THE INDUCTION AND CHARACTERIZATION OF CHROMOSOMAL TRANSLOCATIONS IN ANOPHELES GAMBIAE SPECIES A

by

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ABSTRACT

With the possibility of their eventual use as control agents in mind, attempts were made to isolate and characterize reciprocal chromosomal translocations in species A of the Anopheles gambiae complex. Translocations were induced in males with X-rays and determined by testing for the occurrence of inherited partial sterility. Hatching rate was the principal criterion used for the designation of karyotype. Young males of a Y-autosomal translocation line (TYF-5) were re-irradiated with X-rays at 4,000 rads. Several further interchanges were isolated. Among 120 tested F_1 males, four 3-chromosome double translocation lines were obtained. These lines were found to transmit their characteristic partial sterility (75%) to all their sons when outcrossed to the wild-type, but none of the daughters inherited partial sterility. Comparative studies showed that 3-chromosome doubly translocated males displayed fitness comparable with the wild-type males. Among 92 F_1 daughters tested, 8 lines showing regular inheritance of partial sterility were established. In 5 of these lines the presence of translocations was confirmed cytologically. Four were shown to involve autosome-autosome translocations and one involved an X-autosome interchange. The fertilities of these lines were found to be significantly higher than 50%. The merits of using comparatively low radiation dosage are discussed and tested by attempting to induce translocations using only 1,500 rads. From a resulting number of 154 F_1 males and females which were outcrossed, 7 partially sterile lines were isolated. One of these proved to be a

reciprocal interchange between the two autosomes. The remaining 6 lines, when outcrossed to the wild-type, showed a rather low frequency of semisterility. Efforts were made to produce autosomal translocation homozygotes, but without success. For all the 5 lines investigated, data obtained from hatching rates of intercrossed heterozygotes suggested that translocation homozygotes would be lethal. In future isolation of autosomal translocation homozygotes it will be essential to employ a team of workers to perform all the requisite tasks of creating, selecting and evaluating strains suitable for genetic control purposes. The probable effectiveness of male-linked translocations is discussed in the light of experimental experiences.

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INTRODUCTION

Residual insecticides were thought to be the definitive answer for vector control and the control and eradication of diseases transmitted by vectors. Despite the remarkable successes achieved by using synthetic insecticides during the past 30 years, many problems of vector-borne diseases still remain. In the field of malaria, for example, the success of malaria eradication especially in many temperate areas has been most striking but this disease is still the most common infection of the tropical world. As at September 1974, of the estimated 1,945 million people living in the originally malarious areas of the world, at least 523 million people were still exposed to endemic malaria (WHO, 1975). In many developing countries, technical, operational and socio-economical conditions have hampered the further progress of malaria eradication. For reasons of the general standard of people's health and to provide an environment compatible with economic development, appropriate malaria control measures will have to be undertaken, where the disease forms a health hazard (Wright et al., 1972).

At present malaria eradication programmes mainly rely on residual insecticide spraying of premises to interrupt transmission of malaria by reducing the age of the vector to below that required for the parasite to complete its extrinsic cycle. However, this method has not always been successful. Some of the problems are common to all insecticides, e.g. exophilic mosquitoes can feed without coming into contact with insecticide. Exophagy may also result from the habit of people sleeping out of doors or in huts with incomplete or no walls

(Wright et al., 1972). Resistance to such residual insecticides as dieldrin and DDT now occurs in many Anopheles species (Brown and Pal, 1971) and in some cases prevents the effective use of these insecticides.

In the case of An. gambiae species A and B, important vectors of malaria in Africa, resistance to dieldrin with cross resistance to HCH occurs in much of West Africa and parts of East Africa. DDT resistance has also been reported in species A in parts of Upper Volta and Togo (Hamon et al., 1968; Haridi, 1972) and in species B in the Sudan and Senegal (Haridi, 1972). Although some substitute insecticides are available and free from cross resistance problems, they are not suitable because of their high cost and short residual life. Also there is now considerable concern over environmental contamination by long lasting insecticides. Taking into account all these problems, alternative methods of malaria control need to be studied. In order to overcome some of these problems, efforts are being continued to develop new safer and biodegradable insecticides (WHO, 1971). However, alternative methods of attack against vectors without the use of insecticides are also necessary. Possible alternatives are the use of parasites and predators as biological control agents, the use of traps, attractants, and hormones, general environmental sanitation to reduce breeding sources, and genetic control methods (Davidson, 1972; Hamon, 1970; WHO, 1971).

Genetic control has been defined in its broadest sense as "the use of any conditions or treatment that can reduce the reproductive potential of noxious forms by altering or replacing the hereditary material" (WHO, 1964). There are several ways to manipulate the hereditary material of insects and the possibilities have been summarized

by several authors (Craig, 1963; Curtis, 1971a; Davidson, 1972, 1974 a, b; Davidson and Kitzmiller, 1970; Hamon, 1970; Kitzmiller, 1972; Knipling et al., 1968; Pal and LaChance, 1974; Rao, 1974; Smith and Von Bostel, 1972; Whitten and Pal, 1974; WHO, 1964, 1968 and 1971; Wright et al., 1972).

One outstanding success in genetic control has been the sterile male technique, which has been applied to the screw worm fly in the United States of America. The method involves the production of dominant lethal effects in the sperm by radiation or by the use of chemicals. These insects are then released into natural populations to transmit these lethal effects to other normal insects in the population, which results in their failure to reproduce (Knipling, 1967). This method was established as a practical means of control in 1952 where the screw worm fly Cochliomyia hominivorax was controlled in a small island, Sanibel, two miles off the west coast of Florida. Since then many basic and applied studies have been made and the screw worm fly has been practically eradicated from the whole of the southern United States (Bushland, 1971; Davidson, 1974 a). This method of control is now generally referred to as "SIRM" - the sterile insect release method (Whitten and Pal, 1974).

An important advance in the sterile male technique has been the development of "chemosterilants". The most commonly used chemosterilants are alkylating agents, especially aziridine compounds, such as tepa, apholate and tretamine (for comprehensive review see Campion, 1972). The methods used for applying them to insects by feeding, residues, dipping and topical application are described by LaBreque and Smith (1968).

Chemosterilized Culex pipiens quinquefasciatus were employed in a successful eradication project on Seahorse Key, a small island off the Gulf coast of Florida. A small population of this mosquito was controlled and almost completely eliminated by continued releases of females sterilized by thiotepa for 10 weeks (Patterson et al., 1970). More recently, Lofgren et al., (1974) have reported that the release of about 4.3 million chemosterilized males of An. albimanus at Lake Apastepeque in El Salvador over a 5-month period resulted in 99% reduction in the level of the indigenous population of this species. After the cessation of the release, the population took 4 months to recover to a normal density. Thus, the study clearly demonstrated the applicability of the sterile male release technique.

Insects can be sterilized not only by irradiation or chemicals but also by naturally occurring means. Use of naturally occurring sterility such as cytoplasmic incompatibility and hybrid sterility has also been proposed and developed to the point of field testing. Within some insect species such as Culex pipiens complex, crosses between various populations are shown to be sterile. Sterility is considered to be due to a cytoplasmic factor transmitted through the eggs, which kills the sperm of the incompatible male after its entry into the eggs (Laven, 1967a). The crossing of the members of allopatric populations of the Culex pipiens complex can lead to development of offspring in both directions or in one direction only, or to no offspring in the reciprocal crosses. More than 20 such crossing types are known in the Culex pipiens complex and it is possible to construct one or more incompatible strains for a given population of Culex pipiens fatigans anywhere in the world (Laven, 1967b). Desirable genetic traits can be introduced into a

compatible strain without changing the cytoplasmic incompatibility and thus it is possible to adapt strains from the temperate region to any ecological condition. Such an adapted strain having the cytoplasm of Paris and the genome of Fresno, California, U.S.A. was prepared, and successfully tested in a pilot experiment for suppression of Culex populations in isolated village, Okpo in Burma (Laven, 1967a).

Hybrid sterility sometimes occurs when two closely related species are crossed such as in crosses between the 6 sibling species of the An. gambiae complex (WHO, 1964; Davidson et al., 1967; Davidson and Hunt, 1973; Davidson, 1974a; White, 1974). When crosses between any 2 of these 6 species are made, all produce F_1 male abnormality (not fully sterile in some cases) and some crosses notably between the freshwater species A and B males and saltwater species An. melas and An. merus females result in F_1 progeny predominantly male which are sterile. Results of numerous laboratory-cage competition experiments revealed that these F_1 males are competitive with normal males. Therefore, Davidson (1969) suggested that these F_1 males may be mass produced and released to control wild population. A field trial using this technique was conducted in Upper Volta in 1968. Sterile males produced by crossing the males of species B of the An. gambiae complex with female An. melas were released into an isolated, declining natural population of species A. Unfortunately their employment was unsuccessful due to lack of mating between the hybrid sterile males and wild females (Davidson et al., 1970). The most important factor preventing mating was attributed to the existence of a species mating barrier, possibly exaggerated by the use of a cross

between two species against a third species (Davidson, 1973). The possibility of genetic control by sterile hybrids has also been investigated though in the laboratory so far, in the An. punctulatus complex from New Guinea and adjacent regions, again important malaria vectors (Bryan, 1973). Several other examples in insects of medical importance have been studied (see Davidson, 1974a for review).

Another method of genetic control involves the use of the partial sterility characteristic of translocation heterozygotes. The potential of translocations for insect control was first proposed by Serebrovsky in 1940. His work remained unknown in the Western world for nearly 30 years. In the meantime, Curtis (1968a) ignorant of Serebrovsky's work proposed the production, rearing and release of individuals which are homozygous for a translocation to control natural populations. Mating between the released individuals and the wild population would lead to the production of semisterile heterozygous offspring, with consequent reduction in fertility of the population. Curtis (1968b) also suggested that autosomal translocations might be better utilized as transport mechanism for desirable genes such as the prevention of disease transmission rather than a means of population reduction or eradication. Since then chromosomal translocations for possible use in genetic control of insect pests have attracted a considerable amount of attention. Research is under way by investigators in many parts of the world to explore the translocation method for the control of a number of insect pests. A brief review is given in the following chapters of the history of translocations, genetic and cytogenetic characteristics of translocations, and application of the translocation method in vector control.

Translocations have already been isolated in insects of medical importance in various laboratories but rarely in anopheline mosquitoes. The first intensive study in this group was made by Krafsur (1972a, b), on the principal vectors of malaria in Africa, An. gambiae (species A and species B). He was able to isolate several lines involving male-linked and autosomal interchanges in the heterozygous state but failed to isolate viable translocation homozygotes in spite of repeated attempts. Since translocations to be of efficient use in genetical control must be in the homozygous state, an essential step is to recover appropriate homozygous translocation stocks. In most insect species so far studied a large proportion of induced translocations are either lethal when made homozygous or show severe fitness reduction, e.g. Aedes aegypti (Lorimer et al., 1972), Glossina austeni (Curtis et al., 1972), Drosophila (Burnham, 1962). One of the problems in obtaining a viable homozygous stock in the An. gambiae complex, in the absence of suitable marker genes, is the large-scale fertility testing needed. It therefore appeared worthwhile to re-investigate the possibility of isolating homozygous autosomal translocations in the An. gambiae complex. The work described here is a continuation of the experiments begun by Krafsur and further study of chromosomal translocations in the species A of the complex. It was the hope of the writer that experiments would show genetical changes of more suitable type for use in the field of genetical control of mosquitoes. Although additional chromosomal interchanges have been isolated the full extent of this objective is still to be realized. A combination of Krafsur's and the present study throw some light on the genetical make-up of Anopheles and will be of assistance in any further elaboration of this type of work.

LITERATURE REVIEW

Early historical background

Crosses made in a breeding programme for the improvement of the Florida velvet bean, Stizolobium deeringianumwere found to have about 50% visibly aborted pollen and 50% of a normal seed set. This phenomenon was first recognized by Belling (1914) and he termed it semisterility. About half the offspring were semisterile and half had normal fertility. The fertile ones bred true in the next generation but the semisterile ones again segregated in a 1 : 1 ratio. Blakslee had found that the Jimson weed, Datura stramonium, did not breed in a typical Mendelian manner. In one of them, a small extra chromosome was often attached to each of two pairs of large ones. This led to the conclusion that non-homologous chromosomes could exchange segments (Belling and Blakslee, 1924). Belling (1925) explained that the breeding behaviour of semisterility in Stizolobium was based on 'Segmental interchange between non-homologues'. No cytological observations were ever reported on meiosis in the Stizolobium material, but rings were found later in crosses between certain strains of Datura (Blakslee, 1928). In maize, plants with about 50% pollen abortion were found (Brink, 1927) and further studies showed a breeding behaviour similar to that found by Belling in Stizolobium (Brink and Burnham, 1929). Morphological identification of the maize chromosomes was completed by McClintock (1929). The subsequent work on maize was used to illustrate the various aspects of the behaviour of chromosomal interchange in plants. In cytological examination of semisterile plants, Burnham (1930) observed that plants inheriting semisterility produced a ring of four chromosomes during meiosis.

McClintock (1930) found that there was a 4-armed, cross-shaped configuration at the pachytene stage of heterozygous semisterile plants. Thus, they were able to provide convincing evidence of an exchange of terminal segments of non-homologous chromosomes.

Bridges (1923) wasthe first to discover a translocation in Drosophila melanogaster through a new linkage relationship. Cytological observations were subsequently made by Bridges but at first gave negative results, the piece being apparently too small to be seen by the methods then in use. The first translocation in Drosophila to be analysed cytologically was described as having a piece of the X chromosome attached to one end of the Y chromosome (Stern, 1926). Later Stern (1931) used reciprocal translocations to provide the visual evidence of chromosome exchange. The "Pale" translocation of Bridges (1923), as well as most of the translocations described earlier in Drosophila (Stern, 1926; Painter and Muller, 1929; Dobzhansky, 1930) were simple translocations i.e. a chromosome was broken and one of the resulting fragments was attached to a different chromosome, the recipient chromosome being intact. However, reciprocal translocations have also been found in Drosophila (Sturtevant and Dobzhansky, 1930; Muller, 1930). In these cases two chromosomes were broken, and the resulting four fragments reunited so as to give two "new" chromosomes. The phenomenon of reciprocal translocation is evidently identical with that known as segmental interchange (Belling, 1925).

Synthesis of chromosome translocations

A spectacular discovery was announced by H. J. Muller in 1927 -

that several types of mutation including chromosome aberrations and the corresponding genetic phenomena in Drosophila could be induced by treating the parent with X-rays. His findings were confirmed by Stadler (1930) for plant material (barley and maize), and radiation of various sorts has since been used very extensively for producing mutations in a wide variety of macro- and micro-organisms. Much later, largely due to the work of Auerbach (1943, 1973 for review), it was found that chemical agents, particularly nitrogen mustard and its relatives as well as all carcinogenic agents, could also induce genetic changes of the same sort as radiation. Such substances are said to be radiomimetic (see Begg, 1959 for review).

There is a wealth of later work confirming that reciprocal translocations can be readily produced with physical mutagens e.g. X-rays and gamma-rays. It is also well known that the different stages of spermatogenesis are characterized by different sensitivities to the induction of genetic radiation damage. The translocation and dominant lethal damage produced in the spermatogenic cycle of Drosophila virilis by several types of ionizing radiations were reported by Alexander et al. (1959). Among post-meiotic types of cells, both dominant lethals and translocations were produced with a higher frequency in spermatids than in spermatozoa, and treated spermatogonia gave an extremely low frequency. Successive matings of irradiated males in Drosophila revealed a similar pattern of sensitivity for recessive autosomal and sex-linked lethals and translocations in the successive broods (Chandely and Bateman, 1960). Sobeles (1969) found that sensitivity to the induction of recessive lethals or translocations was greatest in early spermatids, progressively less

in mature spermatozoa and late spermatids, and lowest in spermatogonia.

It should be possible to induce a greater variety of aberration types in diploid cells undergoing meiosis than in the haploid sperm. Yet irradiated Drosophila oocytes show a much lower sensitivity to translocation induction than spermatozoa. In a series of experiments in which spermatozoa and oocytes were irradiated with identical doses of X-rays, namely 2,000 r., only one translocation in 2,500 oocytes was obtained, in comparison to 150 translocations in 2,387 spermatozoa (Glass, 1955). Kannelis and Radu (1943) reported obtaining 2 translocations in 1,813 oocytes given 4,500 r., a frequency of 0.11 per cent (quoted by Glass, 1955). The difficulty in obtaining rearrangements by treatment of Drosophila females might result from a relatively large volume of the oocyte nucleus causing breaks in non-homologous chromosomes to be more widely separated than in sperm. Furthermore, meiotic segregation would limit the recovery of those translocations induced (Parker and McCrone, 1957). Irradiation of females is reported to cause serious reduction of fecundity in onion fly, Hylemya antiqua (Wijnands-stäb and Van Heemert, 1974). They stated that irradiation of females is not advisable for the production of semisterile stocks because few F_1 are produced and many F_1 individuals are either sterile or almost sterile. Moreover, the number of visible rearrangements among them is very small.

Chemical mutagens have also been found to induce translocations. An enormous number of chemicals have been tested on various organisms to determine their capacity for producing hereditary changes (Serra, 1968 for review). Auerbach et al., (1947) were able to show that mustard gas induced chromosomal rearrangements in Drosophila. They obtained 7

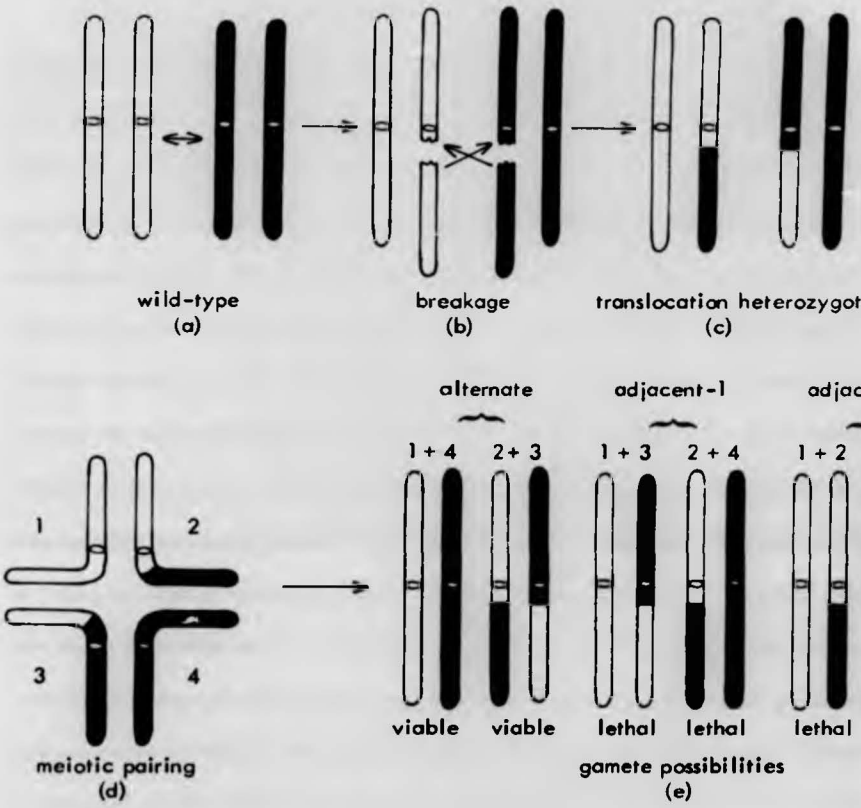
translocations in 816 treated nuclei (0.8%). They also found that mustard gas treatment which gave 9% of sex-linked lethals produced only 0.5 per cent of interchanges between chromosome II and III. This compared unfavourably with high rates (e.g. 6%) of II-III translocations that could be produced with sub-lethal doses of X-rays (3,000 r.) (Auerbach et al., 1947). Watson (1962) tested about 3,500 chromosomes in adult Drosophila males injected with chloroethyl methanesulphonate (CB 1506), and found 11 translocations (approximately 0.3%). A similar result was also found by Reddi (quoted by Watson, 1962), in which treated spermatogonia yielded only 0.24% translocations. Very few translocations induced by CB 1506 were attributed to the delayed mutagenic action i.e. between chemical treatment and the opening of two simultaneous breaks. Jost and Amirkhanian (1971) using 1, 3-propanedioldimethanesulfo nate (PMS) on Culex pipiens reported that 12 out of 376 F_2 egg rafts (3.2%) showed semisterility (between 10% and 80%) and in 3 the presence of translocations was confirmed cytologically. In view of the low frequency of translocations produced by chemicals their wide application in the production of chromosomal interchanges is unlikely.

Translocation genetics

One common result of exposure of a cell to ionizing radiation is chromosome breakage. If two non-homologous chromosomes each break almost simultaneously and the acentric segment of one chromosome becomes attached to the centric segment of the other, while the acentric portion of the latter becomes attached to the centric portion of the former, segmental interchange, i.e. reciprocal translocation, results (See Fig. 1, a. b. c). During meiosis, chromosome pairing occurs

Figure 1. Diagram showing the types of gametes formed by an individual heterozygous for a reciprocal translocation (Above).

The expected fertilities of autosomal translocations in terms of relative frequencies of three classical types of disjunction in heterozygotes (Below).



Ratio of the 3 types of disjunction			Fertility %
Alternate	Adjacent-1	Adjacent-2	
1 + 4 & 2 + 3	1 + 3 & 2 + 4	1 + 2 & 3 + 4	
1	1	1	33.3
1	1	0	50.0
2	1	1	50.0
2	1	0	66.7
3	1	0	75.0

between homologous loci. The four chromosomes in a translocation heterozygote form a complex configuration which resembles a cross like that shown in Fig. 1, d. (In this diagram, the original chromosomes are numbered 1 and 4, the new, translocated chromosomes 2 and 3). There are three kinds of segment in this pairing cross and chiasma formation may occur in any of them. First there are the interchanged segments, second there are the non-interchanged arms and thirdly there are the segments between the centromere and the breakage point (i.e. interstitial segments). Where a single chiasma forms in each of the 4 pairing segments, the pachytene cross opens out at diplotene-diakinesis to form a "ring-of-four" configuration. Where chiasmata form in only three of the four possible sets of pairing segments, a "chain-of-four chromosomes" results. Interchange heterozygotes differ greatly as to the relative frequencies of these "multiple configurations" at diakinesis. There is a general relationship between the frequencies of the various configurations at diakinesis and the relative lengths of the interchange segments. When both interchange segments are long, most of the configurations are rings-of-four. If both interchange segments are short the attraction between the homologues may not be strong enough for multiple configurations to persist from zygotene to diakinesis. "Bivalents" are then formed instead of the crossed and ring configurations. But if one interchange segment is long and the other short, chains-of-four chromosomes are formed as a rule, instead of ring configurations (See Burnham, 1962).

At anaphase 1, the opening of this configuration and its position in the plane of the spindle can proceed in three possible ways which have

been diagrammed and discussed in many publications. The three possibilities are:-

1) Alternate disjunction in which the homologous centromeres go to opposite poles in a twisting fashion producing viable gametes which are either completely normal or contain translocated chromosomes (Fig. 1e. to the left).

2) Adjacent-1 disjunction (adjacent non-homologues) in which homologous centromeres go to opposite poles. This type of segregation leads to the production of gametes which contain duplications for certain chromosome regions and deficiencies in others (Fig. 1e. in the middle).

3) Adjacent-2 disjunction (adjacent homologues) in which the homologous centromeres go to the same pole. This type of segregation also produces duplication-deficiency type gametes (Fig. 1e. right). Thus, there will be six possible kinds of daughter cells with respect to the translocation. Those with a complete gene complement are balanced gametes (orthoploid). Those with deficiencies and duplications are unbalanced gametes (aneuploid).

In the absence of crossing-over between the translocation break point and the centromere, alternate segregation results in balanced chromosome combinations, adjacent segregation results in unbalanced combinations. If crossing over does occur in this interstitial segment, half of the gametes formed as a result of adjacent-1 segregation would be of the duplication-deficiency type and half of them would be of the balanced

type. However, alternate disjunction following crossing over in the interstitial segment is expected to produce gametes half of which are duplication-deficiency types.

The orientation of the ring or chain interchange configuration at metaphase 1 is pertinent to an understanding of both the fertility of translocation heterozygotes and the transmission of genes. If the three types of segregation occurred at random (1 : 1 : 1) a translocation would produce from meiosis one-third fully viable products while the other two-thirds would lead to partial sterility or zygotic non-viability (66% sterility). However, observations in plants and animals have shown that actual sterility or non-viability does not exceed 50%. A number of theories have been put forward in an effort to explain this apparent selection in interchange heterozygotes for those orientations that produce a viable product. As is evident from Burnham's review (1956) and subsequent publications of various authors, the factors controlling segregation of interchange complexes are still imperfectly known. In maize, where orientation types in many different interchanges have received extensive study, alternate segregation in ring complexes involving four chromosomes occurs at a frequency of about 50% (Burnham, 1956). This frequency is approximated in a number of other well studied species, e.g. Mouse (Snell, 1935), Drosophila, (Robinson and Curtis, 1973) and Glossina (Curtis, et al., 1972). The examples are described as being semisterile or as having non-directed segregation. In rye (Thompson, 1956) and barley (Burnham, White and Livers, 1954) and in the multiple interchange complexes of such plants as Oenothera, the frequency of alternate segregation is in excess of 50% and in some it may be as high as 90%. These and other examples (i.e. annual chrysanthemum, (Rana and Jain, 1965))

generally have low sterility and are described as having directed segregation (Burnham, 1956). Burnham (1950) found, in maize interchange heterozygotes involving chromosome 6, a ratio of 1 alternate : 1 adjacent segregations, the latter including adjacent-1 and adjacent-2 types. In these spore mother cells where the configuration is a ring and for which there is little or no crossing over in the interstitial segment, this ratio is close to 2 : 1 : 1 respectively. He considered that a ring may orient itself as an open configuration in two ways (adjacent-1 and adjacent-2) and for each type there is a corresponding alternate arrangement resulting in 50% alternate segregation (Burnham, 1956). In his thesis on some theoretical aspects of selective segregation in interchange complexes, Rickards (1964) emphasises the importance of the interaction of centromeres linked by chiasmata in determining the frequencies of the segregation types. If there are terminalized chiasmata and no crossing-over in the interstitial segment, then each centromere in a translocation heterozygote cross will be linked by chiasmata to two neighbouring centromeres. During meiosis each centromere interacts with one of its neighbours and thus each centromere always has one of its adjacents oriented towards the pole opposite to its own; the other adjacent is oriented to either pole. Taking each centromere in turn there are two possible arrangements: the alternate and the adjacent. But in half of the latter total, homologous centromeres will be oriented to opposite poles (adjacent-1), and in the other half to the same pole (adjacent-2). The ratio alternate : adjacent 1 : adjacent-2 would then theoretically be about 2 : 1 : 1 or as Burnham (1956) recognized in maize, an alternate arrangement for each adjacent arrangement.

Rickards suggests that a cross-over in the interstitial region, between a centromere and a break point, would prevent the occurrence of adjacent-2 segregation because homologous centromeres would always react together because of the proximity of an interstitial chiasma and hence they would invariably co-orient. Thus alternate and adjacent-1 segregation would occur in equal frequency (2 : 2 : 0). The mechanical reasons for co-orientation were discussed by Lewis and John (1966). Co-orientation of non-homologous centromeres in metaphase rings is physically comparable to the co-orientation of homologous ones, each centromere is co-oriented vis-a-vis the two adjacent centromeres with the result that there are equally as many kinds of co-oriented pairs as there are chromosomes in the rings.

In Drosophila, crosses between flies heterozygous for the same interchange have furnished evidence on the kinds of gametes formed and their frequencies. In animals, aneuploid gametes are known to survive and produce viable zygotes because, in matings between two heterozygotes for the same translocation, viable progeny can arise from the union of two duplication-deficiency gametes, provided they are of a complementary type; what is absent or duplicated in one gamete is compensated for in the other to give a full diploid set of genes, e.g. Drosophila (Muller and Settler, 1927), Mice (Snell, 1946; Searle et al., 1971). If a female gamete carrying a duplication and a deficiency is fertilized by one carrying the complementary duplication-deficiency, a viable animal heterozygous for the interchange is produced. The same type of adjacent segregation must occur in male and female if complementary duplication + deficiency gametes are to be available for fertilization. By proper

selection of marker genes, the various kinds of segregations can be recognized phenotypically. Dobzhansky (1933), Glass (1935), Pipkin (1940), Brown (1940) and Hetherington et al. (1968) used this method to estimate the relative frequencies of the three possible types of disjunction in Drosophila. They calculated the frequencies by considering the egg hatchability data and the phenotype frequencies from intercross matings - heterozygous translocation x heterozygous translocation. Attempts were then made to correlate segregation frequencies in interchange heterozygotes with the map length of the interstitial segment - the region between the exchange point and the centromere.

In general, the behaviour of Drosophila chromosomes is similar to that of maize chromosomes. In ring configurations having short interstitial segments and therefore little or no crossing over, alternate, adjacent-1 and adjacent-2 segregation occur to produce six types of gametes. When at least one interstitial segment is long, alternate and adjacent-1 segregations are predominant. However, there appears to be an excess of alternate segregation over the total of the other types in the ring-formers with short interstitial segments, about 60 : 40 or 1.5 : 1. In the chain formers with short interstitial segments there is probably a much greater excess of alternate segregation, 3.5 : 1 or higher. The evidence that fertility in interchange heterozygotes in Drosophila may be of the order of 60% or 70% suggests that alternate segregation is higher than 50%. Thus, an interchange heterozygote characterized by frequencies of alternate to adjacent disjunction of 2 : 1 or 3 : 1 etc. is possible. If this should occur then 2/3 and 3/4 of the gametes pool would be orthoploid rendering heterozygotes 66.7% and 75% respectively as

fertile as their wild-type sibs. The expected fertilities of autosomal translocations in terms of relative frequencies of the three classical types of disjunction are presented in Fig. 1. Glass (1935) found that the segregation of translocation heterozygotes in Drosophila was not at random. He attributed the tendency towards viable combinations to the homologous centromeres possessing a single predetermined axis of segregation. Both Pipkin (1940) and Brown (1940) suggested that a definite correlation exists between the type of disjunction and the amount of crossing over taking place in the translocation segments.

It has been observed that rings may have a different propensity for alternate segregation than chains. Lewis and John (1963 a) and John and Lewis (1965) state that, because of greater flexibility, chains segregate alternately more frequently than rings. This agrees with the observation by Khoshoo and Mukherjee (1966) that in an exchange heterozygote of Canna, rings tend to orientate adjacently, chains orientating alternately. However, Sybenga (1968) reported that rings were found to show alternate orientation more frequently than chains in rye, but Lawrence (1958, 1963) could not demonstrate any such difference between ring and chain quadrivalents in respect of segregation in the same species.

It has been shown that the frequency of disjunctional separations of the chromosomes in rings and chains formed at meiosis in an interchange heterozygote is subject to the control of the genotype. Evidence pointing to a genotypic control of disjunctional orientation of the interchanged chromosomes in rye has been described by Thompson (1956). Consistent with this finding, Lawrence (1958) has shown that selection can be effectively practised in a segregating population of interchange

heterozygote to increase the frequency of disjunction. In fact during an inbreeding programme in rye, the disjunction frequency of interchange heterozygotes increased by about 10 per cent from F_3 to F_6. He attributes this to segregation at a gene locus controlling the character. Rees (1961) has suggested, from selection experiments in rye, a direct role of the genotype in controlling segregation (genotypic control of chiasma position). The finding of Lawrence (1963) that, in rye, newly-induced interchanges may also show a directed segregation of their chromosomes suggests that the genetic constitution has been selected to give a high disjunction frequency.

Very few natural polymorphisms involving chromosome interchanges are known in animals, although this is certainly not true of plants (Burnham, 1956). The reasons are fairly obvious since heterozygotes for translocations will show rings or chains at meiosis and if these orientate in a non-disjunctional (adjacent) way instead of a disjunctional (alternate) way, aneuploid gametes carrying a duplication and a deficiency will be produced. Thus selection against the establishment of translocations that are constantly arising in natural populations is expected to be extremely severe (Wright, 1941). However, some instances of translocation heterozygosity do exist in natural animal populations under exceptional circumstances. The first of these to be described was Piza's demonstration in various Brazilian scorpions. Certain individuals were found to be heterozygous for several translocations leading to the formation of multiple rings of chromosomes at meiosis, similar to those of the plant Oenothera (summarized in White (1973)). Lewis and John (1957) and John and Lewis (1958) have reported translocation heterozygosity in

populations of the American cockroach, Periplaneta americana, from coal mines in South Wales and from laboratory cultures. All the males they examined were heterozygous for at least one translocation. Floating interchanges have also subsequently been found in the same species from Karachi and Lahore. Samples from Pakistan were found to contain interchange multiples of four, six and even eight (John and Qureshi, 1964). Interchange heterozygosity has also been found in laboratory colonies of another species Blaberus discoidalis by John and Lewis (1959). They examined twenty-five individuals among which were 23 heterozygous for one to four interchanges. Evidently translocation heterozygotes were not removed from the population by selection due to semisterility. In most cases a necessary condition for the successful integration of such a translocation into a polymorphic system is the directed segregation of the rings, unless the sterility caused by non-disjunction is greatly over-balanced by the superiority of the heterozygote. In plants, notably Datura, Oenothera and Campanula, many interchange rings undergo directed segregation (Burnham, 1956). This type of chromosomal preadaptation also characterizes Periplaneta chromosomes (John and Lewis, 1959; John and Qureshi, 1964). In the interchanges examined in both species, multiple associations are very frequently formed and no unpaired chromosomes or numerically uneven multiple associations have been found. Chiasmata, when they are formed, are almost always localized near the chromosome ends. Because of this localization the possibility of crossing over in the interstitial and differential segments is remote. It is further restricted by the short length of the interstitial segments in Periplaneta. Meiosis in both cockroaches Periplaneta and Blaberus is characterized by an extensive pre-metaphase stretching of

the chromosomes. Thus it is expected that almost all gametes from such a translocation heterozygote will be orthoploid, although fertility has apparently not been examined by testing hatching rate of oothecae.

Chromosomal preadaptation can thus account for the persistence of interchange heterozygotes in populations,but it does not in itself account for their selective superiority in certain populations. In all the well examined and best understood cases, like Campanula and Oenothera, it has been found that the establishment of interchange heterozygosity is favoured by a change from outbreeding to inbreeding (Darlington and La Cour, 1950). John and Lewis (1959) believe that the populations in which these translocations were encountered had inbred and that selection under such enforced inbreeding is likely to favour structural heterozygosity by maintaining a balanced combination of genes.

Translocations occurring in single individuals of a population cannot be regarded as forming part of an adaptive polymorphic system. However, several examples of spontaneous interchange heterozygosity in animal populations have been recorded, the majority of these being among the Orthoptera (see White, 1973 for review). This is probably a reflection of the greater attention they have received from cytologists. Spontaneous interchanges have been found among many individuals in the Acrididae. For example, John and Hewitt (1963) and Lewis and John (1963 b) have described a spontaneous interchange in the common British grasshopper, Chorthippus brunneus. White (1965) has reported an adult male of the Australian grasshopper, Moraba scurra, heterozygous for a complex translocation involving breaks in four different chromosomes. An individual heterozygous for a reciprocal translocation has been recorded in the grasshopper Gesonula punctiforns from Calcutta

(Sarkara, 1955). Several other cases of spontaneous interchange in the Acrididae have been enumerated by Lewis and John (1963 b). Suomalainen (1946) recorded one individual heterozygous for a single interchange in a sample of twenty individuals of the German cockroach (Blattella (Phyllodromia) germanica) outside Berlin.

Fertility depends on many factors such as the degree of multiple association and the orientation of the multiples at meiotic metaphase. These are affected both by structural properties of the chromosomes and by various genotypically-controlled aspects of chromosome behaviour, such as localization and terminalization of chiasmata (John and Lewis, 1963 a). That chiasma formation is subject to genic control is now beyond dispute and both major and minor genes have been implicated (Rees, 1961). Therefore the relative frequencies of interchange disjunction may be expected to be modified by selection. If by selection, disjunctional properties are genetically altered to any significant degree, then genetical control methods using translocations (semisterility) will hold similar disadvantages of impermanence to those that have already been revealed by the use of chemical control methods.

Recently, Dennhofer (1974) has suggested a mechanism of regulation against sterility due to chromosomal aberration. According to her hypothesis the segregation of the chromosomes involved in a translocation is under strict genetical control by a single, Mendelian factor, called "sg". This factor exists in a series of multiple alleles causing different relations between alternate and adjacent segregation. The allele causing a higher fertility is dominant over the allele that produces less orthoploid gametes. An individual possessing the allele for more fertile gametes - either homozygote or heterozygote - will produce more

offspring than another individual with low-fertility alleles. So the alleles for sterility will be lost in the course of evolution. Finally, only individuals with complete fertility will survive in spite of segmental interchange (like Oenothera, Periplaneta etc.)

Theory of genetic control with translocations

The potential usefulness of translocations for control of insect populations appears to have been first proposed by Serebrovsky (1940). His idea for controlling insect pests was to produce homozygous autosomal translocation stocks (T/T) and to release them into the wild-population ($^{+}/_{+}$). Matings between the released individuals and the wild-population would then produce translocation heterozygotes ($^{T}/_{+}$) which because of their inherited semisterility, would consequently cause a reduction in the fertility of the population. Assuming the introduced strain carrying one viable homozygous autosomal translocation is present in numbers equal to those of the natural population, one would expect a 50% reduction in fertility by the F_2 generation, and 42.2% in the F_3. The percentage of zygotic mortality would finally reach a value of 43% in subsequent generations. Serebrovsky questioned the effectiveness of lowering productivity by only 43% as a means of reducing the whole population. In species regulated by strong density dependent action, the use of a translocation which resulted in only 43% zygotic mortality per generation may not affect the ultimate population size, because surviving progeny having less competition for food and space could obtain an increased likelihood of survival in the environment. Considering technical possibilities for application of the method, Serebrovsky suggested the desirability of obtaining suitably translocated strains of insect pests and discussed the feasibility of rearing and releasing them in sufficient numbers.

If the strain released were to carry two translocations involving four pairs of chromosomes, then each of the translocations would act independently and would produce an overall lethality of 66.4%, i.e. translocation 1 kills 42% of embryos, 58% survive; translocation 2 kills 42% of the 58% leaving 33.6%. On the same reasoning, with three translocations involving six autosomes 80% of all embryos in the population are killed, with four translocations involving eight autosomes about 88% and with five translocations about 93% are killed. Serebrovsky further theorized that permanent lethality of up to 75% could be achieved by using several fully viable translocation homozygotes, each with translocations involving the same pair of chromosomes, and each characterized by 50% sterility in the heterozygous condition. In species with many chromosomes, having possibilities of obtaining a strain with many independent translocations, much higher sterility could be achieved.

Serebrovsky realized, however, from the theory of population genetics, that following the introduction of a translocation into a wild population, a stable polymorphic equilibrium will not necessarily prevail. A temporary equilibrium could exist at a frequency of 50% translocated chromosomes (T) in the population, but once the relative frequencies of either the translocated (T) or non-translocated chromosome (+) changed by chance in one direction or the other, the population is expected to be driven to fixation in favour of the karyotype at the higher frequency. In either event, the population becomes fully fertile again. The explanation of the frequency-dependent selection of karyotype is that at any given frequency of the two sets of chromosomes, the numbers of translocation and normal chromosomes transmitted to the aneuploid gametes, and hence to the inviable zygotes, by translocation heterozygotes

will be equal to each other. Therefore equal numbers of the different types of chromosomes from heterozygous parents will be eliminated in the population at each generation. If the normal chromosomes frequency is larger than the translocation frequency, then the equal numbers of translocation and normal chromosomes in the aneuploid gametes will represent a larger fraction of the total translocation chromosomes and a smaller fraction of the total normal chromosomes (Curtis, 1968 a). If random mating is assumed, individuals homozygous for the rarer chromosomes would mate more often with individuals homozygous for the commoner chromosomes, creating the heterozygote and thus speeding the elimination of the rare chromosome. Ideally, if the equilibrium ratio of translocation to wild type is 1 : 1 the maximum effect for the regulation of population size would be achieved and chromosomes will theoretically remain in the population indefinitely; i.e. a state of unstable genetic equilibrium is attained (Li, 1955). Serebrovsky (1940) discussed several ways of maintaining such an equilibrium between karyotypes and suggested that the optimum ratio of 1 : 1 would be approached closest by releasing small numbers of translocation stock periodically, since the process of their elimination is slow and the effect of population reduction is fairly high.

Long after Serebrovsky's original proposals were published, the theoretical possibility of using translocations in insect control has been elaborated by Curtis (1968 a, b); Curtis and Hill (1968, 1971); Curtis and Robinson (1971); Laven (1969, 1972); Laven et al., (1971 a); McDonald and Rai (1971); Robinson and Curtis (1973); Wagoner et al., (1969, 1971, 1973) and Whitten (1970, 1971 a, b).

Curtis (1968 a) made a theoretical comparison between the effectiveness in tsetse fly control of translocations and the effectiveness of sterile males. He computed that after release of single-translocation homozygotes of both sexes, provided the numbers released were such that the translocation frequency approached the optimum value of 50%, the reduction in tsetse fly population fertility would be prolonged for many generations after releases had finished. The translocation method of control requires only one-half of the adults needed for the sterile male method. It was thus concluded that on certain assumptions, the translocation method might have a considerable economic advantage. However, the reduction in population fertility at each generation that could be achieved by a translocation method was limited and the method would therefore be ineffective against a population whose size was strongly buffered by density-dependent factors. The translocation methods for tsetse fly control were elaborated further by Curtis and Hill (1968, 1971). Their computations made use of more realistic assumptions about the tsetse fly population, since they obtained their data on factors involving viability, density-dependence and migration, from laboratory and field studies. Their study substantially confirmed Curtis's conclusions but emphasized the importance of isolation of the treated population. Curtis and Hill (1971) have shown that translocations causing high sterility in heterozygotes would greatly depress the population for a short time, whereas those causing low sterility would depress it less for a long time. However, eventual results would be similar. This implies that the relative frequency with which adjacent-1 and adjacent-2 segregation occur has been shown to be of minor importance provided that the total frequency of adjacent segregation does not drop below 0.5.

It was also demonstrated that a small reduction in the viability of the translocation homozygote would considerably increase the numbers of insects required for a control prcgramme (Curtis and Hill, 1971).

The potential effect of reduced viability (fitness) of a translocation homozygote on the establishment of an unstable equilibrium has been examined by Robinson and Curtis (1973), using a single translocation homozygote test system in Drosophila. Two cage experiments were carried out with mixed populations of translocation homozygotes and wild-types at a frequency of 9 : 1 in favour of the translocation. Contrary to expectation, translocation chromosomes were eliminated from both experimental cages after seven generations. There was significant reduction in population fertility; population size in the control cage at the end of seven generations being 1943, whereas the populations in the two test cages were 1370 and 1020. The failure of replacement was attributed to the reduced viability (sub-normal fitness) of the translocation homozygotes in the competitive environment. Computer studies showed that the results were consistent with a reduction in fitness of the translocation homozygote to about 0.5, i.e. about the same as the semisterile heterozygote.

In some organisms the translocation alternate (orthoploid) segregation predominates, leading to less reduced or almost completely normal fertility. In such cases interchange is maintained in the population (e.g. Periplaneta, Lewis and John, 1957; Blaberus, John and Lewis, 1959) or even becomes fixed into genetic system (Oenothera, Burnham, 1956, 1962). There is also evidence that the genetic background affects segregation properties of translocation heterozygotes in rye (Thompson, 1956; Lawrence, 1958, 1963; Rees, 1961). Genes which regulate the

meiotic disjunction of chromosomes have been described by Roades in maize (see Dobzhansky, 1951). Therefore the possibility exists that once a translocation is released into a population, natural selection might tend to increase the fertility of translocation heterozygotes. Erk (1960) using a translocation heterozygote in Drosophila found no statistically significant evidence for an increase in fertility after several generations of natural selection during random breeding. Artificial selection experiments designed to determine the evolution of directed chromosome segregation were reported by Hossein using translocations in Drosophila.[1] Only very limited genetic change of this kind was detected.

The use of sufficient multiplicity of translocations so that the heterozygotes are almost sterile was considered by Whitten (1970, 1971 a, b). He pointed out that for most species of insects the genetic load from the release of a strain carrying a single translocation would be too low to reduce population size appreciably. Therefore he stressed the potential role of several multiple-translocation homozygotes, each producing highly or completely sterile heterozygotes with the normal, or with each other. He calculated that a single release of four translocation strains in equal numbers to the wild population can cause a population reduction equivalent to a 20 sterile to normal male release repeated for five generations.

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[1] ESNA European Society of Nuclear Methods in Agriculture, Proceedings 1971. Report of working group 9 Genetical methods of pest control by C. F. Curtis, pp 59-61.

Generally more breaks lead to more sterility, but with increasing numbers of breaks from a single treatment, a viable homozygote becomes more difficult to obtain. Curtis and Robinson (1971) therefore considered only the less demanding case of two reciprocal translocations. Use of double translocation heterozygotes produced by crossing lines homozygous for two translocation stocks was emphasized and various possible types of release strategy were described. The sterility of the double translocation heterozygote will be considerably higher than that of either single heterozygote and hybrid vigour may prevent some of the effect of viability reductions of single homozygotes.

Use of translocations for population replacement

Curtis (1968 b) was the first to suggest that translocations might be better used as transport mechanisms for desirable genes rather than a means of population reduction or eradication. He argued that if translocation homozygotes carrying genes for refractoriness to diseases are introduced into a population and if the translocation chromosome frequency (T) in the wild-population exceeded the equilibrium point (0.5), the population would gradually approach fixation for both translocation chromosomes and the genes for non-infectivity. Thus at fixation the population would be harmless to man. Once genes had become fixed and are closely linked to the translocation points, then any chromosomes bearing the wild-type allele brought in by small numbers of immigrants would be vulnerable. Because these would constitute a minority, they would tend to be disfavoured by natural selection.

Whitten (1970, 1971 a, b) proposed the displacement of field populations using multiple translocation homozygotes; these would have

the advantage over the single translocation system of faster selection of the introduced gene. According to Whitten, the frequency of the translocation chromosome (T) at equilibrium q has the value

$\frac{(W_1 - W_2)}{(W_1 - 2W_2 + W_3)}$ where W_1, W_2 and W_3 are the relative genetic fitness of AA (Wild-type), AT (translocation heterozygote) and TT (translocation homozygote). Ideally, W_1 and W_3 have values near 1 and W_2 near zero so that q is in the vicinity of 0.5. Thus, if TT and AA are mixed together, the least frequent will be replaced in the following generation. Of particular interest to Whitten was the rapidity of the replacement of A by T. Suppose the frequency of T exceeds the equilibrium by as little as 0.05, within six or seven generations A is eliminated from a finite population after a single release of the same size order as the native population. If W_2 is not zero, i.e. the hybrid is not completely sterile, the rate is slower. (e.g. Over 25 generations will be required to introduce a desirable gene by a single homozygous translocation where the hybrid is 50% sterile.) Whitten (1971 a, b) suggested the replacement of a field population with insecticide-susceptible multiple homozygotes. Two translocation strains could be alternately cycled into populations against which three different insecticides would be employed. In his opinion, where insects have become resistant to insecticides, use should be made of genetic manipulation so that insecticides may continue to be used and not, as at present, abandoned. An incentive is therefore provided for the development of better insecticides whose synthesis might otherwise have been economically unattractive. This argument is particularly applicable to mosquito species with their small number of chromosomes. Theoretical arguments, together with computer simulation

studies, suggest it should be possible to substitute desirable genes in small numbers of generations with such insects with a translocation system imparting 50% sterility in heterozygotes(Whitten, 1971 b).

Whitten (1971 a), Foster et al., (1972) and Fitz-Earle et al., (1973) have proposed population replacement by genetic mechanisms which give totally sterile crosses. This proposal, for application in population replacement regimes, uses a special type of translocation termed the compound chromosome which was first described by Rasmussen (1960). Whereas wild-type chromosomes have their homologous arms attached to different centromeres, compound chromosomes have their homologous arms attached to the same centromeres (see Wright, 1970 for review). Progeny arising from crosses between compound and wild-type strains have complete interstrain sterility, since they carry duplications and deficiencies for the arms which render them lethal during the zygotic stage of development. The expected zygotic viability of a compound line will be approximately 25% (see Wright, 1970). On the basis of fertility, compounds will have a fitness of one-fourth that of wild-type. In theory, if the equilibrium ratio of compounds to wild-type is 4 : 1, an increase of the compound frequency will result in the replacement of the wild-types. Thus, compound chromosomes will be used as a vehicle through which other genetic factors may be introduced into pest populations (Foster et al., 1972; Fitz-Earle et al., 1973). Foster et al.,(1972), using bottle cultures, and Childress (1972), using cages, have described the displacement of wild-type D. melanogaster by compounds over discrete generations. More recently, Fitz-Earle et al., (1973) reported successful demonstration of displacement of wild-types by compounds in a population of Drosophila with overlapping generations. Indeed, for

certain strains, cages, in which the initial ratios of compounds to wild-types were as low as 9 : 1, speedily went to fixation of the compounds. Studies of these arrangements are being considered for other insect species, e. g. mosquitoes (Whitten, 1971 a; Foster et al., 1972); and the sheep blowfly (Foster and Whitten, 1974).

One very desirable gene, for possible use in the genetic control of insect vectors involving population replacement, is one making an insect vector incapable of developing and transmitting the infection (Jones, 1957; Craig, 1963; WHO, 1964; Curtis, 1968 b and Whitten, 1971 a, b). There is evidence for genetic variation among vectors and potential vectors of disease in the ability to support the development of parasites (see Macdonald, 1967 for review). The first demonstration of the genetic basis of the host-parasite relationship in malaria was given by Huff (1929) working with Plasmodium cathemerium in Culex pipiens. Later, he studied the inheritance of susceptibility by making appropriate crosses between his selected mosquito stocks and concluded that infection of C. pipiens with P. cathemerium was controlled by a pair of simple Mendelian recessive alleles (Huff, 1931). Ward (1963) studied the genetic aspects of susceptibility of Aedes aegypti to P. gallinaceum. He established a strain of Ae. aegypti highly refractory to P. gallinaceum from a susceptible strain by genetic selection. Susceptibility decreased 98% over a period of 26 generations. Reciprocal crosses between a refractory and a susceptible line gave F_1 and F_2 hybrids that were intermediate. He concluded from back-cross data that a single Mendelian factor or a group of closely linked genes with incomplete dominance was responsible for susceptibility.

In some cases, the genetic basis of such variation is comparatively

simple. Kilama and Craig (1969) established two strains of Ae. aegypti refractory to infection with P. gallinaceum within one generation of selection. Crosses established that the refractory condition is controlled by a simple autosomal recessive factor. Macdonald (1962 a) selected out a strain of Ae. aegypti highly susceptible to infection (mean susceptibility rate 84.8%) with the semi-periodic form of Brugia malayi from a colony which showed only 12 to 31 per cent infectivity rate. Macdonald (1962 b) showed that the vectorial susceptibility to the parasite was controlled by a single sex-linked recessive gene with, as might be expected, some modification by genetic background. Ae. aegypti selected for susceptibility to semiperiodic also becomes susceptible to periodic B. malayi, and to B. pahangi and both periodic and semiperiodic Wuchereria bancrofti. A selected strain refractory to infection with semi-periodic B. malayi has proved to be also refractory to other species of filariae (Macdonald and Wharton, 1963; Macdonald, 1967). A strain of Ae. aegypti highly refractory to Dirofilaria immitis infection was selected by McGreevy et al., (1973). The refractory rate increased from 63% to 100% from the parent to the F_1 generation, but in subsequent generations the refractory rate continued to fluctuate slightly. Study for mode of inheritance showed that the refractory condition was controlled by a sex-linked recessive gene.

There are several experimental studies in which the susceptibility of a population has been altered by selection. Trager (1942) increased the susceptibility of Ae. aegypti to P. lophurae from 60% to 92% through six generations of selection. The selected strain "D", after more than a year of non-selective breeding, retained its character of being more

susceptible to infection with P. lophurae than the stock from which it was derived. Micks (1949), working with P. elongatum in C. p. pipiens increased the level of susceptibility from 13% to approximately 49% within six generations. Al-Mashhadani (1974) applied similar selection pressure to An. gambiae species A to alter its refractoriness to the rodent malaria parasite Plasmodium berghei berghei. After nine generations of selection, the refractory line shows only 5% of mosquitoes developing oocysts and none sporozoites. A parallel line of the same species selected for susceptibility shows 100% sporozoite formation. When both the lines were fed on a chimpanzee infected with P. vivax, the latter again showed 100% sporozoite development while the refractory line showed less than 50%. Thomas and Ramachandra (1970) selected two strains of C. p. fatigans for vector ability to the rural strain of Wuchereria bancrofti. The original susceptibility levels of "A" and "B" colonies were 6.6% and 20% respectively. In strain "A", the susceptibility rates had increased to 66.6% by the 4th generation. Raghavan et al., (1967), selecting from a stock of Ae. aegypti that was 82% susceptible to D. immitis, established a pure susceptible line in 1 generation but failed to establish a pure refractory line after 27 generations of selection.

It should be pointed out however, that some investigators have reported negative results in their attempts to demonstrate the genetic control of mosquito susceptibility to parasites. Partono and Oemijati (1970) applied selection pressure to C. p. fatigans to infection with W. bancrofti. After selecting lines of mosquitoes from susceptible and from refractory individuals for three generations, there was no evidence of the presence of a major gene determining whether or not a mosquito would be refractory. Singh and Curtis (1974) attempted

selection of C. p. fatigans involving Paris cytoplasm and Delhi genome for refractoriness to periodic W. bancrofti. After 5 generations of selection it was found that initial populations of C. p. fatigans did not contain any gene(s) for refractoriness to W. bancrofti.

Another factor which may be selected for in populations is temperature-sensitive lethal mutations. These have been studied extensively in D. melanogaster (Suzuki, 1970), and have also been detected in the housefly Musca domestica (McDonald and Overland, 1972) and in the predaceous wasp, Habrobracon serinopae (Smith, 1971, 1974). Temperature-sensitive lethal genes exist which permit Drosophila to survive at permissive temperatures (e.g. 22 °C) but which lead to death at restrictive temperatures (e.g. heat-sensitive, 29 °C, cold-sensitive, 17 °C). Similarly, houseflies homozygous for heat-sensitive traits survive to maturity when reared at 25 ± 2 °C but die at some immature stage of development when rearing temperatures are 33 ± 2 °C (McDonald and Overland, 1972; Wagoner et al., 1974). Genes preventing diapause could work in the same manner. A mutant unable to enter diapause could be released in areas where diapause is essential for survival. To be useful, such adaptation must be inherited, and they must have a degree of dominance so that they may be expressed in hybrid individuals (Klassen et al., 1970).

The use of inversions to obtain permanent linkage between a gene to be transported and a translocation has been suggested by Curtis (1968 b). For the preparation of such mutant stock, he recommends the following procedures:

" a) Selection of a suitable gene (e.g. conditional lethal gene) and production of homozygotes.

b) Induction of an inversion including the locus of the conditional lethal gene and production of an inversion homozygote stock.

c) Production of a translocation with one break-point within the inversion and production of a translocation homozygote stock. "

Without appropriate genetic markers and crossover suppressors, it would be a monumental task to obtain strains bearing conditional lethal factor-translocation combinations in An. gambiae complex. Recently, McDonald and Overland (1973 b) reported the successful isolation of a homozygous translocation-bearing strain of Musca domestica in which a recessive heat-sensitivity lethal factor was combined with a translocated segment of chromosome 3. Interestingly enough, this strain, T(3 : 5) hs, has been recovered without being linked to the known crossover-suppressing inversions.

Integrated genetic control

With a view to developing an elaborate control system, Laven and Aslamkhan (1970) and Laven (1972) suggested combining bi-directional cytoplasmic incompatibility and a male-linked chromosomal translocation complex to obtain an integrated genetic mechanism to be used for suppression of wild C. fatigans populations. Their proposal was to release both sexes of the "integrated strain" so as to bring about replacement of the wild population by the cytoplasmic incompatibility of the integrated strain, after which the translocation could be capable of completing the process of suppression of the residual populations of the integrated strain. Their thesis was as follows.

Let strain B contain a male-linked translocation with 85% sterility and be incompatible in both directions with a wild population A. If the release of both sexes of this integrated strain B is made into a wild population A at a ratio of 1 : 1 there will be a 50% reduction in A and 92.5% reduction in B. If the release be repeated with the same number as before for two more generations, population A would be eliminated and strain B would replace it. But this strain (B) has a reduced reproductive potential (15% of that of the original population). Under certain circumstances this reduction of mosquito density to 15% of the original density might be sufficient to break the chain of transmission of a disease. Furthermore, this situation of a population with reduced reproductive potential might be preferable to total eradication which creates an open niche that could be filled up by an equally or more dangerous mosquito species. If the reduction to the 15% level does not appear to be sufficient for the interruption of disease transmission or if the biting nuisance still remains, a second round of control with strain C could be applied. This strain C should be incompatible in both directions with B and should carry a translocation system with highest possible sterility (95%). The second round would bring the remaining population down to a negligible density or to total eradication.

Pal (1974) has reported that extensive efforts are being made to construct such incompatible and "integrated strains" at the Institute for Genetics, Mainz University, West Germany and WHO/ICMR Unit, New Delhi, India. An important point being considered in this work concerns the fact that C. fatigans is the vector of W. bancrofti in India. The integrated strains have therefore been made nearly isogenic with the Delhi strain. But the backcrossing procedure might not have been

sufficiently thorough to make the integrated strain completely isogenic with the Delhi strain at gene loci affecting susceptibility to W. bancrofti (Pal, 1974). Other difficulties might conceivably arise from the possibility that vector susceptibility might be affected by cytoplasmic factors. Laven and Aslamkhan realized the biting nuisance and disease-transmission disadvantages of releasing females, and that these females might be more efficient vectors of the disease than the original population. They argued that such objections were to be partly overcome by the fact that exposure to the new strain in full numerical strength would last only for a short time. However, whether or not such counter-argument is accepted, it is clearly desirable to test the new strain for its vector ability before considering its release. If it proves to be more dangerous than the natural population, its vector ability could, perhaps, be reduced by appropriate crosses.

Krishnamurthy and Laven (1972, 1974) gave a detailed description of the production of a strain of C. fatigans with the Delhi genome, incorporating a male-linked translocation complex, combined with Paris cytoplasm. This strain possesses bi-directional incompatibility with the target Delhi wild population and has about 65-70% sterility within the strain (IS-31B). Recently, Krishnamurthy (1974) was able to synthesize a strain (IS-325) having an even higher level of sterility (80%). This was produced by irradiating (3,500 r. gamma-rays) an existing male-linked translocation complex having 65% sterility and then combining this with Paris cytoplasm to produce the "integrated strain". This compound strain also showed cytoplasmic incompatibility with respect to the wild Delhi population, in addition to the high level of sterile matings within the strain.

The comparative susceptibilities to W. bancrofti of the C. fatigans

Delhi strain and of strains cytoplasmically incompatible with it were tested recently by Thomas and Singh (1974). All the strains which were tested were highly susceptible to infection and there was no consistent difference between the strains in their degree of susceptibility. It was thus concluded that there appears to be no need for anxiety that replacement would lead to a great risk of infection by W. bancrofti. Because the males of D3 strain have been found to be incompatible with females of 19 strains collected from various States in West Malaysia, Indonesia, Sabah and Singapore (Thomas, 1971), Thomas reasoned that if incompatible males are released in the field daily in very large numbers over a period of time, it is likely that some females will also inadvertently be released at the same time. Under such circumstances, the incompatible strain of mosquitoes would sooner or later replace the local strain of C. p. fatigans. With this in mind, the efficiency of the cytoplasmically incompatible (D3) strain of C.p. fatigans to infection with the rural strain of W. bancrofti in Malaysia was tested by Thomas (1974), and results showed that this strain, unlike the local strains of C. p. fatigans, has a higher infectivity level than the local strain. The percentage of these mosquitoes infected by the rural strain W. bancrofti was between 75.0 and 86.4, while with the local strains it was between 6.6% and 20.0%. From this result, he concluded that it is not a suitable strain to use as a genetic weapon against local C. p. fatigans.

A series of experiments designed to test the feasibility of the replacement principle was reported by Curtis and Adala (1974). Strains with the cytoplasms of either Delhi or Paris (this strain carried a male-

linked translocation) which are bi-directionally incompatible, were tested in laboratory cages. It was found that there was complete elimination of either the Paris or the Delhi type, depending, as expected, on the relative frequencies of the two types with which the population was initiated.

Additionally, an integrated system with chromosomal translocations has been proposed and discussed by Laven (1969), Rai et al., (1970), Rai (1971) and Suguna and Curtis (1974). Laven (1969) proposed in very general way the integration of the male-linked translocation with a known balanced lethal system in the C. pipiens complex with the aim of forming a line that would produce only males. This would facilitate the production of males (only) or release without the necessity of separating them from females. Similarly, Rai et al., (1970) and Rai (1971) proposed the combination of a translocation system with a recessive lethal factor in Ae. aegypti. If a recessive lethal mutation is induced on the female-determining chromosome in the translocation heterozygote males, daughters of matings of such males with normal females will be heterozygous for the lethal in the F_1 generation. Half of the females formed in subsequent generations arising from matings of males and females heterozygous for this will die. It will be most desirable to have the lethal very tightly linked with the female-determining locus or associated with an inversion (to make recombinants inviable).

Sex-ratios strongly distorted in favour of males have been reported in certain stocks of Ae. aegypti by Hickey and Craig (1966). High male ratios are not due to post fertilization mortality. Therefore there is a driving form of sex-determining locus M (known as M^D gene) which produces an excess of male-chromosome bearing sperm and a deficiency

of female-chromosome bearing sperm. It operates only when present in the heterozygous condition and only when located on the same chromosome as the dominant gene for male, *e.g.* $M^D m^d$. It is favoured at meiosis when associated with a sensitive X-chromosome (m^s*), and the sex ratio shows an excess of males. This system of distortion of segregation ratios is similar to cases of "meiotic drive" in Drosophila (Sandler and Novitski, 1957). Recently, Suguna and Curtis (1974) in collaboration with Wood and Rai, have crossed the Trinidad Ae. aegypti strain carrying M^D with an Indian stock. After backcrossing, distortion sensitive stock with Indian genetic background was isolated, and this produced an average male : female ratio of 6 : 1. Further crosses have incorporated translocations into the stock and a distorter translocation homozygous line ($DT_1\ T_1$) has been established. It produces an average sex-ratio of 15 : 1. A distorter double translocation heterozygote system has been produced by crossing $DT_1\ T_1$ males to distortion sensitive ($m^s\ m^s$) females which are also homozygous for another sex-linked translocation T_3. Double translocation heterozygote males give 61 per cent sterility and a 6 : 1 sex-ratio. Thus the overall female progeny from the cross are 50 per cent sterile, whereas the sterility of the males averages only about 27 per cent but strong (11 : 1) sex-ratio distortion reappears in them if they inherit the m^s gene. Computer simulations based on several simplified assumptions have indicated that the release of males with the distorter double translocation heterozygote system would be more effective for population suppression than the release of the same numbers of chemosterilized males or double translocation heterozygote males without distortion (Suguna and Curtis, 1974).

* m^s = m^d of Hickey and Craig, 1966.

Isolation of translocations suitable for genetic control

Translocations have already been isolated in several pest species of medical and veterinary importance and studied by testing for pseudo-linkage of markers, inherited partial sterility and by making cytological examinations. A brief summary of this work will now be given.

Krafsur (1972 a, b) was the first to isolate radiation induced translocations in the An. gambiae species complex. Translocations were induced with X-rays (3,000 r. - 4,000 r.) in male An. gambiae species A and B and tested for inherited partial sterility. Both sons and daughters of irradiated males were outcrossed to wild-type individuals. Progeny of F_1 matings showing reduced hatching rate (60% or less) were again reciprocally outcrossed to the wild-type and the same procedure of selection for partial sterility with subsequent outcrossing was followed. In the absence of visible genetical markers the isolation of translocations in these species had to be carried out using "semisterility" itself as the criterion. This was achieved, despite variable fertility of the control stock strains. A number of lines of species A and B showing inherited semisterility were established. Five proved to have holandric inheritance, the result of chromosome exchanges involving the Y chromosome and one of the two autosomes. Two were in species B and three in species A. All sons, without exception, were partially sterile while their sisters were normally fertile. The fertility of translocation heterozygotes ranged from 38% to 48%. Another 13 lines were shown to involve autosome-autosome interchanges. One of them was a two-autosome double translocation. Fertilities of the majority of autosomal translocation heterozygotes, when outcrossed to wild-types, were found to be slightly higher than the expected 50% of normal. Two lines were characterized

by significantly different fertilities between females and males, female heterozygotes being more fertile than their translocated brothers (Krafsur and Davidson, 1973). No homozygous translocation lines were successfully isolated despite repeated attempts. The presence of a translocation was confirmed cytologically in 7 out of the 13 lines described above (Hunt and Krafsur, 1972).

Aslamkhan and Aaqil (1970) described the results of irradiating young male An. stephensi with 3,500 r. gamma-ray. Normal females were allowed to mate with F_1 male offspring of irradiated male parents and the presence of translocation was assumed where egg hatch was reduced. Fecundity, fertility, developmental mortality and emergence of adults of 50 matings were recorded. 22 ovipositions showed less than 50% egg hatch and they were regarded as from probable translocation heterozygotes. Only one case was examined cytologically and this was claimed to be a sex-linked translocation. However no detail was given as to whether this line involved an X-autosomal or a Y-autosomal translocation.

Rabbani and Kitzmiller (1972) have presented the methods of induction, screening, maintenance and descriptions of translocations in An. albimanus. Young males irradiated with 4,000 r. of X-rays were mass mated with virgin females. Further mass sib-matings in the first and second generation followed. During this process some gravid females were isolated for egg-deposition and individual families of larvae were reared. Preparations were made of salivary gland chromosomes when larvae reached an early fourth stage and chromosomes were examined for structural changes. Families showing translocations were retained and adults from such families were put together in stock cages. Stocks were maintained by heterozygote x heterozygote and heterozygote x

normal matings. The screening procedure mentioned above was followed in every generation. A total of six reciprocal translocations, three involving autosome-autosome, two involving X-autosome and one involving Y-autosome interchanges have been described precisely with details of chromosome break-points. Fertilities of translocation heterozygotes were around 50% although X-autosomal interchanges have shown slightly higher sterility compared with others. In spite of more than a year of study they have failed to detect any translocation homozygotes.

Two radiation induced translocations have been reported in An. maculipennis by Frizzi and Jolly (1961). These translocations were induced through X-irradiation with a dose of 3,500 r.. However, they were not maintained for further study.

Induction of chromosomal translocations in the C. pipiens complex was first reported by Laven (1969). Young males were exposed to X-rays (4,000 r.) and then crossed to untreated females. F_1 progeny were tested for inheritance of partial sterility measured by reduced hatching rate after outcrossing to normal individuals. Seven lines were found to have patrilineal inheritance of partial sterility. All males were partially sterile, irrespective of the females with which they were mated, while all sister females showed normal fertility when crossed with unrelated males. This indicated that a translocation had occurred between the male-determining chromosome and one or the other of the two autosomes. In Culex mosquitoes the sex determining mechanism is genic, i.e. one of the three metacentric chromosomes pairs carries the sex-determining factor M for maleness and m for femaleness; males are heterozygous M/m, females homozygous m/m, (Gilchrist and Haldane, 1947). The genetic mechanism whereby chromosomal translocations cause

inherited semisterility in mosquitoes was explained by Laven and Jost (1971) and Laven et al. (1971 a, b). Males (2-3 days old) were exposed to various doses of radiation and then mated with untreated females. The incidence of gross chromosomal aberrations, indicated by the percentage of eggs that failed to hatch, rose from about 20% for a dose of 1,000 r. to over 95% for a dose of 8,000 r. The incidence of translocation heterozygotes in the F_1 generation rose from 10% for a dose of 500 r. to 50% for a dose of 5,000 r.

In another experiment (3,500 r. X-rays), Laven et al., (1971 a) studied the F_1 progeny of 136 irradiated sperm samples and 44 translocations were isolated. Sterilities obtained for these 44 translocations in the F_2 generation varied between 12% and 76%. Of thirty one examined 7 were found to have translocations between the M-chromosome and an autosome, 3 had translocations between the m-chromosome and an autosome and the remaining 21 were autosome-autosome translocations. Ten of these lines showing a certain amount of sterility were studied cytologically by Jost and Laven (1971) and it was confirmed that reciprocal chromosomal interchanges were involved. The percentage sterility was 46-52 for the heterozygotes of nine lines with translocation involving two chromosomes and 82 for those of one line with a translocation involving three chromosomes, as compared with 2,25% for the normal strain. An account was given of the behaviour of the chromosomes of heterozygotes during meiosis. Laven (1972) obtained translocation lines with sterilities between 80% and 92% after exposing males of one of the male-linked translocation lines to a second irradiation. Attempts to recover homozygous lines for female-linked and autosomal translocations had only a limited success. Out of 37 such translocations, Laven (1972) claimed isolation of six homozygous

lines but details were lacking.

Extensive data on the isolation of translocations in C. tritaeniorhynchus have been reported by Baker et al., (1970), Sakai et al., (1972) and Baker and Sakai (1974). Males were irradiated with 3,000 r. gamma radiation from a ^{60}Co source and forty-six translocation stocks were isolated. These were studied using genetical markers. Although autosome-autosome translocations were produced they studied only the lines involving sex or sex-linked markers. Fertilities of most of the reciprocal translocations involving two chromosomes were approximately 50%, and 25% for stocks in which all three chromosomes were involved. Sakai et al., (1972) described experiments to increase the sterility of the original stocks by further irradiation (3,000 r. gamma radiation from a ^{60}Co source). A significant enhancement in the sterilities was observed in 30 lines after the second irradiation. The maximum sterility that resulted was 76.6% among females, while among males higher sterilities were found; the highest sterility observed being 89.5%. This was attributed to an increase in the complexity of the chromosomal aberrations. In fact cytological observations in one of the isolated lines showed heteromorphism for all three pairs of chromosomes. They also synthesized doubly heterozygous translocated males. These were produced by crossing males and females which were heterozygous for different complex aberrations. Cross between resultant doubly heterozygous males and wild type females have shown sterilities of over 96%. Attempts to synthesize translocation homozygotes from the stocks described above were unsuccessful (Baker and Sakai, 1974).

Selinger (1972) has also described the results of chromosomal translocations in C. tritaeniorhynchus (derived from X-radiation at a

dosage of 3,500 r.). Out of 125 F_2 progeny examined, 25 were found to have sterilities between 30% and 60%, 9 sterilities between 20% and 30% and 7 between 10% and 20%. The remaining 84 F_2 offspring were found to have normal fertility. In the following generation she studied some of these semisterile lines to determine whether the type of translocation was sex-linked or autosomal-autosomal. She found 9 lines were male-linked translocations and 6 other lines were autosomal interchanges.

All 15 lines were maintained for 16 generations in the heterozygous condition. Viability was found to be as good as in the normal strain and the degree of sterility was constant from generation to generation. The observed fertility in most of the lines had a mean value of 50%. Five out of 9 male-linked translocation lines were examined cytologically and it was found that in two lines the change of chromosome segments had occurred between the smallest chromosome and one of the larger chromosomes, while in 3 other lines which behaved genetically in the same way, the two large chromosomes were involved in the translocations. Selinger therefore postulated that sex determining factors M and m are located on one of the two large chromosomes and thus expressed a different view from that of Baker et al., (1971).

The first record of a translocation in Ae. aegypti is to be found in A sman (1967), where a male-linked translocation was briefly described. The egg hatch associated with this aberration was approximately 30%. Later, Rai et al., (1970), Rai and McDonald (1971, 1972) and McDonald and Rai (1970 a) described two radiation-induced, sex-linked translocations in this species in detail. The translocations were isolated following X-irradiation (5,000 r.) of young adult males from a colony designated ROCK. The irradiated males were crossed with unirradiated ROCK

females and F_1 males thus obtained were mated singly with one or two ROCK females. Using semisterility and the cytogenetic evidence as criteria, cultures showing fertility of 50% or less than the control were selected for cytogenetic examination.

One translocation, (RT (1 : 2)), involved linkage groups I and II with the original break point 0.3 cross-over units from the male-determining allele (M) on group 1 and 1.6 units from the wild-type allele of spot abdomen (S) on group II. The other translocation (RT (1 : 3)) involved linkage group I and III with the original break points 0.4 cross-over units from the wild-type allele of the red-eye gene (re) on group I and 0.6 unit from the normal allele of black-tarsi (blt) on group III. Though originally both these translocations involved the male-determining chromosomes, female translocation heterozygotes have been established by appropriate crosses. Furthermore, two types of males heterozygous for each translocation have been constructed. In one type (M-linked) the male-determining chromosome is translocated (T^M), and in the other (m-linked) the female-determining chromosome is translocated (T^m). In Ae. aegypti sex is determined by a single gene, with Mm being the male genotype and mm the female genotype (McClelland, 1962). The mean fertility for T(1 : 2) was 20% with a range of 4% to 48% while for T(1 : 3) it was 9% with a range of 1% to 21%.

McDonald and Rai (1970 b) described the production of individuals which were heterozygous for both translocations. A genetic exchange in the double heterozygote produced a "new" chromosome bearing parts of the three linkage groups. (RT 1 : 2 : 3). In both the double heterozygotes and the "new" karyotypes, an apparent enhancement of cross-over occurred in the region between the break points of RT (1 : 2) and RT (1 : 3) on

linkage group I. As a result, the double heterozygote produced a high frequency of "new" karyotypes and "new" karyotypes produced a high frequency of single reciprocal translocation heterozygotes. The fertility of double heterozygotes ranged from 7% to 10%.

Lorimer et al., (1972) produced two homozygous interchange lines from more than 40 reciprocal translocations in Ae. aegypti. One was sex-linked and involved chromosomes 1 and 3 (T(1 : 3)b), and the other autosomal and involved chromosomes 2 and 3 (T(2 : 3)c). They were identified on the basis of egg hatch, genetic analysis and cytological evidence. Their fertility was surprisingly low with only 19% hatching rate in T(1 : 3)b and 55% in T(2 : 3)c. The lower than expected mean hatching rate of these translocation homozygotes was interpreted on the basis of low fertility of the parent RED stock, the RED stock showing a reduced hatching rate (62% $\pm$ 21.0) compared with ROCK (84% $\pm$ 5.1) and the wild population (100%) (Lorimer et al., 1972). Recently, Rai et al., (1974) reported that two out of 45 translocations induced in a stock from Delhi have yielded viable homozygotes in Ae. aegypti. Their fertilities are 59% and 55% respectively when sib-mated and 82% and 88% respectively when outcrossed with the Delhi stock.

Bhalla (1973) has described seven sex-linked translocations in Ae. aegypti. The translocations were induced by X-irradiation with 3,500 r. and the three criteria were used for the detection of translocation heterozygotes, namely sterility, linkage alteration and cytological observations of chromosomal configurations. Out of seven, two were T^M type (linked to the M-chromosome) and the remaining five were T^m type (linked to the m-chromosome). Fertilities of seven translocation heterozygotes ranged from a minimum 18% to a maximum of 71%.

Among the higher Diptera of medical and veterinary importance attempts have been made to isolate translocations in the house fly, Musca domestica, the tsetse fly, Glossina austeni, the sheep blow-fly Lucilia cuprina, and the screw-worm, Cochliomyia hominivorax.

Adult male houseflies Musca domestica were treated with various dosages of X-irradiation (2,000 r. to 7,500 r.) and reciprocal translocations have been isolated using the "pseudo-linkage" technique (Wagoner, 1967, 1969; Wagoner et al., 1969). They analysed each strain for egg hatch (fertility), transmission of the translocation, and sex-ratio of progeny. Over 300 stocks of house flies examined contained heterozygous translocations of both autosomal-autosomal and Y-autosomal types. The average fertility of single reciprocal autosomal translocations was 44.6%. The double reciprocal translocations had on average an egg hatch of 34.1% and triple translocation heterozygotes an average of 21.0%. The Y-autosomal heterozygous translocations provided similar results but the average fertilities were slightly higher than with autosome-autosome translocations. The sex-ratios generally approximated the 50 : 50 ratio expected especially for the autosome-autosome translocations. However, about one-third of the Y-autosome translocation stocks produced more males than females (2 : 1). Only five heterozygous strains with chromosomes II or III reciprocally translocated have been made homozygous (Wagoner et al., 1974). Recently, McDonald and Overland (1973 a) included a paracentric inversion in 18 translocations and subsequently recovered four viable translocation homozygotes, three of which showed fertilities above 68%. They used an inversion as a suppressor of genetic recombination to facilitate the recovery of homozygous translocations. (Genetic recombination occurs in the translocation heterozygote females.)

In the tsetse fly Glossina austeni, interchanges have been produced by ^{60}Co gamma radiation with doses of 5,000 r. - 7,000 r. (Curtis, 1969). A total of sixty F_1 males were tested for the occurrence of inherited partial sterility. In the absence of suitable genetical markers, Curtis used reduced larval production as the sole means of identifying individual karyotypes. Eighteen stocks derived from surviving zygotes showed inherited partial sterility in at least a proportion of the male progeny. In 2 stocks the inheritance was patrilineal which suggested that the Y chromosome was involved. The remaining stocks, considered as autosomal translocations showed transmission of partial sterility in a proportion of both sexes. Later, two of the stocks which were originally scored as autosomal interchanges showed a switch-over in pattern of inheritance to the holandric type (Curtis, 1971 b). He explained that in these lines the effect of selection at each generation for semisterile males has been to transfer the sex determining function from the X and Y chromosomes to a locus at or linked to the translocation breakpoints. Since then it has been confirmed cytologically that these were translocations involving the large autosomes or the Y chromosomes (Pell et al., 1972; Curtis et al., 1972). The fertilities varied in different stocks but averaged 50%. One stock with holandric inheritance of extreme sterility (25% of the control fertility) was interpreted as being due to a double translocation involving two autosomes and the Y chromosome.

The descendants of heterozygous autosomal translocation individuals were inbred in an attempt to produce translocation homozygotes (Curtis 1969, 1970, 1971 b). Tests over two generations were used to identify translocation homozygotes among the products of inbreeding.

Over 300 individuals from 7 different translocation stocks were

tested, and translocation homozygotes were identified in three of the stocks. One stock yielded several homozygous individuals. Further inbreeding in these families has yielded some fully fertile progeny. These were studied genetically and for effects on viability (Curtis et al., 1972). It was found that there was strong evidence of reduced viability in translocation homozygotes. Homozygous males proved to be subnormal both as regards survival and mating success. Those female homozygotes which did breed showed a subnormal pupal production. The authors concluded that reduced viability was due to recessive effects of the translocation itself although the influence of linked loci could not be ruled out. These effects would prevent the mass rearing of this particular translocation for a tsetse control project. From the remaining two stocks only single individuals were obtained in the homozygous state.

Childress (1969) has studied six radiation-induced reciprocal translocations in the Australian sheep blowfly, Lucilia cuprina. Translocations were induced by gamma radiation, and irradiated wild-type males were mated to females from the multiple marker stock. F_1 progeny were backcrossed individually to females from the marker stock and their progeny checked for evidence of pseudo-linkage. Because there is no crossing over in male L. cuprina, pseudo-linkage of two or more markers indicated a newly induced translocation between those linkage groups. Polytene chromosome examinations confirmed the presence of autosome-autosome translocations in 3 lines. One X-autosomal and two Y-autosomal translocations were identified from mitotic chromosomes. No figures were given concerning the fertility of the translocation heterozygotes.

Foster and Whitten (1974) reported the isolation of over one hundred autosome-autosome translocations in this species by testing for pseudo-linkage. Thirty of these were screened for homozygous viability by crossing heterozygous males and females and scoring the phenotypes of their progeny. One out of 30 translocations was found to be viable and fertile in both sexes.

The screw-worm C. hominivorax, was apparently the first pest insect in which a translocation was described. One dominant mutant (Brc, black R-cell) which affects the wing venation was derived from untreated laboratory-reared flies, from the progeny of irradiated normal parents and from the progeny of irradiated flies inbred for two generations. The mutant strain was studied over 20 generations. In crosses of Brc x Brc the ratio of mutant to normal progeny was always 3 : 1. The genetic and cytological studies revealed that this mutant was associated with a reciprocal translocation between chromosomes 2 and 4 (LaChance et al., 1964). Heterozygous males were found to be more fertile (66%) than heterozygous females (49%). The genetical data suggests that in males preferential segregation of the chromosomes at the first meiotic division favours alternate disjunction with a consequent increase in the fertility of the males over the expected value. The translocation homozygotes usually survived to the pupal stage and died before emergence. A few adult homozygotes were found but they were of low vigour or completely sterile.

Ullerich (1963) has produced translocations through X-irradiation of varying dosages (3,500 r. - 6,000 r.) in Chrysomia albiceps, C. rufifacies, Lucilia cuprina dorsalis, Calliphora erythrocephala and Phormia regina. His cytological and genetical analysis of these translocations has contributed much to the knowledge of the sex chromosomes

of these flies and of their operation in sex-determination (see Boyes, 1967 for review).

Experimental genetic control

Whereas the theoretical advantages of using homozygous translocations for control programmes have been expounded by many recent authors, in reality only a comparatively few homozygotes have yet been actually isolated in pest insects. Moreover, no-one has yet produced homozygous interchanges having a fitness close to that of the wild-type. As stated by Robinson and Curtis (1973), "the reduced viability of translocation homozygotes which is likely to be found in intensely competitive conditions in the wild, is an obstacle to their use for pest control". It has become clear that prospects for the utilization of translocation homozygotes for pest control with an expectation of high efficiency are slight. Notwithstanding, several workers have considered the possibility of actively using translocation heterozygotes as a means of genetic control (Laven, 1969; Laven et al., 1971 a; McDonald and Rai, 1971; Rai et al., 1970 and Wagoner et al., 1969, 1971, 1973). Their principal achievements are described in the following paragraphs.

Laboratory experiments conducted by Laven (1969) were the first to prove the potential value of translocation. By releasing males bearing various translocations he practically eradicated a caged C. pipiens population after five generations. Release of translocation carriers was repeated each generation. By sustaining the numbers released against a declining target population, the process became more efficient as progress went on. It has to be pointed out, however, that the population was managed in such a way that its size was unbuffered by density dependent factors. Laven (1969) and Laven et al., (1971 a) strongly

recommended the use of male-linked translocations. This is preferable because, as only males are released and the sterility is propagated through male progeny alone, male-linked translocations have never become fixed. However, Whitten (1971 a), Curtis and Hill (1971) and Curtis (1975) have discussed the limitation of this concept. The problem following the release of male-linked partially sterile males is a levelling off in the response so that no advantages are obtained comparable with those afforded by the swamping effect that normally develops after successive releases of fully sterile insects (Whitten, 1971 a). It is pointed out that when normal males and male-linked (Y-linked) partially sterile males are mixed together in a population, there is bound to be a rapid reversion to fixation of normal males (Whitten, 1971 a; Curtis and Hill, 1971). Reasons for this will be discussed again later.

Laven et al., (1971 c, d, 1972) prevented the normal seasonal increase in a small isolated wild population of C. pipiens by releasing male-linked translocation heterozygotes. Their field trial was carried out at the isolated village of Notre Dame near Montpellier in southern France. This particular site was chosen because the wild population was derived from a single breeding source (a well) which allowed accurate daily counting of the numbers of adult mosquitoes produced. Seasonal increase of the natural population started in late June and early July, rising from emergence of a few hundred mosquitoes per day to more than 20,000 per day in mid-August. Release of translocation carriers started on 1st August, but for the first two weeks, the numbers of released males were much lower than the number of wild males emerging from the well. Only from August 18 onwards did the ratio of released males and wild males reach 1 : 1. From then on, however, an average

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of 5 : 1 ratio was maintained for six weeks. Artificial breeding containers were provided and all egg rafts laid in these containers were tested for semisterility. The first egg-rafts with semisterility were found 5 days after the first release and the percentage of such rafts increased steadily to 70% in early September. By the end of September this figure reached 95%. Parallel with the increase of egg-rafts of low fertility, the daily production of wild adult mosquitoes went steadily down from a peak of 20,000 to little more than 100. Observation continued in the following year without release. Laven et al., (1972) then found 365 out of 420 egg rafts laid by females coming out of hibernation to be semisterile. They also noted that the daily egg-raft production in 1971 was very much lower than in the preceding year and remained so until the end of July. This was interpreted as the effect of the translocation in causing an initial reduction in density followed by enhanced pressure on the remainder by a constant predator population. Further observations by Cousserans and Guille (1972) showed this situation to continue until the end of the first week in September.

The claim that the reduced number of egg rafts in 1971, compared with 1970, was attributable to the effect of the translocation has recently been retracted by Cousserans and Guille (1974) (quoted by Curtis, 1975). They point out that this reduction is better attributed to a change in the experimental design between the two years. They also found that the translocation frequency in egg rafts monitored during 1971-1973 showed a steady decline from 80% in April 1971 to less than 1% in September 1973. Recently, all published data on experimental releases of C. pipiens at Montpellier were critically re-examined by Curtis (1975). He concluded that a steady decline in translocation frequency after termination

of releases observed by Cousserans and Guille agreed with the theoretical expectation, because of the selective disadvantage of translocation heterozygote males.

By modifying Laven's original proposition, Rai et al., (1970) suggested that "population suppression following releases of males in which the female chromosome is translocated should be more rapid than for comparable releases of males in which the male determining chromosome is translocated". Their argument rests upon two factors. Firstly, the female progeny of the translocation heterozygote will themselves be semisterile. Secondly, when such a female mates with a male heterozygous for the same translocation a further enhancement in sterility will occur.

Later, McDonald and Rai (1971) evaluated these proposals with the computer by simulation under several different release programmes. These simulations indicated that with T^M (linked to the M-chromosome) single heterozygotes and six introductions in the ratio of 4 : 1 (released to resident males), a resident population with a reproductive potential of one would be eradicated in eleven generations. With T^m (linked to the m-chromosome) males, eradication would be achieved in six generations. With double heterozygotes, eradication would occur in five generations even with only four instead of five releases. The double heterozygote is capable of eradicating in seven generations if the same number of males were used per generation but in a ratio of 5 : 1, whereas a single heterozygote in the same control strategy would not be effective in achieving eradication when the reproductive potential is only doubled.

After these computer simulation tests, Rai et al., (1973) conducted field experiments designed to determine whether an alien genotype can be incorporated into a natural population and maintained for several

generations without any subsequent introductions. Field release of heterozygous translocated males of Ae. aegypti was carried out in a natural population in a tyre dump at Model Basti, near New Delhi, India. Males from the translocation stocks were outcrossed with virgin females of the Delhi strain. The male progeny from this cross were again outcrossed with fresh Delhi females. Males from successive crosses were used for release purposes. The first batch was released in early August and continued for 22 days. Approximately 46,000 translocated males were released during this period. Thus, the release covered approximately one generation span of Ae. aegypti. Samples of females and eggs were collected regularly up to the beginning of October. Reduced hatching rate amongst eggs laid by the females in the laboratory showed that the translocated males did mate with the wild females. The highest percentage of wild females found to have been inseminated by translocated males was 40.9% and significant numbers of females were being inseminated by the translocation males 5 weeks after the last release. These are unlikely to have been the males originally released, which would not live so long, and are therefore evidence that the translocation persisted in the population. Observations were also made on the hatching rate of eggs collected in the area. The average hatch in the wild population was 94.5%, with a range of 80-100% and egg batches showing less than 80% hatch were tentatively considered to be the result of crosses between translocated males and wild females. Such egg batches, with hatches ranging from 30-79%, were collected regularly from mid-August onwards in sites dispersed throughout the area. From these results the authors concluded that with proper manipulation it should be possible to use genetic methods to control

populations of Ae. aegypti.

In the course of a research programme directed against the housefly, Musca domestica, Wagoner et al., (1969) independently proposed the use of autosome-autosome and Y-autosome multiple translocation heterozygotes for control purposes. Wagoner et al., (1971) then conducted cage experiments using two strains (A and B) heterozygous for translocations between three autosomes and one strain (C) heterozygous for reciprocal translocations between the Y chromosome and two autosomes. The strains used had fertilities reduced to 33.5% (A), 30.7% (B) and 27.3% (C) of normal respectively. Translocation-bearing males or males and females were placed in laboratory cages at 9 : 1 ratio with virgin wild-type males and females. The reduction of fertility (fertility coefficient) of resulting matings was assessed. Results of experiments revealed that with translocations A and B, the fertility coefficient ranged from 10.3% to 21.6% of controls in the first cross and the accumulated fertility coefficient through four generations ranged from 1.0% to 6.4%. The corresponding figures for the C translocation were 42%-45% and 34.5%. It was thus concluded that the translocation involving three autosomes was more efficient at reducing fertilities than the translocation in which the Y chromosome and two autosomes were translocated. The authors pointed out that if the greatest reduction in fertility, obtained in one generation (89.7%), were maintained it should hold a fly population static throughout the season.

Several arguments have since been raised against this approach as a practical means of insect control (Whitten, 1971a). These objections are based on the practical difficulties of separating fertile siblings from the translocation heterozygote carriers before release of the latter groups.

The most hopeful method of achieving this would be through the use of appropriate markers. Such techniques are not yet perfected.

Wagoner et al., (1973) later performed several field release experiments against houseflies using a heterozygous chromosomal translocation which reduced fertility to 32.5% of control. These males were released at a poultry house in a pine forest near Gainesville, Florida, U.S.A., during the summer of 1969 to study methods of release and to determine whether released flies would introduce the translocation into the native population. Also, a laboratory simulation of the outdoor release was conducted in a 19-cubic-metre room. When the percentage fertility was based on the number of pupae obtained from a given number of eggs, the average for the control population during the 4 generation test period was 78.2 $\pm$ 1.1%. The flies at the forest release site had a statistically significantly lower average of 63.5 $\pm$ 1.3%, but it was higher than expected. The fertility in the laboratory simulation averaged 27.7%, which was more in line with the theoretical expectation. It was thus concluded that the test was a limited success. However, the genetically-engineered strain did mate with the native strain and did introduce some degree of lower fertility. This provided important information on release methods and on the breeding behaviour of genetically-altered flies in the field.

MATERIALS AND METHODS

Colonies used in the investigations

Two strains of An. gambiae species A from West Africa have been used in the current investigations on chromosomal translocations. The PALA strain, from Upper Volta, has been colonized in the insectary in London since 1969. This strain is homozygous for dieldrin resistance (R^{Dl}/R^{Dl}) and for the dominant gene controlling white collar (C^{+}/C^{+}) in the larvae. The 16C strain, originated from Lagos, Nigeria and has been colonized in London since 1951. This strain is susceptible to dieldrin (R^{+}/R^{+}) and is homozygous for the recessive gene collarless (C/C) in the larvae. Line TYF-5, one of the existing Y-autosomal translocation, derived from the PALA strain, has been maintained in the insectary since 1971. This line was isolated by Dr. E. S. Krafsur from males X-irradiated at 4,000 rads, the result of a chromosome translocation involving the Y-chromosome and one of the two autosomes.

Insectary methods

The adult mosquitoes of the stock strains and the mutant (translocation) lines were housed in 12 and 8 inch-cube mosquito-net cages and were maintained at temperatures of 26 °C-28 °C, and relative humidity of between 70 and 80%. Artificial light was provided by fluorescent ceiling lights and the Venner time switch automatically switched the light on and off with a day : night ratio of 12 : 12 hours. Mating took place readily in these cages under this lighting regime. Cotton wads soaked in 10 per cent glucose solution were provided

Figure 2.

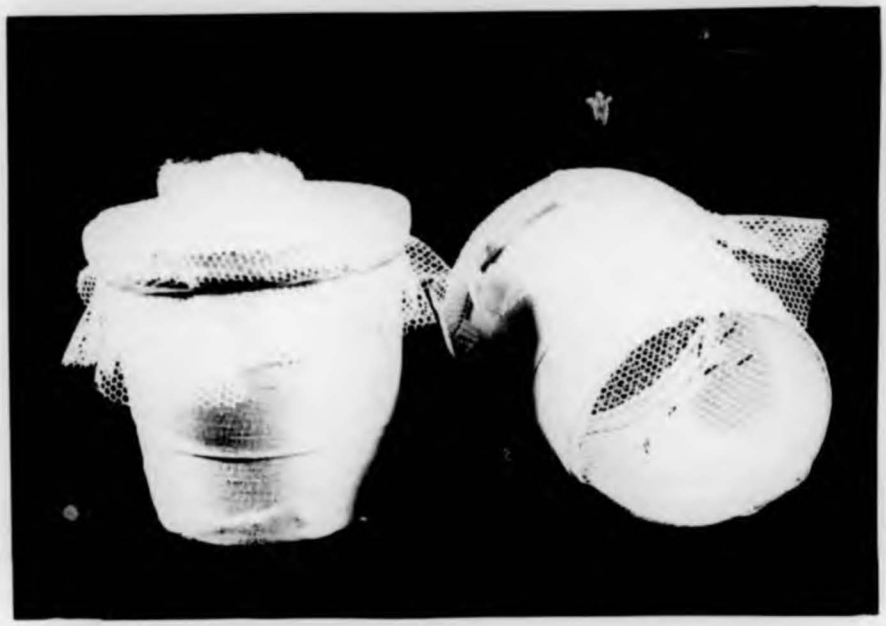
A. Paper cups, covered with mosquito netting at top and bottom, containing mosquitoes ready for feeding on human arm.

B. Method of allowing mosquitoes to feed on human arm while contained in paper cups.

at top and

eding on

man arm while



A

B

continuously and renewed twice weekly. Blood meals were usually provided twice weekly; guinea-pigs anaesthetized with Nembutal® were laid on top of the cages and mosquitoes allowed to feed on them through the netting. If further feeding was required, the mosquitoes were transferred to a paper cup and fed on a human arm (Fig. 2B). Cups were covered with mosquito netting at the top and bottom (Fig. 2A). Oviposition normally occurred 3 days after blood meals and most oviposition occurred during darkness, although some females laid eggs during the day. A 5-inch enamel bowl lined with filter paper containing tap water was introduced into each rearing cage for egg laying. After oviposition the eggs were transferred to the larval rearing room. Hatching occurred within 48 hours. Larval rearing took place in rooms separated from adults. The temperature was maintained at 27°C-30°C (water temperature was approximately 25°C). 100 - 200 newly hatched larvae were reared in white plastic bowls (upper diameter 30 cm, lower diameter 26 cm and depth 10 cm). The rearing bowls were filled with about 1,500 cc of tap water to which was added a piece of grass turf. The turf is thought to provide micro-organisms for the larvae though the principle source of nourishment was in the form of finely ground Farex (a baby food of 2.5% fat, 14.2% protein, 72.7% carbohydrate, 3.6% mineral salts, 0.5% fibre and 6.5% moisture) sprinkled onto the water. The second and subsequent stage larvae were further fed with small amounts of Farex daily. Pupation occurred in the larval medium. Each bowl was covered with mosquito netting and adults were allowed to emerge under these covers and were transferred to cages on the morning following emergence. The duration of the life cycle (egg to adult)averaged 9 days. Parasites such as

viruses, rickettsiae, bacteria, spirochaetes, fungi, protozoans and nematodes are known to be harmful in laboratory colonies of mosquitoes (Kramer, 1964). Krafsur (1972b) showed that a remarkable improvement in longevity and productivity was obtained by observing the following measures. Eggs of stock material, to which mutant lines were crossed, were routinely washed with and left to stand in approximately 0.5% formalin solution for 4-6 hours. Additionally cages and glucose wicks were washed with bleaching solution after their use. These precautions were carried out throughout the entire study.

Cytological technique

Examinations were made of the polytene chromosomes of larval salivary glands prepared by the methods of Coluzzi and Sabatini (1967) and of ovarian nurse cell tissue by the method of Hunt (1973). The half-gravid females were fixed in "Carnoy's" fixative (1 : 3 mixture of glacial acetic acid and absolute ethyl alcohol) for at least 24 hours. After fixation the ovaries were dissected out in a small quantity of Carnoy's fixative and placed in a 50% aqueous solution of propionic acid for 1-2 minutes until they had swollen to approximately twice their original size. The dissection was completed on a clean microscope slide where the ovaries were separated and each one macerated with a dissecting needle in separate drops of 50% propionic acid. A small drop of diluted aceto-orcein was added to each preparation. The stain was prepared according to the method of French et al., (1962). The macerated tissue was agitated in the stain with a dissecting needle to ensure even staining. The stain was then drawn off with absorbent paper and the tissue washed with several changes of 50% propionic acid.

The staining time was approximately half a minute. A siliconised cover slip was placed on each ovary and the preparations were squashed by tapping with a needle.

Insecticide (Dieldrin)-testing

The testing procedure followed was the standard World Health Organization adult mosquito susceptibility test (WHO, 1963). One-day-old male and female adults were introduced into holding tubes, 125 mm long by 44 mm in diameter, lined with clean paper. A maximum of 30 mosquitoes were enclosed in each tube. These were then transferred to the exposure tube lined with the insecticide impregnated paper. Concentrations of 0.4 and 4.0 per cent dieldrin were used. The two concentrations were the discriminating dosages established for dieldrin resistance (Davidson, 1958). The lower dosage of 0.4 per cent dieldrin for one hour killed all susceptibles but not hybrids or homozygous resistant individuals. The upper dosage of 4.0 per cent dieldrin for two hours killed all hybrids, but homozygous resistants survived this dosage. After mosquitoes were exposed to the dieldrin for the required test period they were transferred again to the clean holding tubes. A piece of cotton wool, soaked in 10 per cent glucose solution, was placed on the netting and the tubes kept for twenty-four hours when the mortality was recorded.

Hatching rate studies

Estimates of the fertility of single matings were obtained by hatching rate studies. Gravid females were removed from the cages and placed individually into levelled 2.3 x 7.6 cm glass vials. The vial

was covered with a piece of netting held by a rubber band and a small amount of water was added for oviposition. Racks each containing up to 40 vials were placed in the adult rearing room. After laying, each egg batch was counted and recorded under 3X magnification using a hand tally counter. No formalin treatment was made in case of single egg batch. Untanned and sunken eggs and batches with less than 40 eggs were omitted from the data. Usable egg batches were then left for 48 hours to hatch. The number of first instar larvae was counted for each egg batch with the aid of a finely drawn pipette; larvae were removed from the egg bowl as they hatched.

Method of irradiation

Adult male An. gambiae species A were treated with two different doses of X-radiation for induction of translocations. The open surface of round plastic petri dishes 5 cm diameter x 1.2 cm in depth was covered by a tautly stretched X-ray permeable material (Cellulose Acetate). A centrally-placed cross shaped slit, each line being of approximately one inch, was cut into the surface of this material with a sharp razor blade. It was then possible to place 20-25 virgin adult males into each dish by taking them into a sucking tube and blowing them carefully in by way of this central slit, which moved back into place as the sucking tube was removed. (see Fig. 3A). X-ray treatments were administered to the young adult males held in the petri dishes described above and placed under the ray tube of an X-ray machine, MACHLETT AEG 50, with tungsten target and beryllium window, as shown in Fig. 3B. It was operated at 50 KV_p, 20 cm anode specimen distance with 0.265 mm

Figure 3.

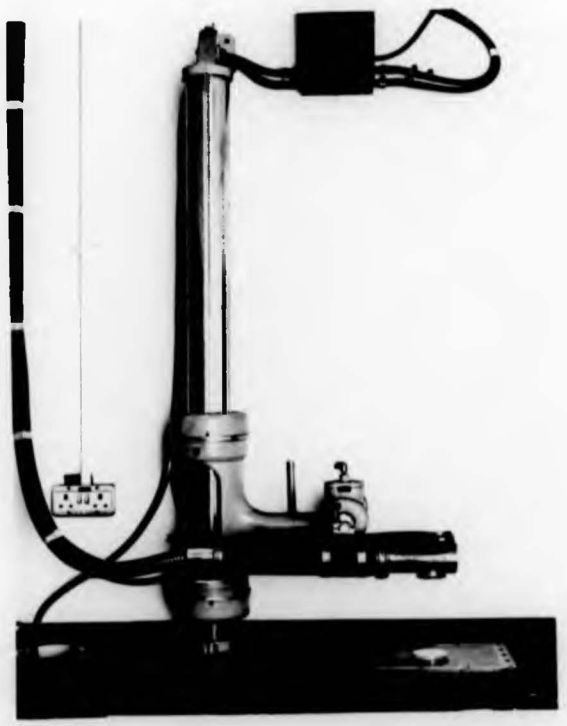
A. Method of placing young adult males in plastic petri dish using a sucking tube.

B. Young adult males held in the petri dish placed under the ray tube of an X-ray machine.

lastic petri dish

placed under

A



B

of Aluminum added filtration. X-radiation of mosquitoes was performed by using a dose rate of 650 r/minute to give a total irradiation dose of 4,000 rads and 1,500 rads respectively. Irradiations were carried out at approximately 19 °C under aerobic conditions.

Production and isolation of partially sterile lines

Following X-radiation irradiated males were mated with approximately equal or greater number of untreated virgin wild-type females, sibs of the irradiated males. Mass ovipositions from first and subsequent blood meals were obtained. Eggs were treated with 0.5% formalin solution as described earlier and then reasonable numbers of eggs were distributed into egg bowls. Hatching rate was recorded to estimate the dominant lethality induced in the sperm by the irradiation. F_1 larvae were reared under uncrowded conditions and F_1 adults were separated by sex at emergence and the yield was recorded. F_1 males were outcrossed to the wild type females (PALA strain) and F_1 females were outcrossed to the wild type males. As many single ovipositions as possible were checked for hatching rate and successive egg batches were obtained from each female, where possible, to see if partial sterility was consistently expressed and to obtain more offspring from the outcrosses. A stock of wild-type newly emerged males and females were always available for use in crossing the resulting offspring of the 2nd and 3rd ovipositions. In the absence of visible genetical markers, the hatching rate of F_1 outcrosses was relied upon to show possibility of the presence of the translocations. The progeny of partially sterile matings were reared to obtain the F_2 generation. Newly emerged male

and female adults were caged separately and crosses between partially sterile lines and the wild type strain were accomplished by mass matings in cages. It has been shown that in An. gambiae species A matings in 8-inch-cube cage require at least 15 males to 10 females to produce adequate insemination (Davidson, personal communication). If this number of adults was unobtainable the line was discarded. F_2 adults were reciprocally outcrossed to the wild-type and selected for partial sterility. The same procedure of selection for partial sterility with subsequent outcrossing was followed until a clear cut pattern of inheritance emerged. This is essentially the same as the method first used by Krafsur (1972 a, b).

The method of isolation of different types of reciprocal translocations

The procedure that was adopted for the isolation of different types of reciprocal translocations was as follows:-

To isolate a three-chromosome double interchange, one of the existing Y-autosome translocation stocks (TYF-5) was subjected to 4,000 rads X-radiation and then the following procedure was carried out:

- F_1 sons of irradiated males were crossed to wild-type females.
- The hatching rate of as many single matings as possible was tested and egg batches showing 30% or less hatch were selected.
- The offspring of the above matings were reared to obtain F_2 progeny.
- F_2 males were crossed to wild-type females and F_2 females to the wild-type males.
- F_2 male outcross matings showing 25% hatching rate or less were selected and reared to the F_3. The hatching rate of outcrossed F_2

females was checked and found to be normal as expected. As many matings as possible were tested in subsequent outcrossed generations.

If all male F_n matings with wild-type females show hatching rate of 25% or less and all F_n sisters show normal hatches when outcrossed, a three-chromosome double interchange with tight linkage to the Y-chromosome is indicated.

To isolate interchanges from daughters of irradiated males the following regime was used:-

- F_1 daughters of irradiated males were crossed to wild-type males.
- The hatching rates of as many single matings as possible were tested and egg batches with a hatching rate of 60% or less were selected and reared to obtain the F_2.
- F_2 adults were separated by sex at emergence and reciprocally crossed to the wild-type parent strain. The hatching rate of the F_2 matings was examined and if approximately one-half of each sex were partially sterile, the outcrossing in the F_3, F_4 etc. was continued.

If partially sterile lines show a close approximation to a 1 : 1 ratio of normal to partial sterile matings of both sexes when outcrossed to the wild type, reciprocal translocation between two autosomes is indicated.

A reciprocal interchange between the X-chromosome and an autosome isolated through outcrossing F_1 daughters of irradiated males behaves similarly to that of an autosomal interchange heterozygote in terms of hatching rate. One half of each sex of the progeny of heterozygous females will themselves be heterozygotes. The presence of

X-autosomal translocations can be detected by outcrossing the progeny of heterozygous males. Should all the daughters prove partially sterile and all their brothers of normal fertility, then an X-autosomal translocation is indicated.

To isolate homozygous autosomal translocations, the following procedure was carried out: After outcrossing several generations, most of the members in both sexes of selected families give a reduced hatching rate. At this stage the progeny of semisterile outcross matings (i.e. of 40-50% hatching rate) can be intercrossed in an attempt to isolate translocation homozygotes resulting from heterozygote-by-heterozygote matings. A single translocation heterozygote would be expected to produce an equal number of $^{+}/+$ and $^{T}/+$* individuals when outcrossed to the wild-type. These progeny, when intercrossed, produce matings in the proportions $\frac{1}{4}$ ($^{+}/+ \times {}^{+}/+$) : $\frac{1}{2}$ ($^{T}/+ \times {}^{+}/+$) : $\frac{1}{4}$ ($^{T}/+ \times {}^{T}/+$). Only one-quarter of the inbred matings are heterozygote by heterozygote. The lowest fertility is expected in $^{T}/+ \times {}^{T}/+$ matings. This fertility could be roughly estimated as the product of the fertilities of the translocation heterozygote males and translocation heterozygote females when mated to wild-type but viable zygotes produced by complementary aneuploid gametes must be taken into account in the estimation. The progeny with least fertile matings (35% hatching rate or less)

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* $^{+}/+$ wild-type

$^{T}/+$ translocation heterozygote

T/T translocation homozygote

were again selected for interbreeding in an attempt to produce pure T/T lines. The relative frequencies of karyotypes present among the progeny of $^{T}/+ \times {}^{T}/+$ matings (i.e. $^{+}/+$, $^{T}/+$ and T/T) would depend upon the ratio of alternate and adjacent segregations in the parents. For example it is frequently the case that 50% alternate segregation and 50% adjacent -1 segregation occurs in both sexes. Therefore, the frequency of the different zygotes produced from such a cross is 1/16 wild type, 4/16 translocation heterzygote, 1/16 translocation homozygote and 10/16 inviable unbalanced zygotes. Assuming that translocation homozygotes are viable and fully fertile (similar to the wild-type), then $^{T}/+ \times {}^{T}/+$ matings would give a hatching rate of 37.5% but where T/T is lethal the hatching rate would be 31.25%. The fertile progeny are either wild-type individuals or translocation homozygotes. Translocation homozygotes can be distinguished from the wild-type by crossing tests. When the fertile F_2 mosquitoes are outcrossed to the wild-type strain, all the progeny are either fertile or partially sterile, indicating that the fertile parents had wild-type chromosomes or homozygous translocation chromosomes, respectively. This method depends entirely on the hatching rate as the criterion used for the designation of karyotype.

Statistical analysis of the results

The percentage hatching was calculated for each mating and hatching rates were grouped in intervals of 5% over a range of 1% to 100%. Egg batches showing no egg hatch were excluded from the grouped data. The arcsin transformation of Bliss (Snedecor and Cochran, 1967), where the transformed angle equals $\text{Sin}^{-1}\sqrt{\%\ \text{hatch}}$, was used to normalise the grouped data for statistical analysis. Statistical analysis was made of the data, where appropriate, using the Olivetti Programma 101 machine

programmed for the Student's t-test, variance ratio (F-test), and Chi-squared test (with Yates' correction (2 x 2 contingency test), when appropriate).

RESULTS

A. Production and isolation of partially sterile lines

Hatching rate studies in stock strains

Egg hatches of PALA and 16C strains (and the intercross strain) used in this experiment were measured to study the natural variation. Results are shown in Table 1 and the frequency distribution of hatching rates is plotted in Fig. 4. A total of 101 matings in the PALA strain had been previously examined by Dr. E.S. Krafsur and found to have an average hatching rate of 87.7%. The proportion of matings that had 'normal fertility' (hatching rate more than 65%) was 90% (91 of these 101 matings). For the 16C strain, the average egg hatching rate was 72.5% in 72 matings. The proportion of these matings that were 'normally fertile' was 67% (48 of these 72 matings). The hatching rate in crosses between PALA and 16C strains was 82.9% in 59 matings. The proportion having 'normal fertility' was 85% (50 of these 59 matings).

The hatching rate in the two strains, PALA and 16C, differs considerably ($F_{100, 71} = 3.18$, $P < 0.01$; $t_{171} = 4.39$, $P < 0.01$) and the proportion of matings having partial sterility (65% hatching rate or less) amounted to 10% in PALA and as much as 33% in the 16C strain. Crosses between PALA and 16C strains showed reasonable fertility, although 15% of those crosses gave partial sterile results. However, it was possible to use the cross PALA x 16C to study linkage between semi-sterility and collar and dieldrin-resistant genes as will be described later (page 194).

Table 1. Showing the frequency distribution of hatching rates in stock strains - PALA, 16C and intercross strain (PALA x 16C).

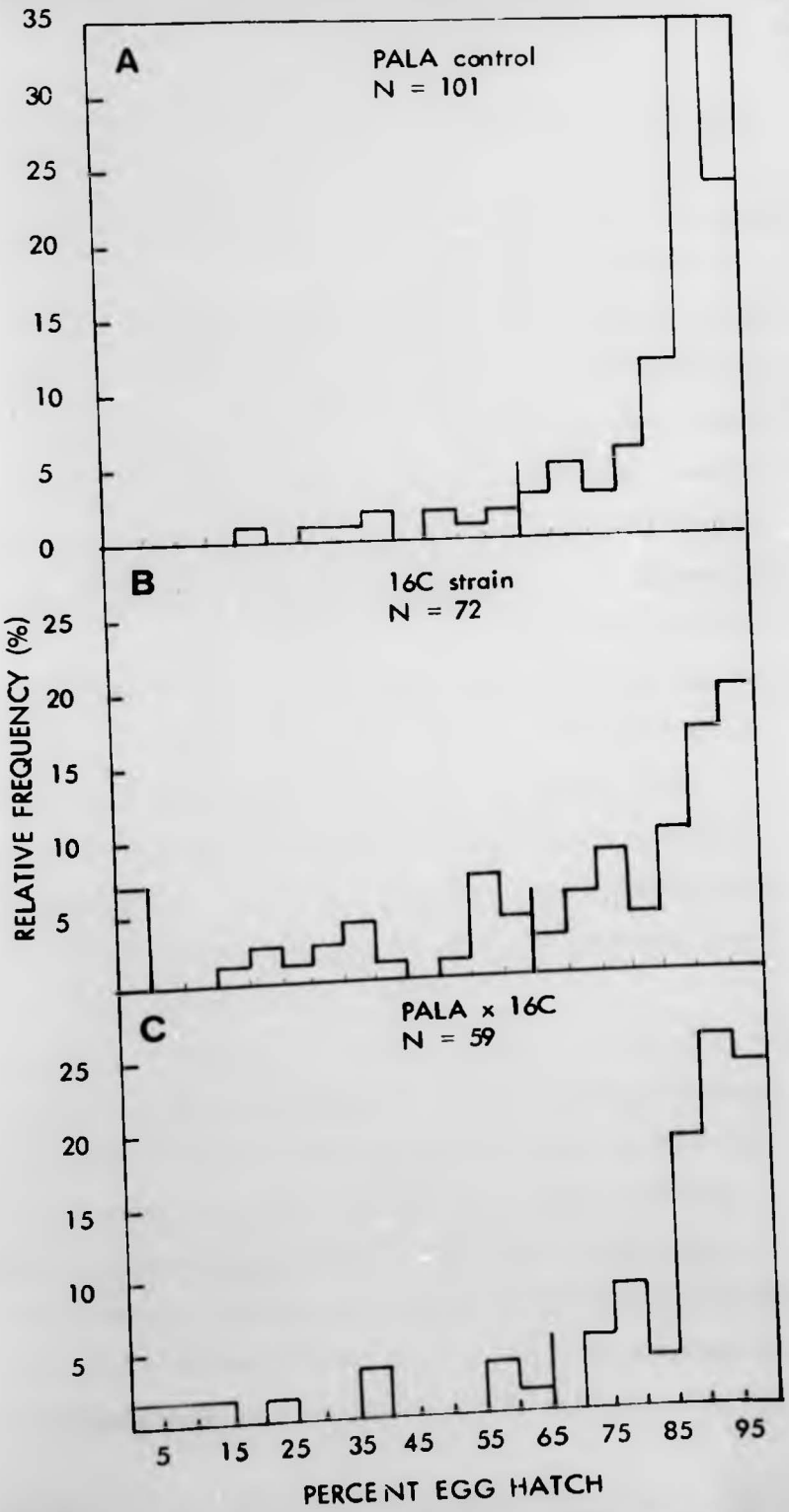
Class interval (%)	No. of matings within class interval		
	PALA strain (after Krafsur, 1972b)	16C strain	Intercross strain (PALA x 16C)
sterile	5	4	3
1-5	0	5	1
6-10	0	0	1
11-15	0	0	1
16-20	0	1	0
21-25	1	2	1
26-30	0	1	0
31-35	1	2	0
36-40	1	3	2
41-45	2	1	0
46-50	0	0	0
51-55	2	1	0
56-60	1	5	2
61-65	2	3	1
66-70	3	2	0
71-75	5	4	3
76-80	3	6	5
81-85	6	3	2
86-90	12	7	11
91-95	38	12	15
96-100	24	14	14
Total No. non-sterile matings	101	72	59
No. eggs	11,609	7,269	6,226
No. larvae hatched	10,178	5,277	5,166
Ave. hatching rate (%)	87.7	72.5	83.0
Relative to PALA (%)	100	82.6	94.6

Figure 4. Frequency distribution of hatching rates among strains of Anopheles gambiae species A.

A. Fertilities of 101 matings of the PALA stock strain (Data from Dr. E.S. Krafsur, 1972b).

B. Fertilities of 72 matings of the 16C strain.

C. Fertilities of 59 matings of the intercross strain (PALA x 16C).


A
PALA control
N = 101
B
16C strain
N = 72
C
PALA x 16C
N = 59
RELATIVE FREQUENCY (%)
PERCENT EGG HATCH
0
5
10
15
20
25
30
35
5
15
25
35
45
55
65
75
85
95

Hatching rate studies on existing Y-linked translocation line (TYF-5) before irradiation

To isolate a 3-chromosome double translocation line, TYF-5, one of the existing Y-autosomal interchanges in An. gambiae species A isolated by Dr. E.S. Krafsur in 1971 was irradiated. However, before irradiation, comparison was made between the original hatching rates of TYF-5, as reported by Krafsur, 1971, and the same colony 7 months later. An attempt was also made to select TYF-5 for higher hatching rate with low variance. Members of different families from matings showing more than 55% hatching among TYF-5 stock were selected and reciprocally crossed and examined for F_1 hatching rate. F_1 matings showing more than 55% hatching rate were again selected and reciprocally crossed. The hatching rate of the F_2 was tested. The object was to lessen the probability of obtaining a false result among the male progeny of irradiated TYF-5 by eliminating those TYF-5 which gave low hatching (30%). It was then expected with confidence that hatching rates below 30% after irradiation would be due, in most cases, to new genetic aberration caused by the irradiation.

Egg hatches observed in the above experiments are shown in Table 2. The average hatching rate of the original TYF-5, isolated by Krafsur, was 43% while after 7 months of laboratory inbreeding it was 44.7%. There was no significant difference in egg hatch between these two samples when variance ($F_{49,55} = 1.39$, $P > 0.10$) and mean ($t_{104} = 0.392$, $P > 0.70$) were considered. F_1 and F_2 hatching rates resulting from reciprocally crossing members of different families selected for more than 55% egg hatch were also compared with that of present TYF-5 stock.

Table 2. Showing comparison of the distributed hatching rates of TYF-5 - original stock, the same colony of 7 months later, F_1 and F_2 resulting from reciprocally crossing members of families resulted in more than 55% hatching rate.

Class interval (%)	No. of matings within class interval			
	Original TYF-5*	Present stock (after 7 months lab. inbreeding)	F_1	F_2
sterile	3	7	1	3
1-5	0	1	2	3
6-10	1	2	2	3
10-15	0	0	1	0
16-20	2	0	2	2
21-25	2	3	1	0
26-30	3	4	2	1
31-35	3	0	5	1
36-40	6	4	10	8
41-45	8	6	10	11
46-50	9	15	15	12
51-55	11	14	12	8
56-60	4	5	3	5
61-65	1	2	1	1
Total no. non-sterile matings	50	56	66	55
No. eggs	-	7,276	7,226	6,544
No. larvae hatched	-	3,252	2,984	2,755
Ave. hatching rate (%)	43.0	44.7	41.3	42.1
Relative to PALA (%)	49.0	50.9	47.1	48.4

*Data from Dr. E.S. Krafsur (1972)

The F and t test statistics for the variances and means of the hatching rate of the stock and F_1 matings were $F_{55,65} = 1.07$, $P > 0.20$; $t_{120} = 1.23$, $P > 0.10$; and the F and t test stastistics for the variances and means of the hatching rate of the stock and F_2 matings were $F_{55,54} = 1.47$, $P > 0.05$; $t_{109} = 1.244$, $P > 0.20$. There was no significant difference in hatching rate between the stock and reciprocal F_1 and F_2 crosses selected for hatching rates over 55%. It was concluded from the variances and means of the arcsin transformed hatching rates, that the matings could have been independent samples drawn from a single population. Thus, it was decided that samples for further irradiation should be chosen from the stock of TYF-5 and need not be selected for high hatching rate.

Result of irradiation at 4,000 rads of X-rays

One- and 2-day old TYF-5 virgin adult males were irradiated at a total of 4,000 rads of X-rays and mass mated the same day to untreated virgin wild-type female sibs. Mass eggs deposited by the mated females were collected after first and subsequent blood meals. They were counted and reared. Hatching rates from crosses between the irradiated TYF-5 males and wild-type females, sex-ratios of F_1 progeny and percentage survival from larvae to adulthood were observed. Results are presented in Table 3.

From mass ovipositions, 799 larvae were obtained from 8,555 eggs. The overall egg hatching rate was 9.3% suggesting an average dominant lethality of 89.4% (dominant lethality = $1 - \frac{0.093}{0.877}$). These larvae produced a total of 535 adult progeny of which 47.1% were males. The level of adult emergence was 66.9%. A chi-square test indicated the sex-ratio did not differ from 1 : 1 ($X_1^2 = 1.8$, $P > 0.15$).

Table 3. Showing hatching rates from crosses between irradiated TYF-5 males and wild-type females, sex ratios of F_1 progeny and percentage survival from larvae to adulthood.

Mass egg batches	Total no. eggs	Total no. larvae hatched	Hatching rate (%)	F_1 progeny		% male	% survival (larva to adulthood)
				male	female		
1st	1,765	200	11.3	46	53	46.5	49.5
2nd	4,237	435	10.2	144	174	45.3	73.1
3rd	752	61	8.1	24	22	52.2	75.4
4th	1,801	103	5.7	38	34	52.8	69.9
Total	8,555	799	9.3	252	283	47.1	66.9

Selection for partial sterile lines

The F_1 males were outcrossed to the wild type virgin females and a total of 120 single ovipositions were obtained with an average hatching rate of 58.6% (6,191 larvae hatched from 10,558 eggs). The distribution of hatching rates was compared with that of TYF-5 parent stock and it was found that there was no significant difference in hatching rate between the two populations, because variances and means did not differ significantly ($F_{55,119} = 1.77$, $P > 0.05$; $t_{174} = 0.55$, $P > 0.65$). The frequency distribution of hatching rate of TYF-5 stock and F_1 sons of TYF 5 males exposed to 4,000 rads are shown in histogram form in Fig. 5 A and B.

Eighteen of the 120 matings initially showed 30% hatching rate or less (see Table 4). Four of these subsequently showed a hatching rate of 40% or greater (22%) while 8 matings left too few progeny for further study. Two matings, namely 1P and 3K showed a hatching rate of 30% or less in the F_1 but when the F_2 outcross progeny were examined, these proved to have a 40% hatch or more. The remaining 4 lines of F_1 males (1C, 3H, 3U and 20J) when outcrossed to the wild type showed a hatching rate of 28% or less in the first oviposition and the same level of hatch continued to occur in subsequent egg batches. These were therefore selected and retained for further study.

The daughters of TYF-5 males treated at 4,000 r., when outcrossed to wild-type, showed an average hatching rate of 80.4% in 92 matings (10,463 larvae hatched from 13,019 eggs). (Fig. 5 C). Of 14 matings initially showing 60% or less hatch (Table 5), three subsequently showed normal fertile ovipositions (21%) and 3 matings could not be studied further due to inadequate number of progeny. Eight of the original 14

Figure 5. Frequency distribution of hatching rates of the Y-autosomal translocation line.

A. TYF-5 stock (7 months of laboratory inbreeding).

B. F_1 sons of TYF-5 males exposed to 4,000 rads.

C. F_1 daughters of TYF-5 males exposed to 4,000 rads.

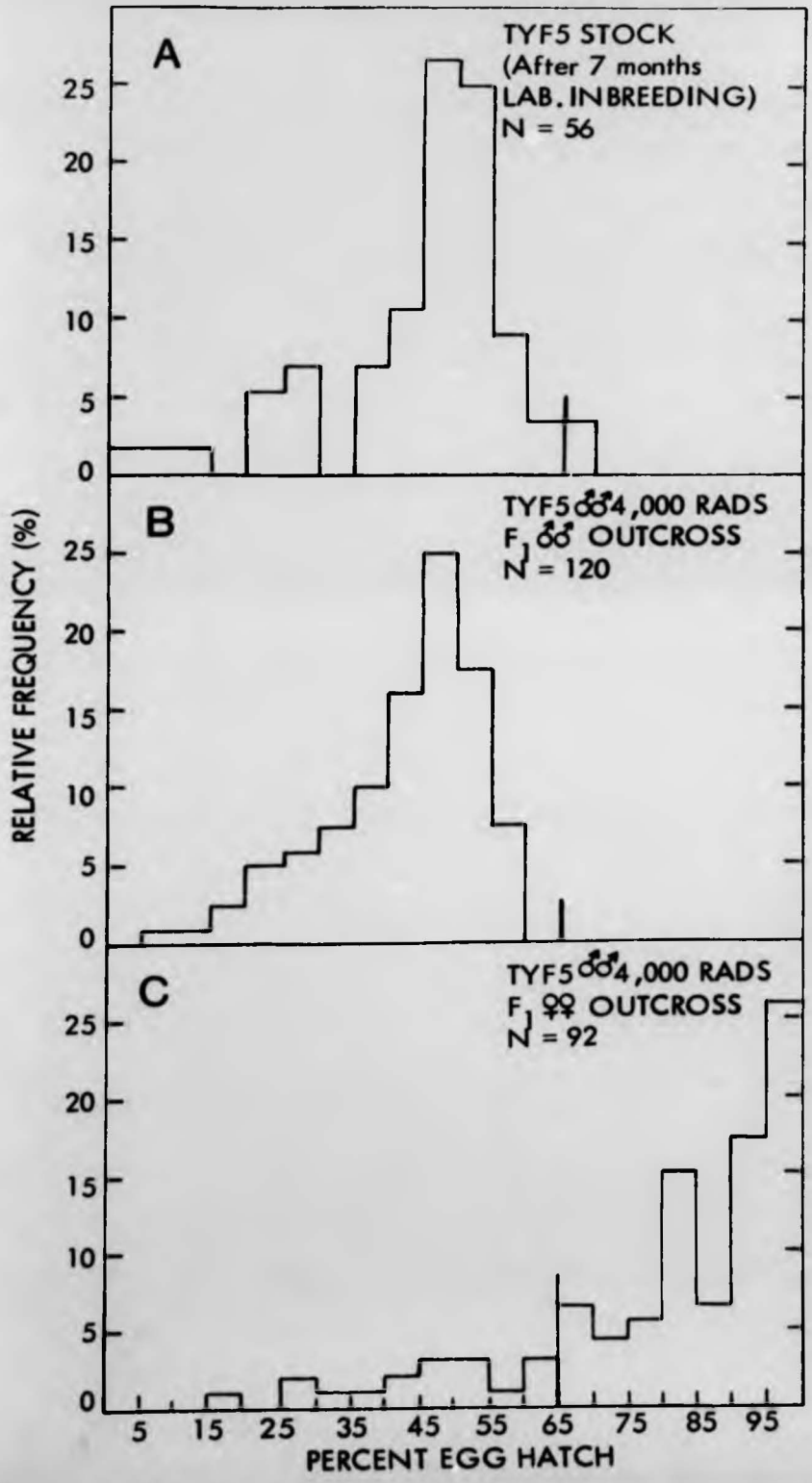

A
TYF5 STOCK
(After 7 months
LAB. INBREEDING)
N = 56
B
TYF5 ♂♂ 4,000 RADS
F1 ♂♂ OUTCROSS
N = 120
C
TYF5 ♂♂ 4,000 RADS
F1 ♀♀ OUTCROSS
N = 92
RELATIVE FREQUENCY (%)
0
5
10
15
20
25
5 15 25 35 45 55 65 75 85 95
PERCENT EGG HATCH

Table 4. The egg hatching percentage of successive batches of eggs in 18 females initially showing less than 30% hatching rate (F_1 sons of irradiated TYF-5 males outcrossed to the wild-type)

Line	First oviposition			Second oviposition			Third oviposition		
	No. eggs	No. larvae	%	No. eggs	No. larvae	%	No. eggs	No. larvae	%
1C	93	24	25.8	95	20	21.0	63	10	20.0
3H	65	18	27.6	85	19	22.3	106	17	16.0
3U	115	24	20.8	70	14	20.0	152	39	25.6
20J	158	23	14.5	143	5	3.4	117	8	6.8
1P	55	16	29.0	50	0	0.0	103	25	24.2
3K	122	34	27.8	74	20	27.0	-	-	-
2F	101	27	26.7	-	-	-	-	-	-
3F	79	14	17.7	-	-	-	-	-	-
3V	111	29	26.1	110	28	25.4	-	-	-
4F	86	9	10.4	-	-	-	-	-	-
5B	75	16	21.3	-	-	-	-	-	-
5F	97	24	24.7	56	8	14.2	73	9	12.3
5G	83	17	20.4	-	-	-	-	-	-
200	83	20	24.0	-	-	-	-	-	-
41	150	35	23.3	93	38	40.8	-	-	-
20S	110	15	13.6	121	64	52.8	-	-	-
20T	130	20	15.3	69	28	40.5	-	-	-
1U	61	15	24.5	34	14	41.0	-	-	-

Table 5. The egg hatching percentage of successive batches of eggs in 14 females initially showing less than 60% hatching rate. (F_1 daughters of irradiated TYF-5 males outcrossed to the wild-type)

Line	First oviposition			Second oviposition			Third oviposition		
	No. eggs	No. larvae	%	No. eggs	No. larvae	%	No. eggs	No. larvae	%
6K	114	54	47.3	89	44	49.4	98	59	60.2
7G	164	78	47.5	139	83	59.7	90	38	42.2
8B	184	79	42.9	84	21	25.0	-	-	-
9B	147	76	51.7	-	-	-	-	-	-
26D	104	62	59.6	174	89	51.1	99	51	51.5
26O	121	36	29.7	111	35	31.5	107	47	43.9
6E	49	24	48.9	-	-	-	-	-	-
8M	85	15	17.6	120	73	60.8	132	58	43.9
6Z	42	22	52.3	84	78	92.0	-	-	-
8L	148	82	55.4	190	168	88.4	-	-	-
8R	46	17	36.9	88	58	65.9	-	-	-
8P	206	69	33.4	-	-	-	-	-	-
8Y	125	32	25.6	-	-	-	-	-	-
26M	128	58	45.3	-	-	-	-	-	-

partially sterile matings (presumably translocations carriers) were tested further for occurrence of partial sterility. Twenty five F_1 females initially showing normal fertility (65% hatch or more) were also examined in the second ovipositions, to see if the same level of hatching rate was consistently expressed. Results are shown in Table 6. As can be seen, in 3 of 25 (12%) heterogeneity was shown in successive ovipositions, - similar to the PALA control stock.

Result of irradiation at 1,500 rads of X-rays

Several attempts have been made to isolate homozygous autosomal translocations in An. gambiae species A, but without success so far. The dosage of X-rays used in the past to induce translocations (4,000 r.) might have been too high. Unpublished work on spider mites by Feldmam (personal communication at working group on genetical methods of pest control, ESNA*, Louvain, 1973) has revealed that lower dosages of 500-1,000 r. produced more viable translocations and that these were associated with fewer undesirable recessive lethals. Such low levels of irradiation might also be more suitable for producing viable translocations in mosquitoes. A new series of experiments was therefore carried out to induce translocations in the PALA strain of An. gambiae species A by irradiating young male adults with 1,500 rads of X-rays.

One-day old PALA adult males were irradiated at 1,500 rads of X-rays and mass mated to untreated virgin wild-type female sibs.

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*ESNA European Society of Nuclear Methods in Agriculture, Proceedings 1973 Report of working group 9. Genetical methods of pest control by R.J. Wood, pp 58-60.

Table 6. The egg hatching percentage of successive batches of eggs in 25 females initially showing more than 65% hatching rate (F_1 daughters of irradiated TYF 5 males outcrossed to the wild-type)

Line	First oviposition			Second oviposition			X^{2*}_c	P
	No. eggs	No. larvae	%	No. eggs	No. larvae	%		
6A	76	49	64.4	121	100	82.6	0.957	N.S.
6I	152	152	100.0	136	124	91.1	0.213	"
7A	152	131	86.1	75	69	92.6	0.046	"
7B	104	68	65.3	47	45	95.7	1.787	"
7C	198	163	82.3	61	42	68.8	0.457	"
7E	121	113	93.3	65	62	95.3	0.000	"
7I	140	135	96.4	77	66	85.7	0.218	"
7J	112	76	67.8	144	88	61.1	0.176	"
7M	164	153	93.2	151	143	94.7	0.001	"
7Q	114	97	85.0	98	91	92.8	0.112	"
7U	127	101	79.5	124	124	100.0	1.329	"
7V	218	218	100.0	60	23	38.3	13.045	< 0.001
7W	134	134	100.0	102	102	100.0	0.000	N.S.
7Z	181	181	100.0	125	123	98.4	0.000	"
8A	240	199	82.9	137	126	91.9	0.342	"
8C	206	191	92.7	77	71	92.2	0.004	"
8F	97	70	72.1	100	17	17.0	23.011	< 0.001
8G	161	161	100.0	132	132	100.0	0.000	N.S.
8I	164	155	94.5	98	77	78.5	0.780	"
8S	127	108	85.0	121	107	88.4	0.013	"
8U	96	86	89.5	176	146	82.9	0.102	"
9E	148	112	75.6	179	113	63.1	0.918	"
26K	159	150	94.3	169	78	46.1	15.637	< 0.001
26Q	147	124	84.3	167	164	98.2	0.712	N.S.
26S	73	51	69.8	63	50	79.3	0.124	"

*2 x 2 contingency N.S. - Not significant.

A total of 3,849 larvae hatched from 5,506 eggs when 30 females were examined. The average hatching rate was 69.9% and the mode was between 70-75% (Fig. 6A). A mass oviposition of 9,416 eggs showed a hatch of 6,142 larvae. Thus, the overall hatching rate was 66.9% (9,991 larvae hatched from 14,922 eggs), suggesting a dominant lethality of 23.7%. This compared with a dominant lethality of 89.4% when an irradiation dose of 4,000 rads was used. A vast number of F_1 larvae hatched and most of the larvae could not be reared and were discarded. Only one thousand eight hundred and ninety larvae (approximately 20% of the entire sample) were randomly selected and reared to the adult stage. From these, 1,351 adults emerged (71%), of which 710 were males and 641 females. The sex-ratio did not differ significantly from 1 : 1 ($X^2_1 = 3.52$, $P > 0.05$).

Selection for partially sterile lines

F_1 males were outcrossed to wild-type females and 120 ovipositions were obtained but only 43 were counted with an average of 82.9% hatching rate (4,024 larvae hatched from 4,855 eggs). (see Fig. 6 B). F_1 sons showed a significantly higher fertility when compared with irradiated P_1 males. Of five matings initially showing 60% hatching rate or less (Table 7), 3 proved to be normally fertile in subsequent ovipositions. Line No. 48 showed a reduced hatching rate in the F_1, but when both sexes were outcrossed to the PALA strain all F_2 matings showed normal egg hatches. Only one of the original 5 semi-sterile F_1 sons of irradiated males showed consistent 1 : 1 ratio of normal to partially sterile progeny among both sexes. This line was designated line 102A and was used for further study.

Figure 6. Frequency distribution of hatching rates of PALA strain.

A. PALA adult males irradiated at 1,500 rads crossed to normal PALA females.

B. F_1 sons from crosses between normal PALA females and PALA males irradiated with 1,500 rads.

C. F_1 daughters from crosses between normal PALA females and PALA males subjected to 1,500 rads of X-irradiation.

es of PALA strain.

crossed to

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PALA females and

radiation.

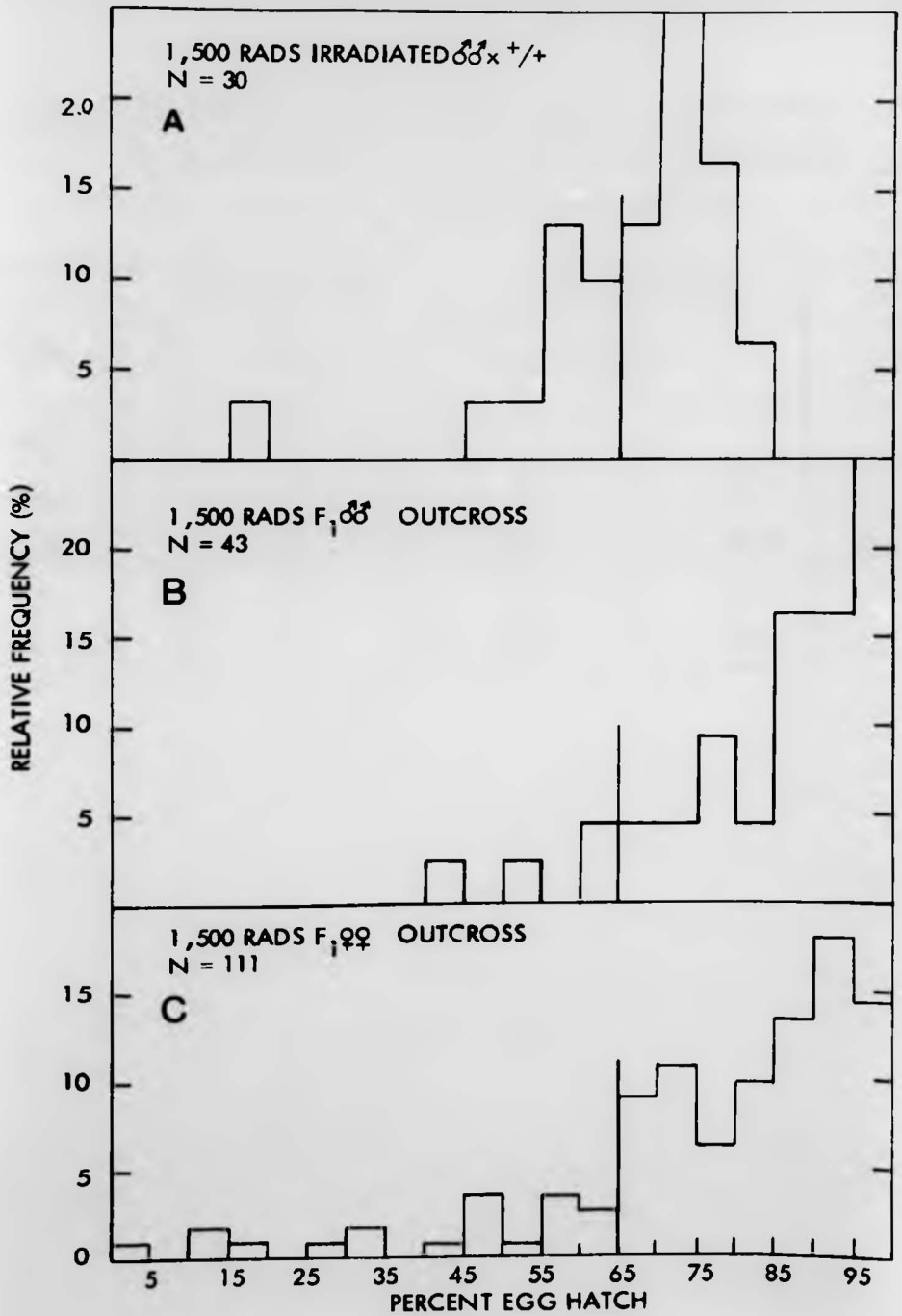

Table 7. The egg hatching percentage of successive batches of eggs in 5 females initially showing less than 60% hatching rate (F_1 sons of irradiated PALA males at 1,500 rads)

Line	First oviposition			Second oviposition		
	No. eggs	No. larvae	%	No. eggs	No. larvae	%
102A	131	57	43.5	-	-	-
104B	102	41	40.1	91	91	100.0
104C	125	14	11.2	86	74	86.0
105H	95	52	54.7	104	95	91.3
No. 48	58	1	1.7	83	42	50.6

F_1 daughters of PALA males exposed to 1,500 rads, when outcrossed to wild-type males, showed an average hatching rate of 78.5% (12,567 larvae hatched from 16,013 eggs) in 111 matings. (see Fig. 6 C). Of 26 matings initially showing 60% hatching rate or less (see Table 8), 9 (29%) subsequently showed more than 60% egg hatch and 2 matings (50V and 54A) left too few progeny for further testing. 8 matings, whose first ovipositions were examined, could not be confirmed by second ovipositions as the females died. Progeny of first ovipositions by these females were therefore not used. One line (55K) showed partial sterility in the F_2 but only normal fertility among 19 F_3 matings. Males and females of the remaining 6 lines were retained for further study.

Table 8. The egg hatching percentage of successive batches of eggs in 26 females initially showing less than 60% hatching rate (F_1 daughters of irradiated PALA males at 1,500 rads)

Line	First oviposition			Second oviposition		
	No. eggs	No. larvae	%	No. eggs	No. larvae	%
50G	83	24	28.9	141	93	65.9
50W	46	21	45.6	79	53	67.0
51L	124	72	58.0	158	142	89.8
52B	89	33	37.0	63	44	69.8
53L	89	12	13.4	115	101	87.9
54B	66	3	4.5	116	72	62.0
54I	89	44	49.4	99	67	67.6
55D	145	82	56.5	102	91	89.2
56T	113	40	35.3	129	88	68.2
50V	80	14	17.5	68	6	8.8
54A	127	14	11.0	50	1	2.0
50E	48	21	43.7	-	-	-
51H	120	61	50.8	-	-	-
53I	127	45	35.4	-	-	-
53V	66	1	1.5	-	-	-
53X	138	66	47.8	-	-	-
54N	102	14	13.7	-	-	-
55L	151	89	58.9	-	-	-
55U	183	104	56.8	-	-	-
55K	145	69	47.5	128	66	51.5
50Q	173	86	49.7	147	79	53.7
53D	111	56	50.4	141	20	14.1
53E	212	61	28.7	168	18	10.7
54E	90	52	57.7	43	12	27.9
54F	128	74	57.8	103	47	45.6
55B	129	42	32.5	85	39	45.8

B. Three-chromosome double translocation lines

Hatching rate studies in male outcrosses

Four lines of F_1 male outcross matings showing 28% hatching rate or less, as described above, were studied further. Outcrosses and selections were repeated for 8 generations in line 1C and 13-15 generations in the other 3 lines (3H, 3U and 20J) for the isolation of 3-chromosome double interchanges. All matings between F_4 and F_7 males crossed with wild-type females in line 1C had shown 25% hatching rate or less, while the rate in the remaining 3 lines in each generation fluctuated in varying degrees (Fig. 7). The average hatching rate was 19.4%, 26.4%, 25.6% and 24.3% in 1C, 3H, 3U and 20J respectively when they were outcrossed to wild-type individuals (Table 9). These figures are 22.1%, 30.1%, 25.7% and 24.3% of the control level respectively. The frequency distribution of egg hatches of these 4 lines are presented in histogram form in Fig. 8 A-D. When sample means were put into an ordered arrangement and t-test was applied on the arcsin - transformed grouped data, the following results were obtained: The means lying above the same horizontal line are not significantly different with $P > 0.05$.

Line	1C	20J	3U	3H
Mean	25.31	26.64	27.56	29.85

The hatching rates of 1C and 20J and also 20J and 3U were found to be from populations with similar means. However, there was a significant difference in hatching rate between 1C and 3U ($t_{274} = 2.50$, $P = 0.015$), 1C and 3H ($t_{312} = 5.48$, $P < 0.001$), 20J and 3H ($t_{271} = 4.05$, $P < 0.001$) and 3U and 3H ($t_{284} = 2.56$, $P < 0.01$). It appeared that

Figure 7. Fertility of four-3-chromosome double translocation lines in successive generations.

translocation

FERTILITY OF FOUR 3-CHROMOSOME-DOUBLE TRANSLOCATION LINES IN SUCCESSIVE GENERATIONS

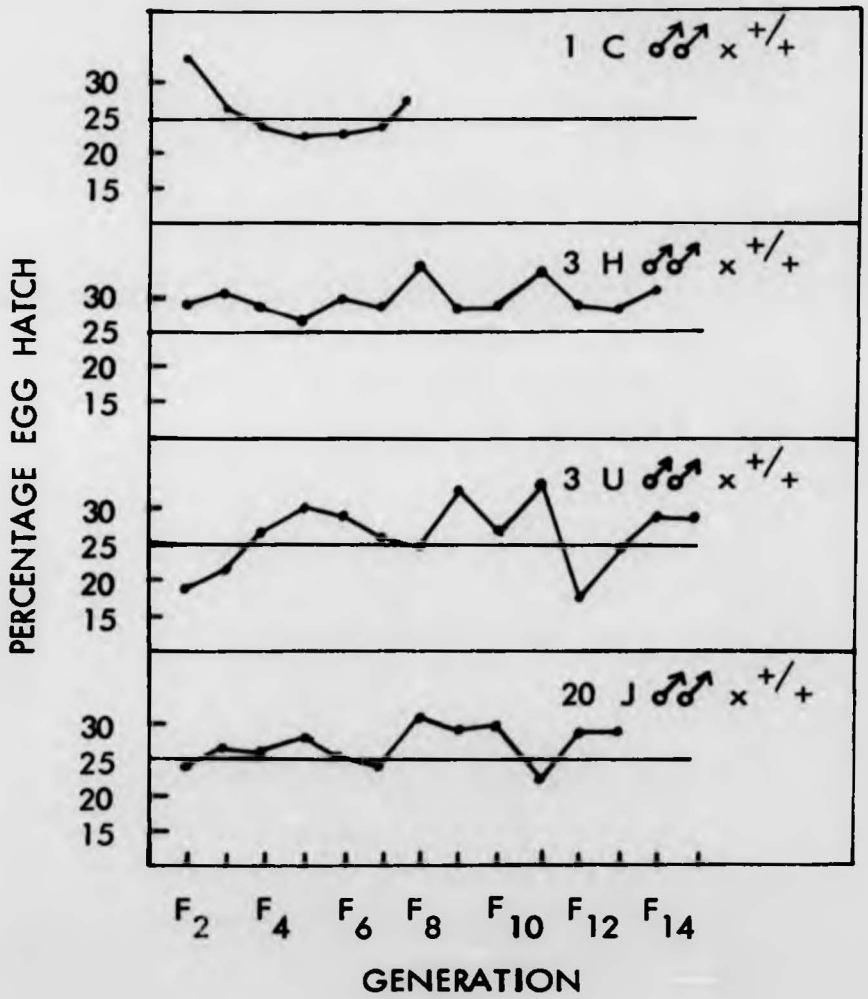

Figure 8. Frequency distribution of hatching rates in four lines of the 3-chromosome double translocation strain.

A. Hatching rates among the outcrossed sons and daughters of line 1C.

B. Hatching rates among the outcrossed sons and daughters of line 3H.

C. Hatching rates among the outcrossed sons and daughters of line 3U.

D. Hatching rates among the outcrossed sons and daughters of line 20J.

Numbers of Females are given in parentheses.

tes in four lines of

ion strain.

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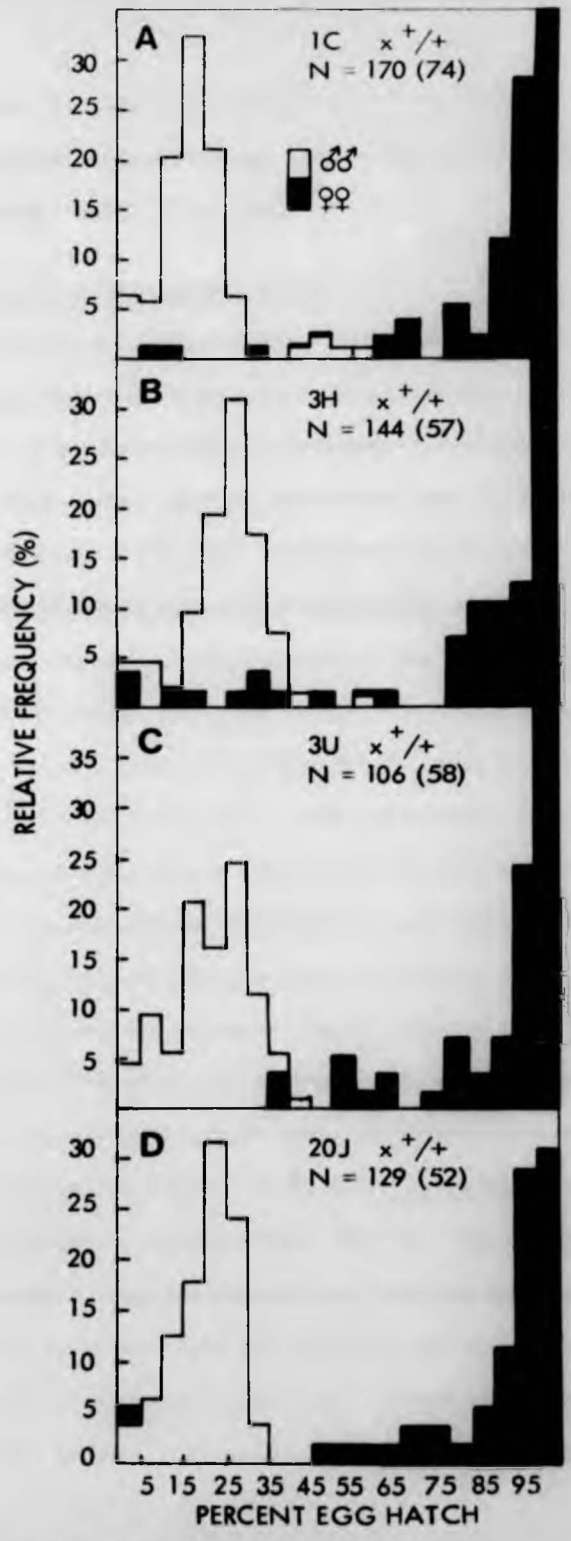


they were samples from different populations. The above results indicated that the mode of meiotic segregation appeared to differ significantly between some of the lines.

Hatching rate studies in female outcrosses

Matings in which daughters of these four lines (1C, 3H, 3U and 20J) were outcrossed to wild-type males, gave hatching rates virtually identical to those of the PALA stock strain (see Table 10). The similarity in the hatching rates in these female outcrosses may be appreciated by glancing at Fig. 8 A-D. Ten of 101 ovipositions in PALA stock showed partial sterility (< 65% hatching rate) a proportion not significantly differing from the proportion when females of 1C, 3H, 3U and 20J were outcrossed to PALA males 9 of 74 in 1C ($X^2_1 = 0.23$, $P > 0.5$), 11 of 57 in 3H ($X^2_1 = 2.79$, $P > 0.08$); 8 of 58 in 3U ($X^2_1 = 0.56$, $P > 0.4$); and 7 of 52 in 20J ($X^2_1 = 0.44$, $P > 0.50$). It was concluded, therefore, that the hatching rates were similar to that of PALA stock and the matings were considered to be of normal fertility, as expected and desired.

Lines 1C, 3H, 3U and 20J have shown holandric inheritance i.e. all the males and none of the females inherited partial sterility. Each strain also exhibited characteristic hatching rate when maintained isolated through several generations after irradiation of existing Y-autosomal translocation males. Wild-type An. gambiae species A has the diploid number of chromosomes, 2n = 6. The chromosome complement includes a long metacentric pair and shorter metacentric pair. Females of the species are the homogametic sex and carry two X chromosomes, whereas in males the X and Y constitute a heteromorphic pair (Mason, 1964; Coluzzi and Sabatini, 1967). Fig. 9 shows, in an

Table 9. The frequency distribution of hatching rates in four lines of 3-chromosome double translocation males outcrossed to wild-type females

Class interval (%)	No. matings within class interval			
	1C males x $^{+}/+$ females	3H males x $^{+}/+$ females	3U males x $^{+}/+$ females	20J males x $^{+}/+$ females
sterile	6	7	0	8
1-5	11	7	5	5
6-10	10	7	10	8
11-15	35	3	7	16
16-20	55	14	22	23
21-25	36	28	17	41
26-30	11	46	26	31
31-35	1	25	12	5
36-40	0	11	6	0
41-45	3	2	1	0
46-50	1	0	0	0
51-55	2	0	0	0
56-60	2	1	0	0
Total no. matings	170	144	106	129
No. eggs	23,086	19,845	13,749	16,271
No. larvae hatched	4,476	5,236	3,104	3,465
Ave. hatching rate (%)	19.4	26.4	22.6	21.3
Relative to PALA (%)	22.1	30.1	25.7	24.3

Note: Sterile matings were excluded from calculation

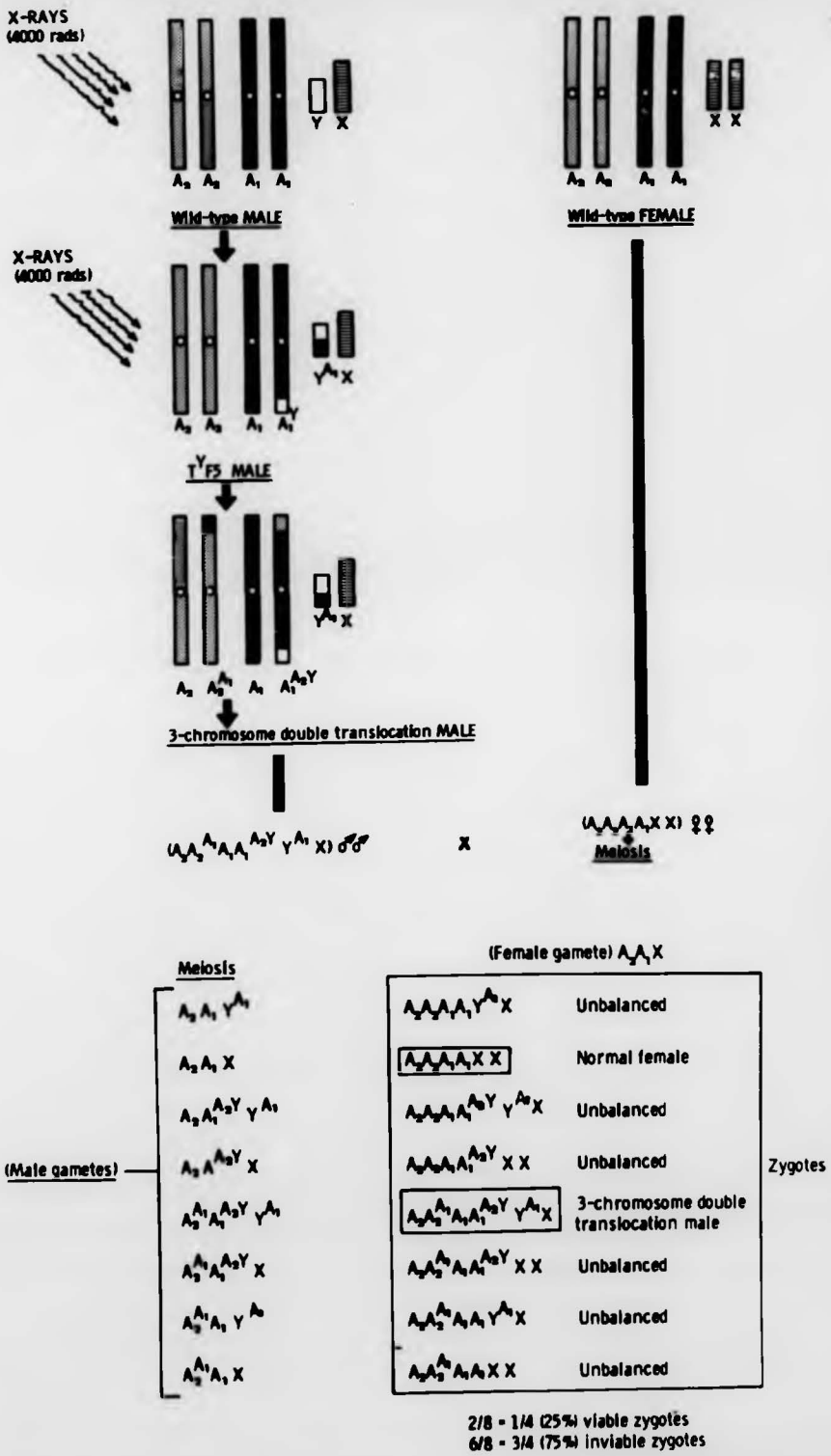
Table 10. The frequency distribution of hatching rates of daughters of 3-chromosome double translocation lines outcrossed to the wild-type males and PALA control stock

Class interval	No. matings within class interval				
	1C females x $^{+}/+$ males	3H females x $^{+}/+$ males	3U females x $^{+}/+$ males	20J females x $^{+}/+$ males	PALA control*
Sterile	3	3	1	3	5
1-5	0	2	0	3	0
6-10	1	0	0	0	0
11-15	1	1	0	0	0
16-20	0	1	0	0	0
21-25	0	0	0	0	1
26-30	0	1	0	0	0
31-35	1	2	0	0	1
36-40	0	1	2	0	1
41-45	1	0	0	0	2
46-50	2	1	0	1	0
51-55	1	0	3	1	2
56-60	0	1	1	1	1
61-65	2	1	2	1	2
66-70	3	0	0	2	3
71-75	0	0	1	2	5
76-80	4	4	4	1	3
81-85	2	6	2	3	6
86-90	9	6	4	6	12
91-95	21	7	14	15	38
96-100	26	23	25	16	24
Total no. non-sterile matings	74	57	58	52	101
No. eggs	9,645	8,876	8,306	6,998	11,609
No. larvae hatched	8,266	7,258	7,457	5,874	10,178
Ave. hatching rate (%)	85.7	81.8	89.8	83.9	87.7
Relative to PALA	97.7	93.2	102.3	95.7	100.00

*Data from Dr. E.S. Krafsur (1972 b)

Figure 9. Diagram showing formation of a three-chromosome double translocation by two steps (Above).

Chart of theoretical zygote formation from 3-chromosome doubly translocated males and normal females, showing the genotypes of zygotes resulting from the various possible combinations of normal female oocytes with 8 types of male spermatozoa (Below).


X-RAYS
(4000 rads)
Wild-type MALE
Wild-type FEMALE
X-RAYS
(4000 rads)
T^YF5 MALE
3-chromosome double translocation MALE
Meiosis
(Male gametes)
Meiosis
(Female gamete) A2A1X
Unbalanced
Normal female
Unbalanced
Unbalanced
3-chromosome double translocation male
Unbalanced
Unbalanced
Unbalanced
Zygotes
2/8 = 1/4 (25%) viable zygotes
6/8 = 3/4 (75%) inviable zygotes

admittedly unconventional but convenient way, the formation of a three-chromosome double translocation. To explain the mechanism, we must denote the two pairs of autosomes by A_2 A_2, A_1 A_1, and the pair of sex chromosomes by XX and YX. The genotype of a normal male is A_2 A_2 A_1 A_1 YX, and normal female A_2 A_2 A_1 A_1 XX. If an interchange between one of the autosomes (A_1) and the Y-chromosomes has occurred in irradiated sperm, then one translocation chromosome (A_1^Y) has a Y segment associated with A_1 chromosome and the other translocation chromosome (Y^{A_1}) has an A_1 segment with the Y-chromosome. Thus, the genotype of such a hemizygous male would be represented as A_2 A_2 A_1 A_1^Y Y^{A_1}X. Line TYF-5 is of this genotype.

A further chromosome exchange has presumably occurred in one of these Y-autosomal translocation males when irradiated at 4,000 rads. The interchange seems to have involved the other (intact) arm of the Y-bearing A_1 autosome and some terminal region of an A_2 chromosome. As a result, the genotype of such a balanced male would be represented as A_2 A_2 $^{A_1}A_1$ A_1 ^{YA_2}Y ^{A_1}X. (see Fig. 9). At meiosis, the male would produce 8 different gametes, only two of which would be balanced while the other 6 would be unbalanced (as in Fig. 9).

When fertilization with a normal female gamete (A_2 A_1 X) occurs, these 8 gametic types form 8 zygotic combinations. Only two, or 25%, of them are viable: normal females (A_2 A_2 A_1 A_1 XX) and 3-chromosome doubly-translocated males (A_2 A_2 $^{A_1}A_1A_1$ ^{YA_2}Y ^{A_1}X). Thus, any mating between a 3-chromosome doubly-translocated male and a wild-type female (such as sibs) should result in a 25% egg hatch and would be expected to produce normal females and doubly-translocated males. The observed data, as shown in Tables 9 and 10, confirm this.

Fitness evaluation studies of 3-chromosome doubly translocated male

As there were possibilities of undesirable biological attributes being associated with the translocations, various parameters that regulate a population were studied. Factors investigated were: egg production, larval survival to adulthood, sex-ratio, upper limit of mating and adult longevity. Observations on 3-chromosome double translocation males were compared with results for normal males. In addition, an experimental comparison was made of the general fitness of the two lines. This test was intended to compare the combined effects of all parameters affecting fitness.

Egg production

Fecundity was assessed by counting numbers of eggs laid by individual PALA females that had been inseminated either by normal PALA males or by 3-chromosome doubly translocated males of the 4 lines. Average numbers of eggs per female were similar in translocated and control groups (Table 11). Translocated sperms had no apparent effect upon the rate of egg-production of normal females.

Table 11. Average numbers of eggs per female in four 3-chromosome double translocation lines and PALA control strain

Line	Total no./ matings examined (non-sterile)	Total no. eggs laid	Average no. eggs per female
1C	170	23,086	135.8
3H	144	19,845	137.8
3U	106	13,749	129.7
20J	129	16,271	126.1
PALA stock*	101	11,609	114.9

*Data from Dr. E.S. Krafsur (1972 b)

Table 12. The percentage of larvae surviving to adulthood and sex-ratio in 3-chromosome double translocation lines and in PALA control strain

Line	No. larvae reared	No. adults emerged	% survival from larvae to adulthood (relative to PALA)	Male	Female	% male	Sex-ratio (1 : 1) χ^2_1	P
1C	1,387	1,148	82.7 (91.8)	609	539	53.0	4.26	> 0.02
3H	1,055	896	84.9 (94.3)	445	451	49.6	0.04	> 0.9
3U	826	590	71.4 (79.3)	298	292	50.5	0.06	> 0.9
20J	1,139	922	80.9 (89.8)	448	474	48.5	0.73	> 0.3
PALA * control	2,600	2,342	90.0 (100)	1,129	1,213	48.2	3.01	> 0.05

*Data from Dr. E.S. Krafsur (1972 b)

Survival to adulthood and sex-ratio

The rate of survival from the stage of newly hatched larvae to adulthood was measured for the 3-chromosome double translocation line and compared with the survival rate of the parent stock. Ideally, numbers of 1st instar larvae in each bowl should have been the same but it was not always possible to achieve this. Thus, comparisons were relative. Sex-ratio of emergent adults was also recorded. Emergence rates (the percentage of 1st stage larvae reaching adulthood) of some lines fluctuated slightly from generation to generation. Specimens dying during emergence or soon after were all counted as adults. Average emergence rates were found to be 82.7%, 84.9%, 71.4% and 80.9% in lines 1C, 3H, 3U and 20J, respectively. (see Table 12). These figures are 91.8%, 94.3%, 79.3% and 89.8% of the control level respectively. Thus all translocated lines showed a rather lower percentage of survival to adulthood than the PALA control. Approximately equal numbers of males and females were produced in all lines. Percentages of males were 53.0% in 1C, 49.6% in 3H, 50.5% in 3U and 48.5% in 20J, while in the PALA strain they were 48.2% (see Table 12). These sex ratios do not deviate significantly from 1 : 1, nor does the overall rate for translocation lines differ significantly from the control strains (Table 12).

Mating ability (Upper limit of mating)

To determine the upper limit of matings, 10 males and 30 females were caged together in 8-inch cube cages for one week. Survival of both sexes and the number of females which were inseminated were recorded for each cage. Five replicates using 1C males with wild-type

females and 3 replicates using wild-type males and females were observed. Table 13 compares the results of mating success of the two groups.

Table 13. Results of mating success of 1C males and wild-type males

Line	Replicates	% survival		% insemination	Matings per male
		Male	Female		
1C	5	90% (50)	97% (150)	61% (143)	1.84
PALA	3	83% (30)	98% (89)	57% (85)	1.75

Note: Number tested in parentheses.

In the relatively short period of comparison (7 days) little difference was observed in mortality of males for strain 1C compared with control males. Neither was there any appreciable difference in survival of the two groups of normal females. Insemination rates and matings per male were also found to be similar in both groups.

Male longevity

The adult survival of the two types of males was compared by placing 50 newly-emerged males of each type in separate 8-inch cube cages and keeping them under the same conditions. Three replicates of each experiment were made and the numbers of males remaining alive after 40 days were counted. After 40 days the mortality rate of 1C males was 55.4% (83/150) while for control males it was 26.0% (39/150). Thus, a highly significantly greater proportion of control males survived for 40 days than was the case for translocated males ($X_1^2 = 22.28$, $P < 0.001$).

Overall fitness

To compare the overall fitness of the three.chromosome double translocation and wild-type males, males of line 1C were taken from its F_7 generation and were outcrossed to wild-type females. Twenty-five males progeny from this cross were placed in an 8-inch cube cage together with 25 wild-type males and 50 wild-type females. After one week mass ovipositions were obtained from first blood-meals taken by the normal females. Samples of the larval progeny were separated in three bowls (200 larvae each) and were carefully reared. From among the emergents, random samples of 50 males and 50 females were caged (8-inch cube cage) for one week. Three such replicates were set-up. Individual females, after blood-meals, were tubed and the numbers showing reduced hatching rates, characteristic of the three-chromosome double translocation, were recorded. The scheme of above fitness study is illustrated in Fig. 10. Assuming random matings between karyotypes, one-fifth of the matings should have been between translocated male and wild-type female. The average hatching rate for the PALA stock was 87.7%, while for 1C males it was 22.0%. The expected mating value between translocated male and wild-type female, based on above, would be $\frac{0.220}{0.877 + 0.220} = 0.20$. Fifteen ovipositions out of 103 matings tested gave a htaching rate of 35% or less as shown in Table 14. Three of the other hatches exceeded 35% but were less than 60%; these were categorised as doubtful. Expected frequency of matings with translocated males would be 20.6 (i.e. 103 x 0.20) in a sample of 103 matings. The observed value (15) was not significantly different from that expected ($X_1^2 = 1.71$, $P > 0.2$), thus suggesting similar mating competitiveness by normal

Figure 10. Scheme for determining the overall fitness of 3-chromosome doubly translocated males and wild-type males.

COMPARISON OF FITNESS OF TRANSLOCATED AND WILD-TYPE MALES

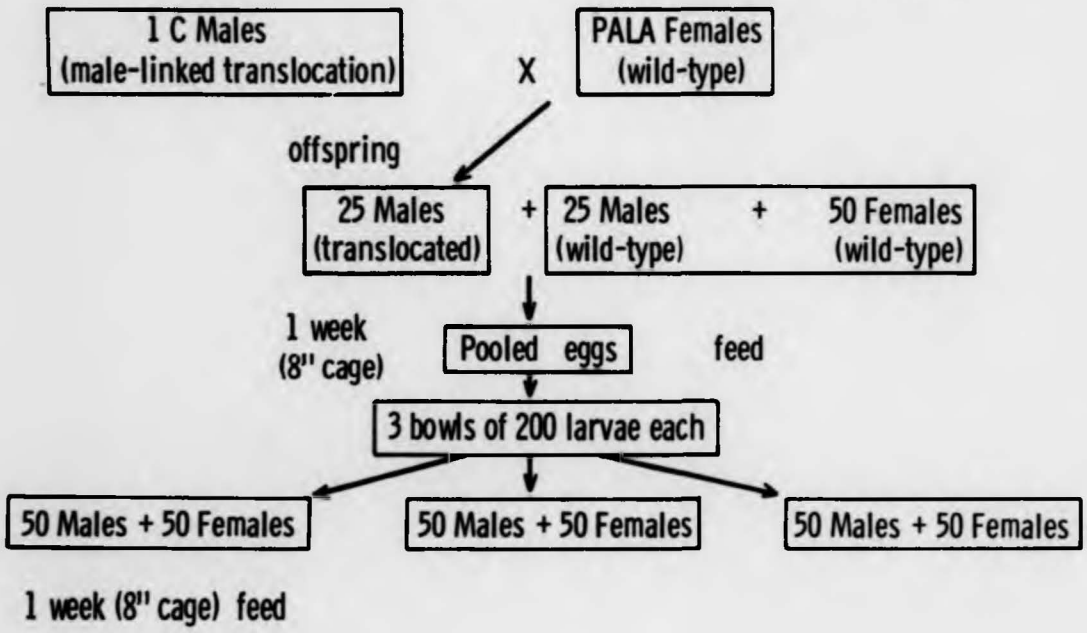


Table 14. Comparison of fitness between the 3-chromosome double translocation males and normal males

Class interval (% hatch)	Replicate			Total
	Experimental cage No. 1	Experimental cage No. 2	Experimental cage No. 3	
1-5	0	0	0	0
6-10	1	1	0	2
11-15	0	0	0	0
16-20	2	0	2	4
21-25	0	0	2	2
26-30	3	1	0	4
31-35	0	1	2	3
36-40	0	0	1	1
41-45	1	0	0	1
46-50	0	0	0	0
51-55	0	0	0	0
56-60	1	0	0	1
61-65	2	1	0	3
66-70	1	1	1	3
71-75	3	2	1	6
76-80	2	2	7	11
81-85	4	2	2	8
86-90	1	7	2	10
91-95	6	7	5	18
96-100	8	8	10	26
Total	35	33	35	103
Expected matings between 1C males and +/+ females	7	6	7	20
Observed	6	3	6	15

and translocated males. This similarity of mating ability occurred in spite of the reduced longevity of the translocated males compared with the wild-type males (see previous section). This indicates that most mating occurs early in the life of the males. This has been confirmed by competition experiments using sterile hybrid males of the An. gambiae complex in which it was found that older males mated less well than younger ones (Davidson and Bryan, personal communication). These findings show that the population of the three-chromosome double translocation was effectively as fit and capable of mating as the normal strain. They also show that larvae of both types survived equally well.

Laboratory inbreeding of 3-chromosome double translocation lines

All four translocated lines (1C, 3H, 3U and 20J) have been maintained until the present time. The fertility of one of them, line 1C, has been tested after one year of brother-sister matings. An average hatching rate of 22.9% was observed when 43 ovipositions were examined (1,294 larvae hatched from 5,634 eggs). There was no significant difference in fertility between this and that recorded at the time of the 8th outcross one year previously ($F_{42,99} = 1.00$, $P > 0.05$; $t_{51} = 0.05$, $P > 0.9$). The mean number of eggs laid per female was 131.0. A sample of 294 larvae were reared from matings with a 25% hatching rate or less. These larvae produced 269 adults (132 males and 137 females). The level of emergence was 91.4% and the sex-ratio approximated the 1:1 ratio expected. The sterility of line 1C has remained close to its original value of 75% during one year of inbreeding.

C. Autosomal translocation lines

Line 6K

Males and females were out crossed to wild-type individuals and selections for semisterility were repeated for 25 generations. Two hundred and twenty matings using 6K males and 305 matings using 6K females were counted for egg number and larval hatch. The percentage hatching was calculated for each mating and hatching rates were subdivided into 5% intervals. The number of matings falling into each of these categories is given in histogram form in Fig. 11 A, B. The ovipositions from outcrossing sons and daughters of semisterile matings showed a bimodal distribution of hatching rate. Table 15 enumerates the average hatching rate and progeny ratio of normal and partially sterile matings.

Table 15. Results of line 6K outcross matings to the wild-type

Cross	No. matings	No. eggs	No. larvae hatched	Average hatching rate (%)	Proportion of matings	
					> 65% hatching rate	< 65% hatching rate
6K males x $^{+}/+$	220	27,345	16,439	60.1	0.34 (74/220)	0.66 (146/220)
6K females x $^{+}/+$	305	43,032	25,522	59.3	0.41 (126/305)	0.59 (179/305)

There was no significant difference in the hatching rate or its variance between 6K males x wild-type females and 6K females x wild-type males ($F_{219, 304} = 1.17$, $P > 0.05$; $t_{523} = 0.14$, $P > 0.9$).

In ovipositions resulting from 146 of 220 outcrossed males (66%) and in 179 from 305 outcrossed females (59%) the hatching rate was 65%

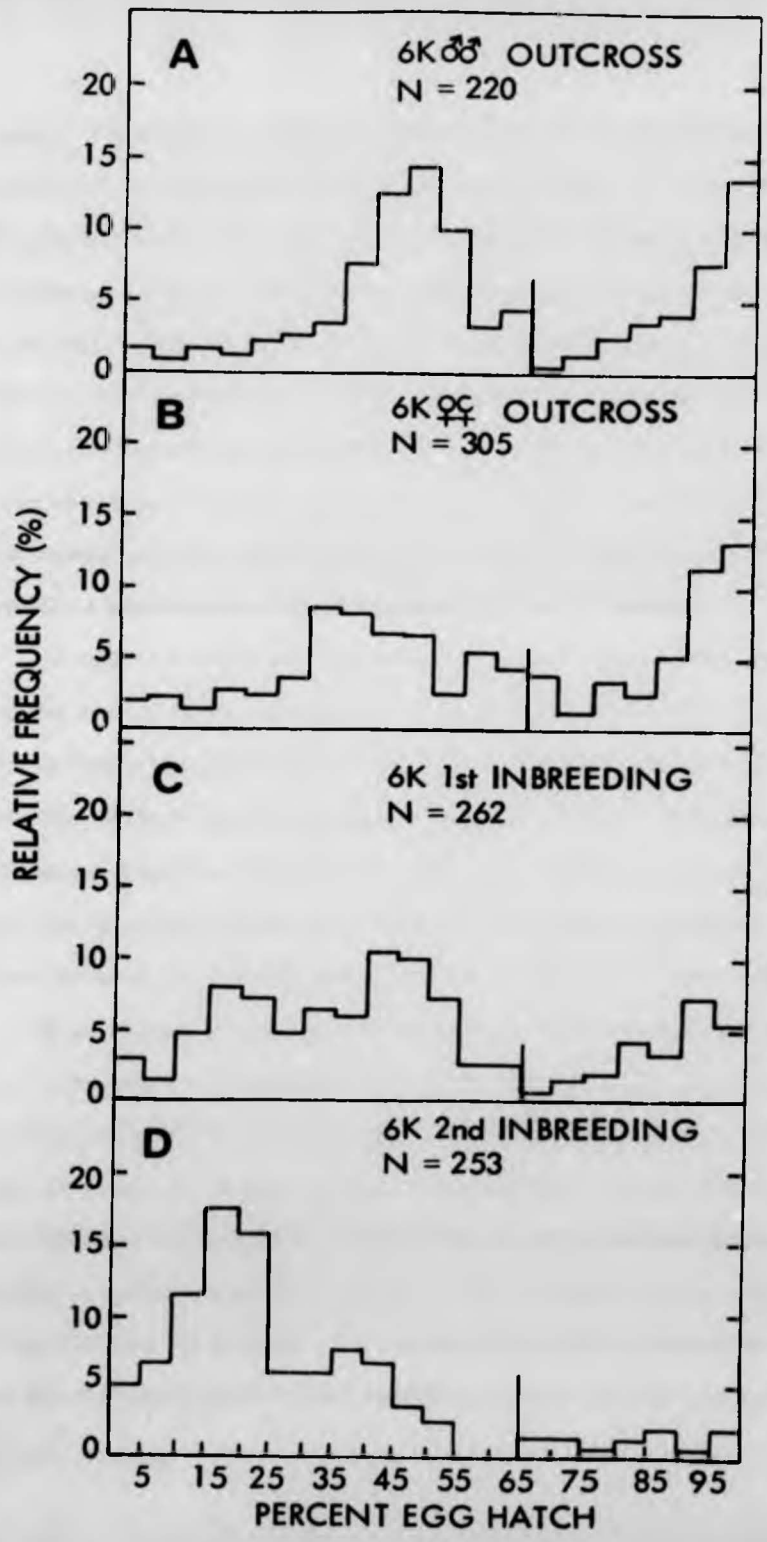
Figure 11. Frequency distribution of hatching rates of line 6K (autosomal translocation).

A. Sons of line 6K heterozygotes outcrossed to the wild-type. There is a bimodal distribution.

B. Outcrossed daughters of line 6K heterozygotes. $^{T}/+$ daughters show modal fertility of 30% - 40% with wide variations.

C. Hatching rates of the first inbreeding of line 6K heterozygotes. There is a trimodal distribution. Fertilities of $^{T}/+ \times {}^{+}/+$ matings appear to be overlapping with the hatches of $^{T}/+ \times {}^{T}/+$ matings.

D. Hatching rates of intercrossed 6K heterozygotes. 20 of the 25 normally fertile matings (65% hatching rate or more) were of the wild-type matings.


A
6K♂♂ OUTCROSS
N = 220
B
6K♀♀ OUTCROSS
N = 305
C
6K 1st INBREEDING
N = 262
D
6K 2nd INBREEDING
N = 253
RELATIVE FREQUENCY (%)
0
5
10
15
20
5 15 25 35 45 55 65 75 85 95
PERCENT EGG HATCH

or less. The expected value was 50% for each of these figures and the observations of 66% and 59% are significantly different from this ($X^2_1 = 23.56$, $P < 0.01$; $X^2_1 = 9.20$, $P < 0.001$). Some of the apparent $T/+$ matings might have been wild-type matings with low hatch, since 10% of PALA control stock showed less than 65% hatching rate (see Table 1). The percentage of larvae surviving to adulthood and sex-ratios were recorded in this and other partially sterile lines. Ideally, larvae should have been reared under standardised conditions (e.g. equal numbers of 1st instar larvae per bowl). It was not always possible to achieve this. Thus comparisons were relative.

Combined results of these observations on 6K and all other partially sterile lines are shown in comparison with the PALA control stock in Table 16. Emergence rates in all 15 lines ranged from 62.4% to 86.2%, while in the PALA control stock it was 87%. In general, the percentage of larvae surviving to adulthood in the partially sterile lines was thus much lower than in the control stock. However, sex ratios did not differ significantly from 1 : 1 except in 2 lines 102A and 50Q.

Examination of ovarian polytene chromosomes confirmed that line 6K contained a reciprocal chromosome translocation involving two autosomes 2R and 3R (Fig. 12). After this line had been outcrossed to the wild-type for 6 generations, the progeny of semisterile outcrosses were inbred in an attempt to produce translocation homozygotes. In the absence of suitable genetical markers, the hatching rate was used as an identification parameter. To isolate translocation homozygotes, each mating has to be assigned accurately to one of three levels of hatching rate, viz normally fertile, semisterile and less than semisterile,

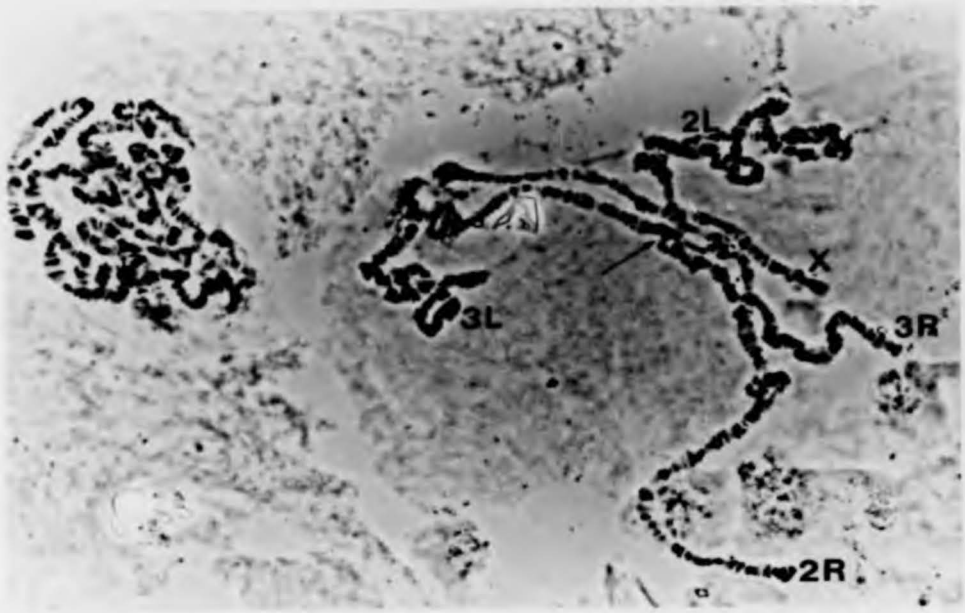
Table 16. The percentage of larvae surviving to adulthood and sex-ratio in 15 partial sterile lines and PALA control strain

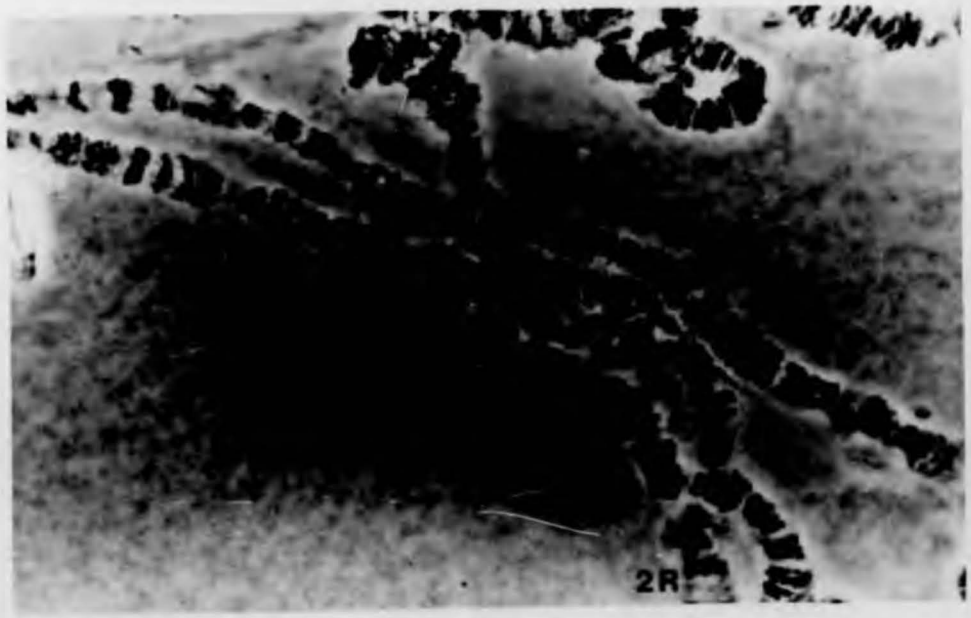
Line	% survival from larva to adulthood	Relative to PALA (%)	Male	Female	% male	Sex-ratio (1 : 1) X_1^2	P
6K	67.3	77.3	860	870	49.7	0.05 >	0.5
26O	75.3	86.5	791	707	52.8	4.71 >	0.03
7G	74.6	85.7	986	952	50.8	0.59 >	0.4
8B	77.8	89.4	1,082	1,075	50.2	0.02 >	0.9
9B	71.3	81.9	339	305	52.6	1.80 >	0.15
6E	77.5	89.0	154	177	46.5	1.60 >	0.2
8M	86.2	99.0	112	121	48.0	0.35 >	0.5
26D	72.8	83.6	291	307	48.6	0.42 >	0.5
102A	74.2	85.2	128	181	41.4	9.09 <	0.01
50Q	62.4	71.7	81	115	41.3	5.89 <	0.02
53D	81.4	93.5	48	53	47.5	0.25 >	0.6
53E	76.8	88.3	94	85	52.5	0.45 >	0.5
54E	63.1	72.5	145	134	52.0	0.43 >	0.5
54F	67.8	77.9	90	113	44.3	2.61 >	0.1
55B	72.4	83.2	134	110	54.9	2.36 >	0.1
PALA * control	87.0	100.0	1,129	1,213	48.2	3.01 >	0.05

*Data from Dr. E.S. Krafsur (1972 b)

Figure 12. Polytene chromosomes from the ovarian nurse cells of Anopheles gambiae species A - line 6K (translocation heterozygote involving two autosomes 2R and 3R). slide and photograph kindly prepared by Dr. J.H. Bryan and Miss J. Chalkley.

2R, 2L, 3R, 3L - autosomal arms. X = X chromosome. Arrow indicates the breakpoint of the translocation.


2L
X
3L
3R
2R


2R

(Robinson, 1971; Krafsur, 1972 b): then each mating type can be defined, namely those which are between wild-type and wild-type ($^{+}/_{+} \times {}^{+}/_{+}$), between heterozygote and wild-type ($^{T}/_{+} \times {}^{+}/_{+}$) and between heterozygote and heterozygote ($^{T}/_{+} \times {}^{T}/_{+}$). An outcrossed heterozygote mating would be expected to produce $^{+}/_{+}$ and $^{T}/_{+}$ offspring in equal numbers. Then, the interbreeding of these offspring would produce mating in the proportions $\frac{1}{4}$ ($^{+}/_{+} \times {}^{+}/_{+}$). : $\frac{1}{2}$ ($^{T}/_{+} \times {}^{+}/_{+}$) : $\frac{1}{4}$ ($^{T}/_{+} \times {}^{T}/_{+}$). The following criteria have been adopted throughout this study for the identification of these 3 types of matings.

1. Matings with 65%-100% hatch are taken as normally fertile matings and are presumed to be $^{+}/_{+} \times {}^{+}/_{+}$.
2. Matings with 35%-65% hatch are taken as semisterile matings and are presumed to be either $^{T}/_{+}$ male x $^{+}/_{+}$ female or $^{T}/_{+}$ female x $^{+}/_{+}$ male matings.
3. Matings with less than 35% egg hatch are taken as less than semisterile and are presumed to be $^{T}/_{+} \times {}^{T}/_{+}$ matings.

The results of the first inbreeding of line 6K are shown in Table 17 and hatching rates are plotted in Fig. 11 C.

Table 17. Result of line 6K first inbreedings

Cross	No. matings	No. eggs	No. larvae hatched	Ave. hatching rate (%)	Proportion of matings		
					with more than 65% hatching rate	with 35-65% hatching rate	with 35% hatching rate or less
Line 6K 1st inbreeding	262	40,358	19,405	48.0	0.24 (64/262)	0.40 (103/262)	0.36 (95/262)

The observed proportion of normally fertile matings was 0.24 (64 of 262 matings), in close agreement with the expected value (0.25). However, 95 of 262 matings (0.36) showed hatches of 35% or less, and this figure differs significantly from the expected ($X_1^2 = 13.28$, $P < 0.001$). Over 40% of progeny of partially sterile 6K female outcrosses showed hatch levels less than 35%. Therefore it was concluded that fertilities of $^{T}/+$ female x $^{+}/+$ male were probably overlapping with those of $^{T}/+$ x $^{T}/+$ matings.

The larvae of some of the ovipositions showing less than 35% hatching rate (potentially $^{T}/+$ x $^{T}/+$) were reared and the resulting adults intercrossed again in an attempt to isolate translocation homozygotes. A total of 253 ovipositions (41,220 eggs) from the 2nd round of intercrosses were obtained and revealed an average hatching rate of 31.94% (13,168 larvae hatched). Twenty five (10%) were found to be normally fertile (showing a hatch of 65% or more) (see Fig. 11 D). The majority of normally fertile offspring were maintained as possible sources of translocation homozygotes and tested by outcrossing to the wild-type. Assuming that translocation homozygotes are viable and fully fertile, then homozygote x wild-type matings would show normal hatches but the offspring would be semisterile and interbreeding of them would show a marked reduction in hatching rate. This procedure was applied to 20 of the 25 normally fertile matings and results obtained are shown in Table 18. These results show clearly that all came from normally fertile individuals, so it was concluded that the original fertile matings were of the wild-type ($^{+}/+$) and not of the translocation homozygote (T/T).

Table 18. Results of testing normal fertile matings in 2nd inbreedings of line 6K for the presence of translocation homozygotes

Normal fertile matings	Hatching rate of parental mating (%)	Hatching rate in test cross *				
		No. matings tested	No. eggs	No. larvae	Ave. hatching rate (%)	No. matings with 65% hatch or less
S_2 6K-1	88.3	7	1,246	1,054	84.5	0
2	97.2	5	422	330	78.1	2
3	74.4	7	908	822	90.5	1
4	84.1	5	823	710	86.2	1
5	96.2	8	793	751	94.7	0
6	65.5	7	897	817	91.0	0
7	100.0	5	529	447	84.4	1
8	74.0	4	335	282	84.1	0
9	78.5	8	681	594	87.2	0
10	95.6	5	337	292	86.6	1
11	80.9	8	874	835	95.5	0
12	94.0	12	1,622	1,406	86.6	1
13	68.3	10	1,265	885	69.9	4
14	93.8	10	824	640	77.6	2
15	100.0	7	895	787	87.9	1
16	86.5	9	695	512	73.6	3
17	86.8	6	552	406	73.5	1
18	87.5	5	495	449	90.7	0
19	81.2	11	1,534	1,379	89.8	0
20	89.8	4	646	427	66.0	1

*S_2 6K-1, 2, etc. x +/+ and inbred.

The frequency of zygotic combinations with the expected viability of each combination could be estimated among the progeny of 6K heterozygote x heterozygote matings if we knew the ratio of alternate and adjacent segregations in the parents 6K translocation heterozygotes. Unfortunately, we do not know this ratio. The data of numerous workers as compiled by Burnham (1962) indicate that in some translocations adjacent-2 disjunction does not occur but only alternate and adjacent-1 types of disjunction. In others the aneuploid gametes consist of the four types (a pair of adjacent-1 types and a pair of adjacent-2 types) with unequal frequencies of the pairs. As there is little information concerning the segregation of chromosomes during meiosis in An. gambiae species A, it is necessary to consider several hypotheses to fit the results observed. The fertility figures of intercrosses between translocation heterozygotes could, in theory, provide information on the segregation of the translocation heterozygote, i.e. the frequency of alternate and adjacent segregation. Since large egg samples were obtained for the measurement of fertility from both outcrosses and intercrosses of this and other lines, an attempt was made to assign numerical frequencies to the different types of gametes produced by lines heterozygous for reciprocal interchanges. With this information, an estimate was made of the frequency of resulting zygotic combinations with the expected viability of each combination among the progeny of heterozygote x heterozygote matings. This approach was based on hypotheses described by LaChance et al., (1964) and Hetherington et al., (1968).

The average hatching rate of 6K male and female outcrosses to the wild-type was approximately 60% (see Table 15). Therefore each of the 6K male and female matings produced orthoploid gametes in the frequency

of 0.30 normal (1 + 4) and 0.30 translocation (2 + 3), 0.40 remaining as duplication-deficiency gametes (1 + 3, 2 + 4) and/or (1 + 2, 3 + 4). (see Fig. 1 for explanation of numbers 1, 2, 3, 4).

Several models were tried to fit the observed results. The best fit was given when the frequency and type of gametes formed by both sexes are approximately 0.30 (1 + 4) + 0.3 (2 + 3) alternate segregation, 0.15 (1 + 3) + 0.15 (2 + 4) adjacent-1 segregation and 0.05 (1 + 2) + 0.05 (3 + 4) adjacent-2 segregation.

The frequencies of the resulting zygotic combinations in the above matings are shown in Table 19. Calculation based on this working model resulted in expected hatching rate of 41.00% if T/T is viable or of 32.00% if T/T is lethal. The observed hatching rate from the second inbreeding (31.94%) did not differ greatly from the expected value where T/T is lethal. ($X_1^2 = 0.054$, $P > 0.80$). Furthermore, seven zygotic combinations shown in Table 19 were viable and were either wild-type ($^{+}/+$) or translocation heterozygotes ($^{T}/+$). Out of 253 matings examined, 25 were normally fertile. Twenty of these were confirmed as wild-type by outcrossing. The expected frequency of the wild-type in 253 matings would be 23 (0.09 x 253 = 22.8); a figure in close agreement with the observed number 25 ($X_1^2 = 0.17$, $P > 0.7$).

Although the hatching rate was the only criterion used for the designation of karyotype, and there were inevitable inaccuracies in these designations, nevertheless the data obtained from egg hatches of intercrossed 6K heterozygotes suggested that translocation homozygotes were lethal.

Table 19. Mating chart showing type and frequency of gametes formed by males and females of 6K translocation heterozygotes and the frequency of resulting zygotic combinations with the expected viability of each combination

			Paternal gametes					
			Alternate disjunction		Adj. -1 disjunction		Adj. -2 disjunction	
			0.30 (1+4)	0.30 (2+3)	0.15 (1+3)	0.15 (2+4)	0.05 (1+2)	0.05 (3+4)
Maternal gametes	Alternate disjunction	0.30 (1+4)	0.09 ($^{+}/_{+}$)	0.09 ($^{T}/_{+}$)				
		0.30 (2+3)	0.09 ($^{T}/_{+}$)	0.09 (T/T)				
	Adj. -1 disjunction	0.15 (1+3)				0.0225 ($^{T}/_{+}$)		
		0.15 (2+4)			0.0225 ($^{T}/_{+}$)			
	Adj. -2 disjunction	0.05 (1+2)						0.0025 ($^{T}/_{+}$)
		0.05 (3+4)					0.0025 ($^{T}/_{+}$)	

Remarks: $^{+}/_{+}$ wild-type, viable, fertile

$^{T}/_{+}$ translocation heterozygote, viable, semisterile

T/T translocation homozygote, lethal

Blank square represent unbalanced zygotes, inviable

Line 26O

An average hatching rate of male outcross matings was 72.7% and female outcross matings 62.9% (Table 20) when line 26O was studied for 24 outcross generations.

Table 20. Results of line 26O outcross matings to the wild-type

Cross	No. matings	No. eggs	No. larvae hatched	Ave. hatching rate (%)	Proportion of matings	
					> 65% hatching rate	< 65% hatching rate
26O males x $^{+}/_{+}$	144	17,559	12,761	72.7	0.61 (88/144)	0.39 (56/144)
26O females x $^{+}/_{+}$	246	33,019	20,780	62.9	0.44 (109/246)	0.56 (137/246)

The frequency distribution of hatching rates of these matings are graphically shown in Fig. 13 A and B. The fertilities of 26O male and 26O female outcross matings were compared. There was no significant difference in variance ($F_{143,245} = 1.17$, $P > 0.05$) but there was a significant difference in the mean ($t_{388} = 3.06$, $P < 0.001$) indicating that 26O male translocation heterozygotes were more fertile than their heterozygous sisters. The proportion of partially sterile matings in male outcrosses 0.39 (56/144) was significantly different from the expected value of 0.5 ($X_1^2 = 7.11$, $P < 0.01$) but in female outcrosses 0.56 (137 of 246) of the matings were semisterile, not significantly different from the expected value of 0.50 ($X_1^2 = 3.18$, $P > 0.05$). Cytological examination of ovarian nurse cells confirmed that this line contained a reciprocal translocation between autosomes 2L and 3L (see Fig. 14).

Figure 13. Frequency distribution of hatching rates of line 260 (autosomal translocation).

A. Male outcross matings.

B. Daughters of 260 heterozygotes outcrossed to the wild-type.

C. Hatching rates of the first inbred progenies of 260 semisterile outcross matings.

D. Selfed progenies of $^{T}/_{+}$ x $^{T}/_{+}$ matings.

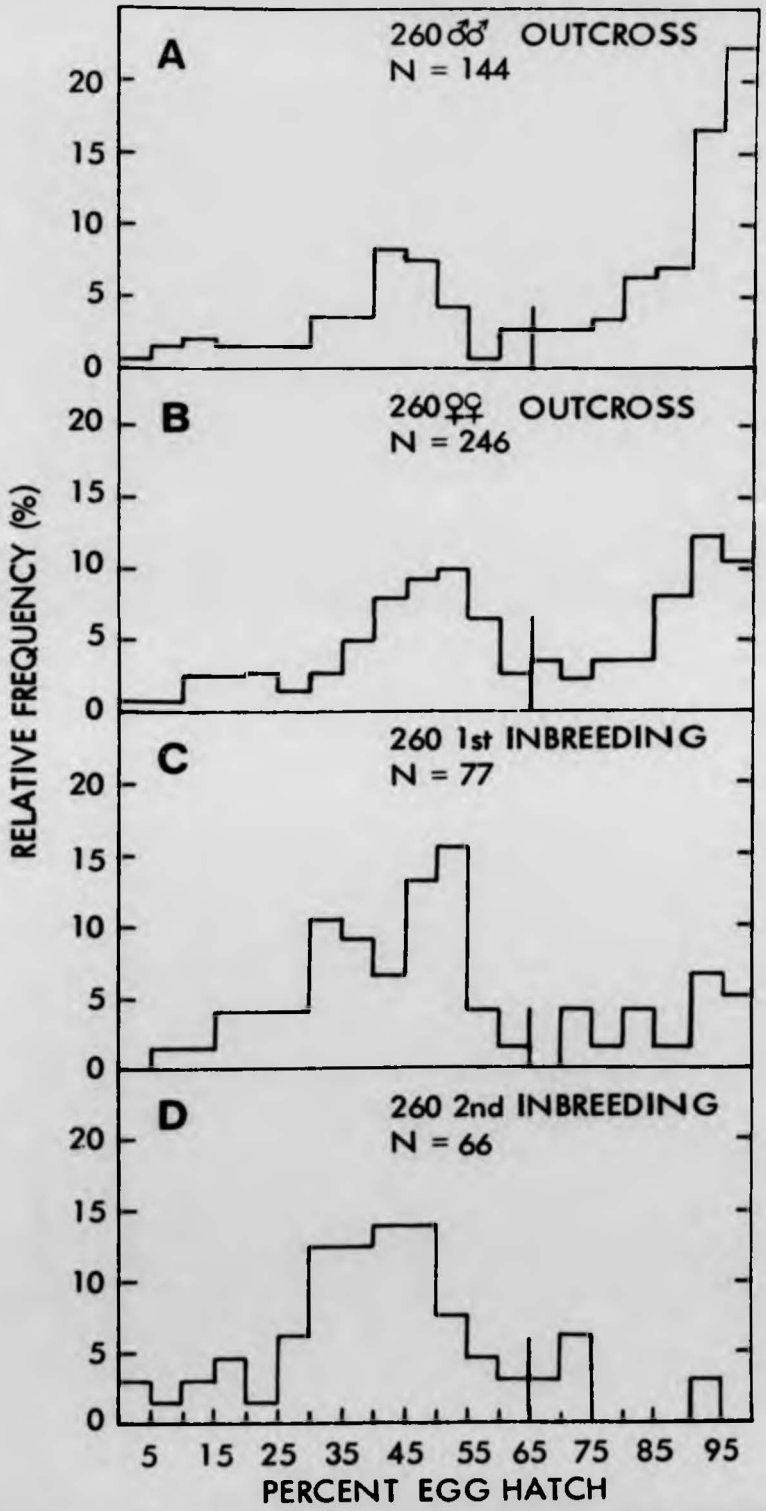
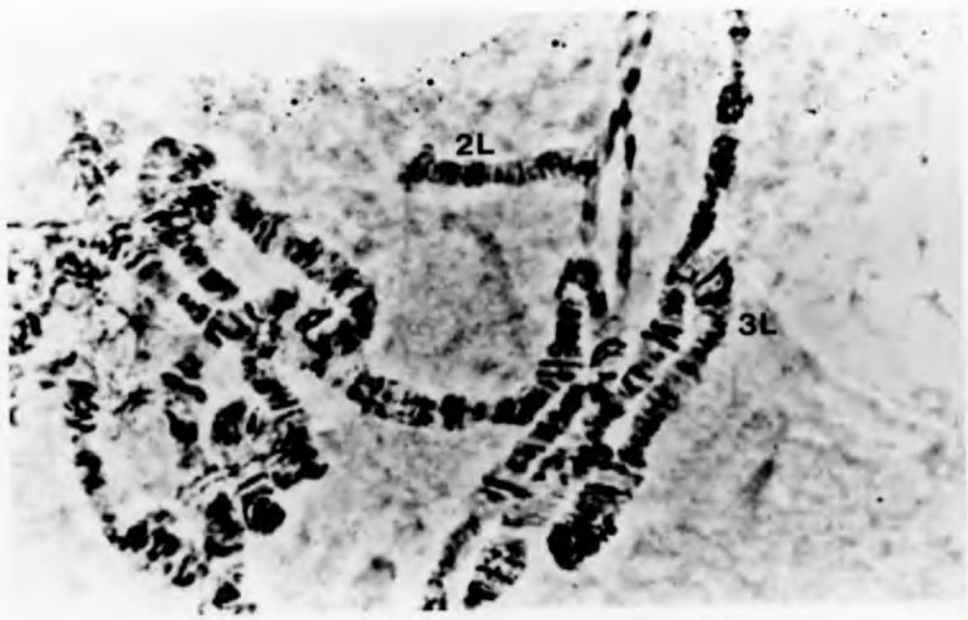

A
260♂♂ OUTCROSS
N = 144
B
260♀♀ OUTCROSS
N = 246
C
260 1st INBREEDING
N = 77
D
260 2nd INBREEDING
N = 66
RELATIVE FREQUENCY (%)
0
5
10
15
20
5 15 25 35 45 55 65 75 85 95
PERCENT EGG HATCH

Figure 14. Polytene chromosomes from the ovarian nurse cells of Anopheles gambiae species A - line 26O (translocation heterozygote involving two autosomes 2L and 3L). Slide and photograph kindly prepared by Dr. J.H. Bryan and Miss J. Chalkley.



he ovarian nurse cells of

- line 260 (translocation

bsomes 2L and 3L).

epared by Dr. J.H. Bryan

The offspring of those outcrosses resulting in partially sterile ovipositions were interbred and produced the 3 categories of hatching rate (>65%, 35-65% and < 35%) in the expected ratio of 1 : 2 : 1 from $^{+}/+ \times {}^{+}/+$, $^{T}/+ \times {}^{+}/+$ and $^{T}/+ \times {}^{T}/+$ matings (see Table 21 and Fig. 13 C).

Table 21. Result of line 260 first inbreedings

Cross	No. matings	No. eggs	No. larvae hatched	Ave. hatching rate (%)	Proportion of matings		
					with more than 65% hatching rate	with 35-65% hatching rate	with 35% hatching rate or less
Line 260 1st inbreedings	77	10,889	5,745	52.7	0.25 (19/77)	0.50 (39/77)	0.25 (19/77)

Further interbreedings were made with the progeny of matings showing 35% hatching rate or less in an attempt to isolate translocation homozygotes. An average hatching rate of 44.55% (3,328 larvae hatched from 7,470 eggs) was observed when 66 matings were examined from this inbreeding and 8 of 66 matings (12%) were found to be normally fertile (Fig. 13 D). The offspring of these 8 normally fertile matings were tested for the presence of translocation homozygotes, but none were found (Table 22).

The fertility of male and female 260 heterozygotes as measured by hatching rate of outcross matings was 73% and 63% respectively - male heterozygotes were more fertile than female thus suggesting different modes of meiotic disjunction between the sexes.

Table 22. Results of testing normally fertile matings in 2nd inbreedings of line 260 for the presence of translocation homozygotes

Normally fertile matings	Hatching rate of parental mating (%)	Hatching rates in test cross				
		No. matings tested	No. eggs	No. larvae	Ave. hatching rate (%)	No. matings with 65% hatch or less
S_2 260-1	70.6	6	611	478	78.2	1
" -2	90.8	10	1,922	1,735	90.3	1
" -3	91.2	5	331	264	79.7	1
" -4	70.1	11	1,217	911	74.8	3
" -5	73.7	5	676	535	79.1	1
" -6	73.5	7	524	416	79.4	1
" -7	67.7	9	725	530	73.1	3
" -8	75.0	9	1,387	1,005	72.4	2

Assuming that the frequency and type of gametes formed by the male are 0.375 (1 + 4) + 0.375 (2 + 3) and 0.125 (2 + 3) + 0.125 (2 + 4) and by the female are 0.333 (1 + 4) + 0.333 (2 + 3), and 0.1667 (1 + 3) + 0.1667 (2 + 4), the ratio of alternate to adjacent-1 is calculated to be approximately 3 : 1 in males and 2 : 1 in females. Thus, crosses involving line 260 heterozygotes would produce eggs with hatches of 54.12% if T/T is viable or of 41.63% if T/T is lethal (see Table 23). The observed hatching rate was 44.55%. This figure differs greatly from either of the expected values - $X_1^2 = 274.8$, if T/T is viable; or $X_1^2 = 26.18$, if T/T is lethal. It is difficult to explain this discrepancy. Possibly some unspecified technical error occurred in the intercross experiment. It is unlikely that the outcross hatching rate is grossly underestimated. However that may be, the result is less discrepant if T/T is treated as lethal rather than viable. This indication is much strengthened by the following. The expected frequency of normally fertile matings in 66 matings would be 0.24975 x 66 = 16.4 assuming that T/T are viable and fully fertile (similar to wild-type). However, the observed number of normally fertile matings was only 8 of which all proved to be wild-type (see Table 22). This figure did not differ from those expected if T/T is lethal based on the above working model (0.124875 x 66 = 8.2). If exact observed values for outcross hatching rates are used, rather than the rounded $\frac{3}{4}$ and $\frac{2}{3}$, no appreciable change in these conclusions ensues.

Table 23. Mating chart showing type and frequency of gametes formed by males and females of 260 translocation heterozygotes and the frequency of resulting zygotic combinations with the expected viability of each combination

Paternal gametes

Maternal gametes		Alternate disjunction		Adjacent-1 disjunction	
		0.375 (1 + 4)	0.375 (2 + 3)	0.125 (1 + 3)	0.125 (2 + 4)
Alternate disjunction	0.333 (1 + 4)	0.124875 ($^{+}/_{+}$)	0.124875 ($^{T}/_{+}$)		
	0.333 (2 + 3)	0.124875 ($^{T}/_{+}$)	0.124875 (T/T)		
Adjacent-1 disjunction	0.1667 (1 + 3)				0.0208375 ($^{T}/_{+}$)
	0.1667 (2 + 4)			0.0208375 ($^{T}/_{+}$)	

$^{+}/_{+}$ - wild-type, viable, fertile

$^{T}/_{+}$ - translocation heterozygote, viable and semisterile

T/T - translocation homozygote, lethal

Blank squares represent unbalanced zygote, inviable

Line 7G

The average hatching rate from male outcrosses was 69.6% and from female outcrosses 70.6% (Table 24). The frequency distribution of their hatching rates is illustrated in Fig. 15 A and B.

Table 24. Results of line 7G outcross matings to the wild-type (25 outcross generations)

Cross	No. matings	No. eggs	No. larvae hatched	Ave. hatching rate (%)	Proportion of matings	
					> 65% hatching rate	< 65% hatching rate
7G $^{+}$ males x +/+	191	21,962	15,297	69.6	0.59 (113/191)	0.41 (78/191)
7G females x $^{+}$/+	251	33,824	23,909	70.6	0.53 (132/251)	0.47 (119/251)

There was no significant difference in hatching rate between male and female outcrosses ($F_{190,250} = 1.21$, $P > 0.05$; $t_{440} = 0.058$, $P > 0.9$). In 78 of 191 outcrosses involving males of line 7G and 119 of 251 outcrosses involving females the egg hatch was 65% or less. The difference from the expected number of semisterile matings (95) was not significant at the 1% level but was significant at the 5% level in male outcrosses ($X^2_1 = 6.41$, $0.02 > P > 0.01$) but female outcrosses gave the expected result ($X^2_1 = 0.67$, $P > 0.4$).

The offspring of semisterile matings were intercrossed and the frequency distribution of hatching rates was examined (Fig. 15 C). The observed proportion of normally fertile matings was 0.256 (10 out of 39), and 0.256 (10 out of 39 matings) showed hatches of 35% or less.

Figure 15. Frequency distribution of hatching rates of line 7G (autosomal translocation).

A. Partial sterile matings resulting from crosses of 7G males to normal females.

B. Partial sterile matings resulting from crosses of 7G females to normal males.

C. Fertilities of the first inbred progenies of 7G semisterile outcross matings.

D. Fertilities of the second inbred progenies of $^{T}/_{+} \times {}^{T}/_{+}$ matings.

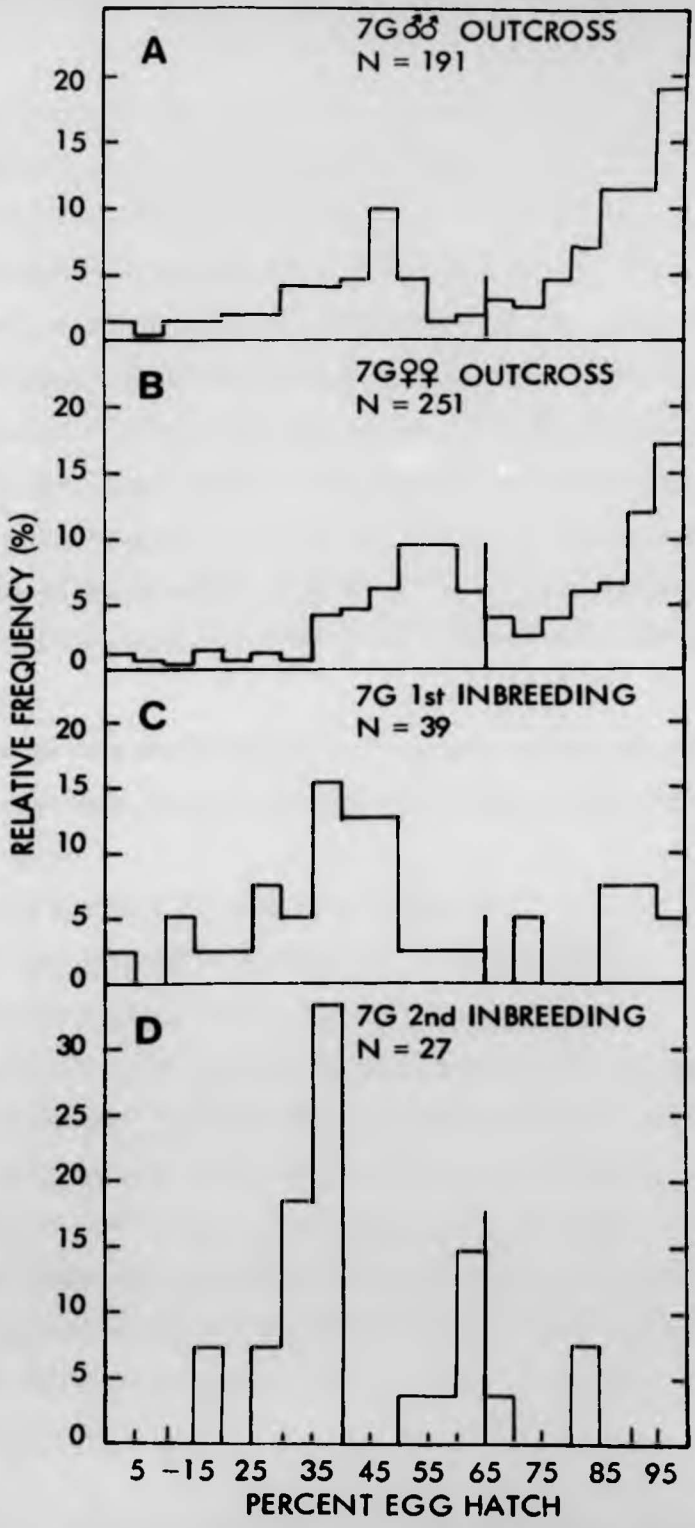
ing rates of line 7G

crosses of 7G males

crosses of 7G females

of 7G semisterile

es of $^{T}/_{+} \times {}^{T}/_{+}$



These figures are in excellent agreement with the expected values of 0.25. Several progeny resulting from ovipositions of less than 35% hatching rate were reared and the offspring were inbred to try to recover translocation homozygotes. A total of 27 matings were obtained with an average egg hatch of 39.61% (1,734 larvae hatched from 4,377 eggs). Two of the 27 matings (11%) were shown to be normally fertile (83.3% and 82.1%) (see Fig. 15 D) and were maintained as possible T/T. Some of them were outcrossed to the wild-type and brother-sister matings of their offspring were made. An average hatching rate of 84.2% and 85.0% respectively was observed and it was concluded that the original parental matings were between wild-type individuals.

From the data of egg hatches it was postulated that the frequencies of the different types of meiotic segregation in both the male and females of line 7G were: -

0.35 (1 + 4) + 0.35 (2 + 3) - alternate segregation

0.0975 (1 + 3) + 0.0975 (2 + 4) - adjacent - 1 segregation

0.0525 (1 + 2) + 0.0525 (3 + 4) - adjacent - 2 segregation.

The expected values based on this working model are that the egg hatch would be 51.45% and the frequency of normal fertile matings would be 0.245, if all karyotypes, i.e. $^{+}/_{+}$, $^{T}/_{+}$ and T/T are viable but if T/T is lethal then 39.20% of eggs would hatch and 0.1225 would be normally fertile (see Table 25). The observed hatching rate of intercrosses was 39.61% and normal fertile matings were 2. These values were in close agreement with expected values if T/T is lethal. ($X^2_1 = 0.310$, $P > 0.6$), (0.1225 x 27 = 3.3).

Table 25. Mating chart showing type and frequency of gametes formed by males and females of 7G translocation heterozygotes and the frequency of resulting zygotic combinations with the expected viability of each combination.

		Paternal gametes: Alternate disjunction		Adjacent-1 disjunction		Adjacent-2 disjunction	
		0.35(1+4)	0.35(2+3)	0.0975(1+3)	0.0975(2+4)	0.0525(1+2)	0.0525(3+4)
Maternal gametes: Alternate disjunction	0.35 (1+4)	0.1225 ($^{+}/_{+}$)	0.1225 ($T/_{+}$)				
	0.35 (2+3)	0.1225 ($^{T}/_{+}$)	0.1225 (T/T)				
Adjacent-1 disjunction	0.0975 (1+3)				0.0095062 ($^{T}/_{+}$)		
	0.0975 (2+4)			0.0095062 ($^{T}/_{+}$)			
Adjacent-2 disjunction	0.0525 (1+2)						0.0027562 ($^{T}/_{+}$)
	0.0525 (3+4)					0.0027562 ($^{T}/_{+}$)	

$^{+}/_{+}$ wild-type, viable, fertile

$^{T}/_{+}$ translocation heterozygote, viable, semisterile

T/T translocation homozygote, lethal

Blank squares represent unbalanced zygotes, inviable

In one of 8 females from preserved material, the polytene chromosomes showed an autosomal-autosomal translocation but unfortunately, the arms involved could not be identified.

Line 8B

This line was tested for the occurrence of inherited partial sterility for up to 25 generations. The distribution of egg hatches from male and female outcrosses are plotted in Fig. 16 A and B. The gross results of these outcross matings are given in Table 26.

Table 26. Results of line 8B outcross matings to the wild-type

Cross	No. matings	No. eggs	No. larvae hatched	Average hatching rate (%)	Proportion of matings	
					> 65% hatching rate	< 65% hatching rate
8B males x $^{+}/_{+}$	207	23,577	16,768	71.1	0.61 (127/207)	0.39 (80/207)
8B females x $^{+}/_{+}$	269	36,055	24,312	67.4	0.54 (144/269)	0.46 (126/269)

Similar fertilities from crosses between 8B males and wild-type females and between 8B females and wild-type males were observed. ($F_{206, 268} = 1.02$, $P > 0.05$; $t_{474} = 1.39$, $P > 0.05$). The proportion of matings with 65% hatching rate or less was found to be low in 8B male outcrosses (0.39) and the difference from the expected value of 0.5 was highly significant ($X_1^2 = 10.67$, $P < 0.01$) but female outcrosses (0.46) gave the expected result ($X_1^2 = 1.07$, $P > 0.3$). In order to recognize $^{T}/_{+}$ x $^{T}/_{+}$ matings semisterile outcrosses of line 8B were inbred and the distribution of egg hatches of their offspring was examined

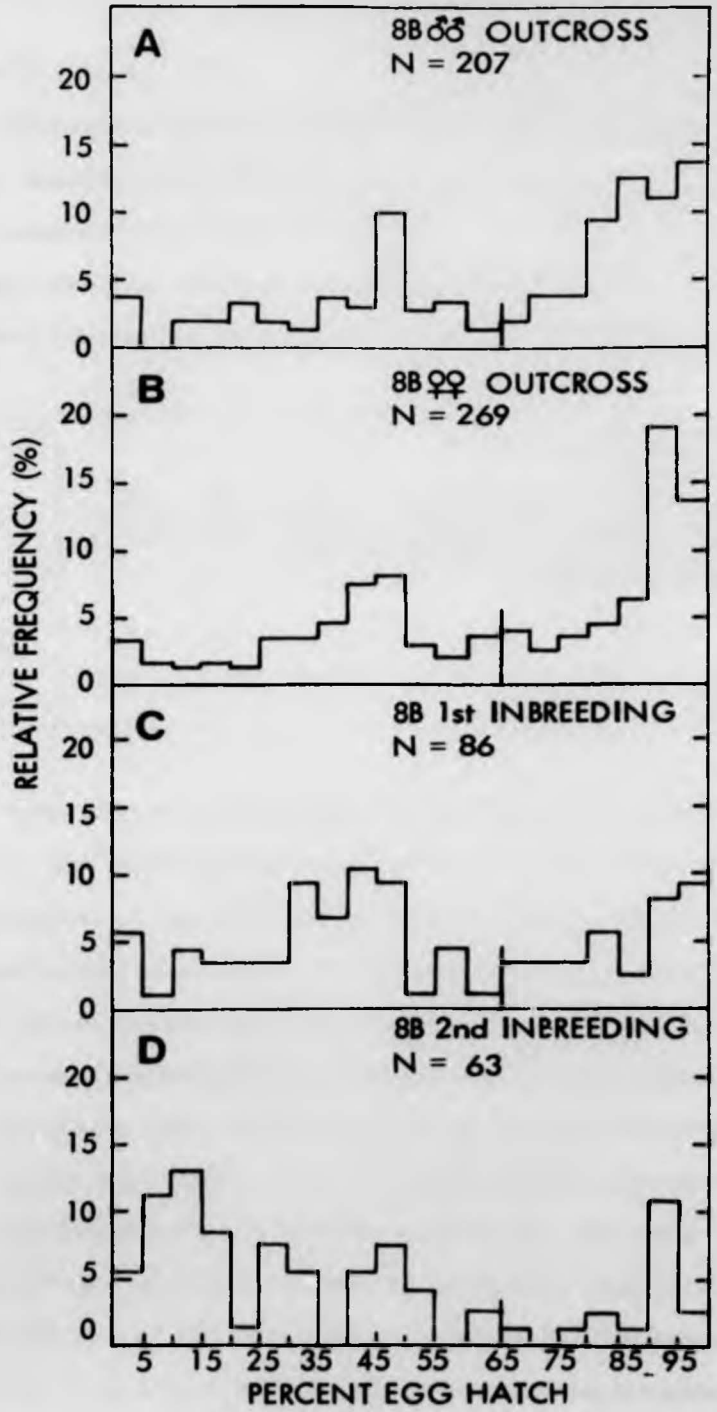
Figure 16. Frequency distribution of hatching rates of line 8B (presumably autosomal translocation).

A. Outcrossed 8B males.

B. Outcrossed 8B females.

C. Fertilities of the first inbred progeny derived from 8B semisterile outcross matings.

D. Fertilities of the second inbred progeny of $^{T}/_{+} \times {}^{T}/_{+}$ matings.


A
8B♂♂ OUTCROSS
N = 207
B
8B♀♀ OUTCROSS
N = 269
C
8B 1st INBREEDING
N = 86
D
8B 2nd INBREEDING
N = 63
RELATIVE FREQUENCY (%)
0
5
10
15
20
5 15 25 35 45 55 65 75 85 95
PERCENT EGG HATCH

(Table 27 and Fig. 16 C).

As can be seen the proportion of normally fertile (65-100% hatching rate), semisterile (35-65% hatching rate), and less than semisterile (35% hatching rate or less) matings were observed to be almost equal in number, but there was no significant departure from the ratio of 1 normal : 3 partially sterile matings ($X_1^2 = 3.09$, $P > 0.05$).

Table 27. Result of line 8B first inbreeding

Cross	No. matings	No. eggs	No. larvae hatched	Average hatching rate(%)	Proportion of matings		
					with more than 65% hatching rate	with 35-65% hatching rate	with 35% hatching rate or less
Line 8B 1st in-breedings	86	11,998	6,521	54.4	0.36 (31/86)	0.33 (28/86)	0.31 (27/86)

Some progeny of the least fertile matings were selected and reared. The offspring were caged together to try to isolate translocation homozygotes. A total of 63 matings were obtained of which 14 showed normal fertility (Fig. 16 D). The average hatching rate was 40.39% (3,811 larvae hatched from 9,435 eggs). A test was carried out to see if there were any translocation homozygotes among the offspring originally showing normal fertile matings but none were found (Table 28).

In this line, males and females might produce gametes with the following frequencies: 0.357 (1+4) + 0.357 (2+3) - alternate; 0.093(1+3) + 0.093 (2+4) - adjacent-1; 0.05(1+2) + 0.05(3+4) - adjacent-2 segregation, as fertility figures were 71% in male outcrosses and 67% in female outcrosses. The ratio of alternate to adjacent segregation was approximately 2.5 : 1 (joint frequency of the two adjacent arrangements). A working model

Table 28. Results of testing normal fertile matings in 2nd inbreeding of line 8B for the presence of translocation homozygotes

Normally fertile matings	Egg hatch of parental matings(%)	Hatching rate in test cross*				
		No. matings tested	No. eggs	No. larvae hatched	Average hatching rate (%)	No. matings with 65% hatching rate or less
S_2 8B-1	91.2	5	426	349	81.9	0
-2	81.6	14	1,271	1,029	80.9	1
-3	80.7	12	1,472	1,213	82.4	2
-4	93.4	6	1,097	959	87.4	0
-5	99.3	8	728	613	84.2	0
-6	94.1	9	1,607	1,309	81.4	2
-7	91.1	11	1,147	1,016	88.5	1
-8	92.5	4	248	230	92.7	0
-9	96.2	9	1,355	1,030	76.0	2
-10	78.8	6	557	442	79.3	1
-11	92.6	9	1,205	1,145	95.0	0
-12	94.6	9	969	809	83.4	1

*S_2 8B -1, 2, etc. x $^{+}/_{+}$ and interbred.

Table 29. Mating chart showing type and frequency of gametes formed by males and females of 8B translocation heterozygotes and the frequency of resulting zygotic combinations with the expected viability of each combination

		Paternal gametes					
		Alternate disjunction		Adjacent-1 disjunction		Adjacent-2 disjunction	
		0.357(1+4)	0.357(2+3)	0.093(1+3)	0.093(2+4)	0.05(1+2)	0.05(3+4)
Maternal gametes: Alternate disjunction	0.357 (1+4)	0.127449 ($^{+}/_{+}$)	0.127449 ($^{T}/_{+}$)				
	0.357 (2+3)	0.127449 ($^{T}/_{+}$)	0.127449 (T/T)				
Adjacent-1 disjunction	0.093 (1+3)				0.008649 ($^{T}/_{+}$)		
	0.093 (2+4)			0.008649 ($^{T}/_{+}$)			
Adjacent-2 disjunction	0.05 (1+2)						0.0025 ($^{T}/_{+}$)
	0.05 (3+4)					0.0025 ($^{T}/_{+}$)	

$^{+}/_{+}$ wild-type, viable, fertile

$^{T}/_{+}$ translocation heterozygote, viable, semisterile

T/T translocation homozygote, lethal

Blank squares represent unbalanced zygote, inviable

based on the above shows the expected egg hatch values of 53.21% or 40.46% depending on the viability of 8B homozygotes (Table 29). The observed hatching rate from second inbreedings was 40.39%, a value in excellent agreement with the expected value where T/T is lethal ($X^2_1 = 0.015$, $P > 0.90$). The expected frequency of wild-type in 63 matings would be 8 (0.1274 x 63 = 8.0), while the observed number of normally fertile matings was 14 of which 12 were proved to be wild-type. This did not differ significantly from the expected at the 1% level but was significant at the 5% level ($X^2_1 = 4.5$, $0.05 > P > 0.03$). The ovarian chromosomes of 23 females from preserved material were examined but translocated chromosomes were not detected.

Line 102A

Line 102A was derived from one of the original 5 semisterile F_1 sons of PALA males irradiated at 1,500 rads. Selection procedure for partial sterility was followed through 6 generations. The frequency distribution of hatching rates is presented graphically in Fig. 17 A and B. Table 30 summarizes the average hatching rate and progeny ratio of normal and partial sterile matings resulting from crosses of 102A males to normal females and 102A females to normal males.

Table 30. Results of line 102A outcross matings to the wild-type

Cross	No. matings	No. eggs	No. larvae hatched	Ave. hatching rate(%)	Properties of matings	
					> 65% hatching rate	< 65% hatching rate
102A males x +/+	63	6,279	4,230	67.36	0.48 (30/63)	0.52 (33/63)
102A females x +/+	73	9,697	6,433	66.34	0.44 (32/73)	0.56 (41/73)

Figure 17. Frequency distribution of hatching rates of line 102A (autosomal translocation).

A. Outcross fertilities of sons of 102A heterozygotes.

B. Daughters of 102A heterozygotes outcrossed to the wild-type.

C. Crosses between the progeny of semisterile outcrosses.

D. Crosses between the progeny of $^{T}/_{+} \times {}^{T}/_{+}$ matings.

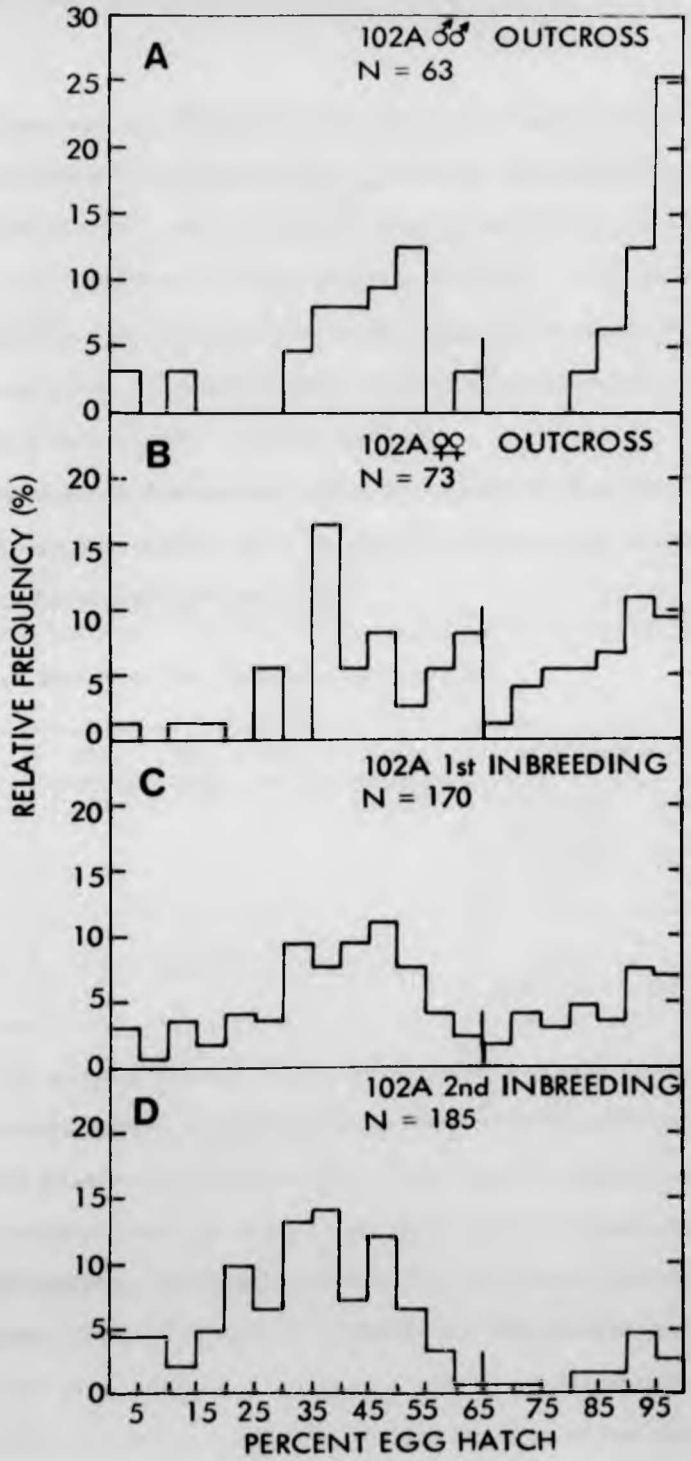
hing rates of line 102A

eterozygotes.

crossed to the wild-type.

sterile outcrosses.

x $^{T}/_{+}$ matings.



There was no difference in fertility or its variance between male and female outcross matings, ($F_{72,62} = 1.45$, $P > 0.05$; $t_{134} = 1.09$, $P > 0.1$) but fertility was significantly higher than 50% of the control level in both outcrosses. Thirty-three of 63 (52%) male by wild-type female crosses and 56% (41/73) of female outcrosses showed 65% or less hatching rate. The segregation ratio of normal to partial sterile progeny approximated 1 : 1 ratios expected.

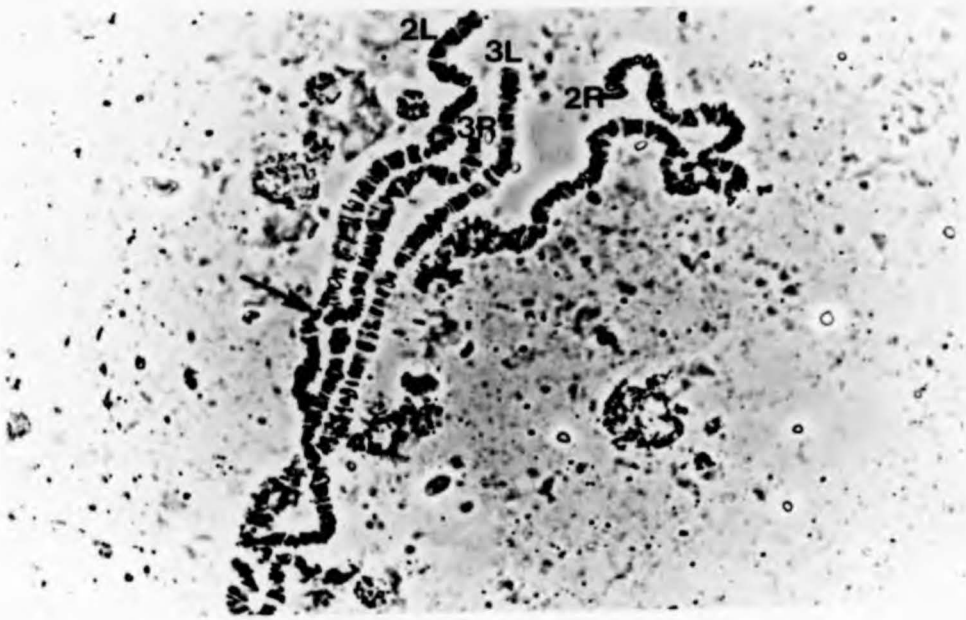
Cytological examination of polytene chromosomes confirmed that line 102A was associated with a reciprocal translocation involving two autosomes 2L and 3R (see Fig. 18).

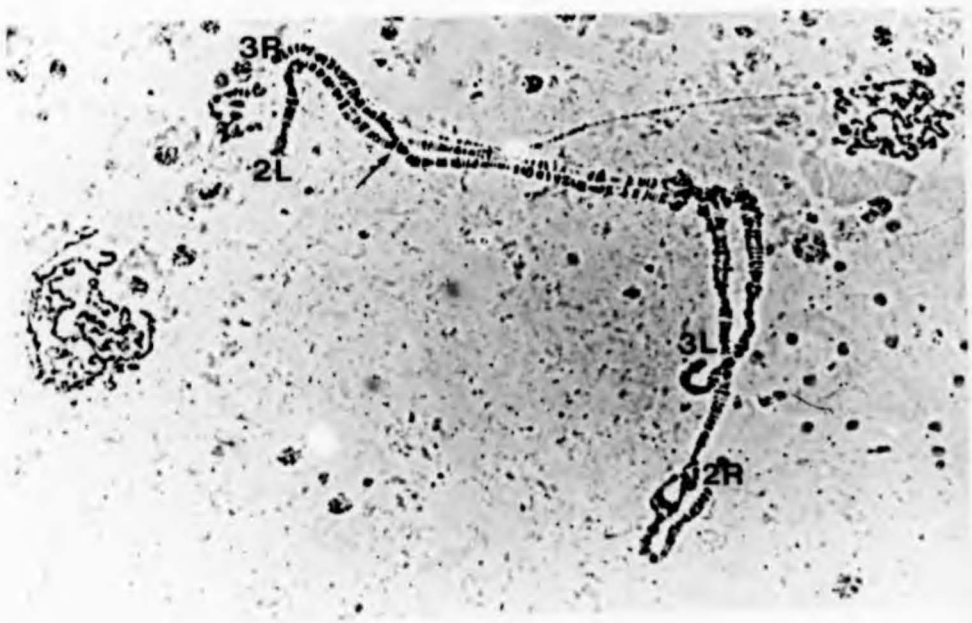
Table 31. Result of line 102A first inbreeding

Cross	No. matings	No. eggs	No. larvae	Ave. hatching rate(%)	Proportion of matings		
					with more than 65% hatching rate	with 35-65% hatching rate	with 35% hatching rate or less
Line 102A	170	23,855	13,199	55.3	0.32 (54/170)	0.42 (72/170)	0.26 (44/170)

The progeny of semisterile outcrosses were intercrossed as previously described, in attempts to produce translocation homozygotes. Inbreeding work in this line was started from the F_2 generation not as in the other 4 lines where at least 6 generations of outcrosses were made before inbreeding. Inbreeding was started early in this line because use of low radiation dose might have produced fewer undesirable recessive lethals, so it was thought unnecessary to have a series of outcrossing for several generations. Secondly, larger numbers of individuals could be tested for homozygosity within a limited time. The expected mating

Figure 18. Polytene chromosomes from the ovarian nurse cells of Anopheles gambiae species A - line 102A (translocation heterozygote involving the autosomes 2L and 3R). Slide and photograph kindly prepared by Dr. J.H. Bryan and Miss J. Chalkley.





the ovarian nurse cells

A - line 102A

volving the autosomes 2L

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Chalkley.

frequencies on inbreeding the line would be the product of the relative proportions of wild-type ($^{+}/_{+}$) and translocation heterozygotes ($^{T}/_{+}$) present in each sex. Accordingly, the expected distribution of fertilities among the first inbreeding was examined (Table 31 and Fig. 17 C). The proportion of matings with 35% hatching rate or less (presumably $^{T}/_{+}$ x $^{T}/_{+}$) was 0.26 (44 of 170 matings), in close agreement with the expected value of 0.25. The progeny of 19 of the matings showing a hatching rate less than 35% were reared and caged together. In these tests the average hatching rate was 42.83% (of 27,902 eggs, 11,952 larvae hatched). In the second round of inbreeding a total of 185 matings were obtained of which 22 were normally fertile (showing a hatch of 65% or more) (see Fig. 17 D). The offspring of these fertile matings were kept as possible sources of translocation homozygotes and 17 of them were tested by outcrossing to the wild-type. However, results obtained show clearly that they were all of normal fertility and it was thus concluded that each line was of the wild-type chromosome arrangement (Table 32).

In order to estimate the expected karyotypes among the progeny of $^{T}/_{+}$ x $^{T}/_{+}$ matings, approximately 2.5 : 1 ratios of alternate to adjacent-1 segregation was assumed in translocation heterozygotes of line 102A. With this assumption, the frequency and type of gametes formed by both sexes are 0.357 (1+4) + 0.357 (2+3) alternate segregation and 0.143 (1+3) + 0.143 (2+4) adjacent-1 segregation, respectively.

The frequency of resulting zygotic combinations in above matings are shown in Table 33. Calculations based on this working model predicted a hatching rate if T/T is viable, of 55.06% or if T/T is lethal of 42.31%. The observed hatching rate from the second inbreeding

Table 32. Results of testing normal fertile matings in 2nd inbreedings of line 102A for the presence of translocation homozygotes

Normally fertile matings	Hatching rate of parental mating	Hatching rate in test cross*				
		No. matings tested	No. eggs	No. larvae hatched	Ave. hatching rate(%)	No. matings with 65% hatching rate or less
$S_2$102A-1	66.6	18	2,683	2,073	77.2	3
-2	92.0	11	1,572	1,442	91.7	1
-3	86.6	7	795	643	80.8	2
-4	95.8	6	698	561	80.3	1
-5	94.0	8	1,233	1,178	95.5	0
-6	86.4	4	555	528	95.1	0
-7	84.0	8	996	968	97.1	0
-8	91.4	8	1,194	1,027	86.0	1
-9	96.2	8	785	714	90.9	1
-10	93.3	8	1,041	909	87.3	2
-11	83.6	6	625	585	93.6	1
-12	92.6	7	778	648	83.2	1
-13	94.4	4	379	351	92.6	0
-14	94.6	8	896	767	85.6	1
-15	98.6	8	977	935	95.7	0
-16	93.5	6	598	559	93.4	0
-17	86.5	8	845	819	96.9	1

*S_2 102A 1,2, etc. x $^+/+$ and interbred

Table 33. Mating chart showing type and frequency of gametes formed by males and females of 102A translocation heterozygotes and the frequency of resulting zygotic combinations with the expected viability of each combination

Paternal gametes

Maternal gametes		Alternate disjunction		Adjacent-1 disjunction	
		0.357 (1+4)	0.357 (2+3)	0.143 (1+3)	0.143 (2+4)
Alternate disjunction	0.357 (1+4)	0.127449 ($^{+}/_{+}$)	0.127449 ($^{T}/_{+}$)		
	0.357 (2+3)	0.127449 ($^{T}/_{+}$)	0.127449 (T/T)		
Adjacent-1 disjunction	0.143 (1+3)				0.020449 ($^{T}/_{+}$)
	0.143 (2+4)			0.020449 ($^{T}/_{+}$)	

$^{+}/_{+}$ wild-type, viable, fertile

$^{T}/_{+}$ translocation heterozygote, viable, partial sterile

T/T translocation homozygote, lethal

Blank squares represent unbalanced zygotes, inviable

(42.83%) did not differ greatly from the expected value where T/T is lethal ($X^2_1 = 3.172$, $P > 0.05$). The expected frequency of wild-types in 185 matings would be 24 (0.127449 x 185 = 23.57) - a figure in close agreement with the observed value of 22 ($X^2_1 = 0.16$, $P > 0.6$). Thus, the data obtained from intercrosses heterozygous 102A suggested that the translocation homozygotes were lethal.

D. An X-autosomal translocation line

Line 26D

Both males and females were outcrossed to wild-type individuals and selected for partial sterility. During this process tests were conducted to ascertain the presence of an X-autosome interchange in this line. Two matings showed very low egg hatches (one of 10% and the other of 20%) when 26D males were outcrossed to wild-type females in the fifth outcross generation. The offspring of these two matings (20 males and 22 females) were outcrossed to wild-type individuals. If all the females outcrossed to normal males showed reduced hatching rates (65% or less) and male outcrosses showed normal egg hatches, then an X-autosomal translocation would be indicated.

Results of the crosses mentioned above are shown in Table 34. It can be seen that all of the 9 daughters of semisterile males, outcrossed to wild type males proved to be semisterile and 11 of 14 of their brothers were normally fertile. Unfortunately 3 partially sterile matings among 14 were not tested in their second ovipositions to see if they were normally fertile. Nevertheless, data obtained from this experiment clearly indicated that an X-autosomal interchange had taken place. Later cytological examination of polytene nuclei confirmed that this line was indeed associated with a reciprocal chromosome translocation involving the X-chromosome and one of the autosomes 2L. The formation of an X-autosomal translocation can be expressed as follows:

Table 34. Results of tests for the presence of an X-autosomal translocation in line 26D

Females derived from F_5 26D males x $^+/+$ females crossed with wild-type males				Males derived from F_5 26D males x $^+/+$ females crossed with wild-type females			
Serial No.	No. eggs laid	No. larvae hatched	hatching rate (%)	Serial No.	No. eggs laid	No. larvae hatched	hatching rate (%)
1	110	53	48.1	1	156	148	94.8
2	84	45	53.5	2	129	128	99.2
3	224	126	56.2	3	94	92	97.8
4	147	74	50.3	4	92	84	91.3
5	139	26	18.7	5	99	95	95.9
6	174	77	44.2	6	57	44	77.1
7	64	34	53.1	7	113	62	54.8
8	41	21	51.2	8	72	72	100.0
9	42	22	52.3	9	94	13	13.8
				10	116	95	81.8
				11	132	103	78.0
				12	116	88	75.8
				13	102	25	24.5
				14	46	39	84.7
Total	1,025	478	46.63	Total	1,418	1,088	76.72

If the two pairs of autosomes are denoted by A_2A_2, A_1A_1, and the pair of sex chromosomes by XX, and XY, the genotype of the normal female will be $A_2A_2A_1A_1XX$ and the normal male $A_2A_2A_1A_1YX$. If an interchange between one of the autosomes (A_1) and an X-chromosome has occurred in an irradiated X-chromosome bearing sperm, then one translocation chromosome (A_1^x) has an X segment associated with A_1 chromosome and the other translocation chromosome (X^{A_1}) has an A_1 segment with an X-chromosome. Thus the genotype of such a hemizygous male would be represented as $A_2A_2A_1A_1^xYX^{A_1}$. If there is random assortment among non-homologous centromeres and regular disjunction between homologous ones, then one-half of the gametes would receive a balanced haploid complement, A_2A_1Y and $A_2A_1^xX^{A_1}$ and one-half an unbalanced complement, $A_2A_1X^{A_1}$, or $A_2A_1^xY$. Consequently, the following zygotic combinations would be produced when fertilization with a normal female gamete - (A_2A_1X) occurs:-

Gametes	Zygotes (with A_2A_1X)
A_2A_1Y	$A_2A_2A_1A_1YX$ - normal male
$A_2A_1X^{A_1}$	$A_2A_2A_1A_1X^{A_1}X$ - unbalanced
$A_2A_1^xY$	$A_2A_2A_1^xA_1YX$ - unbalanced
$A_2A_1^xX^{A_1}$	$A_2A_2A_1^xA_1X^{A_1}X$ - heterozygous female

Of the 4 resulting zygotes only two, or 50%, are viable and are either a normal male ($A_2A_2A_1A_1YX$) or a translocation heterozygous female ($A_2A_2A_1A_1^xXX^{A_1}$).

Matings between the resultant heterozygous female ($A_2A_2A_1A_1^xX^{A_1}X$) and wild-type male should result in a 50% hatching rate and would be expected to produce normal and hemizygous males in equal numbers and normal and heterozygous females, in equal numbers as is shown in the

following:

Maternal gametes	Paternal gametes: $A_2 A_1 Y$	Paternal gametes: $A_2 A_1 X$
$A_2 A_1 \overset{X}{} \overset{A_1}{} X$	$A_2 A_2 A_1 A_1 \overset{X}{} \overset{A_1}{} Y X$ (hemizygous male)	$A_2 A_2 A_1 A_1 \overset{X}{} \overset{A_1}{} X X$ (heterozygous female)
$A_2 A_1 \overset{X}{} X$	$A_2 A_2 A_1 A_1 \overset{X}{} Y X$ (unbalanced zygote)	$A_2 A_2 A_1 A_1 \overset{X}{} X X$ (unbalanced zygote)
$A_2 A_1 X \overset{A_1}{}$	$A_2 A_2 A_1 A_1 Y X \overset{A_1}{}$ (unbalanced zygote)	$A_2 A_2 A_1 A_1 X \overset{A_1}{} X$ (unbalanced zygote)
$A_2 A_1 X$	$A_2 A_2 A_1 A_1 Y X$ (normal male)	$A_2 A_2 A_1 A_1 X X$ (normal female)

However, matings between the resultant normal male (offspring from the previous crosses) and wild-type female should produce normal egg hatches. The observed data (Table 34) confirm this.

This line was studied for 7 outcross generations. (Fig. 19 A and B). The average egg hatches of 26D male outcross matings and 26D female outcross matings were 62.1% and 69.9% respectively. (Table 35). Egg hatches appeared to be higher than the expected 50% in both sexes.

The distribution of egg hatches was compared between the 2 types of outcrosses. There was a significant difference in variance ($F_{99,47} = 1.996$, $P < 0.01$), but there was no significant difference in the mean ($t_{146} = 1.783$, $P > 0.05$). As can be seen from Fig. 19, the resultant matings with less than 65% hatch in male outcrosses showed a wide spread

F

A

B

C

D

Figure 19. Frequency distribution of hatching rates of line 26D (an X-autosomal translocation).

A. Outcrossed males (Note a wide spread of hatching rates).

B. Outcrossed females (Note a clear bimodal distribution of fertility).

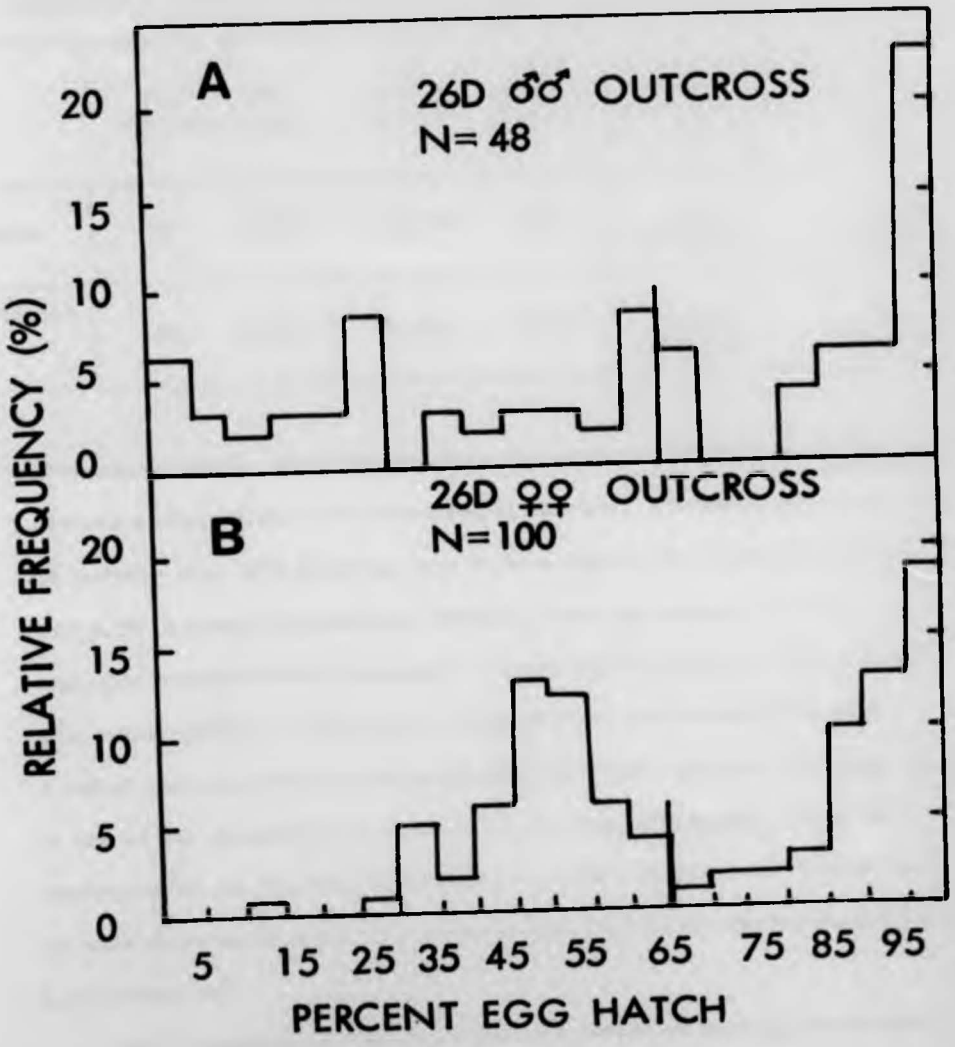

A
26D ♂♂ OUTCROSS
N= 48
B
26D ♀♀ OUTCROSS
N=100
RELATIVE FREQUENCY (%)
0
5
10
15
20
5 15 25 35 45 55 65 75 85 95
PERCENT EGG HATCH

Table 35. Results of line 26D outcross matings to the wild-type

Cross	No. matings	No. eggs	No. larvae hatched	Ave. hatching rate(%)	Proportion of matings	
					> 65% hatching rate	< 65% hatching rate
26D males x +/+	48	6,337	3,936	62.1	0.46 (22/48)	0.54 (26/48)
26D females x +/+	100	12,811	8,961	69.9	0.50 (50/100)	0.50 (50/100)

of hatching rates, while the daughters of semisterile outcross matings showed a clear bimodal distribution of hatches. However, the proportion of matings with 65% hatching rate or less was 0.54 in male outcrosses and 0.50 in female outcrosses showing close agreement with the expected proportion of 1 normal : 1 semisterile matings. Rabbani and Kitzmiller (1972) reported that a distortion of sex-ratio (2 females : 1 male) was observed in the progeny from a heterozygote x normal cross in one of the X-autosomal translocation in An. albimanus. This was attributed to the inability of hemizygous males to survive. There was no such difference in the sex-ratio in line 26D which approximated 1 : 1 (see Table 16).

All X-autosomal translocations are inherited through males and females and only females can become homozygous for such translocations. A scheme illustrating gamete formation in hemizygous male and heterozygous female and different zygotes resulting from different combinations of these gametes is shown in Fig. 20. It must be pointed out, however, that homozygous X-autosome translocation, if viable, will always show 50% egg hatch, because the males, by definition, will

Fi

A.

B.

C.

D.

Figure 20. Formation of an X-autosomal translocation homozygote; showing how zygotes of different genotypes are formed by the various types of gametes produced by hemizygous male and heterozygous female.

nslocation homozygote;
genotypes are formed
produced by hemizygous

$A_2A_2A_1A_1{}^{X}X^{A_1}Y$ (Hemizygous male)

$A_2A_2A_1A_1{}^{X}X^{A_1}X$ (Heterozygous female)

Gametes from female / Gametes from male	$A_2A_1X^{A_1}$	A_2A_1X	$A_2A_1{}^{X}X^{A_1}$	$A_2A_1{}^{X}X$
A_2A_1Y	$A_2A_2A_1A_1X^{A_1}Y$ (Unbalanced)	$A_2A_2A_1A_1X\,Y$ (Normal male)	$A_2A_2A_1A_1{}^{X}X^{A_1}\,Y$ (Hemizygous male)	$A_2A_2A_1A_1{}^{X}X\,Y$ (Unbalanced)
$A_2A_1X^{A_1}$	$A_2A_2A_1A_1X^{A_1}X^{A_1}$ (Unbalanced)	$A_2A_2A_1A_1X^{A_1}\,X$ (Unbalanced)	$A_2A_2A_1A_1{}^{X}X^{A_1}\,X^{A_1}$ (Unbalanced)	$A_2A_2A_1A_1{}^{X}X^{A_1}\,X$ (Heterozygous female)
$A_2A_1{}^{X}Y$	$A_2A_2A_1A_1{}^{X}X^{A_1}\,Y$ (Hemizygous male)	$A_2A_2A_1A_1{}^{X}X\,Y$ (Unbalanced)	$A_2A_2A_1{}^{X}A_1{}^{X}X^{A_1}\,Y$ (Unbalanced)	$A_2A_2A_1{}^{X}A_1{}^{X}X\,Y$ (Unbalanced)
$A_2A_1{}^{X}X^{A_1}$	$A_2A_2A_1A_1{}^{X}X^{A_1}\,X^{A_1}$ (Unbalanced)	$A_2A_2A_1A_1{}^{X}X^{A_1}\,X$ (Heterozygous female)	$A_2A_2A_1{}^{X}A_1{}^{X}\,X^{A_1}\,X^{A_1}$ (Homozygous female)	$A_2A_2A_1{}^{X}A_1{}^{X}X^{A_1}\,X$ (Unbalanced)

be heterozygous, $A_2A_2\ A_1A_1^{X}YX^{A_1}$. No test was made to determine the viability of homozygous X-autosomal translocation during this experiment.

E. Partial sterility due to unknown causes

Lines 9B, 6E and 8M

These 3 lines were derived from 8 of the original 14 partially sterile F_1 daughters of TYF-5 males treated at 4,000 rads. The lines 9B, 6E and 8M were studied for 10, 6 and 4 outcross generations respectively but were not promising as they had low frequencies of semisterile individuals. Fertilities of these lines were significantly higher than 50% of the control level in both sexes (see Table 36). However, there was no significant difference in fertility between male and female outcross matings (9B : $F_{75, 131} = 1.11$, $P > 0.05$; $t_{206} = 1.79$, $P > 0.05$; 6E: $F_{49, 73} = 1.28$, $P > 0.05$; $t_{122} = 0.77$, $P > 0.5$; 8M: $F_{31, 55} = 1.97$, $P > 0.01$; $t_{86} = 1.42$, $P > 0.1$).

Table 36. Results of lines 9B, 6E and 8M outcross matings to the wild-type

Cross	No. matings	No. eggs	No. larvae hatched	Ave. hatching rate(%)	Proportion of matings	
					> 65% hatching rate	< 65% hatching rate
9B males x +/+	76	9,831	7,933	80.6	0.71 (54/76)	0.29 (22/76)
9B females x +/+	132	20,541	14,561	70.8	0.58 (76/132)	0.42 (56/132)
6E males x +/+	50	7,042	5,640	80.0	0.78 (39/50)	0.22 (11/50)
6E females x +/+	74	10,863	8,811	81.1	0.77 (57/74)	0.23 (17/74)
8M males x +/+	32	3,796	3,116	82.0	0.78 (25/32)	0.22 (7/32)
8M females x +/+	56	7,456	5,350	71.7	0.73 (41/56)	0.27 (15/56)

The distribution of egg hatching rates from outcrosses involving lines 9B, 6E and 8M are also graphically presented in Fig. 21, and 22. Clearly, the skewness of the distribution was more to the right than to the left. Testing against the hypothesis of a 1 : 1 ratio of normal and semisterile matings revealed that the shortage of semisteriles was highly significant in both sexes in all the lines except 9B female outcrosses as follows:-

Outcrosses	Proportion of matings χ^2_1	(Exp. 1 normal : 1 semisterile) P
9B male x $^{+}/_{+}$	13.47	< 0.01
9B female x $^{+}/_{+}$	3.03	> 0.05
6E male x $^{+}/_{+}$	15.68	< 0.01
6E female x $^{+}/_{+}$	21.62	< 0.01
8M male x $^{+}/_{+}$	10.12	< 0.01
8M female x $^{+}/_{+}$	12.07	< 0.01

The progeny of 9B semisterile matings, derived from female outcrosses were inbred in an attempt to produce translocation homozygotes. Results of this inbreeding revealed that 11 of 23 matings were normally fertile and 11 matings showed a hatching rate between 35% and 65%, he result expected from matings between wild-type and translocation heterozygotes ($^{T}/_{+} \times {}^{+}/_{+}$). Matings between translocation heterozygotes ($^{T}/_{+} \times {}^{T}/_{+}$) would have given less than 35% hatching rate but only one such oviposition was obtained (see Fig. 21 C) and no further inbreeding was attempted.

These lines were abandoned. Unfortunately no cytological examinations were made of these lines, and / whether or not the cause of partial sterility was due to chromosomal translocations is not known.

Fi

Figure 21. Frequency distribution of hatching rates of line 9B.

A. Outcrossed males.

B. Outcrossed females.

C. Inbred progeny of outcrossed females.

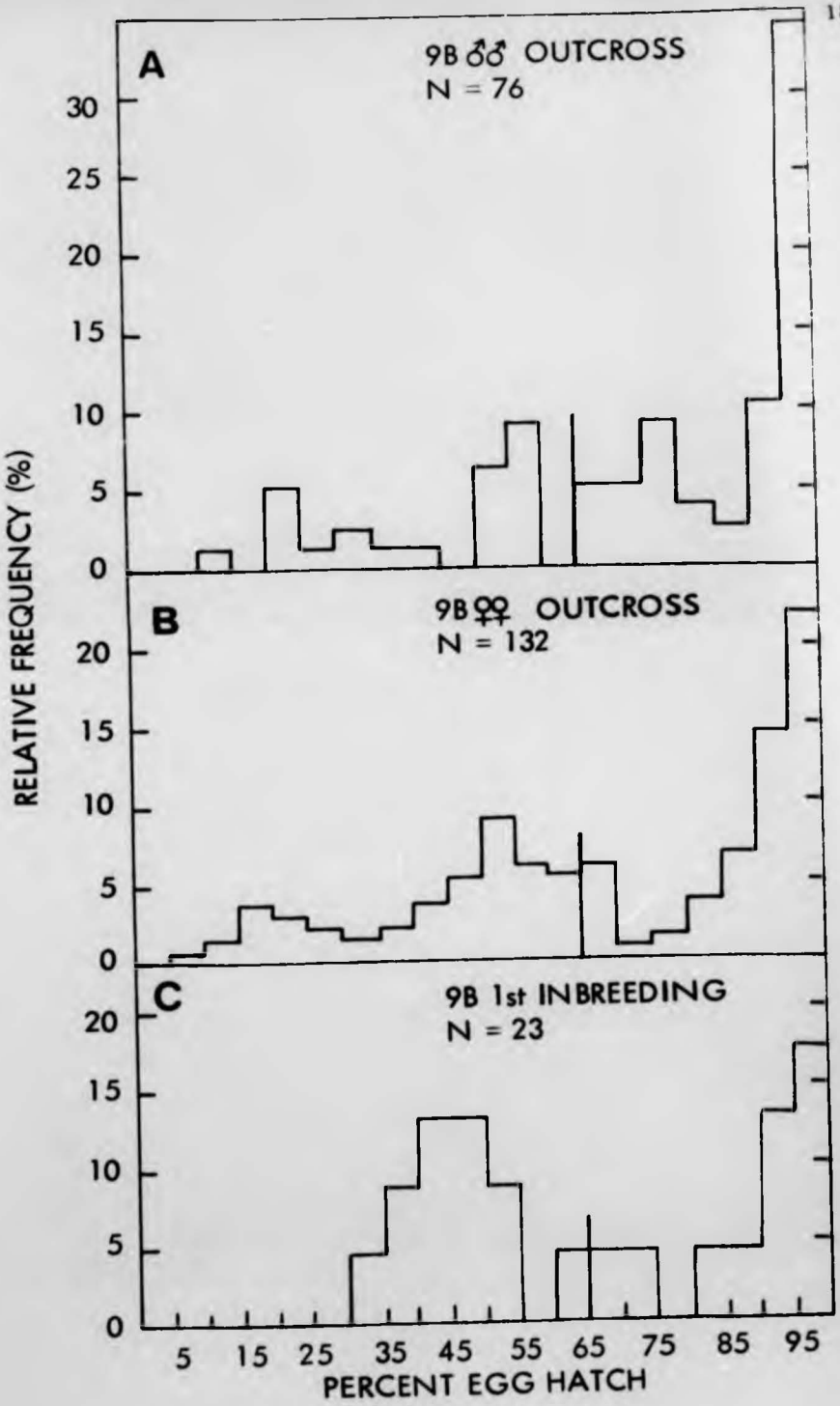
A.

B.

C.

D.

hing rates of line 9B.



Fi

Figure 22. Frequency distribution of hatching rates from outcrosses involving lines 6E and 8M.

A. Sons and daughters of line 6E.

B. Sons and daughters of line 8M.

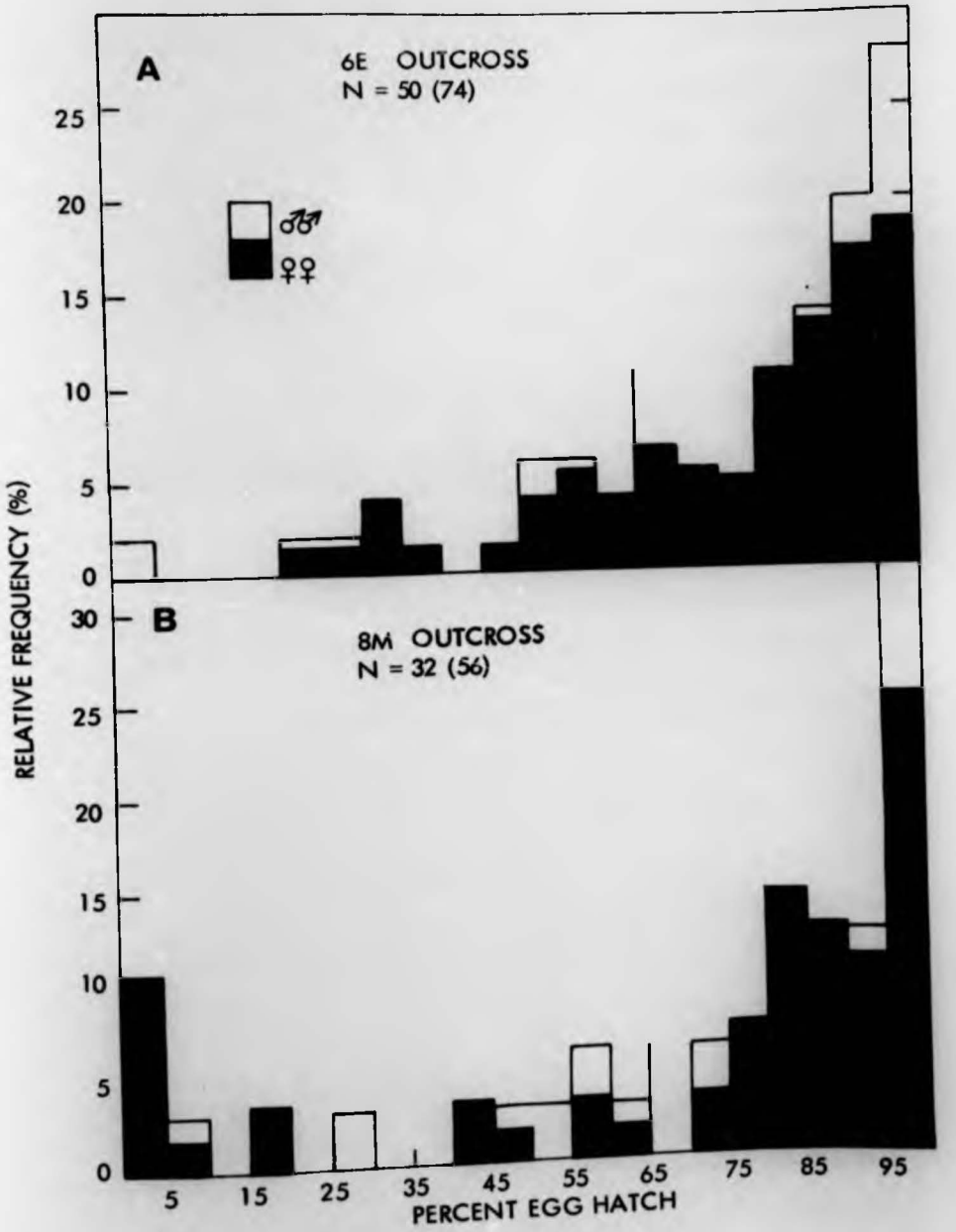
Numbers of Females are given in parentheses.

A.

B.

C.

D.


A
6E OUTCROSS
N = 50 (74)
♂♂
♀♀
25
20
15
10
5
0
B
8M OUTCROSS
N = 32 (56)
30
25
20
15
10
5
0
RELATIVE FREQUENCY (%)
5
15
25
35
45
55
65
75
85
95
PERCENT EGG HATCH

Lines 53D, 53E, 54F, 54E, 50Q and 55B

As described earlier, among 111 F_1 daughters of irradiated PALA males exposed to 1,500 rads, 6 partially sterile lines were isolated. These lines were maintained by outcrossing to both sexes of the wild-type and selecting for semisterility for 4-7 generations. The egg hatching rates and the relative frequencies of the four categories of egg hatch among PALA control and the 6 lines are tabulated in Table 37. As can be seen, fertilities of these lines were found to be very high and both male and female outcrosses of lines 53D, 53E and 54F showed an extremely low frequency of partial sterility. Testing against the hypothesis of a 1 : 1 ratio of partially sterile to normal hatches (the expected result of outcrossing translocation heterozygotes) revealed that the shortage of semisteriles was highly significant in both sexes in these lines. These lines were not considered promising and were hence abandoned. In the remaining three lines, namely, 54E, 50Q and 55B, male matings to wild-type females showed a very low frequency of partial sterility, while approximately 40% of reciprocal crosses showed 65% egg hatch or less. The offspring of line 50Q female outcross matings showing less than 50% hatching rate were inbred and the frequency distribution of hatching rates examined. Forty-one of 74 matings showed a hatching rate of 65% or more, an excess of apparent wild-type matings. The difference from the expected 1 : 3 ratio of normal to partial sterile was significant ($X_1^2 = 54.72$, $P < 0.0001$). Thus, further inbreeding to produce translocation homozygotes was not made and this line was discarded. Cytological study of ovarian nurse cell polytene chromosomes from preserved material failed to show any chromosomal aberration.

Table 37. Fertilities and the proportion of egg layings showing 70% or less hatching rates in control and progeny of 6 partially sterile lines of An. gambiae species A

Line	No. matings	No. eggs	No. larvae hatched	Ave. hatching rate (%)	Proportion of egg layings showing				1 partially sterile : 1 normal (Expected)	
					<70%	<65%	<55%	<50%	X_1^2	P
53D male x $^{+}/_{+}$	37	4,433	3,650	82.3	0.21	0.18	0.16	0.16	14.29	<0.001
53D female x $^{+}/_{+}$	36	6,086	5,194	85.3	0.11	0.08	0.05	0.05	25.00	<0.001
53E male x $^{+}/_{+}$	42	4,724	3,843	81.3	0.14	0.11	0.11	0.11	24.38	<0.001
53E female x $^{+}/_{+}$	31	5,479	4,987	91.0	0.12	0.06	0.06	0.00	23.51	<0.001
54F male x $^{+}/_{+}$	47	4,740	3,727	78.6	0.27	0.21	0.10	0.06	15.51	<0.001
54F female x $^{+}/_{+}$	55	8,452	6,561	77.6	0.27	0.25	0.23	0.21	13.25	<0.001
54E male x $^{+}/_{+}$	54	6,168	5,056	81.9	0.20	0.18	0.16	0.14	21.41	<0.001
54E female x $^{+}/_{+}$	63	10,335	7,229	69.9	0.44	0.41	0.41	0.33	1.92	>0.10
50Q male x $^{+}/_{+}$	44	4,449	3,519	79.0	0.18	0.13	0.13	0.13	23.27	<0.001
50Q female x $^{+}/_{+}$	61	8,142	5,521	67.8	0.47	0.45	0.36	0.31	0.80	>0.4
55B male x $^{+}/_{+}$	49	5,517	4,809	87.1	0.08	0.04	0.04	0.04	41.32	<0.001
55B female x $^{+}/_{+}$	64	9,177	6,028	65.6	0.48	0.43	0.35	0.32	1.00	>0.30
PALA control*	101	11,609	10,178	87.7	0.12	0.09	0.05	0.05	-	-

*Data from Dr. E.S. Krafsur (1972 b)

F. Attempt to produce translocation homozygotes with the aid of morphological markers

General consideration

In addition to the use of hatching rate as an identifying measurement, the "pseudo-linkage" method used by Krafsur (1972 b) was applied in an attempt to produce translocation homozygotes. He reasoned that "reciprocal translocation of chromosome segments will create new linkage relationships among the chromosomes involved. The fact that both members of a reciprocally translocated pair of chromosomes must be present for zygotic viability allows us to 'mark' genetically one or both of them and so recognize its translocated progeny, an advantage in efforts to produce translocation homozygotes. Should locus A become translocated to the chromosome carrying locus B, A and B will thereafter be linked with each other and with semisterility. If, on the other hand, locus A is not included in the translocated segment, or if both loci A and B are mutually exchanged, 'pseudolinkage' between A, B and semisterility will obtain, in appropriate crosses. The degree of 'linkage' between A, B and semisterility whether apparent or real, will depend on the three frequencies of recombination between A, B and the translocation breakpoint in animals heterozygous for all 3 factors."

For this method, it is necessary for the linkage between A and the breakpoint to be close and also for the linkage between B and the breakpoint to be close.

Progeny of partially sterile outcrosses (dominant phenotype C^+/C^+ (manifested in the larva) and dieldrin resistance (R^{Dl}/R^{Dl})) were crossed with genic marker stock "16C", homozygous for the recessive mutant collarless (C/C) and for the recessive mutant dieldrin-susceptibility

(R^+/R^+). Mason (1964) studied linkage between the C locus and the dieldrin resistance locus in An. gambiae species A. His result showed that the collar and dieldrin loci assort independently of each other and of sex. They may therefore be on different autosomes (see also Haridi, 1971; Krafsur, 1972 b). The semisterile F_1, heterozygous for the interchange and for the marker genes, were backcrossed reciprocally to 16C and the fertility of single matings was tested. In the absence of recombination (not the true situation, unfortunately) translocated F_2 progeny should be also heterozygous for the genic markers, i.e. $^{T}/_{+}$; R^{Dl}/R^{+}; C^{+}/C; while F_2 of wild-type karyotype should be collarless and dieldrin susceptible. Translocation homozygotes, if viable, would be produced among the F_3 by intercrossing those F_2 that could be identified as translocation heterozygotes by means of their heterozygosity for collar and dieldrin resistance. Such translocation homozygotes could, in principle, be identified as they would be collared, homozygous dieldrin-resistant individuals (R^{Dl}/R^{Dl}), surviving a two-hour exposure to 4.0% dieldrin, $\frac{T}{+}\ \frac{R^{Dl}}{R}\ \frac{C^{+}}{C} \times \frac{T}{+}\ \frac{R^{Dl}}{R}\ \frac{C^{+}}{C} \rightarrow \frac{T}{T}\ \frac{R^{Dl}}{R^{Dl}}\ \frac{C^{+}}{C^{+}} + \ldots\ldots$.

Experiment with line 6K

Progenies of 6K partial sterile outcross matings were backcrossed reciprocally to a strain 16C, homozygous for the recessive mutant collarless (C/C) and for the recessive mutant dieldrin-susceptibility (R^+/R^+). Their partially sterile progeny were selected for collar (C^+/C) and screened on 0.4% dieldrin for one hour when adults emerged to ensure that they were dieldrin-resistant (R^{Dl}/R^{+}). F_1 survivors were then backcrossed to double-recessive wild-karyotype 16C. Of 69 F_1 backcross

matings of this type, 29 backcross matings (16 of 30 F_1 males and 13 of 39 F_1 females) showed partial sterility (65% hatching rate or less). Some of the progeny of partially sterile F_1 backcrosses were reared and their phenotypes were examined in order to test for independent segregation among their progeny. The detailed results of backcross segregation of collar and dieldrin-resistance among the progeny of the crosses $6K \frac{T}{+} \frac{R^{Dl}}{R^{+}} \frac{C^{+}}{C} \times 16C \frac{+}{+} \frac{R^{+}}{R^{+}} \frac{C}{C}$ are shown in Table 38.

On the basis of independent assortment of dieldrin resistance and collar, 1 : 1 ratios of resistant to susce ptible phenotypes would be expected in the collarless and collared classes in the F_1 backcross progeny. Linkage between the R and C loci would be indicated by a significant departure from the 1 : 1 ratios. Chi-square was applied to test the expected 1 : 1 ratio in the segregation of C^{+} to wild-type (C) and also resistant (R^{Dl}) to wild-type (R^{+}), in each sex, each family and the total segregation. It was clear from the table that there was some loss in the collar phenotype class among the F_2 males but the total segregation revealed no significant departure from the 1 : 1 ratios of collar, collarless ($X_1^2 = 0.72$, $P > 0.4$) or dieldrin-resistant, susceptible ($X_1^2 = 0.26$, $P > 0.6$). However, a chi-square heterogeneity test showed 3 of 7 families to be highly heterogeneous in the latter case ($X_6^2 = 18.89$, $P < 0.01$). The source of disturbance in this segregation was an excess in the resistant phenotype class in two of 3 families (No. 3 and No. 6) and a shortage in that class in one family (No. 7). The R-C recombination fraction was 276 of 556 individuals or 49.6% (53.9% in F_2 males and 46.0% in F_2 females). This recombination value was close to 50% - the figure corresponding to independent assortment.

$X_1^2 = 4.86$ $0.05 > P > 0.02$

Table 38. Backcross segregation of Collar (C^+) and Dieldrin resistance (R^{Dl}) among the progeny of the cross $6K \frac{T}{+} \frac{C^+}{C} \frac{R^{Dl}}{R^+} \times 16C \frac{+}{+} \frac{C}{C} \frac{R^+}{R^+}$

Family No.	No. eggs	No. larvae hatched	Ave. hatching rate (%)	No. adults emerged	Male				Female				Parental	Recombinant		Parental	Test for 1 : 1				Linkage	
					$R^{Dl}C^+$	$R^{Dl}C$	R^+C^+	R^+C	$R^{Dl}C^+$	$R^{Dl}C$	R^+C^+	R^+C	$R^{Dl}C^+$	$R^{Dl}C$	R^+C^+	R^+C	C^+ X^2_1	C^+ P	R^{Dl} X^2_1	R^+ P	X^2_1	P
1	148	62	41.8	60	5	2	7	12	9	9	6	10	14	11	13	22	0.60	>0.4	1.66	>0.2	2.40	>0.1
2	170	96	56.4	86	9	11	11	10	14	11	10	10	23	22	21	20	0.04	>0.8	0.18	>0.7	1.16	>0.2
3*	234	94	53.9	76	9	18	6	5	15	5	4	14	24	23	10	19	0.84	>0.4	4.26	<0.05	1.31	>0.25
4*	212	100	24.0 54.2	74	4	11	6	9	11	8	12	13	15	19	18	22	0.67	>0.3	0.67	>0.5	0.01	>0.9
5	190	63	40.0 33.1	49	3	6	6	8	6	6	8	6	9	12	14	14	0.18	>0.7	1.00	>0.3	0.18	>0.6
6	224	137	61.1	131	13	16	11	12	29	23	19	8	42	39	30	20	1.29	>0.2	7.33	<0.01	0.61	>0.4
7	265	80	30.1	80	5	12	13	12	6	8	11	13	11	20	24	25	1.25	>0.2	4.05	<0.05	0.80	>0.3
Total	1433	632	44.1	556	48	76	60	68	90	70	70	74	138	146	130	142	4.87		19.15		6.47	

	D.F.					
Deviation	1	0.72	>0.4	0.26	>0.6	0.03	>0.9
Heterogeneity	6	4.15	>0.5	18.89	<0.01	6.44	>0.3

*Progeny of 2 partially sterile family were reared together

Male

$X^2_1 C^+$, C = 5.14, 0.05>P>0.02

$X^2_1 R^{Dl}, R^+$ = 0.06, P > 0.8

$X^2_{Linkage}$ = 1.59, P > 0.25

Female

$X^2_1 C^+$, C = 0.84, P>0.35

$X^2_1 R^{Dl}, R^+$ = 0.84, P>0.35

$X^2_{Linkage}$ = 1.89, P > 0.15

Male + Female

$X^2_1 = C^+$, C = 0.72, P > 0.4

$X^2_1 = R^{Dl}, R^+$ = 0.26, P > 0.6

$X^2_{Linkage}$ = 0.03, P > 0.9

Sex ratio (exp. 1 : 1)

X^2_1 = 4.86 0.05 > P > 0.02

The formula for the detection of linkage was applied (Mather, 1963) and it was found that chi-square linkage tests did not show a significant departure from the expected on the basis of independent assortment of R^{D1} and Collar ($X^2_1 = 0.03$, $P > 0.9$) and families were homogeneous ($X^2_6 = 6.44$, $P > 0.3$). These results lead to the conclusion that one or other of the only two autosomal markers available turns out to be far away from either of the two translocation breakpoints. The R and C loci recombine freely before and after translocation. It is therefore evident that a homozygote for this particular translocation is unlikely to be identified by this method.

Experiment with line 26O

A similar study was also made in line 26O and results are given in Table 39. Chi-square to test for the single factor segregation of $\underline{C}^+$: $\underline{C}$ and $\underline{R}^{D1}$: $\underline{R}^+$ showed insignificant departure from the expected 1 : 1 ratios in both males and females. The R - C recombination fraction was 177 of 329 individuals or 53.7% (55.1% in F_2 males and 52.4% in F_2 females) - a figure close to 50%. The linkage test indicated independent assortment of $\underline{C}$ and R^{D1} in line 26O as was found in line 6K. One of the 4 families tested showed evidence of linkage but the test for heterogeneity between the families was insignificant ($X^2_3 = 6.0$, $P > 0.1$).

This means that segregation of these loci has not been affected by the 2L-3L translocation either because they are far apart on the same chromosome (and were not separated by the translocation) or they occur on the translocated chromosomes but remained far from each other with a crossover value near 50%. It was impossible to tell which of these conditions were operating, without the aid of further markers.

As discussed earlier, markers are only of use when close to the breakpoints

Table 39. Backcross segregation of Collar (C^+) and Dieldrin resistance (R^{Dl}) among the progeny of the cross 26O $\frac{T}{+}\ \frac{C^+}{C}\ \frac{R^{Dl}}{R^+}$ x 16C $\frac{+}{+}\ \frac{C}{C}\ \frac{R^+}{R^+}$

Family No.	No. eggs	No. larvae hatched	Ave. hatching rate(%)	No. adults emerged	Male				Female				Parental	Recombinant		Parental	Test for 1 : 1		Linkage	
					$R^{Dl}C^+$	$R^{Dl}C$	R^+C^+	R^+C	$R^{Dl}C^+$	$R^{Dl}C$	R^+C^+	R^+C	$R^{Dl}C^+$	$R^{Dl}C$	R^+C^+	R^+C	C^+ , C X_1^2	P	R^{Dl} , R^+ X_1^2	P	X_1^2	P
1	297	116	39.0	95	13	11	11	10	12	19	7	12	25	30	18	22	0.85	>0.35	2.36	>0.1	0.01	> 0.9
2	283	107	37.8	94	14	14	18	6	5	13	15	9	19	27	33	15	1.06	>0.3	0.04	>0.9	7.19	< 0.01
3	218	58	26.6	54	5	10	5	4	6	4	10	10	11	14	15	14	0.07	>0.9	0.07	>0.9	0.29	> 0.6
4	195	95	48.7	86	10	11	11	12	9	2	16	15	19	13	27	27	0.42	>0.4	5.62	>0.01	0.41	> 0.5
Total	993	376	37.8	329	42	46	45	32	32	38	48	46	74	84	93	78	2.40		8.09		7.90	

	D.F.			
Deviation	1	0.07	>0.8	0.51 >0.3	1.89	> 0.1
Heterogeneity	3	2.33	>0.4	7.58 >0.05	6.0	> 0.1

Male

$X_1^2\ C^+$, C = 0.49, P > 0.3

$X_1^2\ R^{Dl}, R^+$ = 0.73, P > 0.3

$X^2_{Linkage}$ = 1.75, P > 0.1

Female

$X_1^2\ C^+$, C = 0.09, P>0.3

$X_1^2\ R^{Dl}, R^+$ = 3.51, P>0.05

$X^2_{Linkage}$=0.39, P > 0.5

Sex-ratio (exp. 1 : 1)

X_1^2 = 0.001 P> 0.99

Male + Female

$X_1^2\ C^+$, C = 0.07, P > 0.8

$X_1^2\ R^{Dl}, R^+$ = 0.51, P > 0.3

$X^2_{Linkage}$ = 1.89, P > 0.1

of translocation. Unfortunately this necessary requirement for close linkage between the markers and interchange points was not realized.

DISCUSSION

Some background information is of importance to the discussion of these translocation lines derived from the PALA strain of An. gambiae species A. During chromosomal translocation research in the An. gambiae complex, Krafsur (1972 b) studied the fertility of different stocks of untreated An. gambiae species A and species B. He found that there was a significant variation in fertility in species B stocks, but rather less so among species A. Thus in species B, the method of scoring chromosomal translocation by hatching rate alone was extremely difficult and often inaccurate. Therefore, the PALA stock of An. gambiae species A was chosen for further attempt to isolate and characterize translocations.

When test crosses yielded very large numbers of progeny it was not feasible to examine the fertility of them all. Sometimes it was not possible to assess a fully satisfactory sample of single pair matings. For instance, in the second experiment, using 1,500 r., initial screening of the F_1 should have been carried out on enormous numbers to identify the few which were semi-sterile. For practical reasons, however, only a small proportion could be examined (6% of F_1 males and 17% of F_1 females). Semisterility is usually considered to be the inevitable effect of reciprocal translocations or pericentric inversions, but, in fact, these are sometimes compatible with an almost normal fertility. In Drosophila, maize and a number of other organisms, it has been shown that when whole chromosome arms are exchanged, normal disjunction of homologues takes place and individuals containing

such reciprocal translocations do not show any signs of sterility (Dubinin, 1964). The criterion used for distinguishing partial sterility from normal fertility in the present experiments was specified at a 65% hatching rate or less. Thus, some translocations were undoubtedly missed when F_2 cultures were scored. In addition, a substantial fraction of females initially giving rise to semisterile egg batches showed significantly greater fertilities in subsequent ovipositions. This phenomenon was first detected by Krafsur (1972 b) and he concluded that much of the apparent zygotic lethality among the F_1 matings was not due to inherited chromosomal aberration. Disruption in some aspect of reproductive physiology has presumably occurred in these F_1 mated females, such as a failure of sperm transfer or egg fertilisation. Differential mortality of sperm could be responsible for the difference in fertility in successive ovipositions, since abnormal sperm may not be as competitive as normal sperm. Thus, as the days pass, such abnormal sperm might die leaving a higher proportion of normal sperm for transmission to the female.

Because formalin was used to cleanse eggs, it is unlikely that pathogens could be factors causing changes in fertility. That parasites can cause damage to gonads is well known (Imms, 1949). Microsporidia have been found parasitizing a wide range of mosquito species. Fox and Weiser (1959) concluded, on pathological grounds, that these parasites (e.g. Nosema stegomyiae) have a harmful effect, especially on the reproductive capacity, on the infected mosquitoes (see also Reynolds, 1970). The observation that fertility tended to rise, rather than fall, does not indicate that the strains tested were affected by

pathogens. The precise nature of this mechanism remains to be solved. Comparatively little attention appears to have been paid to this aspect of variation in other insects. Hence, the translocation detection method used in this work is not completely reliable since semisterility does not always indicate chromosomal aberration. It is for these reasons that no quantitative estimation of the radiation effect could be made.

In spite of these difficulties the method of assigning the karyotypes by counting the eggs and larvae has shown itself, in this and other studies, to be capable of yielding reasonably repeatable and consistent results that have proved useful for isolation of stocks carrying chromosomal interchanges. The recognition of translocations is much easier in populations carrying good scorable markers. For example, in Drosophila Patterson et al., (1934) studied 10,000 fertile F_2 cultures after irradiating sperm with 4,452 r. of X-rays. By employing a genetical technique (using markers), they were able to isolate all possible translocations involving any pair of the four chromosomes. Out of 10,000 cultures, 1,992 translocations were found. Wagoner (1967) recovered 177 different translocations in 3,865 tests in the housefly Musca domestica. These large numbers of identifications were possible owing to the availability of clearly scorable markers. The need for good markers in members of the An. gambiae species complex was stressed by Kitzmiller and Mason (1967). Few such markers are yet available in An. gambiae despite several investigations during recent decades. The profitable use of markers as an aid to isolation of translocations in An. gambiae is therefore unlikely at present.

In one of the Y- autosomal translocations (TYF-5) there can be little doubt that a further chromosome exchange has occurred after the second irradiation. The interchange seems to have involved the other arm of the Y-bearing A_1 autosome and some terminal region of an A_2 chromosome (see Fig. 9). No cytological attempt was made to confirm this deduction. The best evidence concerning the effects of treatment comes from the four lines of the least fertile males which were analysed for fertility. All 4 lines were found to transmit their characteristic partial sterility (75%) to all their sons when outcrossed to the wild-type, but none of the daughters inherited partial sterility. A fertility of 25% for 3-chromosome doubly-translocated males is expected from the fact that three quarters of their gametes bear unbalanced chromosome combinations. Direct evidence that re-irradiation causes an increased number of chromosomal aberrations, and that sterility is thus enhanced, has been reported in C. tritaeniorhynchus (Sakai et al., 1972) and C. pipiens (Laven, 1972; Krishnamurthy and Laven, 1974). In a study of C. tritaeniorhynchus Sakai and co-workers found that the fertilities of individuals bearing a translocation heterozygote involving three non-homologous chromosomes without other chromosomal aberrations, e.g. inversions, ranged from 23.4% to 33.9%. Krishnamurthy and Laven (1974) recorded fertility levels as low as 25-30% in C. fatigans when single male-linked translocation lines (T^M3 and T^M10) were re-irradiated with X-rays at a dosage of 3,500 r.

It is interesting to find that in one of the 3-chromosome double translocation lines (1 C), sterility has remained close to its original value of 75% during one year of inbreeding. The fact that the high

sterility factor did not break down might indicate that there is no crossing over in the differential segment. In fact a chiasma here would be equivalent to a reverse interchange because it leads to the breakdown of the multiple translocation complex (John and Lewis, 1965). Krishnamurthy and Laven (1974) reported that a multiple male-linked translocation strain (1S - 31B) of C. fatigans had an initial mean fertility of 30 31% when placed under conditions of mass rearing. However, after 5 months of mass rearing, the percentage of fertility within the strain had increased to an average of 33%. Although these workers did not themselves attempt to explain this slight increase, Curtis (1975) speculated that small increases of fertility may arise from "the evolution of an enhancement of fertility in the translocation heterozygotes above their initial value". There was no such increase in fertility in line 1C as observed in the present work.

In other insects so far investigated, i.e. Musca domestica (Wagoner et al., 1969), Glossina austeni (Curtis, 1969) and Drosophila (Robinson and Curtis, 1973), a majority of single translocation hetero - zygotes appear to have a fertility very close to the expected value of 50%. In the present work with An. gambiae species A, all six supposed translocation heterozygote lines had fertilities significantly higher than 50%; fertilities ranged from 59.3% to 72.2%. Of these the karyotypes of five lines (6K, 26O, 7G, 102A and 26D) were cytologically confirmed as translocations. In the nine other lines doubtfully considered to carry translocation heterozygotes, there were much higher fertilities (62.4% - 82.1%). Similar relatively high fertility of translocated lines was found in An. gambiae species A by Krafsur (1972 b). In all but one of 6 presumably T/+

lines, there was no significant difference in the fertility of the two sexes. The exception was found in line 26O, where male heterozygotes were more fertile than their translocated sisters (see Table 20).

Fertility in excess of 50% of the control value would be expected if there is a greater tendency for alternate rather than adjacent chromosome segregation. Indeed, the data on Drosophila show there was some deviation in favour of alternate segregation, and fertilities in interchange heterozygotes could be as high as 60% to 70% (Burnham, 1962). Thus the present results are not really unexpected. Alternatively, aneuploid zygotes (duplication-deficiency zygotes) may have survived the early stage of larval development, as suggested by Baker and Sakai (1974). This has already been reported in Glossina austeni (Curtis et al., 1972) and in the onion fly, Hylemya antiqua (Van Heemert, 1974). Usually however the aneuploid zygote is arrested very early, causing failure of egg-hatch. The possibility remains that the lines in which the observed fertility was higher than expected may represent cases in which first instar larval development was completed, although all aneuploid zygotes perished at some later stage. In fact in all the lines studied in this experiment, the percentage of larvae surviving to adulthood was significantly lower than in the control stock (71.7% to 99% of the control level). However, the experiment was not designed for this comparison so not too much weight should be attached to this finding. Indeed, Krafsur (1972 b) found no significant difference in viability between his translocated lines and the wild-type. Significantly higher fertility occurred in male translocation heterozygotes compared to female heterozygotes in Cochliomyia hominivorax (LaChance et al., 1964). LaChance and co-workers

considered that since no chiasma formation or crossing-over occurs in male screw-worm flies, the translocation complex is quite flexible. This favours alternate segregation over adjacent segregation. In An. gambiae, unlike C. hominivorax, crossing-over occurs in both sexes (Mason, 1964; Haridi, 1971). In contrast Krafsur (1972 b), reported that two translocation lines in An. gambiae species A showed higher percentage fertility in the female sex. He postulated that orientation of the aneuploid products of meiosis more frequently go into the non-functional polar body nuclei. These speculations could only be confirmed or refuted by a detailed cytogenetic study of meiosis which was outside the scope of this project.

Unfortunately, because of concentration of effort on obtaining progeny from semisterile outcross matings for the isolation of autosomal translocation homozygotes, cytological analysis could not be accomplished at the same point in time. Cytological analysis was made only from individuals which had been preserved for many months previously. In a number of cases, characterisation of the chromosomes was not possible, simply because the preserved material resulted in poor chromosome preparations which could not be read.

The attempts of Krafsur to produce translocation homozygotes in An. gambiae species complex were as unsuccessful as were the present ones. Although hatching rate was the only criterion used for the designation of karyotype (and undoubtedly there were inevitable inaccuracies in these designations), nevertheless the data obtained from hatching rates of intercrossed heterozygotes of all the 5 lines studied, suggested that translocation homozygotes might be lethal. If interchanges are the result of mere exchanges of segments, one might expect the

homozygotes to be viable as they possess almost the full genetic complement. In fact, many translocations, at least in insects, seem to be lethal when homozygous. In Drosophila, studies by Patterson et al. (1934) of radiation induced translocations showed that out of 332 single reciprocal translocations only 40% were viable when homozygous. Indeed, those involving chromosome 2 and 3 were only viable in 15% of cases. Similar but less extreme results were compiled from the literature by Burnham (1962); less than half of the 53 different homozygotes for translocations involving chromosome 2 and 3 were viable. The others were lethals or lower than normal in viability or in fertility. More recently Robinson (1971) reported that 24 out of 30 newly induced reciprocal translocations were lethal when homozygous and only 2 of the six non-lethal homozygotes were fertile. Although insects of medical importance have been studied much less than Drosophila, the available data on them shows even less viability in the homozygous state (see literature review).

Factors affecting translocation homozygote viability are poorly known. However, two theories to explain the effect have been postulated so far. One theory suggests that mutations tend to be induced especially at, or very near to the chromosome break points on the translocated chromosomes. When homozygous, some of these are lethal. The other theory is that the rearrangement itself may cause lethality due to position effects. It was thought that genes relocated in a new position could interfere with one another owing to reaction between the gene products when these, under the new arrangement, were produced in the neighbourhood of one another (Muller, 1935). In a variant of this

hypothesis, it was postulated that competition for a common substrate could take place if two genes were located next to one another, but not if the same genes occupied distant loci (Waddington, 1939). In his observations on homozygous viability of translocations in Drosophila, Sobels (1972) reported that 84 out of 135 (62.2%) were lethal when homozygous. He has calculated that about 28% of the fatal effect was due to recessive lethals and a little over one half was due to the translocation per se. Then he suggested that deletions, or break up of the contiguity of gene clusters, or separation of linked genes with their relocation to different sites, could result in some kind of recessive lethal position effect. No experimental discrimination between the two hypotheses has been done, most workers have accepted these two theories to explain reduced viability or lethality in homozygous translocations (e.g. Curtis et al., 1972; Ives and Fink, 1962; Krafsur, 1972 b; Rai et al., 1970; Robinson and Van Heemert, 1974; see also Dobzhansky, 1951; White, 1973 for review).

It has been claimed that repeated outcrossing or backcrossing may remove recessive lethal genes from translocated chromosomes through crossing-over (Rai et al., 1970), so all the lines except one were outcrossed to the wild-type for at least 6 generations before any inbreeding, but without success. The reason for this lack of success is not clear but one possibility is the following : At least in Drosophila, the frequency of crossing-over in translocation heterozygotes is reduced especially in the vicinity of the break points (Dobzhansky, 1931). The absence of close pairing during meiosis in the region of the break points might make the removal, by outcrossing, of recessive lethals within this particular chromosome segment extremely difficult (Robinson and

Van Heemert, 1974). It was hoped that the use of a relatively low irradiation dose would give a better chance of recovery of a viable translocation homozygote on the assumption that less damage would occur at the break points and fewer recessive lethals would be produced. Even this proved to be unsuccessful in this study, though admittedly the total number of lines investigated was small. The value of this approach in obtaining a viable translocation homozygote remains to be worked out in a large scale experiment.

The possibility of isolating autosomal translocation homozygotes, however, should not be abandoned until a more thorough investigation is made over a longer period of time. With limited resources and time, the goal is difficult to attain. In future attempts to produce viable T/T stocks, it would probably be wise to combine cytological analysis with fertility testing. Experience has shown that there is no difficulty in inducing autosomal translocations in An. gambiae species complex by treating 2-3 day-old males with 1,500 r. - 4,000 r. X-rays. The An. gambiae complex can be easily maintained in the laboratory and it shows good polytene chromosomes, both in the larvae and in the adults (Coluzzi, 1970). If the assistance of a cytologist is available, structural changes in chromosomes may easily be observed in the polytene complement and should prove far more reliable in detecting translocations than the use of hatching rate alone. To perform all the requisite tasks of creating, selecting and evaluating strains suitable for genetic control purposes a team composed of entomologist, cytologist and technicians is considered essential. Only with many pairs of hands can the problems of obtaining and testing appropriate translocation homozygote stocks be solved. The effort in developing the strains may be

considerable, but in comparison to developing new insecticides, or inventing other control approaches, it may prove well worthwhile (Whitten, 1971 a). Although objects of the work have not been fully realized, "genetic control" has distinct possibilities as stated in the introduction. Further research should therefore be continued and even intensified in the laboratory and finally in the field. There are positive examples of successful isolation of viable translocation homozygotes in insects of medical importance. This is the case in Ae. aegypti (Lorimer et al., 1972; Rai et al., 1974) and Musca domestica (McDonald and Overland, 1973 a, b).

Apart from slight reduction in larval survival rates and shorter mean longevity of males compared with the control, the 3-chromosome doubly translocated males displayed good general vigour. Fitness tests showed that translocated males were comparable with the wild-type males. However, the method of quantifying fitness may not accurately reflect events which would occur in the field. If 3-chromosome doubly translocated males are repeatedly outcrossed to the indigenous strain of An. gambiae species A, males having similar qualities in terms of adaptation to the environment, mating behaviour etc., to the indigenous strain may be obtained.

Since a strain with 3-chromosome doubly translocated males has been produced having 75% sterility, it is worthwhile discussing its probable effectiveness for field release. The release of male-linked translocations would have an effect no greater than the release of the same number of sterile males, assuming equal mating competitiveness (Curtis, 1971 a). If we apply the formula used by Whitten (1971 a) for a case in which $s = 0.75$, where s represents sterility, then if 3-

chromosome double translocation males are released in the ratio r : 1 into a natural population, then the proportion of their progeny which survive is 1-s, and the genetic load induced is $r.s/(r + 1)$. If we assume it is feasible to supply large numbers of males for release, the continued release of these males cannot increase the zygotic mortality above 0.75 since $r.s/(r + 1) \rightarrow s$ as $r \rightarrow \infty$. It is therefore evident that an increase in release numbers (r) from 10 to 10,000 does not greatly increase the zygotic mortality (0.68 → 0.75). Release of Y-linked translocation heterozygotes will definitely produce a reduction in population fertility. However, since a Y-linked translocation cannot be made homozygous, it will be eliminated quickly from the population by natural selection, unless the mating competitiveness of translocation males were permanently raised (Curtis and Hill, 1971; Whitten, 1971 a). This is because of the selective disadvantages of translocation heterozygotes. That such disadvantages do exist has been clearly shown by Curtis (1975), using results obtained by Laven et al., (1971 c, d; 1972) and Cousserans and Guille (1972, 1974 (quoted by Curtis, 1975)) in their experimental release of C. pipiens carrying a male-linked translocation in a village near Montpellier, France.

For practical purposes, planned releases may best be made either into an isolated population of known size, or against a more widespread population commencing when the population is at its lowest density. It would always be advantageous to employ genetic methods following initial reduction of the target population by conventional means (Knipling, 1967). In any event, released individuals of reduced fertility will make their greatest impact when density of the natural

population is so low that wild individuals might experience difficulty in finding a mating partner. General field experiences, such as with species A at Pala in West Africa (Davidson, 1974 a) and with both species A and B in East Africa (White et al., 1972), have revealed that densities of the An. gambiae complex become very low indeed during dry seasons. To reduce the reproductive efficiency of a population by introducing males of 75% sterility might cut the dry season densities of An. gambiae to a level below the natural threshold required for population survival, if the released males can survive the adverse climatic conditions of the dry season and successfully compete in mating with wild females. If normal males could be completely replaced by translocated males, then a fixed depression of the target population would be achieved due to the sustained effects of inherited partial sterility (Whitten, 1971 a). When such a situation has been established a very small number of immigrants could upset the balance (Curtis, 1975). Let us consider the consequences if there is an immigration of one wild-type male per thousand translocated males. Using the formula of Curtis and Hill (1971) and Curtis (1975) the frequency of karyotypes among males in the next generation would be as shown in Table A. Under the circumstances that no more fertile males immigrate, but releases of translocation carriers are not repeated, the theoretical situation arising after 8 generations is given in Table B.

After the first generation, the proportion of normal males shows a 4-fold increase. Further increases would be expected in each subsequent generation, so that after 8 generations (Table B) the frequency of translocated and normal males will be virtually reversed. Displacement of the 3-chromosome doubly translocated males by wild-type male would theoretically occur in the 10th generation. In the light of

Table A.

Type of male	Frequency among male parent	Frequency of matings (assuming equal competitiveness)	Fertility of matings	Relative number of male progeny	Frequency among males in the next generation
3-chromosome doubly translocation	0.999	0.999	0.25	0.999 x 0.25 = 0.24975	0.24975/0.25075 = 0.996
Normal	0.001	0.001	1.0	0.001 x 1.0 = 0.001	0.001/0.25075 = 0.004
		Total of viable progeny		0.25075	

Table B. (situation after 8 generations of breeding)

3-chromosome doubly translocation	0.013	0.013	0.25	0.00325	0.003
Normal	0.987	0.987	1.0	0.987	0.997
		Total of viable progeny		0.99025	

present knowledge of the bionomics of An. gambiae (i.e. its wide distribution, high reproductive potential, probable buffering effect of density-dependent factors and its high vectorial efficiency) it must be admitted that the use of 3-chromosome doubly translocated males is highly unlikely to be effective. The inefficiency of such a mechanism has been demonstrated by Rai et al., (1973) with Ae. aegypti. In that project, released translocated males were able to mate with native females and certainly introduced some degree of lower fertility, but there was no evidence that any numerical effect on the total population was produced.

REFERENCES

Alexander, M.L., Bergendahl, J. and Brittain, M. (1959).
Biological damage in mature and immature germ cells of Drosophila virilis with ionizing radiation.
Genetics, 44, 979-999.

Al-Mashhadani, H.M. (1974).
Selections of Anopheles gambiae species A for susceptibility and refractoriness to malaria parasites.
Trans. roy. Soc. trop. Med. Hyg., 68, 267-268.

Aslamkhan, M. and Aaqil, M. (1970).
A preliminary report on the gamma-induced translocations and semisterility in the malaria mosquito, Anopheles stephensi.
Pak. J. sci. Res., 22, 183-190.

Asman, M. (1967).
Inherited semisterility in Aedes aegypti.
Proc. Calif. Mosq. Ass., 35, 103.

Auerbach, C. (1943).
D. melanogaster. New mutants.
Chemically induced mutations and rearrangements.
Drosoph. Inf. Serv., 17, 48-50.

Auerbach, C. (1973).
History of Research on Chemical Mutagenesis. Chapter 24, pp. 1-19. In: Hollaender, A. (Eds.). Chemical Mutagens, Principles and Methods for their Detection, Vol. 3. Plenum Press, New York, London.

Auerbach, C., Robinson, J.M. and Carr, J.G. (1947).
The chemical production of mutations.
Science, N.Y., 105, 243-247.

Baker, R.H., Sakai, R.K. and Mian, A. (1970).
Stock list of genetic strains of Culex tritaeniorhynchus.
Unpublished Mimeographed document, WHO/VBC/70. 245.

Baker, R.H., Sakai, R.K. and Mian, A. (1971).
Linkage group - chromosome correlation in a mosquito.
Inversions in Culex tritaeniorhynchus.
J. Hered., 62, 31-36.

Baker, R.H. and Sakai, R.K. (1974).
Genetic studies on Culex tritaeniorhynchus. Chapter 8. pp. 133-182. In: Pal, R. and Whitten, M.J. (Eds.). The use of genetics in insect control. Elsevier/North-Holland.

Begg, C.M.M. (1959).
Introduction to Genetics.
The English Univ. Press Ltd., London.

Belling, J. (1914).
The mode of inheritance of semisterility in the offspring of certain hybrid plants.
Z. induk. Abstamm. u. Vereb Lehre, 12, 303-342.

Belling, J. (1925).
A unique result in certain species crosses.
Ibid, 39, 286-288.

Belling, J. and Blakslee, A.F. (1924).
The configurations and sizes of the chromosomes in the trivalents of 25-chromosome Datura.
Proc. natn. Acad. Sci. U.S.A., 10, 116-120.

Bhalla, S.C. (1973).
Sex-linked translocations, semisterility and linkage alternations in the mosquito Aedes aegypti.
Can. J. Genet. Cytol., 15, 9-20.

Blakslee, A.F. (1928).
Genetics of Datura.
Verh. V. Int. Kongr. Vererbungswiss. 1927 (Berlin), 1, 117-130.

Boyes, J.W. (1967).
The cytology of muscoid flies. Chapter 10, pp 371-384.
In: Wright, J.W. and Pal, R. (Eds.). Genetics of insect vectors of diseases.
Elsevier, Amsterdam.

Bridges, C.B. (1923).
The translocation of a section of chromosome-II upon chromosome III in Drosophila.
Anat. Rec., 24, 426-427.

Brink, R.A. (1927).
The occurrence of semisterility in maize.
J. Hered., 18, 266-270.

Brink, R.A. and Burnham, C.R. (1929).
Inheritance of semisterility in maize.
Amer. Nat., 63, 301-316.

Brown, A.W.A. and Pal, R. (1971).
Insecticide Resistance in Arthropods.
Wld Hlth Org., Monogr. Ser., 38

Brown, M.S. (1940).
The relation between chiasma formation and disjunction.
Univ. Texas Publ., 4032, 11-64.

Bryan, J.H. (1973).
Studies on the Anopheles punctulatus complex. III. Mating behaviour of the F_1 hybrid adults from crosses between Anopheles farauti No. 1 and Anopheles farauti No. 2.
Trans. roy. Soc. trop. Med. Hyg., 67, 85-99.

Burnham, C.R. (1930).
Genetical and cytological studies of semisterility and related phenomena in maize.
Proc. natn. Acad. Sci. U.S.A., 16, 269-277.

Burnham, C.R. (1950).
Chromosome segregation in translocations involving chromosome 6 in maize.
Genetics, 35, 446-481.

Burnham, C.R. (1956).
Chromosomal interchanges in plants.
Bot. Rev., 22, 419-552.

Burnham, C.R. (1962).
Discussion in Cytogenetics.
Burgess Publ. Co., Minneapolis.

Burnham, C.R., White, F.H. and Livers, R. (1954).
Chromosomal interchanges in barley.
Cytologia (Tokyo), 19, 191-202.

Bushland, R.C. (1971).
Sterility principle for insect control: Historical development and recent innovations.
International Atomic Energy Agency Publication: STI/PUB/265, Vienna, pp. 3-14.

Campion, D.G. (1972).
Insect chemosterilants: a review.
Bull. ent. Res., 61, 577-635.

Chandely, A.C. and Bateman, A.J. (1960).
Mutagenic sensitivity of sperm, spermatids, spermatocytes, and spermatogonia in Drosophila melanogaster.
Heredity, 15, 363-375.

Childress, D. (1969).
Polytene chromosomes and linkage group-chromosome correlations in the Australian Sheep Blowfly. Lucilia cuprina (Diptera: Callipholidae).
Chromosoma (Berl.), 26, 208-214.

Childress, D. (1972).
Changing population structure through the use of compound chromosomes
Genetics, 72, 183-186.

Coluzzi, M. (1970).
Sibling species in Anopheles and their importance in malariology.
Misc. Pub. ent. Soc. Amer., 7, 63-77.

Coluzzi, M. and Sabatini, A. (1967).
Cytogenetic observations on species A and B of the Anopheles gambiae complex.
Parassitologia, 9, 73-88.

Cousserans, J. and Guille, G. (1972).
Experience de lutte genetique contre Culex pipiens dans la Region de Montpellier. 2e annee d'observations.
Bull. Biol. Fr. Belg., 4, 337-343.

Craig, G.B. (1963).
Prospects for vector control through genetic manipulation of populations.
Bull. Wld Hlth Org., 29, suppl., 89-97.

Curtis, C.F. (1968 a).
A possible genetic method for the control of insect pests with special reference to tsetse flies (Glossina spp.).
Bull. ent. Res., 57, 509-523.

Curtis, C.F. (1968 b).
Possible use of translocations to fix desirable genes in insect pest populations.
Nature (Lond.), 218, 368-369.

Curtis, C.F. (1969).
The production of partially sterile mutants in Glossina austeni.
Genet. Res., 13, 289-301.

Curtis, C.F. (1970).
An attempt to produce stocks homozygous for chromosome translocations in Glossina austeni.
Proc. 1st Int. Symp. on Tsetse Fly Breeding, (Lisbon), 355-360.

Curtis, C.F. (1971 a)
Induced sterility in insects. Vol. 5, pp. 119-165.
In: Bishop, M.W.H. (Eds.). Advances in Reproductive Physiology.
Logos Press Ltd., London.

Curtis, C.F. (1971 b).
Experiments, on breeding translocation homozygotes in tsetse flies.
International Atomic Energy Publication: STI/PUB/265, Vienna, pp. 425-435.

Curtis, C.F. (1975).
The behaviour of male-linked translocations in populations.
Unpublished Mimeographed document, WHO/VBC/75. 513.

Curtis, C.F. and Hill, W.G. (1968).
Theoretical and practical studies on a possible genetic method for tsetse fly control.
International Atomic Energy Agency Publication: ST/PUB/166, Vienna, pp 233-247.

Curtis, C.F. and Hill, W.G. (1971).
Theoretical studies on the use of translocations for the control of tsetse flies and disease vectors.
Theoret. pop. Biol., 2, 71-90.

Curtis, C.F. and Robinson, A.S. (1971).
Computer simulation of double translocations for pest control.
Genetics, 69, 97-113.

Curtis, C.F., Southern, D.I., Pell, P.E. and Craig-Cameron, T.A. (1972).
Chromosome translocations in Glossina austeni.
Genet. Res., 20, 101-113.

Curtis, C.F. and Adala, T. (1974).
Population replacement in Culex fatigans by means of cytoplasmic incompatibility. 1. Laboratory Experiment with Non-overlapping Generations.
Unpublished Mimeographed document, WHO/VBC/74. 481.

Darlington, C.D. and La Cour, L.F. (1950).
Hybrid selection in Campanula.
Heredity, 4, 217-248.

Davidson, G. (1958).
Studies on insecticide resistance in anopheline mosquitoes.
Bull. Wld Hlth Org., 18, 579-621.

Davidson, G. (1969).
The potential use of sterile hybrid males for the eradication of member species of the Anopheles gambiae complex.
Ibid, 40, 221-228.

Davidson, G. (1972).
Alternative measures to insecticides for mosquito control.
Pestic. Sci., 3, 503-514.

Davidson, G. (1973).
Hybrid sterility and Anopheles gambiae control. A field experiment and the aftermath.
Proc. 9th Int. Cong. trop. Med. Malar. (Abst.), 1, 262-263.

Davidson, G. (1974 a).
Genetic Control of Insect Pests.
Academic Press, London.

Davidson, G. (1974 b).
Genetic control of insects. pp 162-177.
In: Price Jones, D., and Solomon, M.E. (Eds.). Biology in pest and disease control.
Blackwell Sci. Publ., Oxford.

Davidson, G., Paterson, H.E., Coluzzi, M., Mason, G.F. and Micks, D.W. (1967).
The Anopheles gambiae complex. Chapter 6, pp 211-250.
In: Wright, J.W. and Pal, R. (Eds.). Genetics of insect vectors of disease. Elsevier, Amsterdam.

Davidson, G. and Kitzmiller, J.B. (1970).
Genetic control of anophelines.
Misc. Publ. ent. Soc. Amer., 7, 118-129.

Davidson, G., Odetoyinbo, J.A., Colussa, B. and Coz, J. (1970).
A field attempt to assess mating competitiveness of sterile males produced by crossing 2 member species of the Anopheles gambiae complex.
Bull. Wld Hlth Org., 42, 55-67.

Davidson, G. and Hunt, R.H. (1973).
The crossing and chromosome characteristics of a new, six species in the Anopheles gambiae complex.
Parassitologia, 15, 121-136.

Dennhofer, L. (1974).
Über die durch chromosomale Aberrationen verursachte Sterilität.
Theor. appl. Genet., 44, 311-323.

Dobzhansky, Th. (1930).
Translocation involving the third and fourth chromosomes of Drosophila melanogaster.
Genetics, 15, 347-399.

Dobzhansky, Th. (1931).
The decrease of crossing-over observed in translocations and its probable explanation.
Amer. Nat., 65, 214-232.

Dobzhansky, Th. (1933).
Studies on chromosome conjugation. II. The relation between crossing over and disjunction of chromosomes.
Z. indukt. Abstamm. u. VerebLehre, 64, 269-309.

Dobzhansky, Th. (1951).
Genetics and the Origin of Species.
Columbia Univ. Press, New York and London.

Dubinin, N.P. (1964).
Problems of radiation genetics.
Oliver & Boyd, Edinburgh & London.

Erk, F.C. (1960).
A study of genetic control over segregation in a translocation heterozygote.
Genetics, 46, 986.

Fitz-Earle, M., Holm, D.G., and Suzuki, D.T. (1973).
Genetic control of insect populations. I. Cage studies of chromosome replacement by compound autosomes in Drosophila melanogaster.
Ibid., 74, 461-475.

Foster, G.G., Whitten, M.J., Prout, T. and Gill, R. (1972).
Chromosome rearrangements for the control of insect pests.
Science, N.Y., 176, 875-880.

Foster, G.G. and Whitten, M.G. (1974).
The development of genetic methods of controlling the Australian sheep blowfly, Lucilia cuprina. Chapter 2, pp 19-43.
In: Pal, R. and Whitten, M.J. (Eds.). The use of genetics in insect control.
Elsevier/North Holland.

Fox, R.M. and Weiser, J. (1959).
A microsporidian parasite of Anopheles gambiae in Liberia.
J. Parasit., 45, 21-30.

French, W.L., Baker, R.H. and Kitzmiller, J.B. (1962).
Preparation of mosquito chromosomes.
Mosquito News, 22, 377-383.

Frizzi, G. and Jolly, M.S. (1961).
Mutazioni indotte con raggi X in Anopheles maculipennis.
Atti Ass. genet. ital., 6, 285-290.

Gilchrist, B.M. and Haldane, J.B.S. (1947).
Sex-linkage and sex determination in a mosquito, Culex molestus.
Hereditas (Lund.), 33, 175-190.

Glass, H.B. (1935).
A study of factors influencing chromosomal segregation in translocations of Drosophila melanogaster.
Univ. Missouri Agr. Res. Bull., 231, 1-28.

Glass, H.B. (1955).
A comparative study of induced mutation in the oocytes and spermatozoa of Drosophila melanogaster. 1. Translocations and inversions. Genetics, 40, 252-267.

Hamon, J. (1970).
Ecological factors important in insecticidal and alternative means of mosquito control.
Unpublished Mimeographed document, WHO/VBC/70. 205.

Hamon, J., Subra, R., W Sales, S., and Coz, J. (1968).
Presence dans le sud-ouest de la Haute-Volta d'une population d'Anopheles gambiae A resistant au DDT.
Med. trop., 28, 222-228.

Haridi, A.M. (1971).
DDT and Dieldrin Resistante in Species A and Species B of the Anopheles gambiae complex.
Ph.D. Thesis, University of London (Unpublished).

Haridi, A.M. (1972).
Inheritance of DDT resistance in species A and B of the Anopheles gambiae complex.
Bull. Wld Hlth Org., 47, 619-626.

Hetherington, C.M., Whittington, W.J., Hossain, M.A. and Peat, W.E. (1968).
The genetics of the Xasta mutant of Drosophila melanogaster.
Genet. Res., 12, 285-294.

Hickey, W.A. and Craig, G.B. (1966).
Genetic distortion of sex-ratio in a mosquito, Aedes aegypti.
Genetics, 53, 1177-1196.

Huff, C.G. (1929).
The effects of selection upon susceptibility to bird malaria in Culex pipiens Linn.
Ann. trop. Med. Parasit., 23, 427-442.

Huff, C.G. (1931).
The inheritance of natural immunity to Plasmodium cathemerium in two species of Culex.
J. prev. Med., Baltimore, 5, 249-259.

Hunt, R.H. (1973).
A cytological technique for the study of Anopheles gambiae complex.
Parassitologia, 15, 137-139.

Hunt, R.H. and Krafsur, E.S. (1972).
Cytological demonstration of some heterozygous reciprocal translocations in Anopheles gambiae species A.
Trans. roy. Soc. trop. Med. Hyg., 66, 23-24.

Imms, A.D. (1949).
A general Text-Book of Entomology.
Methuen & Co. Ltd., London.

Ives, P.T. and Fink, G.R. (1962).
Comparison of translocation and cross over chromosomes produced by gamma rays.
Genetics, 47, 963.

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

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

John, B. and Lewis, K.R. (1965).
The meiotic system.
Protoplasmat. (Wien), 6, 1-335.

John, B. and Hewitt, G.M. (1963).
A spontaneous interchange in Chorithippus brunneus with extensive chiasma formation in an interstitial segment.
Chromosoma (Berl.), 14, 638-650.

John, B. and Quraishi, H.B. (1964).
Studies on Periplaneta americana IV. Pakistan populations.
Heredity, 19, 147-156.

Jones, S.A. (1957).
Possible replacement of malaria mosquitoes.
Trans. roy. Soc. trop. Med. Hyg., 51, 469-470.

Jost, E. and Amirkhanian, J.D. (1971).
Comparative studies of the effect of X-rays and 1, 3-propane-dioldimethanesulfo nate on the mosquito Culex pipiens L.
Mut. Res., 13, 49-57.

Jost, E. and Laven, H. (1971).
Meiosis in translocation heterozygotes in the mosquito Culex pipiens.
Chromosoma (Berl.), 35, 184-205.

Kannelis, A. and Radu, Gh. (1943).
Uber die Auslösung von Translokationen durch Röntgen-bestrahlung weiblicher Gemeten von Drosophila melanogaster.
Naturwissenschaften, 31, 390.

Khoshoo, T.N. and Mukherjee, I. (1966).
A translocation heterozygote in garden Canna.
Genetica, 37, 255-258.

Kilama, W.L. and Craig, G.B. Jr. (1969).
Monofactorial inheritance of susceptibility to Plasmodium gallinaceum in Aedes aegypti.
Ann. trop. Med. Parasit., 63, 419-432.

Kitzmiller, J.B. (1970).
Genetic control of mosquitoes.
Amer. J. trop. Med. Hyg., 21, 764-771.

Kitzmiller, J.B. and Mason, G.F. (1967).
Formal Genetics of Anophelines. Chapter 1, pp 3-14.
In: Wright, J.W. and Pal, R. (Eds.).
Genetics of Insect vectors of disease. Elsevier, Amsterdam.

Klassen, W., Knipling, E.F. and McGure, J.U. (1970).
The potential for insect-population suppression by dominant conditional lethal traits.
Ann. ent. Soc. Amer., 63, 238-255.

Knipling, E.F. (1967).
Sterile Technique - Principles Involved, Current Application, Limitations and Future Application. Chapter 20, pp 587-616.
In: Wright, J.W. and Pal, R. (Eds.). Genetics of insect vectors of disease. Elsevier, Amsterdam.

Knipling, E.F., Laven, R., Craig, G.B., Pal, R., Kitzmiller, J.B., Smith, C.N. and Brown, A.W.A. (1968).
Genetic control of insects of public health importance.
Bull. Wld Hlth Org., 38, 421-438.

Krafsur, E.S. (1972 a).
Production of reciprocal translocations in Anopheles gambiae species A.
Trans. roy. Soc. trop. Med. Hyg., 66, 22-23.

Krafsur, E.S. (1972 b).
Production and isolation of reciprocal translocations in the Anopheles gambiae species complex.
Ph.D. Thesis, University of London (Unpublished).

Krafsur, E.S. and Davidson, G. (1973).
The translocation method as applied to the control of Anopheles gambiae species.
Proc. 9th Int. Cong. trop. Med. Malar., (Abst.) 1, 264-265.

Kramer, J.P. (1964).
Parasites in laboratory colonies of mosquitoes.
Bull. Wld Hlth Org., 31, 475-478.

Krishnamurthy, B.S. (1974).
Construction of "integrated" strains of Culex pipiens fatigans Weid. for Genetic control.
J. com. Dis., 6, 76-79.

Krishnamurthy, B.S. and Laven, H. (1972).
Preparation of a cytoplasmically incompatible strain of Culex pipiens fatigans for use in genetic control in India.
Unpublished Mimeographed document, WHO/VBC/72. 389.

Krishnamurthy, B.S. and Laven, H. (1974).
Development of cytoplasmically incompatible and integrated (translocated incompatible) strains of Culex pipiens/fatigans for use in genetic control.
Unpublished Mimeographed document, WHO/VBC/74. 496.

LaBreque, G.C. and Smith, C.N. (1968) (Eds.)
Principles of insect chemosterilization.
Appleton-Century-Crofts, New York.

LaChance, L.E., Riemann, J.G. and Hopkins, D.E. (1964).
A reciprocal translocation in Cochliomyia hominivorax (Diptera: Calliphoridae). Genetic and cytological evidence for preferential segregation in males.
Genetics, 49, 959-972.

Laven, H. (1967 a).
Eradication of Culex pipiens fatigans through cytoplasmic incompatibility.
Nature (Lond.), 216, 383-384.

Laven, H. (1967 b).
Speciation and Evolution in Culex pipiens. Chapter 7, pp. 251-275.
In: Wright, J.W. and Pal, R. (Eds.). Genetics of insect vectors of disease.
Elsevier, Amsterdam.

Laven, H. (1969).
Eradicating mosquitoes using translocations.
Nature (Lond.) 221, 958-959.

Laven, H. (1972).
Genetic Mechanisms for Pest Control: Cytoplasmic Incompatibility and Translocations.
Indian Council med. Res., tech. Rept. Ser., 20, 95-109.

Laven, H. and Aslamkhan, M. (1970).
Control of Culex pipiens and C. p. fatigans with integrated genetical systems.
Pak. J. Sci., 22, 303-312.

Laven, H. and Jost, E. (1971).
Inherited semisterility for control of harmful insects 1. Productions of semisterility due to translocations in the mosquito, Culex pipiens L. by X-rays.
Experientia (Basel), 27, 471-473.

Laven, H., Jost, E., Meyer, H. and Selinger, R. (1971 a).
Semisterility for insect control.
International Atomic Energy Agency Publication:
STI/PUB/265, Vienna, pp. 415-423.

Laven, H., Meyer, E., Bieniok, R., Guille, G. and Ohmann, J. (1971 b).
Inherited semisterility for control of harmful insects II. Degree of sterility and types of translocations in the mosquito Culex pipiens L.
Experientia (Basel), 27, 968-969.

Laven, H., Cousserans, J. and Guille, G. (1971 c).
Inherited semisterility for control of harmful insects III. A first field experiment.
Ibid, 27, 1355-1357.

Laven, H., Cousserans, J. and Guille, G. (1971 d).
Experience de Lutte Genetique contre Culex pipiens dans la Region de Montpellier.
Bull. Biol. Fr. Belg., 4, 357-367.

Laven, H., Cousserans, J. and Guille, G. (1972).
Eradicating mosquitoes using translocations: a first field experiment.
Nature (Lond.), 236, 456-457.

Lawrence, C.W. (1958).
Genotypic control of chromosome behaviour in rye. VI: Selection for disjunction frequency.
Heredity, 12, 127-131.

Lawrence, C.W. (1963).
The orientation of multiple associations resulting from interchange heterozygosity.
Genetics, 48, 347-350.

Lewis, K.R. and John, B. (1957).
Studies on Periplaneta americana II. Interchange heterozygosity in isolated populations.
Heredity, 11, 11-22.

Lewis, K.R. and John, B. (1963 a).
Chromosome Marker.
Churchill, London.

Lewis, K.R. and John, B. (1963 b).
Spontaneous interchange in Chorthippus brunneus.
Chromosoma (Berl.), 14, 618-637.

Lewis, K.R. and John, B. (1966).
The co-orientation of non-homologous chromosomes.
Heredity, 22, 692-694.

Li, C.C. (1955).
Population Genetics
University of Chicago Press, Chicago.

Lofgren, C.S., Dame, D.A., Breeland, S.G., Weidhass, D.E., Jeffery, G., Kaiser, R., Ford, H.R., Boston, M.D. and Baldwin, K.F. (1974).
Release of chemosterilized males for the control of Anopheles albimanus in El Salvador III. Field Methods and population control.
Amer. J. trop. Med. Hyg., 23, 288 297.

Lorimer, N., Hallinan, E. and Rai, K.S. (1972).
Translocation homozygotes in the yellow fever mosquito, Aedes aegypti
J. Hered., 63, 158-166.

Macdonald, W.W. (1962 a).
The selection of a strain of Aedes aegypti susceptible to infection with semi-periodic Brugia malayi.
Ann. trop. Med. Parasit., 56, 368-372.

Macdonald, W.W. (1962 b).
The genetic basis of susceptibility to infection with semi-periodic Brugia malayi in Aedes aegypti.
Ibid, 56, 373-382.

Macdonald, W.W. (1967).
The influence of genetic and other factors on vector susceptibility to parasites. Chapter 19, pp. 567-584.
In: Wright, J.W. and Pal, R. (Eds.), Genetics of insect vectors of disease. Elsevier, Amsterdam.

Macdonald, W.W. and Wharton, R.H. (1963).
Genetic control of susceptibility to filariae in Aedes aegypti.
Trans. roy. Soc. trop. Med. Hyg., 57, 4.

Mason, G.F. (1964).
Cytogenetics and genetics of strains in the Anopheles gambiae complex.
Ph.D. Thesis, University of London (unpublished).

Mason, G.F. (1967).
Genetic studies on mutations in species A and species B of the Anopheles gambiae complex.
Genet. Res., 10, 205-217.

Mather, K. (1963).
The measurement of linkage in heredity.
Methuen & Co. Ltd., London.

McClelland, G.A.H. (1962).
Sex-linkage in Aedes aegypti.
Trans. roy. Soc. trop. Med. Hyg., 56, 4.

McClintock, B. (1929).
Chromosome morphology of Zea mays.
Science N.Y., 69, 629-630.

McClintock, B. (1930).
A cytological demonstration of the location of an interchange between two non-homologous chromosomes of Zea mays.
Proc. natn. Acad. Sci. U.S.A., 16, 791-796.

McDonald, I.C. and Overland, D.E. (1972).
Temperature-sensitive mutations in the house fly: the characterization of heat-sensitive recessive lethal factors on autosome III.
J. econ. Ent., 65, 1364-1368.

McDonald, I.C. and Overland, D.E. (1973 a).
House Fly Genetics. I. Use of an inversion to facilitate the recovery of translocation homozygotes and to reduce genetic recombination on translocated third chromosomes.
J. Hered., 64, 247-252.

McDonald, I.C. and Overland, D.E. (1973 b).
House Fly Genetics. II. Isolation of a heat-sensitive translocation homozygote.
Ibid, 64, 253-256.

McDonald, P.T. and Rai, K.S. (1970 a).
Correlation of linkage groups with chromosomes in the mosquito, Aedes aegypti.
Genetics, 66, 475-485.

McDonald, P.T. and Rai, K.S. (1970 b).
Aedes aegypti: Origin of a "New" chromosome from a Double Translocation Heterozygote.
Science N.Y., 168, 1229-1230.

McDonald, P.T. and Rai, K.S. (1971).
Population Control Potential of Heterozygous Translocations as Determined by Computer Simulations.
Bull. Wld Hlth Org., 44, 829-845.

McGreevy, P.B., McClelland, G.A.H. and Lavoipierre, M.M.J. (1973).
Inheritance of susceptibility to Dirofilaria immitis infection in Aedes aegypti.
Ann. trop. Med. Parasit., 68, 97-109.

Micks, D.W. (1949).
Investigations on the mosquito transmission of Plasmodium elongatum Huff, 1930.
J. nat. Malar. Soc., 8, 206-218.

Muller, H. J. (1927).
Artificial transmission of the gene.
Science N.Y., 66, 84-87.

Muller, H.J. (1930).
Oenothera-like linkage of chromosomes in Drosophila.
J. Genet., 22, 335-357.

Muller, H.J. (1935).
The position effect as evidence of the localization of the immediate products of gene activity.
Proc. 15th Int. Physiol. Congr. (Leningrad), 21, 587-589.

Muller, H.J. and Settler, F. (1927).
The non-functioning of genes in spermatozoa.
Z. indukt. Abstamm. u. VerebLehre., 43, 285-301.

Painter, T.S. and Muller, H.J. (1929).
The parallel cytology and genetics of induced translocations and Deletions in Drosophila.
J. Hered., 20, 287-298.

Pal, R. (1974).
WHO/ICMR programme of genetic control of mosquitoes in India.
Chapter 5, pp. 73-95.
In: Pal, R. and Whitten, M.J. (Eds.). The use of genetics in insect control. Elsevier/North Holland.

Pal, R. and LaChance, L.E. (1974).
The operational feasibility of genetic methods for control of insects of medical and veterinary importance.
A. Rev. Ent., 19, 269-291.

Parker, D.R. and McCrone, J. (1958).
A genetic analysis of some rearrangements induced in oocytes of Drosophila.
Genetics, 43, 172-186.

Partono, F. and Oemijati, S. (1970).
Susceptibility of Culex pipiens fatigans to Wuchereria bancrofti in Djakarta, Indonesia.
SE Asian J. trop. Med. publ. Hlth, 1, 516-518.

Patterson, J.T., Stone, W., Bedechek, S. and Suche, M. (1934).
The production of translocations in Drosophila.
Amer. Nat., 68, 359-369.

Patterson, R.S., Weidhass, D.E., Ford, H.R. and Lofgren, C.S. (1970).
Suppression and elimination of an island population of Culex pipiens quinquefasciatus with sterile males.
Science N.Y., 168, 1368-1370.

Pell, P.E., Southern, D.I. and Curtis, C.F. (1972).
Correlation of cytogenetic and fertility data on translocations in Glossina austeni.
Trans. roy. Soc. trop. Med. Hyg., 66, 321.

Pipkin, S.B. (1940).
Segregation and crossing-over in a 2,3 translocation in Drosophila melanogaster.
Univ. Texas Publ., 4032, 73-125.

Rabbani, M.G. and Kitzmiller, J.B. (1972).
Chromosomal translocations in Anopheles albimanus Wiedemann.
Mosquito News, 32, 421 432.

Raghavan, N.G.S., Das, M., Mammen, M.L., Singh, N.N. and Wattal, B.L. (1967).
Genetic basis of differential susceptibility of Aedes aegypti to dirofilarial infection. Part 1: Preliminary observations on selection of Aedes aegypti strains susceptible and refractory to Dirofilaria immitis infection.
Bull. Indian Soc. Malar. com. Dis., 4, 318-323.

Rai, K.S. (1971).
The prospects for genetic control of filariasis vectors.
Jap. J. Genet., 46, 207-214.

Rai, K.S., McDonald, P.R. and Asman, S.M. (1970).
Cytogenetics of two radiation induced sex-linked translocations in the yellow-fever mosquito, Aedes aegypti.
Genetics, 66, 635-651.

Rai, K.S. and McDonald, P.T. (1971).
Chromosomal translocations and genetic control of Aedes aegypti pp. 437-451.
International Atomic Energy Agency Publication: STI/PUB/265, Vienna.

Rai, K.S. and McDonald, P.T. (1972).
Application of radiation-induced translocations for genetic control of Aedes aegypti.
Indian Concil Med. Res. techn. Rept. Ser., 20, 77-94.

Rai, K.S., Grover, K.K. and Suguna, S.G. (1973).
Genetic manipulation of Aedes aegypti: incorporation and maintenance of a genetic marker and a chromosomal translocation in natural populations.
Bull. Wld Hlth Org., 48, 49-56.

Rai, K.S., Lorimer, N. and Hallinan, E. (1974).
The current status of genetic methods for controlling Aedes aegypti.
Chapter 7, pp. 119-132.
In: Pal, R. and Whitten, M.J. (Eds.) The use of genetics in insect control. Elsevier/North Holland.

Rana, R.S. and Jain, H.K. (1965).
Adaptive role of interchange heterozygosity in the annual chrysanthemum.
Heredity, 20, 21-29.

Rao, T.R. (1974).
Research on genetic control of mosquitoes in India: Review of the work of the WHO/ICMR research Unit. New Delhi.
J. com. Dis., 6, 57-72.

Rasmussen, I.E. (1960).
Attached-2R;2L.
Drosoph. Inf. Serv., 34, 53.

Rees, H. (1961).
The consequences of interchange.
Evolution, 15, 145-152.

Reynolds, D.G. (1970).
Laboratory studies of the microsporidia Plistophora culicis (Weiser) infecting Culex fatigans Wied.
Bull. ent. Res., 60, 339-349.

Rickards, G.K. (1964).
Some theoretical aspects of selective segregation in interchange complexes.
Chromosoma (Berl.), 15, 140-155.

Robinson, A.S. (1971).
A study of chromosome translocations in Drosophila.
Ph.D. Thesis, University of Bristol (Unpublished).

Robinson, A.S. and Curtis, C.F. (1973).
Controlled crosses and cage experiments with a translocation in Drosophila.
Genetica, 44, 591-601.

Robinson, A.S. and van Heemert, C. (1974).
Preliminary radiobiological studies on Hylemya antiqua (Meigen) and data on three radiation induced (0.5 krad) chromosomal rearrangements. pp. 23-36.
In: Van Heemert, C. (1974). Chromosomal rearrangements in the onion fly Hylemya antiqua (Meigen), induced and isolated for genetic insect control purposes. Wageningen.

Sakai, R.K., Baker, R.H. and Mian, A. (1971).
Linkage group-chromosome correlation in mosquito Culex tritaeniorhynchus.
J. Hered., 62, 90-100.

Sakai, R.K., Baker, R.H., Mian, A. and Said, S. (1972).
Sterility enhancement in the mosquito, Culex tritaeniorhynchus.
Can. J. Genet. Cytol. 14, 215-220.

Sandler, L. and Novitski, E. (1957).
Meiotic drive as an evolutionary force.
Amer. Nat., 91, 105-110.

Sarkara, I. (1955).
A translocation heterozygote in the grasshopper Gesonula punctifrons.
J. Hered., 46, 157-160.

Searle, A.G., Ford, C.E. and Beechey, C.U. (1971).
Meiotic disjunction in mouse translocations and the determination of centromere position.
Genet. Res., 18, 215-235.

Selinger, R. (1972).
Inherited Semisterility for Control of Harmful Insects. V. Translocations in Culex tritaeniorhynchus.
Experientia (Basel), 28, 481-482.

Serebrovsky, A.S. (1940).
On the possibility of a new method for the control of insect pests.
Zool. Zh., 19, 618-630. (in Russian, translated by C.F. Curtis).

Serra, J.A. (1968).
Modern Genetics, Vol. 3.
Academic Press, London.

Singh, K.R.P. and Curtis, C.F. (1974).
Attempt to select a strain of Culex pipiens fatigans Wied. Non-susceptible to Infection with Periodic Wuchereria bancrofti.
J. com. Dis. 6 (2), 88-90.

Smith, R.H. (1971).
Induced conditional lethal mutations for the control of insect populations.
International Atomic Energy Publication: STI/PUB/265, Vienna, pp. 453-465.

Smith, R.H. (1974).
Temperature-sensitive mutations in Habrobracon.
Genet. Res., 23, 63-74.

Smith, R.H. and Von Brostel, R.C. (1972).
Genetic control of insect population.
Science N.Y., 178, 1164-1174.

Snedecor, G.W. and Cochran, W.G. (1967).
Statistical Methods. 6th Ed.
Iowa State Univ. Press, Ames.

Snell, G.D. (1935).
The induction by X-rays of hereditary changes in mice.
Genetics, 20, 545-567.

Snell, G.D. (1946).
An analysis of translocations in mouse.
Genetics, 31, 157-180.

Sobel, F.H. (1969).
A study of the causes underlying the differences in radiosensitivity between mature spermatozoa and late spermatids in Drosophila.
Mutat. Res., 8, 111-125.

Sobel, F.H. (1972).
The viability of II-III translocations in homozygous condition.
Drosoph. Inf. Serv., 48, 117.

Stadler, L.J. (1930).
Some genetic effects of X-rays in plants.
J. Hered., 21, 3-19.

Stern, C. (1926).
Eine neue chromosomabenation von Drosophila melanogaster und ihre Bedentung fur die Theorie der Anordnung der Gene.
Biol. Zbl., 46, 505-508.

Stern, C. (1931).
Zytologisch-genetische Untersuchungen als Beweise für die Morgansche Theories des Faktoraustauchs.
Biol. Zbl., 51, 547-587.

Sturtevant, A.H. and Dobzhansky, Th. (1930).
Reciprocal Translocations in Drosophila and Their Bearing on Oenothera Cytology and Genetics.
Proc. natn. Acad. Sci. U.S.A., 16, 533 536.

Suguna, S.G. and Curtis, C.F. (1974).
Sex Ratio Distorter Strains in Aedes aegypti.
J. com. Dis., 6(2), 102-105.

Suomalainen, E. (1946).
Die Chromosomenverhältnisse in der spermatogenese einiger Blattarien.
Ann. Acad. Sci. Fenn. A., 4, 10, 1-60.

Suzuki, D.T. (1970).
Temperature-sensitive mutations in Drosophila melanogaster.
Science N.Y., 170, 695-706.

Sybenga, J. (1968).
Orientation of interchange multiples in Secale cereale.
Heredity, 23, 73-79.

Thomas, V. (1971).
Studies on cytoplasmic incompatibility in South East Asian Culex pipiens fatigans.
SE Asian J. trop. Med. publ. Hlth, 2, 469-473.

Thomas, V. (1974).
Efficiency of the cytoplasmic incompatible (D3) strain of Culex pipiens fatigans to infection with the rural strain of Wuchereria bancrofti.
Med. J. Malaysia, 29, 73-77.

Thomas, V. and Ramachandran, C.P. (1970).
Selection of Culex pipiens fatigans for vector ability to the rural strain of Wuchereria bancrofti - a preliminary report.
Med. J. Malaya, 24, 196-199.

Thomas, V. and Singh, R. P. (1974).
Comparative susceptibility to Wuchereria bancrofti of Culex fatigans Delhi strain and of strains cytoplasmically incompatible with it.
Unpublished Mimeographed document. WHO/VBC/74.491.

Thompson, J.B. (1956).
Genotypic control of chromosome behaviour in rye II: Disjunction at meiosis in interchange heterozygotes.
Heredity, 10, 99-108.

Trager, W. (1942).
A strain of the mosquito Aedes aegypti selected for susceptibility to the avian malaria parasite Plasmodium lophurae.
J. Parasit., 28, 457-465.

Ullerich, F.H. (1963).
Geschlechtschromosomen und Geschlechtsbestimmung bei einigen Calliphorinen (Calliphoridae, Diptera).
Chromosoma (Berl.), 14, 45-110.

Van Heemert, C. (1974).
Chromosomal rearrangements in the onion fly Hylemya antiqua (Meigen), induced and isolated for genetic insect control purposes. Studies on cytogenetics and fertility, with emphasis on an X-linked translocation. Doctoral thesis,
Wageningen.

Waddington, C.H. (1939).
An introduction of Modern Genetics.
Allen & Unwin, London.

Wagoner, D.E. (1967).
Linkage group karyotype correlation in the house fly determined by cytological analysis of X-ray induced translocations.
Genetics, 57, 729-739.

Wagoner, D.E. (1969).
Linkage group-karyotype correlation in the house fly Musca domestica L., confirmed by cytological analyses of X-rays induced Y-autosomal translocations.
Ibid, 62, 115-121.

Wagoner, D.E., Nickel, C.A. and Johnson, O.A. (1969).
Chromosome translocation heterozygotes in the house fly.
J. Hered., 60, 301-304.

Wagoner, D.E., Johnson, O.A. and Nickel, C.A. (1971).
Fertility reduced in a caged native house fly strain by the introduction of strains bearing heterozygous chromosomal translocations.
Nature (Lond.), 234, 473-475.

Wagoner, D.E., Morgan, P.B., LaBrecque, G.C. and Johnson, O.A. (1973).
Genetic manipulation used against a field population of house flies 1. Males bearing a heterozygous translocation.
Envir. Ent., 2, 128-133.

Wagoner, D.E., McDonald, I.C. and Childress, D. (1974).
The present status of genetic control mechanisms in the house fly, Musca domestica L. Chapter 9, pp. 183-197.
In: Pal, R. and Whitten, M.J. (Eds.). The use of genetics in insect control.
Elsevier, North Holland.

Ward, R.A. (1963).
Genetic aspects of the susceptibility of mosquitoes to malaria infection.
Expl. Parasit., 13, 328-341.

Watson, W.O.F. (1962).
The production of translocations in spermatogonial cells of Drosophila by chloroethyl methanesulphonate.
Genet. Res., 3, 467-471.

White, G.B. (1974).
Anophcles gambiae complex and disease transmission in Africa.
Trans. roy. Soc. Trop. Med. Hyg., 68, 278-301.

White, G.B., Magayuka, S.A. and Boreham, P.F.L. (1972).
Comparative studies on sibling species of the Anopheles gambiae Giles complex (Dipt., Culicidae): bionomics and vectorial activity of species A and species B at Segera, Tanzania.
Bull. ent. Res., 62, 295-317.

White, M.J.D. (1963).
Cytogenetics of the grasshopper Moraba scurra. VIII.
A complex spontaneous translocation.
Chromosoma (Berl.), 14, 140-145.

White, M.J.D. (1973).
Animal cytology and Evolution (Third edition).
Cambridge Univ. Press, Cambridge.

Whitten, M.J. (1970).
"Genetics of Pests in their management", pp. 119-135.
In: Rabb, R.L. and Guthrie (Eds.). Concepts of Pest Management.
N.C. State University, Raleigh.

Whitten, M.J. (1971 a).
Use of chromosome rearrangements for mosquito control.
International Atomic Energy Agency Publication, STI/PUB/265.
Vienna, pp. 399-413.

Whitten, M.J. (1971 b).
Insect control by genetic manipulation of natural populations.
Science, N.Y., 171, 682-684.

Whitten, M.J. and Pal, R. (1974).
Introduction, Chapter 1, pp. 1-16.
In: Pal, R. and Whitten, M.J. (Eds.). The use of genetics in insect control.
Elsevier/North Holland.

WHO (1963).
Adult Mosquito Susceptibility Test. Report of a WHO Scientific Group.
Wld Hlth Org. techn. Rep. Ser., 265.

WHO (1964).
Genetics of vectors and insecticide resistance. Report of a WHO Scientific Group.
Ibid., 268.

WHO (1968).
Cytogenetics of Vectors of Disease of Man.
Report of a WHO Scientific Group.
Ibid., 398.

WHO (1971).
WHO Chronicle.
Wld Hlth Org., 25 (5), 199-245.

WHO (1975).
The work of WHO, 1974. Annual Report of the Director General.
Official records of the World Health Organization, 221.

Wijnands-Stäb, K.J.A. and Van Hemert, C. (1974).
Radiation Induced Semi-sterility for genetic control purposes in the onion Fly Hylemya antiqua (Meigen). 1. Isolation of Semi-sterile stocks and their cytogenetical properties.
Theor. appl. Genet., 44, 111-119.

Wright, J.W. and Pal, R. (Eds.). (1967).
Genetics of insect vectors of disease, Elsevier, Amsterdam.

Wright, J.W., Fritz, R.F. and Haworth, J. (1972).
Changing concepts of vector control in Malaria eradication.
A. Rev. Ent., 17, 75-102.

Wright, S. (1941).
On the probability of fixation of reciprocal translocations.
Amer. Nat., 75, 513-522.

Wright, T.R.F. (1970).
The genetics of embryogenesis in Drosophila.
Advanc. Genet., 15, 261-395.