg batch				$\hat{b}$	χ^2	P
	1	2	3	4			
No. eggs laid	1093	1886	1293	507	−0.04	18[illegible]	<0.01***
No. eggs hatched	42	201	[illegible]	[illegible]	[illegible]		

$\hat{b}$ = Slope

The results show that there was a highly significant linear trend in proportions downwards from egg batch No. 1 to egg batch No. [illegible] ($P < 0.01$), and it may be concluded that F_2 hens from the 4 parental matings had significantly different fertilities, with a trend towards reduced fertility in the later batches, reflecting differences due to birth order of these F_1 hens. Proportions of these 84 F_2 normal matings were completely sterile compared with only [illegible] of the 34 parental matings and the proportions of sterile, semi-sterile and fully fertile F_2 normal

crosses have been calculated for eggs of the 4 batches, the results of which are shown in Table 4.11.

Table 4.11. Proportions of sterile, semi-sterile and fully fertile bugs for 86 F_1 X normal matings from 4 parental egg batches.

Parental egg batch	No. of F_1 pairs	Sterile		Semi-sterile		Fully fertile	
		No.	%	No.	%	No.	%
1	14	2	14.3	11	78.6	1	7.1
2	39	18	46.2	21	53.9	0	0
3	20	13	65.0	6	30.0	1	5.0
4	13	10	76.9	3	23.1	0	0
T o t a l	86	43	50.0 ± 0.8	41	46.7 ± 0.7	2	2.3 ±0.2

The results show that 2.3% (2) of the 86 matings were fully fertile, 50% (43) were completely sterile and 46.7% (41) were semi-sterile. Furthermore, it is clear that the proportions of completely sterile bugs increased with birth order from 14.3% in egg batch 1 to 76.9% in egg batch 4, while the proportions of semi-sterile bugs decreased from 78.6% in egg batch 1 to 23.1% in egg batch 4. These data indicate a strong correlation between birth order of F_1 bugs and the proportions carrying dominant lethal mutations.

An interesting comparison was made between F_1 ♂ X normal♀ and F_1 ♀ X normal♂ fertilities. As such a comparison it would not be used simply to contrast the mean percentage egg hatch of the 2 groups since the analysis must allow for family relationships within groups, i.e. ♂ and ♀ F_1 parents of the same sibship must be compared with each other. The method adopted for this comparison --- [illegible] analysis, the details of which are given in Cox (197[illegible] Chapter 3. The data for this analysis were taken from Tables 4.6 - 4.7 and the results of the calculation are shown in Table 5.11.

Table 5.11. Weighted logistic analysis of F_1♂ x normal♀ and ♀ x normal♂ fertility data. Only fertile or semi-fertile crosses included.

Cross	No. of pairs	No. of eggs laid	No. of eggs hatched	Fertility	Weight		
F_1♂ X normal♀	24	1787	277	15.5	[illegible]	[illegible]	3.63 <[illegible]***
F_1♀ X normal♂	19	1977	[illegible]	23.6	[illegible]	[illegible]	

Z = Standard normal deviate

The results show that in a comparison of 43 fertile or semi-fertile crosses, F_1 female hens were significantly ($P < 0.001$) better in terms of egg hatchability than F_1 males when mated with normal partners. Also, of the 777 eggs which had come from the 24 F_1 ♂ x normal female matings,

only 153 (55.2%) came through successfully to adults as against 33[illegible] (70.7%) reaching adult of the 467 larvae hatching from the 19 F_1 ♀ X normal male matings.

The sex of these 'F_2' bugs was tested for departures from the 1:1 expected ratio and the results showed that the proportions of male to female was almost exactly 1:1; viz., 153 bugs raised from F_1 ♂ X normal ♀ 79 were male and 74 were female, and, of the 80 bugs raised from F_1 ♀ X normal ♂ crosses, 142 were male and 138 were female.

Twenty-eight of these 'F_2' bugs were matings involved with normal males, these 28 being chosen from among the F_1 crosses with the lowest fertilities. These 'F_2' pairings were each given 3 blood-meals and the eggs produced were scored for [illegible]. The results show that the mean fertility of [illegible] 'F_2' 2-normal matings was 47.8%, with family fertilities ranging from [illegible]. The fertility of 25 of the 'F_2' 2-normal crosses was greater than that of their respective parents' fertilities except for families $21_{4,1}$ and $28_{4,1}$ which had slightly reduced fertilities in comparison with their parents.

Surprisingly, 6 of the 28 'F_2' crosses were fully fertile even though all crosses were deliberately selected from the F_1 families with the lowest fertilities. For comparative purposes the mean fertilities and ranges in fertility for all families raised from parental, F_1 and 'F_2' crosses are shown in Table 4.14.

Table 4.13. Fertility of 28 'F_2' X normal crosses, data pooled for all eggs laid after 3 blood-meals. Parental and grand-parental fertilities are included for comparison.

Family No.			Sex	Eggs		Corrected % fertility			Parental egg batch
P	F_1	F_2		Laid	Hatched	F_2	F_1	P	
4	2	1	♂	25	19	82.72	[illegible]	[illegible]	
			♂	[illegible]	22	36.28	3.81	26.27	
			♂	54	3	6.05	3.80	26.27	
			♂	[illegible]	16	20.67	[illegible]	[illegible]	
		1	♂	39	15	41.86	3.46	35.44	
			♂	62	5	8.77	8.92	33.53	
6	2	1	♂	77	8	11.30	4.58	10.73	
		1	♂	59	57	100.00	[illegible]	[illegible]	
		2	♂	87	6	7.50	6.40	[illegible]	
		2	♂	102	100	100.00	[illegible]	[illegible]	
		4	♂	died					
		5	♂	80	3	4.08	3.72	26.94	
5	1	1	♂	47	18	41.68	[illegible]	[illegible]	
	2	1	♂	59	5	9.22	8.16	57.93	
			♂	[illegible]	13	23.98	8.16	57.93	
		1	♂	70	17	26.43	3.47	57.93	
		2	♂	39	1	2.79	3.47	57.93	
		3	♂	22	4	19.79	3.47	[illegible]	3
		1	♂	56	6	11.66	4.87	57.93	
			♂	48	47	100.00	4.87	[illegible]	
		3	♂	54	0	0	4.87	57.93	
		4	♂	died					
37	2	1	♀	100	93	100.00	1.05	26.94	
11	[illegible]	1	[illegible]	63	61	100.00	[illegible]	25.83	
[illegible]	3	2	♂	36	8	24.19	15.55	[illegible]	
		3	♂	33	9	29.68	26.85	43.54	4
		4	[illegible]	69	62	97.80	26.85	43.54	
				49	47	100.00	26.85	43.54	
Total				1809	660	44.64	5.44	28.58	

Table 4.14. Corrected fertility for 3 generations of families of *H. proximus* to compare fertility of all families with fertility of selected families.

Generation	All families		Selected families	
	Mean fertility %	Range %	Mean fertility %	Range %
P	23.9	7.9 - 57.9	28.6	10.7 - 57.9
F_1	12.6	1.1 - 38.6	5.4	1.1 - 10.9
'F_2'	44.6	2.8 - [illegible]	44.6	[illegible]

The results show that the 16 F_1 families selected for breeding had a mean fertility of 5.4% (range 1.1% - [illegible]) which was much lower than the overall mean for F_1 families of 12.6% (range 1.1 - 38.6%). These data show not only that following irradiation with 6 K rad X-rays *H. proximus* males had a much reduced fertility compared with control matings, but also that the fertility of the F_1 progeny was lower than normal bugs and much less than that of their parents. Furthermore, when 'F_2' bugs (specially reared from eggs of F_1 females of low fertility) were crossed with normal mates, the fertility of this 'F_2' generation is seen to have returned to [illegible] 2 normal.

Only a few 'F_3' bugs were reared to adult and these all came from a 'F_2' families descended from the first parental egg batch. These bugs were not mated and therefore no fertility data were produced for this generation.

Cytogenetic studies

It is now appropriate to consider the cytogenetic findings with

relation to these fertility data, and these were derived from progeny bugs of the above experiments, after male parents had been withdrawn from their mating period. Fig. 4.1 shows a metaphase I nucleus from a normal male spermatocyte of R. [illegible], the diploid number of which is 2n = 20 autosomes + XY. The karyotype of R. [illegible] was first described by Ueshima (1966) to consist of 3 longer pairs of autosomes and 7 shorter pairs, although it was not possible from that investigation to distinguish individual bivalents, in meiotic preparations, only the sex univalents being readily identifiable. The 10 pairs of autosomes are usually associated by a single chiasma in each element.

Lacto-acetic-orcein squash preparations were made from the fixed testes of F_1 males, their sons ('I_{-1}') and grandsons ('I_1'). Forty-nine of the 51 F_1 males were examined cytogenetically after mating with normal females, the other 2 males having died before the date of fixation. Eighty-nine 'F_2' males were examined cytogenetically, of these F_1 δ x normal φ crosses and 40 from F_1 φ x normal δ crosses. Only 11 'F_2' males were used for cytogenetic examination. In scanning the testis squashes any departures from the normal 10 bivalents + XY configurations at metaphase I were noted and photographs taken for compiling cytogenetic data.

All 49 F_1 males examined revealed chromosomal abnormalities in their spermatocytes so that in every male at least one interchange was evident, while many [illegible] and several had 5+. In addition, several cells showed [illegible] fragments, presumably [illegible]. The cytogenetic results obtained by examination of F_1 male testes have been summarised and are shown in Tables 4.1 - 4.18, together with fertility data. It must be emphasized that although patterns of

Table 9.15. Chromosomal associations at metaphase I in F_1 male *D. virilis* from irradiated male parents X normal female parents - first egg batch. Except where indicated, multivalents associated as chains.

F_1 male number	Metaphase I associations	Abnormalities	% fertility F_1	Parental fertility
1,1	8 II + 1 III + I	1 Translocation	0	29.8
4,1	8 II + 1 III + I	1 "	16.6	26.3
4,1	6 II + 2 III + 2 I	2 "	3.7	26.3
19,1	6 II + 2 III + 2 I + F	2 "	35.1	16.8
29,1	8 II + 1 III + I	1 "	34.4	35.4
29,1	8 II + 1 III + I	1 "	3.5	35.4
30,1	8 II + 1 III + I	1 "	64.4	23.5
30,2	8 II + 1 III + I	1 "	8.9	23.5

F = Fragments

Table 4.16. Chromosomal associations at metaphase I in F_1 male *A. anilinus* from irradiated male parents X normal female parents - second egg batch. All multivalents associated as chains, except where stated.

F_1 male number	Metaphase I associations	Abnormalities	% fertility F_1	Parental fertility
1,1	6 II + 1 IV + 1 III + 1 I	2 Translocations	2.3	29.8
2,6	8 II + 1 III + I	"	0	36.8
6,1	8 II + 1 III + I	"	20.6	10.7
9,1	4 II + 3 III + 3 I	"	0	19.[illegible]
9,3	8 II + 1 III + I + 1	"	[illegible]	19.2
13,1	6 II + 1 IV + 1 III + 1	"	[illegible]	21.8
14,2	6 II + 2 III + 2 I + 1	"	[illegible]	20.2
28,1	4 II + 1 IV + 2 III + 2 I	"	0	57.9
28,2	6 II + 2 III + 2 I	"	6.4	57.9
32,1	8 II + 1 III + I	"	[illegible]	25.4
34,2	8 II + 1 III + I	"	[illegible]	19.8
35,1	8 II + 1 III + I	"	4.9	[illegible]
37,2	6 II + 2 III + 2 I	"	0	26.9
37,3	6 II + 1 IV RING + 1 III + I	"	45.5	26.9
37,4	6 II + 2 III + 2 I	"	[illegible]	26.9
37,5	6 II + 2 III + 2 I	"	1.6	26.9
38,2	8 II + 1 IV	"	7.2	[illegible].2
40,1	6 II + 2 III + 2 I	"	0	43.5
40,2	8 II + 1 III + I	"	0	43.5

Table 4.17. Chromosomal associations at metaphase I in F_1 male *S. stellatus* from irradiated male parents X normal female parents - third egg batch. All multivalents associated as chains except where stated.

F_1 male number	Metaphase I associations	Abnormalities	% fertility F_1	Parental fertility
5,4	6 II + 2 III + 1 I	2 Translocations	0	33.8
10,1	8 II + 1 III + 1	1 "	0	19.6
13,1	6 II + 2 III + 2 I	2 "	0	25.8
18,1	6 II + 2 III + 2 I	2 "	0	19.8
28,1	8 II + 1 III + I	1 "	4.6	[illegible]
28,2	8 II + 1 III + I	1 "	8.2	57.9
28,3	8 II + 1 IV	1 "	0	57.9
28,4	6 II + 2 III + 2 I	2 "	3.5	57.9
28,5	6 II + 2 III + 2 I	2 "	4.9	[illegible]
28,6	8 II + 1 IV	1 "	0	[illegible]
28,7	8 II + 1 III + I	1 "	0	57.9
34,2	8 II + 1 IV	1 "	0	19.8
34,3	8 II + 1 III + I	1 "	0	19.8
36,2	6 II + 1 IV + 1 III + I	2 "	0	15.5
37,1	8 II + 1 III + I	1 "	0	26.9
37,2	8 II + 1 III + I	1 "	1.0	26.9

Table 4.18. Chromosomal associations at metaphase I in testis squashes of F_1 male *S. proliferus* from irradiated male parents x normal female parents - fourth egg batch.

F_1 male number	Metaphase I associations	Abnormalities	fertility F_1	Parental fertility
2_1	8 II + 1 III + I	1 Translocation		36.[illegible]
[illegible]	4 II + 1 III + I + 2 IV	3 "		36.[illegible]
13^2	8 II + 1 III + I	"	15.5	25.[illegible]
37^1	6 II + 2 III + 2 I	2 "	0	26.[illegible]
40^1	4 II + 3 IV	3 "	0	43.[illegible]
40^2	8 II + 1 III + I	"	16.5	43.[illegible]

Table 4.19. Metaphase I associations and translocation frequencies for 49 [illegible] males from 4 parental egg batches (*R. prolixus*).

No. of translocations	Metaphase I associations	Parental egg batch No.				Total	%
		1	[illegible]	3	4		
1	8 II + 1 III chain + 1	6	7	7	3	23	46.9
	8 II + 1 IV chain		1	3		4	8.2
	8 II + 1 IV ring						
	Sub total	6	8	10	3	27	55.1
2	6 II + 2 III chains + 2 I	2	[illegible]	5	1	14	28.6
	6 II + 1 IV chain + 1 III chain + I		2	1		3	6.1
	6 II + 1 IV ring + 1 III chain + I		1			1	2.0
	Sub total	[illegible]	[illegible]	6	1	18	36.7
3	4 II + 3 III chains + 3 I		1			1	2.0
	4 II + 1 IV chain + 2 III chains + 2 I		1			1	2.0
	4 II + 3 IV chains				1	1	2.0
	4 II + 2 IV chains + 1 III chain + 1				1	1	2.0
	Sub total	0	2	0	2	4	8.2
	Total for 4 egg batches	8	19	16	6	49	

metaphase I chromosome association often showed some degree of variation between cells within individual testis squashes, no attempt has been made to quantify this variation, the associations presented in the Tables of results represent either the only pattern seen or else the most common association within a particular testis. The numbers of translocations per individual shown in Tables 4.15- 4.18 were inferred from the patterns of association seen at metaphase I. These interchange data were used to compare the translocation frequencies of the F_1 males from the 4 parental egg batches. The translocation frequencies are shown in Table 4.19. The results show that of the 49 F_1 males examined, 27 (55.1%) showed a single translocation, 18 (36.7%) showed evidence of 2 translocations and 4 bugs (8.2%) showed evidence of having 3 translocations. Analysis of the distribution of translocations with parental egg batch did not reveal any statistically significant differences, probably because of the small numbers in each group, but it can be noted by inspection, that the majority of males from the first egg batch had only a single translocation while in later batches 2 and even 3 translocations became more frequent.

The most striking feature of these analyses of multivalent associations is that of the 49 F_1 males examined, only one bug showed a ring multiple association (Bug No. 37, from egg batch No. 2), the rest all having multivalents associated at metaphase I as chains of III plus 1 univalent, or else as chains of IV. The production of these chain multiples may be explained by reference to Figs. 4.1 - 4.4. It has already been explained in the introduction, how the chromosomes of an F_1 male, heterozygous for a single interchange must form a zygotene cross at meiosis to ensure regular pairing (Fig. 4.1), but the pattern of association at metaphase I will depend on numbers and

positions of chiasmata formed within the cross, and the numbers of chiasmata formed will in turn be dictated by the morphology of the chromosomes involved in the interchange. If chiasmata are formed in all 4 pairing segments of the zygotene cross, a ring of 4 chromosomes will be produced at metaphase I as illustrated in Fig. 4.21a1. It must be noted that there can be no interstitial segments (i.e. the section of a multiple lying between the centromere and point of exchange in a monocentric multiple) in multiples formed by holokinetic organisms, so that the special associations produced by interstitial crossing-over (Lewis and John, 1963) need not be considered here. The genetic consequences of ring formation are illustrated in Fig. 4.21a2. The standard mode of nomenclature used for monokinetic organisms has been adopted here although the terms 'alternate' and 'adjacent', which they reserve for centromere position, cannot strictly be applied to holokinetic species. Although no centromeres are illustrated in these line drawings, it is still clear that only 1— 'alternate' mode of orientation can lead to the production of genetically balanced gametes and that this involves a twisting of the multiple at metaphase I.

If, however, one of the arms of a zygotene cross is short, then chiasma formation may fail in that arm, resulting in the production of a chain of IV at metaphase I (Fig. 4.21b1), rather than a ring multivalent. Similarly, if there are two consecutive short arms in the zygotene cross, there may be a failure in the formation of two chiasmata resulting in a chain of III plus a univalent (Fig. 4.21c1). It also follows that if 2 short arms of the zygotene cross alternate with 2 long arms, then chiasmata formation in the long arms alone would result in the production of 2 bivalents at metaphase I. The arrangement of chromosomes within a chain will obviously depend on which arms

of the zygotene cross are chiasmata. Thus, for a single interchange there are 4 possible arrangements of the chromosomes in a chain multiple and these are shown in Fig. 4.3(A) for both a chain of II and a chain of III + I. The genetic consequences of chain formation will, however, depend on the orientation of the multiple in relation to the spindle at metaphase I. The possible modes of orientation of chains of IV and III + I are illustrated in Fig. 4.4. Lewis and John (1963) have shown that it is possible for a chain of IV to orientate in 4 ways: linear, convergent, indifferent and parallel, while a trivalent chain of III + I can only orientate in 3 of these ways, the parallel mode being impossible. When disjunction of these chain multiples occurs, only the convergent orientation type leads to the production of balanced gametes (Lewis and John, loc. cit.).

It may be seen from the summary of results presented in Table 4.1 that the most frequent association found in *C. brevicaudatus* F_1 males with a single interchange was 8 II + 1 III chain + 1. Plate 4.1 shows, for comparative purposes, a normal metaphase I cell from adult male *C. brevicaudatus* (Fig. 4.1 shows a metaphase plate in). Plate 4.2 shows a metaphase spermatocyte from F_1 ♂ No. 4_1 (egg batch No. 1) with a single interchange associated as a chain of III + I, the chain being clearly orientated.

Four F_1 males were found with chain of IV associations of a single interchange, one of which is illustrated in Plate 4.3 which shows a cell from F_1 ♂ No. 38_2 (egg batch No. 2) with chain of IV loosely orientated. Not a single ring of IV association was found in any of the F_1 males with a single translocation.

The results given in Table 4.1 show that 6 of the 7 males examined showed metaphase configurations which indicated that their chromosomes were involved in 2 interchanges. Plate 4.4 shows a metaphase I spermatocyte from F_1 bug No. 19_1 (egg batch No. 1) associated

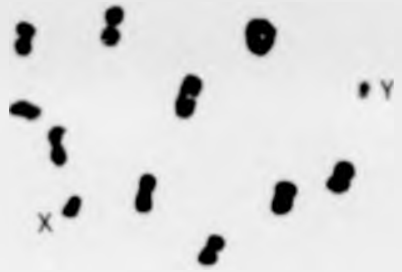

Plate 4.1. Normal metaphase I spermatocyte from untreated adult male [illegible].

Plate 4.2. Metaphase I spermatocyte from F_1 male [illegible] showing single translocation associated as 8 II + 1 III + 1 [illegible].

Plate 4.3. Metaphase I spermatocyte from F_1 male (No. 38_2) *R. prolixus* showing single translocation associated as 8 II + 1 chain of IV linearly orientated. Fertility - 7.2%. X 2500.

Plate 4.4. Metaphase I spermatocyte from F_1 male (No. 19_2) *R. prolixus* showing two translocations associated as 6 II + 1 III + 1 IV, one chain orientated linearly (L) the other multidimensionally (M). Fertility - 34.4%. X 2500.

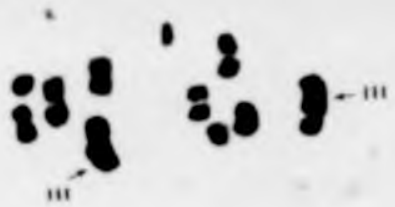

Plate 4.5. Metaphase I spermatocyte from F_1 male (No. 37_4) R. prolixus showing two translocation both associated as linear chains of III + I. Fertility = 0%. X 2500.

Plate 4.6. Metaphase I spermatocyte from F_1 male (No. 14_1) R. prolixus showing two translocations associated as (i) chain of IV and (ii) chain of III + I. The chain of IV shows parallel orientation and the chain of III indifferent orientation. Fertility = 0%. X 2500.

Plate 5.7. [illegible] from F_1 male No. 37 [illegible] showing two translocations, one associated as a chain of III + I linearly orientated, the other [illegible] as a ring of IV with alternate orientation. Fertility = [illegible]. X 1000.

Plate 5.8. [illegible] from F_1 male No. 3 [illegible] showing three translocations all associated as chains of III + I and all linearly orientated. Fertility = [illegible]. X [illegible].

Figure 4.9. Metaphase I spermatocyte from 'F_1' male (No. $38_{1,3}$) *R. prolixus* showing three translocations associated as chains of III + I, one being linearly orientated, the other showing [illegible]. X 2000.

Figure 4.10. Metaphase I spermatocyte from 'F_2' male (No. $6_{2,1}$) *R. prolixus* showing two translocations, one being a chain of IV in parallel orientation, the other a chain of III + I in [illegible] relationship. Fertility = 11.3%. X 2000.

Plate 5.11. [illegible] showing a single chromosomal fragment. Fertility - [illegible]. X 2500.

Plate 5.12. [illegible] showing two chromosomal fragments. Fertility - 41.7%. X 2500.

as 6 II + 2 III + . I, the . chains of III showing different forms of orientation, one being linear the other bottleneck. Plate 4.5 shows a cell from F_1 male 37_4 (egg batch No. 2) with 2 translocations in the form 6 II + 2 III + I but in this case both chains of III are linearly orientated.

Three F_1 males showed chain of IV associations together with a chain of III plus a univalent in the same cell. This type of configuration involving 2 translocations is illustrated by Plate 4.6 which shows a metaphase I cell from F_1 male No. 14_1 (egg batch No. 2) in the association 6 II + 1 IV + 1 III . I, the chain of IV showing parallel orientation and the arm of III showing bottleneck orientation.

Only one male was found showing a multivalent consisting of 5 rings, and this was in bug No. 37_3 (egg batch No. 2) which had 5 translocations. Plate 4.7 shows a metaphase I spermatocyte from F_1 male 37_3 with a ring of IV and a linear chain of III plus a univalent, the ring showing the twisted configuration necessary for alternate orientation. The formation of this ring multivalent association, although not located once in the present experiment, is important since it demonstrates that ring formation is not precluded in H. pruinosa.

Four F_1 males were found to show evidence of 3 translocations, all of which showed chains with linear orientation. Plate 4.8 shows a metaphase I cell from F_1 bug No. 9_2 (egg batch No. .) with the association 4 II + 3 III + 3 I, the chains all linearly orientated.

As the photomicrographs depict, the mode of association of multivalents at metaphase I and also, should orientation on the equatorial plate vary, this variation occurring between cells within testes. The results show that the most frequent association in these F_1 males was of the form 8 II + 1 III chain + I suggesting that most of the viable

translocations produced in the parental males by irradiation resulted in the formation of zygotene crosses with 2 short arms which could result in the failure of chiasma formation in these arms and the subsequent chain of III plus univalent associations. The cytological data also show that the majority of the chains were orientated linearly on the metaphase spindle as shown in Plate 4...

The cytogenetic results from 'F_2' male testis squash preparations are presented in Tables 4.20 - 4.25 and Table 4.26 contains a summary of these results.

The summary of the 'F_2' male cytogenetic data in Table 4.26 shows that of the 87 male examined, 34 (39.1%) had an identifiable chromosomal abnormalities, which is in sharp contrast to the F_1 males which all showed some degree of abnormality. Fifty-three of the 87 'F_2' males tested, however, were evidence of normal meiosis (61 to 100.0%) with a single translocation, 8 (9%) with 2 translocations, and one with 3 translocations. These figures are very different from the F_1 males, 94.7% of which had 2 translocations and 5.3% had 3 translocations. Plate 4.4 shows a metaphase I configuration from the only 'F_2' male with 3 translocations (see No. $3a_{1,2,3}$) associated as 4 II + 1 III + 2 I, one chain showing linear orientation but the other 2 showing convergent orientation. The observation of chains showing convergent orientation is important for, as already stressed, only this type of chain orientation may lead to the production of balanced gametes (see Fig. 4.4) which are essential for the maintenance of a translocation in a population.

Twenty-eight of the 34 'F_2' males with a single translocation were found to be associated in the form of a chain of III plus a univalent while 6 were associated as a chain of IV. The modes of orientation of these chains in 'F_2' males again showed variation both between and within

Table 4.20. Chromosomal associations at metaphase I in testis squashes of 'F_2' male *R. prolixus* from F_1 male (first egg batch) X normal female. All multivalents associated as chains except where stated.

Bug number F_1	Bug number F_2	Metaphase I associations	Abnormalities	% fertility F_2	F_1 parental fertility %
4_1	2	All cells normal	None		
	3	8 II + 1 III + I/8 II + 1 IV	1/2 Translocations		
	4	8 II + 1 IV	1 "		
4_3	1	8 II + 1 III + I	1 "	6.0	3.7
19_1^3	1	1 Fragment	1 Fragment		
	2	8 II + 1 III + I	1 Translocation		
29_1	4	All cells normal	None		
	5	8 II + 1 III + I	1 Translocation		
	6	All cells normal	None		
	8	All cells normal	None		
30_1	1	All cells normal	None		
	12	8 II + 1 III + I	1 Translocation		
	14	All cells normal	None		
	16	8 II + 1 III + I	1 Translocation		
	17	All cells normal	None		
30_2	2	8 II + 1 III + I	1 Translocation	8.8	8.9
29_3^2	1	All cells normal	None	41.3	3.5

Table 4.21. Chromosomal associations at metaphase I in testis squashes of 'F_2' male *R. prolixus* from F_1 female (first egg batch) X normal male. All multivalents associated as chains except where stated.

Bug number F_1	Bug number F_2	Metaphase I associations	Abnormalities	% fertility F_2	F_1 parental fertility %
1_2	2	All cells normal	None		
	3	"	"		
	5	"	"		
	9	"	"		
	10	1 Fragment	1 Fragment		
2_1	6	All cells normal	None		
	7	"	"		
	9	"	"		
	11	"	"		
	15	"	"		
4_2	1	1 Fragment	1 Fragment	82.7	3.8
	2	1 Fragment	"	36.3	3.8
9_1	1	1 Fragment	"	20.7	2.6

Table 4.11. Chromosomal associations of metaphase I in testis squashes of 'F_2' male R. prolixus from F_1 male egg batch No. 25 x normal female. All calculations mentioned in chains except where stated.

Bug number F_1	Bug number F_2	Metaphase I associations	Abnormalities	'F_2' fertility %	F_1 parental fertility %
6_1	1	6 II + 2 III + 2 I	2 Translocations		
9_5^1	1	All cells normal	None		
	2	8 II + 1 III + I	1 Translocation		
28_2	2	6 II + 2 III + 2 I	"	44	64
32_1^2	1	8 II + 1 IV	"		
37_3^1	4	8 II + 1 III + I	"		
	7	8 II + 1 III + I	"		
	9	8 II + 1 III + I	"		
	10	All cells normal	None		

Table 4.13. Chromosomal associations at metaphase I in testis squashes of [illegible] male *R. prolixus* from F_1 female (egg batch No. 2) X normal male. All multivalents associated as chains except where stated.

Bug number F_1	Bug number F_2	Metaphase I associations	Abnormalities	'F2' fertility %	F_1 parental fertility %
1_3	1	8 II + 1 IV	1 Translocation		
	3	8 II + 1 III + I + F	1 Translocation + F		
2_1	2	8 II + 1 III + I	1 Translocation		
	3	8 II + 1 III + I	"		
	6	8 II + 1 III + I	"		
3_3	3	8 II + 1 III + I	"		
	6	8 II + 1 III + I	"		
	7	All cells normal	None		
6_2	1	6 II + 1 IV + 1 III + I/8 II + 1 III + I	1/2 Translocations	11.3	4.5
	2	6 II + 1 IV + 1 III + I/8 II + 1 III + I	1/2 "		
	4	8 II + 1 IV	1 "		
9_4	5	All cells normal	None		
	7	All cells normal	"		
	10	8 II + 1 III + I	1 Translocation		
	17	8 II + 1 III + I	1 "		
14_3	1	All cells normal	None	96.6	1.6
37_6	10	All cells normal	"		
	11	1 Fragment	1 Fragment		
	13	1 Fragment	"		
	17	1 Fragment	"		

..... Continued.

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Table 4.13. (Continued)

Bag number F_1	Bag number F_2	Metaphase I associations	Abnormalities	'F_2' fertility %	F_1 parental fertility %
37_7	2	All cells normal	None	100.0	3.7
	5	8 II + 1 III + I	1 Translocation	4.0	
25_7	3	4 II + 3 III + 3 I/6II + 2 III +	2/3 "		
	6	6 II + 1 IV + 1 III +	2 "		
	7	8 II + 1 III + I + F	1 Translocation + 1 F		
29_3	1	8 II + 1 III + I	1 Translocation		
	2	1 Fragment	1 Fragment		
	7	8 II + 1 III + I	1 Translocation		
	8	6 II + 1 IV + 1 III + I	2 "		
	10	8 II + 1 III + I	1 "		
	13	1 Fragment	1 Fragment		
	14	8 II + 1 III + I	1 Translocation		

F = Fragment

Table 4.24. Chromosomal associations at metaphase I in testis squashes of 'F_2' male *H. prolixus* from (a) F_1 male (egg batch No. 3) X normal female and (b) F_1 female (egg batch No. 3) X normal male. All multivalents associated as chains except where stated.

Bug number F_1	Bug number F_2	Metaphase I associations	Abnormalities	F_2 fertility %	F_1 parental fertility %
(a) 13_2	2	All cells normal	None		
	3	All cells normal	"		
	4	All cells normal	"		
	5	All cells normal	"		
28_2	1	1 Fragment	1 Fragment	9.2	8.2
	2	8 II + 1 III + I	1 Translocation	23.9	8.2
28_4	2	6 II + 2 III + I	2	2.8	3.5
	3	8 II + 1 IV	1 "	19.8	3.5
28_5	1	8 II + 1 III + I	1 "	11.7	4.9
	2	All cells normal	None	100.0	4.9
	3	1 Fragment	1 Fragment		4.9
(b) 5_1	1	8 II + 1 III + I / Fragments	1 Translocation + F	41.7	2.2

F = Fragment

Table 4.23. Chromosoral associations at metaphase I in testis squashes of 'F_2' male R. prolixus from F_1 male (egg batch No. 4) X normal female. All multivalents associated as chains except where stated.

Bug number F_1	Bug number F_2	Metaphase I associations	Abnormalities	'F_2' fertility %	F_1 parental fertility %
13_1	1	All cells normal	None	100.0	15.5
	2	8 II + 1 III + I	1 Translocation	24.2	15.5
40_3	2	8 II + 1 III + I	1 "	29.7	26.6
	3	All cells normal	None	97.8	26.6
	4	All cells normal	"	100.0	26.6
	5	All cells normal	"	-	-

Table 4.26. Summary of results from Tables 4.20 - 4.25 to show chromosomal abnormalities of 89 'F_2' male R. prolixus from 4 parental egg batches.

Abnormality	Parental egg batch								Total	%
	1		2		3		4			
	♂	♀	♂	♀	♂	♀	♂	♀		
None	8	9	2	6	5		4		34	38.2
1 Fragment	1	4		5	2				12	13.5
1 Translocation	7		5	16	3	1	2		34	38.2
2 Translocations	1		2	4	1				8	9.0
3 Translocations				1					1	1.1
T o t a l	17	13	9	32	11	1	6	0	89	100.0

♂ = Progeny of F_1 ♂ X normal ♀

♀ = Progeny of F_1 ♀ X normal ♂

individual bugs, although the most frequently encountered orientation assumed by the chain was linear, i.e. parallel to the long axis of the spindle. As with F_1 males, where 2 translocations occurred in the same cell the multivalent could assume different modes of orientation as in Plate 4.10 which shows a spermatocyte from 'F$_2$' male [illegible] with 3 translocations associated as 6 II + 1 IV chain + 1 III chain + 1, the chain of IV showing parallel orientation and the chain of III linear orientation. In examining the squashes from testes of all these 'F$_2$' males not a single ring association multivalent was seen. These results suggest that the majority of heritable translocations passed on from F_1 parents to 'F$_2$' males were probably small interchanges producing zygotene configurations with one small arm, which would be achiasmate, producing the chain of III + I associations in these bugs.

The results show that in 12 of these 'F$_2$' males, the only detectable chromosomal abnormality was the presence of one or more chromosomal fragments. Plate 4.11 shows a metaphase I spermatocyte from 'F$_2$' male No. $2B_{6,3}$ with a single fragment and Plate 4.12 shows a metaphase spread from F_2 male No. $5_{7,1}$ with 3 chromosomal fragments. The presence of these fragments in meiotic cells of 'F$_2$' males is an important finding in relation to the structure of *R. prolixus* chromosomes. If *R. prolixus* were monocentric, such radiation induced fragments would have been lost since they would have been acentric and therefore unable to attach to the spindle; the persistence of these fragments to the 'F$_2$' generation offers good evidence for the holokinetic nature of triatomine chromosomes.

The results of the cytogenetic examinations of the 'F$_3$' bugs raised in this experiment are shown in Table 4.7. These results show that none of the 11 'F$_3$' males examined showed any detectable chromosomal abnormalities.

Table 4.27. Results of cytogenetic examinations of 11 'F_3' male *R. prolixus* descended from F_1 males of the first parental egg batch.

Body No.		Chromosome classification	Fertility %	Embryonic mortality %
F_F	F_3			
$^{2}_{1,1}$	1	[illegible]	[illegible]	[illegible]
	2	"	"	"
$^{4}_{1,1}$	1	"	[illegible]	"
	2	"	"	"
	3	"	"	"
	4	"	"	"
	5	"	"	"
$^{8}_{3,1}$	1	"	6.0	[illegible]
$^{29}_{3,1}$	1	"	41.9	[illegible]
	2	"	"	"
	3	"	"	"

Correlation of fertility and cytogenetic results

The F_1 generation of R. prolixus bred from irradiated male parents and normal female parents had a much lower mean fertility than their parents when they were crossed with normal males or females or approximately and cytogenetic examination of the testes of the F_1 males showed that each was carrying at least one translocation. The 27 F_1 males with a single translocation mated with normal females resulted in 1718 eggs, 150 of which hatched successfully giving a mean fertility of 8.7%. The 18 F_1 males shown to have 2 translocations when mated with normal females resulted in 1151 eggs, 69 of which hatched giving a mean fertility of 6.0% and the 4 F_1 males with 3 demonstrable translocations when mated with normal females produced a yield of 172 eggs none of which hatched. Although there were no demonstrably significant differences between the mean values for fertility of males carrying 1 or 2 translocations, it is more informative to examine the proportions of bugs in these 2 groups which were completely sterile. From the data presented in Table 4.17 - 4.18 it can be seen that of the 27 F_1 males with a single translocation, 12 (44.4%) were completely sterile and of the 18 males with 2 translocations 10 (55.5%) were sterile, and that all of the bugs with 3 translocations were completely sterile. These results do suggest a correlation between degree of chromosomal abnormality and fertility.

The fertility results for F_1 males show that their fertility may be related to their birth order. Those males from later parental egg batches being significantly more fertile than those from earlier egg batches. The cytogenetic results presented in Table 4.19 show that the number of detectable chromosomal abnormality increased with F_1 male birth order. Thus, from the first parental egg batch 8 sterilized males were examined, and 6 had one translocation each and 2 had 2 translocations. Examination

of the other egg batches shows that males from later egg batches had greater degree of chromosomal abnormality and this is closely reflected in their reduced fertility and in the increased proportions of completely sterile matings.

F_1 male bugs were found to be significantly less fertile than their female sibling[illegible] and [illegible] but, since only males could be examined for chromosomal abnormalities, a cytogenetic origin cannot be readily ascribed to these differences between male and female F_1 bugs in fertility.

The 28 'F_2' bugs crossed with normal mates had a mean fertility of 44.64 which was much greater than the mean fertility of the F_1 generation of 12.64. [illegible] 28 selected 'F_2' bugs were raised, 27 were examined cytogenetically. These preparations showed [illegible] chromosomal abnormalities in the bugs of this generation were much reduced when compared with the abnormalities of the F_1 males, [illegible] F_1 males showed some abnormality, only [illegible] of 'F_2' males had detectable chromosomal aberrations. Moreover, 5 of the 'F_2' males which were crossed with normal females were fully fertile and examination of their testes revealed no detectable abnormalities. These 6 bugs were numbered $14_{3,1}$; $37_{7,2}$; $28_{5,2}$; $13_{1,1}$; $40_{3,2}$ and $41_{1,2}$. [illegible] produced from F_1♀ X normal ♂ [illegible], and, therefore, [illegible] cytogenetic data not being corrected for their parents. However, the other 4 males were produced from F_1 ♂ X normal ♀ crosses and the results show that the father of $28_{5,2}$ had a detectable translocations and the fathers of the other 3 males [illegible]. These results suggest that the translocations of these 4 F_1 parental males were inviable and were not transmitted to their progeny.

Only 2 of the 28 'F_2' bugs crossed with normal mates showed evidence of having reduced fertility in comparison with their parents and these

were bugs Nos. $30_{2,2}$, which showed evidence of carrying a single translocation, and $28_{2,2}$ which had 2 translocations, their parents showing configurations which indicated 1 and 2 translocations respectively. The apparent stability of these translocations over 2 generations suggests that the chain associations found in these bugs were capable of convergent orientation in prometaphase. Unfortunately, these families were not crossed to produce further generations, so that the further stability of these translocations was not tested.

It has been shown that the mode of orientation of chain multiples is critical in determining their viability and, although these orientation patterns were not quantified, it appears from the photomicrographic evidence that the majority of the F_1 males showed multiples which were orientated linearly which would inevitably lead to low fertility, and many of the translocations would not be passed on to the next generation. The cytogenetic results are in accordance with this, as it was that while all F_2 males tested [illegible] 100% of F_2' males sometimes showed no evidence of any abnormality. Furthermore, only 2 of the 23 F_2' males examined [illegible] carried 2 translocations, compared with the 26.7% of F_1 males with 2 translocations. This result probably reflects the increasing improbability that 4 multivalents would align in a viable mode in the same cell.

The cytogenetic results also demonstrate that even a single chromosomal fragment can have a wide ranging effect on the fertility of the male carrying it; thus 'F_2' male $4_{2,2}$ [illegible] a single fragment and a fertility of 92.7% while 'F_2' $28_{2,2}$ [illegible] with a single fragment was in many respects, completely [illegible], and 'F_2' $5_{2,2}$ with 2 fragments [illegible] had a fertility of 92.7%. In the

case of 'F_2' $28_{5,3}$ the single fragment clearly had the effect of a dominant lethal mutation.

The overall increase in 'F_2' fertility has been seen to be a reflection of a decrease in the amount of detectable chromosomal abnormality in the males of that generation. However, cytogenetic examination of the 'F_2' males provided no explanation for the superiority of the F_1 female over the F_1 male in terms of fertility. The results in Table 4.26 show that, in the first egg batch, 8 'F_2' males from F_1 ♂ X normal crosses carried translocations while none of the males from the F_1 ♀ X normal crosses carried any translocations. These findings were reversed in the second egg batch in which males from F_1 ♀ X normal crosses showed the highest translocation frequencies.

Only 14 'F_3' males, all descended from the first parental egg batch, were produced for cytogenetic examination and none of them showed any sign of any chromosomal abnormality in their spermatocytes. It may reasonably be assumed that their fertility would have been nearly normal had it been assessed. 'F_3' male No. $5_{3,1,1}$ is of special interest in this group in that its male parent 'F_2' $5_{3,1}$ had a single translocation and fertility of 8.7%, and its male grandparent F_1 5_3 had 3 translocations associated with a fertility of 1.7%. This is a clear demonstration of how a bug with a large amount of chromosomal abnormality can, by generations of outbreeding to the wild-type, produce males without any detectable abnormalities in their meiotic chromosomes.

DISCUSSION

The dose-response curve produced as a preliminary requirement for this experiment revealed differences, both in amount of radiation required for complete sterility and in rate of response, between the curves

produced [illegible] by Shaver (1963) and in the present work. Much higher doses were required by these workers to produce complete sterility in adult male [illegible] and the reason for this most probably lies in the different radiation [illegible]. [illegible] that there is a qualitative difference between γ-rays and X-rays of the same dosage; they found X-rays to be twice as effective as ^{60}Co γ-rays of the same energy in producing chromosomal aberrations in [illegible]. Similarly, Searle et al. (1968), in studying translocation [illegible] in mouse spermatogonia, found that at low dose rates X-rays induced twice the frequency of translocations as did γ-rays of the same energy while at high doses this difference was reduced. A [illegible] interpretation of these results is difficult. Searle et al. (loc. cit.) suggested that the kinetics of translocation induction was different for the 2 types of irradiation. The differences in dose-response curves demonstrated here serve to confirm that variation in dose-rate and radiation source can produce widely differing changes in the same organism.

E. wollcoti males irradiated with 6 K rad γ-rays were crossed with [illegible]. [illegible] of these F_1 progeny were maintained by [illegible]. The resultant 'F_2' adults were then maintained as normal [illegible]. Fertility and [illegible] were recorded for all generations. Representative F_1, 'F_2' and 'F_3' males were examined cytogentically and the results of these studies of meiotic preparations were compared with the fertility data. The results show that the reduction in fertility of the 2 generations of males correlated well with the degree of abnormality demonstrable in their spermatocytes. The most frequent chromosomal abnormalities encountered were translocations and, in the F_1 generation, males carrying the greatest number of translocations were generally less

fertile. The maximum number of translocations seen in F_1 males was 3 and all bugs carrying this number were completely sterile. These results are similar to those obtained by Bauer (1967) and LaChance et al. (1970) working with other holokinetic insect species. The high recovery rate of translocations in the F_1 males in this experiment is in agreement with Bauer's (1967) theory that dicentric chromosomes cannot be produced in holokinetic species, so that all forms of interchange should be viable.

When mated with normal bugs, the F_1 generation in general, whether male or female, resulted in lower fertility of eggs than their siblings mated to normals (normal males). This result parallels the 'delayed sterility' demonstrated by various workers in Lepidoptera for example, Boyle and Bell (1928) showed that F_1 male hybrid Lepidoptera were more sterile than their fathers which had been given sub-sterilizing doses of radiation. However, LaChance et al. (1970) found no delayed sterility effect in their experiments with irradiated milkweed bugs.

The cytogenetic evidence from the present work has shown that the greatly reduced fertility of F_1 males may be related to the form of association and resultant orientation difficulties of multivalents in these translocation heterozygotes. The majority of interchange associations at first metaphase, are chain trivalent (III) plus a univalent (I), which must have arisen due to the failure of chiasma formation in 2 adjacent arms of the cygotene pairing bivalent during meiotic prophase. Chains of IV were also commonly found in F_1 interchange heterozygotes. This presumably arose because of the failure of a single chiasma. Lyon and Meredith (1966) in a study of translocations in mice, found that translocations producing sterility showed a higher frequency of chain associations than did those which did not lead to sterility. The high frequency of multivalent chain associations seen in F_1 males in the

present experiment was associated with a very high degree of sterility (84.5% mean sterility) calculated from the proportions of eggs which hatched. The genetic consequences of chain formation depend on the mode of orientation of the multiple in relation to the spindle and, in a monocentric species, it would be expected that the linear, convergent, indifferent and parallel modes would be assumed with equal frequency. However, R. prolixus normally shows linear orientation of its multiple bivalents (Buck, 1967) with each centromere in line with the spindle, so that it would be expected that linear orientation would be favoured by chain multivalents. The results show that this is the case; most of the chain multiples seen in the present study were linear. Lewis and John (1963) have shown that only the convergent orientation of chain multiples allows the production of balanced gametes, other modes leading to duplication and deficiency of genetic material and subsequent zygotic death. The much reduced fertility of R. prolixus found in this experiment is therefore in agreement with both the cytological evidence and theoretical expectations.

LeTreux et al. (1971) demonstrated the preferential production of chain multiples in the bug O. pallidipennis, but did not comment on the orientation of these chains; judging from their published photomicrographs, the multiples showed a mainly linear orientation in the translocation heterozygotes. The present work has shown that, although linear orientation of chain multiples is favoured in R. prolixus, the 3 other position modes of orientation (convergent, indifferent and parallel) were also assumed, if not as frequently. Indeed, in individuals heterozygous for more than one translocation, a mixture of orientation modes was sometimes found within the same cell (Fig. 5.5). Only one interchange heterozygote was found in this experiment with a

demonstrable ring association in metaphase. But this result is important because it shows that although such associations are exceptional, their formation is not precluded in triatomine bugs. Bauer (1967) found that ring multiple associations were most common in Lepidopteran interchange heterozygotes. Only rarely were such multiples being found. The striking difference between organisms with apparently similar holokinetic chromosomes must be partly a function of chromosome morphology. The apparent infrequency of ring formation in R. prolixus suggests that most chromosomes known were small, probably telocentrics (rodlets with short arms and, since chiasmata frequency in R. prolixus is normally low, only one chiasma per bivalent being formed (Ueshima, 1966), the formation of chains is likely to be encouraged in this bug. Both authors suggest that the genotypic properties may also influence the type of orientation of interchange multiples formed in different organisms which may account for the differences between the present results and those of Bauer (1967) and LeChance et al. (1970).

The fertility studies of F_1 R. prolixus showed a significant linear trend downwards in fertility, related to the birth order of 4 separate batches of F_1 bugs which had been reared from 4 successive egg batches laid by their mothers after mating to randomised partners. Cytogenetic examination of the F_1 males from these 4 successive egg batches revealed that the males from the later egg batches with lower fertilities also showed the greatest amount of chromosomal abnormality. If it is accepted that these insects do not suffer the effects of parity that a viviparous animal would, then these chromosomal differences would most likely account for the major part of the reduced fertility. The increased chromosomal damage of males of later egg batches may be explained by reference to the experimental design by which the parental females were

fed 4 times at 14 day intervals, the eggs being collected to form one egg batch prior to the next feed. It is known that adult *M. prolixus* of both sexes mate repeatedly (Baldwin and Shaver, 1963) and therefore the first egg batches were probably fertilized by sperm which were mature spermatozoa at the time of irradiation, while the later egg batches were probably fertilized by sperm which at the time of irradiation may have been spermatocytes or spermatogonia. This being so, the chromosomes of prophase spermatocytes or spermatogonia would be in a less condensed state than those in the sperm head and would have been subject to more hits, which would increase the frequency of breaks and interchanges. This could account for the differences in fertility of the 4 batches of males reared from eggs laid over a period of 8 weeks after the initial irradiated parent male X normal female crosses.

An interesting feature of the analyses of fertility data for F_1 *M. prolixus* was the superior fertility of F_1 females when mated to normal males, over that of F_1 males mated to normal females. Unfortunately, cytological examination of 'F_2' males raised from these 2 types of crosses revealed no obvious cytogenetic cause for this result, and cytogenetic examination of F_1 females was not possible. Snell (1946) in a study of radiation-induced translocations in the mouse found that for 5 of the 6 induced translocations, males had a higher fertility than females carrying the same abnormality. John and Lewis (1965) sought to show that this difference was due to the reduced frequency of multivalent disjunction in females because of their higher chiasma frequency. However, it had been assumed that chiasma frequency was greater in female mice because of the higher recombination frequency in that sex (Green, 1966), and it has since been shown (Henderson and Edwards, 1968) that chiasma frequency is in fact higher in female mice than in males. It

would appear that chiasma frequency may account for this result in mice, but as the chiasma frequency of female R. prolixus is not known, it is not possible to relate this character to the present results. John and Lewis (1968) have further suggested that the time available for orientation of a multiple could influence the mode of orientation taken up, and that this was perhaps a genotypically related factor. It may be that the time available for multiple orientation is greater in the egg than in sperm of R. prolixus because of the much slower rate of egg production compared with sperm production, and that this allows time for more genetically stable egg nuclei to be produced in females. However, in the absence of further cytological information, this explanation can only be speculative.

The increased fertility of 'F_2' males compared with that of their fathers was found to correlate with cytogenetic differences between the 2 generations. Thirty-four of the 89 'F_2' males examined showed no evidence of any chromosomal abnormality in their spermatocytes, and since every F_1 male examined had at least one translocation, this reduction in observable chromosomal abberations must account for the superiority of the 'F_2' bug fertility. This would suggest further that it is difficult for an interchange to persist from one generation to the next in this species, probably because of the holokinetic nature of its chromosomes linked with a predilection for axial orientation and a low chiasma frequency. However, 43 'F_2' males did show evidence of carrying translocations suggesting that linear orientation of chain multiples is by no means compulsory in this system and that it may be possible to establish a heritable interchange in a population of bugs.

Several F_1 males, as well as exhibiting translocations, showed chromosomal fragments in their spermatocytes and 12 of the 'F_2' males examined showed no other chromosomal abnormality than a fragment. The

persistence of such fragments through 2 generations is evidence of their mitotic and meiotic stability and provides proof of the holokinetic nature of K. prolixus chromosomes which has up till now been inferred from ultrastructural studies (Buck, 1967). [illegible] were found to have a variable effect on the fertility of males carrying them, so that a male with a single fragment may have been completely sterile while others with 2 fragments resulted in approximately 50% sterility. Schwarz et al. [illegible] found that a fragment which induces complete sterility in the milkweed bug, but this was not found to be true in K. prolixus. It seems that the relative importance of fragments may depend more on the degree of genetic importance of that part of the genome incorporated within them, their action being that of dominant lethal mutations by either genetic duplication or deficiency.

None of the 11 'F_1' males examined showed any evidence of meiotic or cytogenetic abnormalities, suggesting that the translocations present in their parents' spermatocytes were stable. However, it may be presumptious on the basis of this limited number of observations to assume that this would be the fate of all translocations induced in triatomine bugs. For example, 2 F_2 males [illegible] interchanges had only slightly reduced fertilities compared with their parents (bugs nos. $B_{1,7}$ and $B_{4,1}$) and it may be that some particular translocations were relatively stable; but unfortunately these bugs were not bred by a further generation so this must remain a speculation.

Since K. prolixus is an important vector of Chagas' disease in [illegible], the results of this experiment must be considered in relation to the control of this pest by genetic means. Davidson et al. (1963, 1964) and Baldwin and Chant (1970) ruled out the use of sterile male release as a control measure for K. prolixus populations because of the

high radiation doses required to completely sterilize males, which resulted in bugs of greatly reduced fitness. Baldwin and Chant (loc. cit.) experimented with irradiation of R. prolixus in an atmosphere of nitrogen and found that this improved the fitness of these bugs compared with those irradiated in air. Because of the holokinetic nature of the chromosomes of R. prolixus demonstrated in the present work, it would seem appropriate to assay control measures which take advantage of the peculiarities this confers on triatomine bugs, advantages which have been usefully employed in controlling Lepidoptera, since the 'delayed sterility' demonstrated here for R. prolixus given sub-sterilizing doses of X-rays, is an effect characteristic of holokinetic insects and first observed by Proverbs (1962) in the codling moth, Laspeyresia pomonella. A further useful feature of giving sub-sterilizing doses of radiation to Lepidoptera was found to be a distortion of the sex-ratio in the F_1 so that the majority were males (Proverbs, loc. cit.). This sex-ratio distortion in favour of males which is so desirable for genetic control programmes has also been demonstrated in irradiated Glossina morsitans by Parker et al. (1972) who suggested that it was related to the extra large X-chromosome of female-determining sperm being more susceptible to radiation damage. The present work has shown that irradiation does not distort the sex-ratio of the progeny in R. prolixus and this may be due to the very small size of the sex chromosomes in this species.

Despite this drawback related to reproduction, the present work has shown that sub-sterilizing doses of radiation will induce delayed sterility in R. prolixus and, as Knipling (1967) has shown for Lepidoptera, it would be more effective in controlling populations of this species if partially rather than fully sterile males were employed. Although

K. prolixus may mate repeatedly, this may not be a disadvantage as von Borstel (1960) has pointed out that monogamous matings are not essential for the success of sterile male control programmes, for the true competition is between normal and lethal sperm within the female spermatheca and it is therefore irrelevant how many times an insect mates.

The present experiment has demonstrated that while the linear orientation of multivalents is favoured in K. prolixus, leading inevitably to the loss of translocations, it is possible to obtain multivalents to orientate in a stable fashion and also for ring multivalents to be formed in this bug. A [illegible] could perhaps produce a translocation which preferentially orientated in a stable way during meiosis, but the long [illegible] bugs may prove to be an obstacle to such a scheme.

GENERAL DISCUSSION

The triatomine bugs of South and Central America are of great medical, and with that [illegible] importance as vectors [illegible] which seriously affect the lives of large numbers of people. It is therefore appropriate that the results of the present work should be considered in relation to the problems associated with reducing the incidence of this disease. The elimination, or reduction in size [illegible] vector population whether triatomines or vectors of pathogens other than T. cruzi [illegible] of a disease. Insecticides, particularly indoor residual deposits of contact insecticides such as DDT and other chemicals more recently developed, revolutionized, [illegible] vector control for many insect-borne diseases over the past 30 years. For triatomines, indoor residual deposits of organochlorines (BHC and dieldrin) in rural and urban housing throughout much of the South and Central America have been particularly successful in eliminating or reducing domiciliary infestations of vectors of Chagas' disease [illegible] where financial resources were adequate, improved housing standards have also contributed to control of the vector and the disease. Re-infestations of treated premises do, however, occur with species which have, in part, [illegible] indoors, or due to accidental re-introduction of bugs.

In general, medical usage of toxic insecticides [illegible] is much less contaminative of the general environment than methods used in agriculture such as aerial spraying of crops, although such problems do sometimes necessitate outdoor sprayings of this kind. [illegible] control methods, the polluting effects of insecticides have attracted considerable criti[illegible] in recent years. As a result, attention has been directed to [illegible]

methods for pest and vector control, which could circumvent hazards

[illegible] It may also be

said that by insecticide control elimination of a vector is seldom

complete, although often providing reduction in population density

consistent with virtual eradication or effective reduction in the

[illegible]

Results of this order have been achieved over large areas against

[illegible] T. [illegible] and in part T. [illegible], [illegible]

[illegible] In spite of their [illegible], [illegible]

of these species has yet been controlled thoroughly and, moreover,

[illegible] T. [illegible] provide a warning that, as in other insect pests and vectors, this

serious consequence of repeated chemical treatment may become a

practical problem. Other control measures warrant investigation on

this account alone. Genetic methods of control necessitate thorough

investigation at laboratory level if they are to be adequately

[illegible] before the principles of genetics can be applied to the regulation

of insect populations, as much information as possible must be gathered

[illegible]

[illegible] For

example, the [illegible] although the adult [illegible] indistinguishable, and while some strains

[illegible] to act as malarial vectors, others were not. By crossing

these strains, Hackett (1937) found that hybrid crosses were sterile

thus demonstrating that distinctive sub-divisions of this species

exist[illegible]. [illegible] to as [illegible] complex. It

is clear from this example that taxonomy, even at the egg-stage, can play an important role in studies of vectors. More recently, an elaboration from the classical criteria of morphology, has been the [illegible]. In the case of the [illegible], [illegible] the existence of the species complex, other stages remained morphologically indistinguishable until it was shown that the salivary gland chromosomes of the larvae provided an excellent means of differentiating the members of the complex [illegible]. The [illegible] salivary glands showed complex banding patterns when stained [illegible], and the bands were found to be highly characteristic. Cytogenetic taxonomy was also developed to differentiate members of the [illegible] complex, in which [illegible] were certain vectors; the discovery of polytene chromosomes in the nurse cells of the ovaries of the adult females of these mosquitoes being of particular convenience for identification of adult members of the 6 sub-species (Coluzzi and Sabatini, 1967).

Hybrid viability studies of the Triatominae have been made, and indeed are still being carried out (Perlowagora-Szumlewicz, 1974), but such genetic experiments are very slow because of the lengthy life-cycle of the bugs. Furthermore, unlike mosquitoes, the tissues of [illegible] possess polytene chromosomes to assist in their taxonomy. The present study of [illegible] chromosomes in triatomine bugs confirmed that [illegible]. [illegible] are not sufficient evidence on which to base taxonomy, the only distinguishing feature between certain species being a variation [illegible] in the number of B-chromosomes. The cytotaxonomy of the Triatominae appeared to be an intractable problem

improved during the researches of this thesis by application of techniques of chromosome differentiation developed for mammalian somatic cells. It is [illegible], when stained by the A.F.G. [illegible] technique [illegible] et al., 1971). [illegible] banding for Triatoma infestans and Rhodnius prolixus which are species specific. This banding technique deserves to be investigated for a wider range of Triatomine species, with the prospect of adding to triatomine taxonomy some of the precision which the study of polytene chromosomes has given to the study of mosquito species and complexes of medical importance.

The general study of spermatogenesis, in R. [illegible], in the second part of this thesis, has shown that starvation and feeding can affect the rate at which spermatocytes develop. In particular, the process of spermatogenesis has been shown to be inhibited by starvation in fifth instar larval males, and it was found that spermatocytes spend longer periods in the 'diffuse stage' of meiosis during such periods of starvation. Although this particular aspect of the study was not directly related to considerations of insect control, it is nevertheless worthwhile to consider these results in relation to this problem. The results show that meiosis and spermatogenesis are influenced by the [illegible] environment of the fly. Although the nature of the control mechanism, whether hormonal or some other factor, is not clear, except that it is linked in some way to the nutritional status of the bug. If further research revealed that this control mechanism was chemical, then it is possible that the process of

spermatogenesis could be inhibited to provide an alternative method of sterilizing either wild populations, or laboratory-reared or wild-caught males for a sterile-male control programme.

The third part of this thesis examined the genetics of bug susceptibility to infection with T. cruzi, a study which has obvious applications since xenodiagnosis still occupies a central role in the clinical diagnosis of chronic Chagas' disease. A highly susceptible strain of triatomine bug would improve the sensitivity of this diagnostic test, and also fewer bugs than the 10 bugs used on each of the 4 occasions customarily recommended at present for each patient tested would be required to be fed on each patient, thereby decreasing the discomfort and, in some individuals, serious possibility which this test feeding inevitably imposes for the patient. The results of the present study have shown that the intensity of faecal infection of individual bugs is, in part, genetically determined. Therefore, on the basis of quantitative differences in faecal infection, produced in three generations of two discrete populations of R. prolixus, one group excreting significantly greater numbers of trypanosomes than the other, indicating differences in susceptibility to infection. The results also revealed a most interesting difference between sexes of R. prolixus, males excreting significantly greater numbers of trypanosomes than females, despite the fact that females ingested more blood (and thereby trypanosomes) of the infected meal. These results clearly refute the suggestion that faecal infection in bugs is determined by the amount of trypanosomes ingested. For example, Tibayrenc et al. (1973) related the percentage of bugs with positive faeces at xenodiagnosis to the amount of blood ingested by the bug, but the present work confirms the conclusion of Bertram and Phillips (1967) that the numbers

[illegible] Further, [illegible] recently shown that Panstrongylus megistus supernumerary larvae, produced by juvenile hormone treatment, had much lower infection rates than control bugs when fed on chronic Chagas' infected patients, despite the fact that they [illegible] at the infecting feed; this supports the present conclusion that blood-meal size is not directly related to subsequent faecal infection in bugs. The present experiment demonstrated that it is possible to breed bugs with increased susceptibility to T. cruzi infection, and it is suggested that inbred populations of susceptible R. prolixus should be bred for use in xenodiagnosis and preferably only males of such colonies should be used.

The results obtained by irradiating male R. prolixus, mating them with normal females and following the results through successive generations of out-crossing, must be considered in relation to the control of wild populations of this vector species. The results have demonstrated that sub-sterilising doses of γ-irradiation produce F_1 generation offspring males with reduced fertility compared with their irradiated fathers. This delayed effect of sterility has been related, by parallel studies of chromosomal abnormalities, to the holokinetic structure of bug chromosomes, and is similar to the situation encountered in Lepidopteran pests. Knipling (1970) has suggested that where an insect species demonstrates a high level of radio-resistance, [illegible] control is more effective when semi-sterile rather than completely sterile [illegible] are released. Two reasons are cited: Firstly to sterilize fully the males would make them unfit and unable to compete with normal males at mating and, secondly, the delayed effect on

fertility of releasing semi-sterile males would produce a longer-term suppression of population numbers. In the present experiment, however, the fertility of most of the progeny of semi-sterilized male P. prolixus was returning to normal by the second generation of outcrossing to normal mates. [illegible] practical dynamics which would arise in such a situation must be taken into account in terms of repeated releases into a population of [illegible] and its vectors have social consequences and constraints. Since male [illegible], the release of large numbers of males in a control project would not be [illegible] is more feasible to suppose that such releases could be justified to replace the natural population in an area with one carrying a translocation linked to, say, genes for insecticide [illegible] in the event of insecticide resistance becoming a serious public health problem. This, although so far a matter of little practical [illegible] health authorities. Conceivably, male releases, whether of semi-sterile irradiated males or males carrying translocations linked with [illegible] authorities if these control attributes were also genetically coupled with use only of males of a strain minimally susceptible to infection with the pathogenic organism, Trypanosoma cruzi.

Clearly much still remains to be done in experimental genetics

[illegible] tentative prospects can be elaborated to realistic appraisals of the potentialities of genetic manipulation in triatominae for the reduction or eradication of Chagas' disease by genetic control [illegible] vector insects.

SUMMARY

PART ONE

1.1. A study of meiosis in males of 12 species of triatomine bug (Triatoma brasiliensis, T. infestans, T. lenti, T. maculata, T. tibiomaculata, T. phyllosoma, T. protracta, T. vitticeps, T. pseudomaculata, T. sordida, Rhodnius neglectus and R. prolixus) from South and Central America showed that the basic complement for this genus was 20 autosomes + XY. The greatest amount of variation between species was in the number of X-chromosomes which varied from X_1Y to X_1X_2X_3Y. These X_nY systems are thought to have originated by fragmentation of an archetypal X-chromosome. One further species, Panstrongylus megistus was however found to only have 18 autosomes + XY.

1.2. Female meiosis was observed in a single species, R. prolixus, the first maturation division in an egg being observed about the time of oviposition.

1.3. A technique is described for the production of G-bands in the chromosomes of [illegible] from triatomine embryos. The results obtained with R. prolixus and T. infestans suggest that the technique will provide genetic markers of value in elucidating problems in the systematics, biology and genetic control of these two important vectors of Chagas' disease, and of other triatomine species of South and Central America.

1.4. Meiosis in F_1 males of the cross T. pseudo-maculata X T. sordida and in males from the reciprocal cross was found to be characterized by multiple associations and univalent formation. The orientation of the chain multiples made the survival of hybrid interchanges seem improbable, and the sterility of these F_1 bugs most probably related to these chromosomal events.

SUMMARY

PART TWO

2.1. Examination of squash preparations from testes of fourth and fifth instar larval R. prolixus showed that testis differentiation would only occur in fifth instar larvae and it is suggested that some genetic switch mechanism associated with age may be necessary to stimulate testis differentiation and that juvenile hormone may be involved in this process.

2.2. It was found that spermiogenesis was inhibited in diapausing fifth instar larvae, the primary spermatids produced by these bugs becoming 'aborted' and probably removed by autolysis.

2.3. Following a blood meal, meiotic divisions and spermiogenesis were rapidly initiated in fifth instar larvae, mature spermatozoa being found in their testes prior to the moult to the adult.

2.4. The rate of meiosis was compared in fed and diapausing fifth instar larvae and adults by ^{3}H-thymidine labelling of spermatocytes. Meiosis occupied 21 days in unfed fifth instar larvae and 12 days in fed larvae. Similar differences were demonstrated among fed and unfed adult testes, the difference in time taken being largely accounted for by variation in the length of time spent by spermatocytes in the 'diffuse stage' of meiosis.

2.5. Incubation of larval testes in ^{3}H-uridine demonstrated that RNA was being actively synthesized in 'diffuse stage' nuclei of both fed and diapausing testes. It is proposed that during the 'diffuse stage' the chromosomes, which are extended and may, possibly, have a functional significance similar to that of lampbrush chromosomes, could be engaged in the synthesis of messenger RNA in response to the nutritional status of the bug and its age, so governing the further stages of spermatogenesis.

SUMMARY

PART THREE

3.1. Susceptibility rate and intensity of infection were measured for 2 populations of R. prolixus selected over successive generations for susceptibility and refractoriness to 2 different strains of T. cruzi.

3.2. Selection for 2 generations with the Peru strain of T. cruzi, and for 3 generations with Strain 7 T. cruzi, failed to demonstrate continuous variation which could be ascribed to major gene differences controlling susceptibility rate in R. prolixus populations.

3.3. Selection for differences in intensity of infection in individual bugs produced the following quantitative changes in the experimental bug populations:-

i. Highly significant differences were found between similar populations of R. prolixus infected with 2 different strains of T. cruzi, suggesting that trypanosome genotype can affect the level of intensity of infection in the bug.

ii. Significant differences in mean values of intensity of infection were found among selected families of bugs infected with the Peru strain or Strain 7 T. cruzi, and for all generations raised. These family differences reflected a degree of resemblance between relatives characteristic of a continuously variable character under polygenic control.

iii. Analyses of male and female results revealed significant differences between sexes, males having consistently higher levels of intensity of infection than females, suggesting either sex-linkage or sex-limitation of the character.

3.4. i. Two-way selection for high and low levels of intensity of infection among bugs of the T. cruzi Strain 7 group produced, in the F_3 generation, 2 clearly separated groups of families with significant differences in mean scores between families of the 2 selection lines.

ii. Response to selection when plotted against selection differential for selected families of the Strain 7 group of bugs produced highly significant regressions which were used to calculate the realized heritability for the character. The heritability for intensity of infection measured from the divergence of the two regressions of response on selection differential was found to be 5.5%.

iii. The response to selection was asymmetrical being twice as great in the downward direction (increasing refractoriness) as in the upward direction (increasing susceptibility). It is suggested that this asymmetry may have been caused by the dominance of genes for increasing intensity of infection and/or by inbreeding depression of a fitness-correlated character.

3.5 It is suggested that the efficiency of xenodiagnosis may be improved by the production of an inbred line of R. prolixus selected for high intensity of infection with T. cruzi and that only male bugs of such a population should be used titrimetrically.

SUMMARY

PART FOUR

4.1 Eggs from crosses of 40 adult male R. prolixus irradiated with 6 K rad γ-rays with normal females had a mean fertility of 23.9%, only 2 being completely sterile. The mean fertility for control eggs of normal matings was 97.8%. The 36 F_1 progeny of the irradiated male x normal female matings had a mean egg fertility of 22.8%, and 15 of these matings were completely sterile.

4.2 Twenty-eight F_1 bugs reared from F_2 normal females were mated with normal partners and had a mean fertility of 44.6%, 6 of these being fully fertile, a reversal towards normal fertility.

4.3 These findings of initial reduction of fertility followed by recovery of fertility by the 'F_2' generation, following irradiation of the males of the original parental generation [illegible] changes in chromosomal configurations as observed by cytogenetic examination of preparations of male meiosis.

4.4 The survival to the 'F_2' generation of chromosomal fragments confirmed the holokinetic nature of triatomine chromosomes.

The very high recovery rate of translocations in F_1 generation males can be related to the holokinetic chromosome system of these bugs which precludes the formation of dicentric chromosomes which are inviable in monocentric species.

4.5 In F_1 and TT_1T males the modality of translocations were ascertained as [illegible] III + I or as chains of IV. Only one bug was found with a ring of IV chromosome association and it is suggested that chromosome morphology combined with a low chiasma frequency favours chain association formation.

4.6 Most chain multivalents showed linear orientation which may lead to [illegible] translocations in *T. prolixus* will be significant, since bivalents normally orientate linearly so that multivalents will similarly be biased towards this mode. However 'parallel', 'indifferent' and the more stable 'convergent' modes of chain orientation were also all observed indicating that survival of some translocations in this species may be possible.

4.7 It is suggested that semi-sterile males would prove more effective than releases of completely sterile males for reducing wild populations of *T. prolixus*, because of the delayed effects of sterilizing radiation which is consequent upon the holokinetic structure of triatomine chromosomes.

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Giemsa banding of metaphase chromosomes in triatomine bugs

CONSIDERABLE progress has been made in developing the formal genetics and cytogenetics of several insect vectors of disease[1–4], notably with mosquitoes, houseflies and tsetse flies in which the polytene chromosomes provide suitable material for detailed analysis of chromosome morphology. The triatomine bugs (Hemiptera, Reduviidae) are medically important as vectors of Chagas' disease in the Americas, yet cytogenetic information on these insects is meagre[5,6]. These bugs present the same problems which, until recently, limited developments in mammalian cytology in that they possess a large number (typically $2n = 22$) of small, almost indistinguishable chromosomes[7]. Further, since their chromosomes are also holokinetic[8] (that is with non-localised centromeres) they do not show any primary constrictions and it is correspondingly difficult to recognise arms of a chromosome which are readily seen in chromosomes of organisms possessing discrete centromeres. This difficulty with triatomine material has now been overcome by applying the Giemsa staining methods, which have been effectively developed by mammalian cytologists[9–13], to embryonic cells of bugs with metaphase configurations. The technique I describe here makes possible the identification of individual chromosomes within the complements of different species of triatomine bug.

The procedure is as follows: 5–7-d-old embryos are dissected out from bug eggs, placed in hypotonic sodium citrate solution (1% for 8 min) and the cells dispersed in 0.25% trypsin in 0.02% versene. The suspension is washed in insect ringer, centrifuged and fixed in alcohol–acetic acid. Slides are prepared by air drying in the normal way[14]. Dry slides are kept for 1 week at 20° C before heating at 60° C for 1 h in 2 × SSC (0.3 M sodium chloride; 0.03 M trisodium citrate). After rinsing in absolute ethanol, preparations are stained in 2% Giemsa (Gurrs 'improved' R 66) at pH 6.8 for 30 min, dried and mounted. This ASG (acetic–saline–Giemsa) technique[15] has proved to be a more reliable method for the production of G bands in bug chromosomes than other methods which have also proved effective with mammalian cells.

Figure 1 shows banding and differentiation of metaphase chromosomes in *Triatoma infestans*, and indicates the results obtainable with this technique. Similar preparations have been obtained for other *Triatoma* species and also *Rhodnius prolixus*. Preliminary analyses indicate that there are prospects for production of chromosome maps of triatomine species which could be of value in further investigations of chromosome markers and their application to problems in genetic characterisation and manipulation in the vectors of Chagas' disease.

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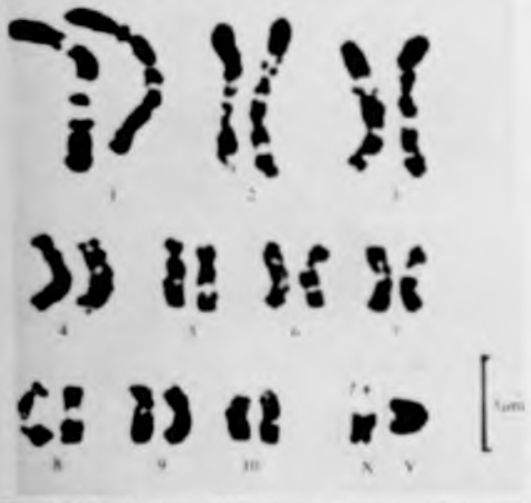

Fig. 1 Metaphase neuroblast from a *Triatoma infestans* male embryo 6 d old (2n = XY).

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