PROGRESS IN THE USE OF CHROMOSOMAL TRANSLOCATIONS FOR THE CONTROL OF INSECT PESTS

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I. INTRODUCTION

Since the historic demonstration of the eradication of the screw-worm fly, Cochliomyia hominivorax (Coquerel), from the south-western United States by the method of releasing sterile insects (Bushland, 1971) there has been a rapid expansion in the investigation of genetic methods for insect control. Two other factors have also contributed: firstly concern over the environmental effect of insecticides and secondly the development of insecticide resistance (Brown & Pal, 1971); in 1946 there was only one reported case of a disease vector being insecticide-resistant but in 1971 the number was 110 (Pal & LaChance, 1974). The technique to be described here of utilizing chromosomal translocations is only one of many genetic approaches being considered (Smith & von Borstel, 1972; Pal & Whitten, 1974; Journal of Communicable Diseases, 1974; Davidson, 1974; Whitten & Foster, 1975).

The inheritance of a condition in which 50% of zygotes aborted was first recorded in the plant, Stizolobium deeringianum by Belling (1914), and he named this condition ‘semi-sterility’. In 1940 Serebrovskii realized that this inherited reduction in fertility, which is a property of chromosomal translocations, could be utilized for the control of insect pests. Some 25 years later interest in chromosomal translocations for pest control was again stimulated (Rai, 1967; Curtis, 1968a; Laven, 1968) and that interest
has been sustained. This review is specifically concerned with the application of chromosomal translocations to insect control but reference will also be made to non-pest insects, plants and mammals where particular points require amplification.

II. GENETICS OF TRANSLOCATIONS

The use of the word 'translocation' in this review will be restricted to the mutual exchange of chromosome sections between terminal segments of non-homologous chromosomes. In Fig. 1 are shown the three karyotypes present in a simple translocation system, i.e. the wild type, the translocation heterozygote and the translocation homozygote. During meiosis in the translocation heterozygote, the synaptic forces between homologous loci result in the formation of a cross-shaped figure (Belling & Blakeslee, 1924). It is the subsequent segregation pattern of this configuration which can lead to reduced fertility of translocation heterozygotes and which may be utilized for insect control. There are three patterns of segregation possible (see Fig. 1): firstly alternate segregation, when alternate centromeres pass to the same anaphase pole; secondly adjacent 1 segregation when non-homologous centromeres pass to the same pole; and thirdly adjacent 2 segregation when homologous centromeres pass to the same pole. A study of the types of gamete shows that only alternate segregation leads to the formation of orthoploid gametes; both adjacent types produce aneuploid
Chromosomal translocations and pest control

<table>
<thead>
<tr>
<th>Adjacent 1</th>
<th>Alternate</th>
<th>Adjacent 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2'</td>
<td>1' 2</td>
<td>1 1' 2'</td>
</tr>
<tr>
<td>1' 2</td>
<td>2' T/+c</td>
<td>1' 1' 2</td>
</tr>
<tr>
<td>1 2</td>
<td>1' T/+c</td>
<td>2' 1' 2'</td>
</tr>
<tr>
<td>2 2'</td>
<td>1' T/+c</td>
<td>2' 1' 2'</td>
</tr>
</tbody>
</table>

Fig. 2. Zygote types resulting from matings between translocation heterozygotes. The blank squares represent inviable combinations. The translocation heterozygotes (T/+) followed by c are derived from complementation.

Many factors have been reported to affect segregation of translocation heterozygotes and hence affect their sterility, amongst these factors are the morphology of the synaptic complex, the relative sizes of the exchanged segments of chromosome, the position of the centromeres, the flexibility of the chromosomes and the frequency and distribution of chiasmata (Lewis & John, 1963; Rickards, 1964; Sybenga, 1972). In many species there has been found to be an equal number of alternate and adjacent segregants from translocation heterozygotes leading to a fertility of about 50%, e.g. in *Zea mays* (Burnham, 1956), the mouse (Snell, 1934; Slizynski, 1957) and *Drosophila* (Painter & Muller, 1929). However, in plants there are a few cases of newly induced translocations showing fertilities significantly higher than 50% (Rana, 1965; Soriano, 1957; Lawrence, 1958). Dennhöfer (1974) attempted to interpret the different levels of fertility of translocations as being due to the control by a single Mendelian factor responsible for segregation of the chromosomes in translocation heterozygotes. The crucial property of the inheritance of semi-sterility by translocations follows from the segregation of translocation gametes and wild-type gametes from translocation heterozygotes (see Fig. 1); consequently, in matings between translocation
heterozygotes and wild types, half the surviving progeny are translocation heterozygotes and half are wild types.

As can be seen in Fig. 1 meiosis does not have serious consequences for translocation homozygotes; all the gametes produced from such an individual are orthoploid. Translocation homozygotes can be found amongst the progeny from mating between translocation heterozygotes. In such matings certain combinations of aneuploid gametes may be complementary, i.e. in a zygote the duplication and deficiency of one gamete is complemented by the deficiency and duplication of the other thus producing a viable embryo (see Fig. 2). Complementation always produces translocation heterozygotes and in this way the proportion of translocation heterozygotes from such matings is increased above the Mendelian expectation (Curtis, 1968a). Further, the survival of such complementary zygotes increases the fertility of matings between translocation heterozygotes above that which would be expected from simply taking the product of the female and male heterozygote fertilities. For example, if male and female translocation heterozygotes have fertilities of 50% when test-crossed to wild-types, then, assuming no complementation, the fertility of matings between translocation heterozygotes would be 25%. The occurrence of complementation raises this fertility by an amount depending on the frequency of the gametic types segregating from the translocation heterozygotes.

Complementation has been demonstrated by the use of marker genes in *Drosophila* (Muller & Settles, 1927) and mice (Snell, 1946) and has been inferred from fertility data in *Glossina austeni* (Curtis, Southern, Pell & Craig-Cameron, 1972), *Hylemya antiqua* (Robinson & van Heemert, 1975) and *Drosophila* (Robinson, 1971).

III. TRANSLOCATIONS IN PEST INSECTS

(a) Production and isolation

Translocations, like all mutations, occur naturally and their frequency can be increased by the application of mutagenic agents. Ionizing radiation is usually employed, but translocations can be produced by treating germ cells with chemical mutagens (Schalet, 1955; Watson, 1962; Cacheiro, Russell & Swartout, 1974). Observations on *Drosophila* males have shown wide differences in susceptibility of the different stages of spermatogenesis to the induction of translocations (Savhagen, 1960) and if translocations are to be isolated for use in insect control it would be most efficient to sample sperm which was at the sensitive stage at the time of radiation. It appears that in *Drosophila* the early spermatids are most sensitive to the induction of translocations (Sobels, 1969). Early experiments with *Drosophila* females (Glass, 1955) seemed to suggest a very low frequency of induced translocations when oocytes were irradiated; but Traut (1967) by restricting irradiation to mature oocytes was able to induce and isolate many translocations. As oocytes are pre-meiotic any induced rearrangement must still pass through meiotic segregations and as such will be subject to germinal selection.

There are currently two main techniques available for the isolation of chromosomal translocations in the *F*₁ generation following irradiation in the parental generation.
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Firstly the pseudo-linkage technique; this was developed by Muller & Altenburg (1930) and it involves the mating of irradiated wild-type males to females which are homozygous for recessive and visible genes on two or more chromosomes. The $F_1$ heterozygous males are then back-crossed individually to the same type of female and the $F_2$ generation cultures examined. If there is independent assortment of the two or more characters then the $F_1$ male did not have a translocation between the marked chromosomes. If, however, an $F_1$ male had a translocation between two of the marked chromosomes then the mutant characters would segregate together. The technique is dependent on the absence of genetic crossing-over in the Drosophila male so that in the $F_2$ generation the markers remain tightly linked to the non-translocated chromosomes. However, the technique is not absolutely restricted to those species in which crossing-over is absent in one sex because such tight linkage is produced when the translocation breakpoints are in close proximity to the marker genes. Translocations have been isolated by this technique in Musca domestica (Wagoner, Nickel & Johnson, 1969; Wagoner & Nickel, 1971), Aedes aegypti (Rai & McDonald, 1971, 1972; Rai, McDonald & Asman, 1970; Bhalla, 1973), Culex tritaeniorsynchus (Sakai, Baker & Mian, 1971) and Lucilia cuprina (Foster & Whitten, 1974). The pseudo-linkage technique is of course extremely efficient in the isolation of translocations and if each pair of chromosomes is suitably marked then all induced translocations can be isolated.

Secondly, reduced fertility can be used as a marker for translocations when visible markers are not available. $F_1$ progeny from irradiated parents are individually out-crossed to wild-type insects and the fertility of such matings is measured. In cases where fertility is reduced, the progeny of such matings are retained and they are again outcrossed to the wild-type stock. If approximately half of the progeny again show reduced fertility then this gives good evidence for the presence of a translocation, although it is not conclusive because inversions can also lead to inherited reduced fertility (Robinson & van Heemert, 1975; Robinson, 1975; Jost & Laven, 1971; Baker, Sakai & Mian, 1971; Bhalla, 1970). By means of such fertility measurements, translocations have been isolated in Glossina austeni (Curtis et al., 1972), Hylemya antiqua (Wijnands-Stäb & van Heemert, 1974; Robinson & van Heemert, 1975; van Heemert & Wijnands-Stäb, 1975), Culex pipiens (Laven, Jost, Mayer & Selinger, 1971b; Laven & Jost, 1971), Culex tritaeniorsynchus (Selinger, 1972), Anopheles albimanus (Rabbani & Kitzmiller, 1972), Anopheles gambiae (Akiyama, 1973) and Blatella germanica (Ross & Cochran, 1973). In the two-spotted spider mite, Tetranychus urticae, chromosome mutations which behave genetically very much like translocations have been isolated by measurements of fertility (van Zon & Overmeer, 1972; Feldmann, 1975).

A very important and perhaps crucial sequel to the isolation of translocations by either of these methods is the development of cytological techniques for their confirmation and characterization. This has now been achieved with a high degree of sophistication in many of the above species. In the species listed below, meiotic and mitotic preparations are routinely used for this purpose, Culex pipiens (Jost & Laven, 1971), Musca domestica (Wagoner, 1967), Glossina austeni (Curtis et al., 1972), Lucilia
cuprina (Foster & Whitten, 1974), Hylemya antiqua (van Heemert, 1973), Blatella germanica (Cochran & Ross, 1969), Culex tritaeniorhynchus (Baker, 1968) and Aedes aegypti (McDonald & Rai, 1970a). In addition, preparations of polytene chromosomes have been produced in Anopheles albimanus (Rabbani & Kitzmiller, 1972), Lucilia cuprina (Childress, 1969) and G. m. morsitans (Southern, Pell & Craig-Cameron, 1973).

(b) Fertility

Fertility in this discussion is defined as the percentage of eggs which hatch; the value of such a restricted definition will be commented upon later (p. 8). As suggested on p. 3, the fertility of translocation heterozygotes theoretically averages around 50% and this has been found to be true for most translocations in pest insects. In Table 1 are shown the mean fertilities of the translocation heterozygotes studied so far in pest insects. Only those examples are included in which reciprocal translocations were positively identified either by cytology or pseudo-linkage. In compiling the table no discrimination has been made between sex-linked and autosomal translocations and with the exception of Cochliomyia hominivorax the data from males and females have been combined. The translocation studied in C. hominivorax by LaChance, Riemann & Hopkins (1964) was unusual in that the translocation heterozygote had a phenotypic effect on wing pigmentation. LaChance et al. (1964) inferred from a combination of genetic and cytological data that the males exhibited preferential alternate segregation and hence the fertility was in excess of 50%; segregation in females was random. From Table 1 it can be seen that three species exhibit a high degree of stability in the fertility of translocation heterozygotes, i.e. Culex tritaeniorhynchus, Anopheles albimanus, and Blatella germanica. In B. germanica the range of fertilities is exaggerated by the inclusion of one translocation with an extremely high fertility; the remaining 14 of the translocations are in the range of 47-58% fertility. The rest of the species shown in Table 1 show a degree of variability in the fertility of individual translocations, however, even in these cases the fertility generally approximates to 50%; the most notable exception being C. pipiens (Laven & Jost, 1971). In Table 1 is also shown the technique used for the isolation of the translocations. It is probably not coincidental that in the three species listed above as showing a high degree of stability in the fertility of the translocation heterozygotes, fertility measurements were used for isolation. These translocations therefore constitute a selected group. Conversely where pseudo-linkage was used for isolation a much wider range of fertility was usually observed.

For insect control it is obvious that the lower the fertility of the translocation heterozygotes the better. However, low fertility places restrictions on the laboratory rearing of such translocations in certain species, due either to low numbers of offspring per female, e.g. Glossina spp., or to problems associated with rearing techniques, e.g. Hylemya antiqua. Conversely, Culex pipiens male-linked translocations, with fertilities as low as 15%, can be successfully reared with a surplus for release (Laven & Aslamkhan, 1970).

It might be suggested that the fertility of translocations could be altered by selection, i.e. that segregation is under genetic control. This has been shown to occur in plants
Table 1. Fertility and homozygous viability of translocations in pest insect

<table>
<thead>
<tr>
<th>Species</th>
<th>No. tested*</th>
<th>Heterozygous fertility</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. viable</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>adult</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>N.A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. tritaeniorhynchus</td>
<td>15 F</td>
<td>0.497 ± 0.9</td>
<td>39.7-54.3</td>
</tr>
<tr>
<td>C. tritaeniorhynchus</td>
<td>46 P.L.</td>
<td>0.53 ± 0.7</td>
<td>47.0-70.0</td>
</tr>
<tr>
<td>C. pipiens</td>
<td>31 F</td>
<td>0.66 ± 1.56</td>
<td>27.0-92.0</td>
</tr>
<tr>
<td>A. aegypti</td>
<td>24 P.L.</td>
<td>0.41 ± 0.11</td>
<td>14.0-60.0</td>
</tr>
<tr>
<td>A. aegypti</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. aegypti</td>
<td>7 P.L.</td>
<td>0.45 ± 1.6</td>
<td>30.0-59.0</td>
</tr>
<tr>
<td>A. albimanus</td>
<td>6 F</td>
<td>0.53 ± 0.30</td>
<td>50.3-58.8</td>
</tr>
<tr>
<td>M. domestica</td>
<td>18 P.L.</td>
<td>N.A.</td>
<td>23.0-61.0</td>
</tr>
<tr>
<td>M. domestica</td>
<td>127 P.L.</td>
<td>0.44 ± 0.8</td>
<td>22.4-80.2</td>
</tr>
<tr>
<td>B. germanica</td>
<td>15 F</td>
<td>0.52 ± 0.50</td>
<td>47.0-68.0</td>
</tr>
<tr>
<td>G. austeni</td>
<td>5 F</td>
<td>0.40 ± 0.55</td>
<td>32.7-46.7</td>
</tr>
<tr>
<td>H. antiqua</td>
<td>2 F</td>
<td>0.70 ± 0.2</td>
<td>61.9-78.4</td>
</tr>
<tr>
<td>C. hominivorax ♀</td>
<td>1</td>
<td>0.49 ± 0.12</td>
<td>29.2-61.5</td>
</tr>
<tr>
<td>C. hominivorax ♂</td>
<td>1</td>
<td>0.66 ± 0.9</td>
<td>7.0-77.8</td>
</tr>
<tr>
<td>T. urticae†</td>
<td></td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>L. cuprina</td>
<td></td>
<td>N.A.</td>
<td></td>
</tr>
</tbody>
</table>

**Homozygous viability**

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. viable</th>
<th>No. as tested adult</th>
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</thead>
<tbody>
<tr>
<td>N.A.</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>Baker &amp; Sakai (1974)</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>Laven et al. (1971)</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Rai &amp; McDonald (1972)</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>Rai et al. (1974)</td>
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<td>2</td>
</tr>
<tr>
<td>Lorimer et al. (1972)</td>
<td>40</td>
<td>2</td>
</tr>
<tr>
<td>Rabbani &amp; Kitzmiller (1972)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>McDonald &amp; Overland (1973a)</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>Ross (pers. comm.  )</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Curtis (1971)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>van Heemert (pers. comm.)</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>LaChance et al. (1964)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>van Zon &amp; Overmeer (1972)</td>
<td>34</td>
<td>13</td>
</tr>
<tr>
<td>Foster &amp; Whitten (1974)</td>
<td>30</td>
<td>1</td>
</tr>
</tbody>
</table>

N.A. = Data not available.
* Translocations isolated by fertility (F) or pseudo-linkage (P.L.).
† Data for structural chromosome mutations, not confirmed translocations.
(Rees & Sun, 1965) but Erk (1960) could find no evidence for genetic control of segregation in a translocation in *Drosophila*. Conversely, Hossain, Curtis & Jaffe (1974) were able to demonstrate a significant effect of selection on the fertility of translocation heterozygotes in *Drosophila*, although the selection limit was reached within 3–4 generations; Hossain & Curtis (1975) also found a similar effect with translocation heterozygotes in *Musca domestica*. However, neither change in fertility was large.

At the beginning of this section, fertility was defined as the percentage of eggs that hatch. This is perhaps a somewhat arbitrary measure determined mainly by the stage of development that is most easily monitored. However, there are now instances in the literature of duplication and deficiency zygotes produced from translocations surviving to the larval stage, e.g. *Culex tritaeniorhynchus* (Sakai et al., 1971) and *Hylemya antiqua* (Robinson & van Heemert, 1975), to the pupal stage (*Glossina austeni* (Curtis et al., 1972)) and even to the adult stage as in *Aedes aegypti* (Ved Brat & Rai, 1974). The survival of such duplication and deficiency zygotes would indicate that a more realistic measure of the fertility of translocation heterozygotes would be the percentage survival of all zygotes to the next generation. It is also possible that the fertilities of those translocations recorded in Table I that were above 50% might be attributable to the survival through the egg stage of a certain proportion of duplication and deficiency zygotes.

From the practical point of view, if it is the larval stage of the insect which is damaging and hence has to be controlled, then the survival of such ‘unbalanced’ zygotes is undesirable.

(c) Homozygous viability

For the maintenance of a translocation stock in the laboratory and for maximum efficiency in the use of translocations in the field it is preferable to have the translocation as a homozygote. Theoretically the homozygote should be as fit and fertile as the wild type as it experiences no meiotic difficulties and it possesses the full genome (see Fig. 1). However, experience with many insects has demonstrated that translocations are usually lethal, sterile or have very low viability as homozygotes.

Data from *Drosophila* indicate various values for the percentage of translocations viable as homozygotes. Ives & Fink (1962) found that 84 out of 102 translocations between chromosomes 2 and 3 were lethal as homozygotes. Of 332 translocations tested for homozygous viability by Patterson, Stone, Bedichek & Suche (1934) 40% were viable as homozygotes and of these 83% were fertile. However, the authors showed that the percentage of viable and fertile translocations was dependent on which chromosomes were involved. Sobels (1972) and Ytterborn (1970) also showed that approximately 40% of translocations in *Drosophila* were viable as homozygotes. Ytterborn made the significant observation that the percentage of translocations viable as homozygotes was inversely related to the original dose of radiation applied; when 3500 rad were applied then 80% of translocations were lethal as homozygotes, but when 500 rad were applied then only 20% were lethal. In contrast to the data from *Drosophila*, translocations in mice (Carter, Lyon & Phillips, 1956) and in plants
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(Burnham, 1962) can be reared as homozygotes with a much higher frequency. A basic difference between animals and plants in relation to the viability of translocation homozygote probably lies in the fact that in plants viable translocation heterozygotes are a selected group, since a translocation having a lethal or sub-lethal effect tends to be eliminated in the haploid gametophyte generation (Dobzhansky, 1936). Further, Muller (1954) observed that animal species showing strong somatic chromosome pairing are liable to show reduced viability when structural rearrangements, such as translocations and inversions, are made homozygous; he advanced no explanation of this correlation. The causes of reduced viability of translocations can be position effects, damage at the translocation breakpoint or linked radiation-induced recessive lethals. Sobels (1972) calculated the relative importance of these different aspects in Drosophila and concluded that over half the lethality he observed was due to the translocation itself.

Table 1 also shows the data so far available on the homozygous viability of translocations in pest insects; only a small proportion are viable and even then their fitness can be reduced. The data of McDonald & Overland (1973 a) were obtained by the use of a genetic stock which prevented crossing-over of a certain portion of the chromosome material in the female of the house fly. With this stock homozygous translocations could be phenotypically distinguished in the progeny of translocation heterozygous matings. The use of such stocks greatly increases the efficiency in screening for translocations. In Tetranychus urticae van Zon & Overmeer (1972), Overmeer & van Zon (1973) and Feldmann (1975) have been able to isolate many homozygous structural chromosome rearrangements. As this species reproduces parthenogenetically (unfertilized females producing only haploid males) the use of haploid males to propagate the lines with rearrangements selects out those rearrangements having a recessive lethal effect; the surviving rearrangements will thus have an increased chance of being viable as homozygotes.

The difficulty in obtaining viable translocation homozygotes has been a major stumbling block in the development of translocations for insect pest control for two main reasons. Firstly, for maintaining a stock in the laboratory it is preferable that it should remain pure and be relatively fertile. Secondly, when different translocation homozygotes are available, various combinations of heterozygotes can be synthesized to produce higher levels of sterility (see below).

To improve the fitness of translocation homozygotes several techniques have been suggested: firstly, the use of as low a dose of radiation as is practically possible for the initial induction of translocations (Ytterborn, 1970; Feldmann, 1975); secondly, repeated backcrossing of the translocation heterozygote to remove all radiation-induced recessive lethals; and thirdly, the use of as broad a genetic base as possible for the two inbreeding generations necessary for the synthesis of a homozygous stock.

(d) Multiple translocations

There are three ways to obtain multiple translocations: from multiple break-and-join events after irradiation; from re-irradiation of existing single translocations; and from crossing lines that have different single translocations. Examples of all three
Chromosome breaks and exchanges

Meiotic associations

Fig. 3. Two-chromosome double translocations (A, B, C). The centromeres are numbered and represented by blank squares and the chromosome arms have been given letters. The position and size of the exchanged segments are shown together with the meiotic association following the synapsis of homologous loci in the double-translocation heterozygote.

Techniques will be given below. As a general rule, the more chromosomes involved in a translocation in an individual, the lower will be the resulting fertility. It is therefore preferable and perhaps essential to think in terms of the use of multiple translocations for insect control.

Two different translocations in a cell can involve 2, 3 or 4 chromosomes (Gopinath & Burnham, 1956; Curtis & Robinson, 1971). Fig. 3 shows three types of double translocation involving the same two pairs of chromosomes, i.e. two-chromosome double-translocations; it depicts the exchanged pieces of chromosome and also the meiotic configuration in the two-chromosome double-translocation heterozygote resulting from the synapsis of homologous loci. The differences between the three types shown in Fig. 3 (A, B, C) are related to the position of the chromosome breaks.
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relative to the centromeres. In these types of double translocations there are segments of the same chromosome between two chromosome breaks; these are so-called differential segments (Darlington, 1936). In Fig. 3 they are represented by the curved parts of the chromosome and, depending on the position of the chromosome breaks relative to the centromere, they include two pairs of centromeres (A), one pair of centromeres (B) or no centromeres (C). In the absence of genetic crossing-over within a differential segment the fertility of two-chromosome double-translocation heterozygotes would be the same as the fertility of single-translocation heterozygotes. However, crossing-over within the differential segment would generate extra aneuploid gametes and hence the fertility could be further reduced. This means that in the cyclorrhaphid diptera, where crossing-over is restricted to females (Wagoner, 1967; LaChance, Dawkins & Hopkins, 1966), the fertility of such two-chromosome double-translocation heterozygotes in females can be less than in males. This has been demonstrated experimentally for a two-chromosome double-translocation in Drosophila (Robinson & Curtis, 1972). There is little information on two-chromosome double-translocations in pest insects. However, in Aedes aegypti, four such translocations have been studied (Rai, Lorimer & Hallinan, 1974). The authors showed that the fertilities of these four rearrangements were not lower than the fertility of single translocations and they concluded from this that the breakpoints of the translocations were close enough to prevent crossing-over. A contributing factor to the dearth of information on two-chromosome double translocations could be that if the pseudo-linkage technique is used for their isolation, then their segregation pattern can be identical with that of single translocations.

In Fig. 4 are shown four types of double translocation involving three pairs of chromosomes. As with two-chromosome double translocations the difference between the types is related to the position of the chromosome breaks relative to the centromeres. Types A, B and C in Fig. 4 all have differential segments signified by ‘d’. Fig. 4D is a rotational or cyclic translocation where the occurrence of three breaks, one in each chromosome, is followed by rotation and rejoining of the three chromosome segments.

For three-chromosome double translocations many more data are available and frequently both sexes have exhibited decreased fertility compared to single translocations. In Culex tritaeniorhynchus Sakai et al. (1971) recovered six, three-chromosome double translocations from multiple break-and-join processes following radiation. However, in only three was the fertility less than the fertility of single-translocation heterozygotes. In one of these three the fertility of the male was significantly less than the fertility of the female, and in this species crossing-over is restricted to the male (Baker & Rabbani, 1970). Thus the lower fertility of the male might be attributable to the occurrence of crossing-over in the differential segment. By re-irradiation of existing single-translocation stocks, Sakai, Baker, Mian & Said (1972) were able to generate many three-chromosome double translocations and, in the majority, the fertility was significantly reduced below the fertility of single-translocation heterozygotes. Further, by crossing two different three-chromosome double translocations they were able to produce a proportion of males that were heterozygous for two independent
translocation complexes involving all the chromosomes; such males were 96 % sterile in outcrosses to normal insects (Baker & Sakai, 1974). By crossing two different single-translocation heterozygotes, in *Aedes aegypti*, each with a fertility of about 30 %, McDonald & Rai (1970b) were able to produce a three-chromosome double-translocation heterozygote of the type shown in Fig. 4B with a fertility of 10 %. By the use of genetic markers the authors were able to show that there was an increased amount of crossing-over occurring in the differential segment. In a subsequent paper (Rai *et al.*, 1974) four more three-chromosome double translocations were studied and they all
had fertilities well below the level of single-translocation heterozygotes; the range was 13–20%.

In the same insect a three-chromosome double-translocation heterozygote has been produced by multiple break-and-join processes (Bhalla, 1973). With this double heterozygote there was a significant difference between the fertility of males, 45%, and females 18%. This difference cannot be explained on the ground of differential crossing-over, as in this insect both sexes have crossing-over. With a three-chromosome double translocation in *Blatella germanica* Ross & Cochrane (1973) obtained results which they interpreted as indicating that the segregation of the synaptic complex was under genetic control. They observed that in the first generation, the fertility of the three-chromosome double translocation was 30% and in subsequent generations it rose to 55%. In the housefly, 16 three-chromosome double translocations have been studied by Wagoner *et al.* (1969) and they had an average fertility of 38%. In *Culex pipiens* (Laven, 1969) and *Glossina austeni* (Curtis, 1969) three-chromosome double translocations had fertilities around 20%. The three-chromosome double translocation shown in Fig. 4D can only be produced by multiple break-and-join events following irradiation, and the double translocations described by Laven (1969) and Wijnands-Stäb & van Heemert (1974) are of this type.

### IV. USE OF TRANSLOCATIONS IN INSECT CONTROL

The genetics and relevant properties of translocations and their production in pest insects has now been documented and the use of such chromosomal rearrangements in insect control must now be considered.

(a) *Theoretical assessments*

Chromosomal translocations can function in two ways in insect control. Firstly, they can subject the population to an increased death rate of zygotes through the segregation of aneuploid gametes from translocation heterozygotes (Serebrovskii, 1940). This increase in the proportion of lethal zygotes is generally referred to as genetic load. Secondly, they can effect gene manipulation of pest species (Curtis, 1968b) either to replace the pest species with a less noxious form, e.g. the incorporation of refractor genes for disease transmission (McDonald, 1962; Ward, 1963; Rodriguez & Craig, 1973) or to achieve population suppression, e.g. by the incorporation of temperature-sensitive lethals (Suzuki, 1970; Whitten, 1971b) or ‘non-diapausing’ genes (Klassen, Knipling & McGuire, 1970). The use of translocations to effect gene manipulation follows from the population genetics of translocations in natural populations. A mixed population of wild types, translocation heterozygotes and translocation homozygotes exists as a negatively heterotic system, i.e. the translocation heterozygote is less fit than either the wild type or the translocation homozygote. Because of this relationship there is theoretically an equilibrium chromosome frequency when both the translocation and the wild-type chromosomes are continuously maintained in the population. However, this equilibrium point is unstable;
consequently, if the frequency of either the translocation or the wild-type chromosome is made to exceed this equilibrium frequency, then natural selection will favour the chromosome type in the majority and the other chromosome type will be eliminated. Therefore if a particular gene is linked to a translocation and the translocation frequency is made to exceed the unstable equilibrium frequency then natural selection will increase the frequency of the translocation until it becomes the only chromosome type, i.e. it is fixed in the population and along with it, the particular gene. The linkage of a temperature-sensitive lethal to a translocation has already been achieved in the house fly (McDonald & Overland, 1973b).

Curtis & Hill (1968), using a theoretical model specifically adapted for populations of tsetse-fly, investigated the effects of viability, density dependence and migration on the genetic load generated in a population by the release of single translocation homozygotes. They concluded that small decreases in the viability of the released strain could in principle be overcome by the release of more flies. However, when density-dependent regulation and migration are considered, there is a critical level above which it is impossible to reduce the population by means of a single translocation. In a further paper, Curtis & Hill (1971), computed the effects of using translocations with different levels of fertility as heterozygotes and with different levels of viability of the translocation homozygotes. They also considered the use of male-linked translocations. They demonstrated that the lower the fertility of translocation heterozygotes the more rapidly would fixation occur. Small reductions in the viability of the translocation homozygotes greatly increase the number of insects needed for release in order to get a reduction in population size. If the viability of the translocation homozygote is below that of the semi-sterile translocation heterozygote then an unstable equilibrium cannot be obtained and natural selection will always act against the translocation (Robinson & Curtis, 1973). Male-linked translocations, since they cannot become homozygous, do not demonstrate frequency-dependent selection and they would be rapidly eliminated from the population unless continuous releases were made or the males with translocations had superior competitiveness.

The limitations in the use of single translocations for insect control, i.e. a limited amount of sterility and the difficulty in achieving the optimum translocation frequency, led to the development of theoretical models for the use of multiple translocation systems for insect control. As they exhibit higher sterility, multiple translocations can lead to faster selection and fixation. Whitten (1970, 1971 a, b) described a theoretical scheme utilizing multiple translocation homozygotes (the multiple heterozygotes being sterile) for population replacement and suppression. His scheme involved the periodical replacement of an insecticide-resistant population by an insecticide-sensitive one, using multiple translocations as the transporting mechanism. He also indicated that the size of the genetic load produced during the replacement process might itself prove a limiting factor on population density.

In all systems of population replacement the particular gene to be introduced in the natural population must be tightly linked genetically to the transporting mechanism. This can be achieved in two ways; firstly, by using a translocation-homozygous strain which leads to the production of fully sterile translocation heterozygotes or secondly,
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by ensuring that the gene is closely linked to the translocation breakpoint either by position or by the inclusion of an inversion (Curtis, 1968b). As indicated on p. 14 this close linkage of a conditional lethal gene to a translocation has already been achieved in the house fly (McDonald & Overland, 1973b).

As mentioned earlier (p. 8) a serious problem in the experimental work has been the reduced viability of translocation homozygotes. However, Whitten (1970) argued that in nature there are many cases of translocations becoming established (White, Blackith, Blackith & Cheney, 1967; John & Lewis, 1965) so that the process should be repeatable in the laboratory. Conversely, Wright (1941) concluded that "fixation [of translocations] can hardly occur under exclusive sexual reproduction except in species in which there are numerous isolated populations that pass through phases of extreme reduction in numbers".

The reduced viability of homozygous translocations led to a consideration of the release of heterozygotes, specifically double heterozygotes, into natural populations (Curtis & Robinson, 1971; Curtis, 1971, discussion). If two translocation homozygotes can be reared in the laboratory even though they have reduced viability, then double heterozygotes could be produced by crossing them and their use should show a high degree of sterility. Provided that the defects in viability of the translocations are recessive, the hybrid should be fully competitive and it could even benefit from heterosis. Further there would be no opportunity for selection to increase the fertility of the heterozygote (Curtis, 1975, personal communication). Curtis & Robinson (1971) used computer models to simulate double heterozygote releases, using different release strategies and different types of double translocations with different levels of fertility. They concluded that any reduction in viability of the single-translocation homozygotes would make extremely attractive the use and release of double-translocation heterozygotes. Similarly McDonald & Rai (1971) simulated the effects of release of double heterozygotes and single heterozygotes into populations of Aedes aegypti with various growth rates. The fertility of the double heterozygote was 12.5% and the release of this strain in a ratio of 4:1 to the natural population over six generations could effect eradication of a population with a reproductive potential of five times per generation; as expected, the double heterozygote was many times more effective than the single heterozygote. Computer models for the introduction of translocations into natural populations of Hylemya antiqua have been investigated by Wijnands-Stäb & Frissel (1973). They introduced the idea of releasing translocations into a field population of Hylemya antiqua which had been considerably reduced in numbers by the method of releasing sterile insects (Theunissen et al., 1973). By this technique it might be possible to maintain the population for many generations at a level below the threshold at which economic damage is caused without the release of further insects.

(b) Experimental and field assessments

There are so far only a few cage experiments incorporating translocations. Erk (1955), using a 2–3 translocation in Drosophila, studied the competition of this translocation, associated with a dominant homozygous lethal marker, in cage experiments
with wild-type insects. From an initial translocation frequency of 25\%, the final frequency was 0.5\% and it was maintained at this level probably because of heterosis of the translocation heterozygote. Using a viable translocation homozygote in Drosophila, Robinson & Curtis (1973) were unable to demonstrate frequency-dependent selection in favour of the translocation even when the translocation was introduced into the cage at a ratio of 9:1 with wild-type insects. The translocation homozygote used had excellent viability when assessed in isolation, but in mixed populations its viability was greatly reduced. With Aedes aegypti, Rai & McDonald (1972) tested single translocation heterozygotes in various ratios against wild types in both laboratory and field population cages. In the laboratory experiments the two translocations that they tried were either as or more competitive than the wild type. However, in the field experiments one of the translocations showed reduced competitiveness. As the experiments had a duration of only one generation, the competitiveness measured took into account aspects of male survival and mating ability but excluded the overall competitiveness of the translocations after many generations of breeding under competitive conditions.

Using cage populations of houseflies, Wagoner, Nickel & Johnson (1971) studied the effect of three different multiple-translocation heterozygotes on population fertility. They were able to generate high genetic loads in mixed populations (up to 89\% in one generation) using releases in a ratio of 9:1 in favour of the translocation heterozygote. A single-translocation heterozygote with a fertility of about 50\% has been studied in populations of the German cockroach, Blatella germanica (Ross, 1975). In one population a single release of translocation-heterozygous males in a ratio of 2:1 to the wild-type males resulted in a reduction of the size of the F1 and F2 generations by about a half. In another population the repeated introduction of translocation-heterozygous males reduced considerably but did not prohibit population growth over a 6-month period.

The first results of releases of translocations into field-cage populations of pest insects were published by Laven (1969) when he demonstrated the eradication of an artificial population of Culex pipiens by release into it of a male-linked translocation; the population was maintained with the exclusion of density-dependent factors. In a continuation of the work, Laven, Cousserans & Guille (1971a) described the release during 1970 of a male-linked translocation into a natural isolated population of Culex pipiens. The male-linked translocation had a fertility of 50\% and it was possible to inject the translocation into the natural population so that more than 95\% of the males in the population had the translocation. With the translocation at this frequency in the population the number of emerging adults/day dropped to 100 at the end of September from 20000 on 12 August. During 1971 the same population was monitored (Laven, Cousserans & Guille, 1972) but no more releases were made. In the first egg rafts of 1971, 89\% showed semi-sterility. Throughout the breeding cycle until June the number of egg rafts/week never rose above 20 compared with a maximum the previous year of 411. The authors concluded that the presence of the translocation was the main factor preventing the seasonal increase in the population in 1971 aided by increased predator and parasite pressure on the already diminished population.
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However, Cousserans & Guille (1974) have since retracted this conclusion and attributed the fall in the number of egg rafts sampled in 1971 to a change in the experimental design between 1970 and 1971. Further, from 1971 to 1973 the translocation frequency dropped from 89% to less than 1%, but there was no change in the number of egg rafts collected in the ovitraps (Cousserans & Guille, 1974).

Wagoner, Morgan, Labrecque & Johnson (1973) made field releases in 1969 of three-chromosome double-translocation heterozygous males of *Musca domestica* into a poultry house near Gainesville, Florida, at a ratio of 5:1 in favour of the translocation-bearing males (although the goal was 9:1). In the first generation after the release, a fertility of 46% was recorded in the wild-type females; that of the control was 94.0%. However, in subsequent generations the fertility rose and the average over four generations was 63% compared with the control value of 78%. They concluded that the natural population was saturating the available environmental niche and consequently the released males were dispersing very quickly, probably before mating with the wild females. In a second experiment with the same translocation, releases were made into a natural population of house flies in a pig-rearing installation. The natural population, when the releases were made, had passed its initial seasonal peak population level (Morgan, Wagoner & Fye, 1973). In contrast to the previous experiment both males and females were released and also the recessive homozygous mutants used to maintain the translocation. This procedure was followed in order to eliminate the time-consuming process of sexing and separating the translocation-bearing flies from the mutant phenotypes. The flies carrying the recessive homozygous mutants were unable to survive and reproduce in the wild. The releases were started when the natural population numbered 20,000 and, surprisingly, it remained at that level even though between 1500 and 5000 marked flies were released daily. The overall fertility in the treated population was 78.7% and in the control it was 86.5%. They concluded that as in the previous experiment the released flies rapidly dispersed from the area of release as the natural population had saturated the environment.

Release of a male-linked single translocation has been made into a natural population of *Aedes aegypti* (Rai, Grover & Suguna, 1973). The authors showed that the translocation was incorporated into the natural population with a consequent reduction of fertility. They also showed, as did Laven *et al.* (1972), that the translocation persisted in the population for several generations after the cessation of releases.

**(c) Factors militating against the use of translocations for insect control**

Generally such factors can be divided into two types: there are factors related only to the properties of the released insects, and there are factors resulting from the interaction of the released insects with the wild population. The former alone can be influenced in a positive way by the experimenter.

Strains of insects to be used in any experiments in genetic control necessarily have to be reared in the laboratory for many generations before release. Further, when translocations are to be used, each translocation must first be isolated in a single individual and subsequently multiplied. During its sojourn in the laboratory the insect can undergo significant changes in its behaviour and competitiveness in relation
to the population in the field, and these changes are generally to the detriment of the laboratory insect. To minimize these deleterious effects of laboratory colonization, geneticists, nutritionists and behaviourists must devise ways of maintaining genetic variability – for example, by varying regimes in the laboratory, by the incorporation of genetic material from the target population and perhaps even by the use of mutagenic agents (Wagoner, McDonald & Childress, 1974).

When a translocation strain is released into a field population there is an increase in the genetic load, but if the population can compensate for this load after the time of genetic death – for example, by the increased survival rate in the remainder – then the population may not be significantly reduced. This compensation of the imposed genetic load by increased survival is regulated by density-dependent factors. If the applied genetic load does not exceed the natural density-dependent regulation then the size of the population will be unaffected. Of course, where translocations are used as a transporting mechanism for gene manipulation of populations, density-dependent regulation is irrelevant.

In a field population into which translocation homozygotes have been released there would be a strong selective pressure for the evolution of assortative mating to prevent the generation of semi-sterile heterozygotes. The evolution of assortative mating for mutant traits has already been observed in laboratory populations of *Drosophila* (Thoday & Gibson, 1962; Parsons, 1965) but no such development was observed in laboratory populations containing translocation homozygotes and wild-type flies (Robinson, 1971). Further, as the segregation and hence the fertility of the translocation heterozygote is under genetic control and can therefore be modified by selection, there is a possibility that natural selection would tend to increase the fertility of the translocation heterozygote once it had been released into a natural population.

These last points serve to indicate that relatively simple interactions between natural selection and the applied genetic control technique are of crucial importance in determining the success or failure of the latter.

### V. CONCLUSIONS

Control of insect pests by the use of chromosomal translocations requires a knowledge of most of the data that are needed for conventional procedures for insect control. However, additional crucial data are necessary. Firstly, a detailed knowledge of the ecology of the target species, in all its aspects, is essential; as the principle of genetic control is to interfere with natural reproductive processes, such processes must be well understood. Secondly, it is an advantage but not a prerequisite if something of the genetics of the target species is known; it is not a coincidence that the species providing, at the moment, the most promise for genetic control are those well studied genetically.

Since the successful eradication of the screw-worm fly from the south-western United States, attention has been focused on the eradication of pests rather than on
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their management even though eradication represents an extremely unstable situation (Kojima, 1971). Certainly for agricultural pests the only requirement for control is the reduction in the numbers of the pest below a level which causes economic damage. The maintenance of a pest population at a low density can perhaps be achieved by the incorporation into that population of translocations that exert a long-lasting genetic load.

Any procedure for genetic control is species-specific, and this is an important advantage over conventional methods. However, the control procedure for each environmental situation and species has to be specifically adapted to that situation. The full potential of measures for genetic control can only be realized in the future when the talents of geneticists, entomologists and ecologists are focused on each particular species in relation to its ecological niche.

VI. SUMMARY

1. The successful eradication of the screw-worm fly by the release of sterile insects, the increased concern about the environmental effects of insecticides and the phenomenon of resistance to insecticides have created a favourable climate for the development of alternative methods for insect control. Chromosomal translocations could provide such a method.

2. The potential use of chromosomal translocations follows from the segregation of aneuploid gametes from translocation heterozygotes since this leads to a reduction in fertility. Translocation heterozygotes in crosses with normal individuals transmit the translocation to half of their progeny: therefore the reduced fertility is inherited.

3. Translocation heterozygotes can be produced by irradiation and isolated by fertility measurements or by the pseudo-linkage technique. For both of these procedures, cytological confirmation is essential. In general, the fertility of translocation heterozygotes in pest insects has been found to be around 50%, although there are exceptions.

4. Translocation homozygotes can be found among the progeny of matings between translocation heterozygotes. For ease of rearing and for maximum efficiency in the field it is an advantage to maintain the translocation as a homozygote. Translocation homozygotes should theoretically be fully fertile and viable. However, in many species of insects this has not been found. The reduced viability of translocation homozygotes has been the main drawback to the widespread development of translocations for insect control.

5. Multiple translocations involving more than two chromosomes lead to higher levels of sterility. In many species of insect, multiple translocations have now been isolated and studied.

6. Theoretical studies have illustrated the two ways in which translocations could function in insect control. Firstly, they could act as agents capable of causing the death of an appreciable number of zygotes in a natural population, i.e. they could exert a considerable genetic load. Secondly, they could function in the genetic manipulation of natural populations by acting as a transport system for the incorporation of particular genes.
7. Once translocations are released into a natural population the interaction between the applied genetic load and the natural mortality rate, influenced by density-dependent regulation, is crucial. If the applied genetic load does not exceed this natural mortality then the size of the natural population will be unaffected. However, when translocations are used as a transporting mechanism for the incorporation of particular genes the amount of natural mortality is irrelevant.

8. Field experiments have so far been limited. Experiments with small isolated populations of *Culex pipiens* and native populations of *Musca domestica* have indicated that translocations can be incorporated into field populations with a subsequent reduction in fertility of the field population. In these two situations some indications of the potential of translocations as agents for the control of insect pests have been revealed. However, there has not yet been any successful large-scale field experiment utilizing chromosomal translocations in insect control.

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VII. REFERENCES


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