STUDIES ON THE INDUCTION OF HIGH STERILITY MALE LINKED TRANSLOCATIONS IN CULEX P. FATIGANS, THEIR LEVEL OF STERILITY AND EFFECTS ON MATING COMPETITIVENESS

by

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ABSTRACT

Male Culex pipiens fatigans carrying a male linked translocation were irradiated and lines with 70-80% male linked partial sterility were derived from the progeny. These levels of sterility were maintained if selection was applied for high sterility, but where selection was relaxed the sterility levels gradually declined to 50%. Crosses were carried out to try to determine whether these declines were due to break up of translocation complexes or to changes in the genetic background causing directed segregation at meiosis. Males from two of the translocation lines were tested in field cages for their mating competitiveness in comparison with a Delhi wild type strain.

Introduction

Several methods have been proposed of using chromosome translocations for insect pest control (for review see Davidson, 1974). One of the types of translocations which have been considered are male linked translocations and these have been introduced into wild populations of Culex pipiens pipiens (Laven et al., 1972; Cousserans & Guille, 1973). Natural selection, however, resists the establishment of heterozygous male linked translocations in populations (Curtis & Hill, 1971; Cousserans & Guille, 1974; Curtis, 1975). Such translocations would behave differently if integrated with a cytoplasm bi-directionally incompatible with the target wild population (Laven & Aslamkhan, 1970). Release of males plus a small proportion of females of such an "integrated strain" should lead initially to a high level of sterility due to incompatibility and, eventually, to replacement of the residual wild population by a small population of the integrated strain. If the sterility due to the translocation was sufficient to balance or overcome the recovery potential of the population (and if the population was isolated), the replaced population would be prevented from recovering to its original density. There are indications that the recovery potential of C. p. fatigans populations is high, at least in some seasons (Weidhaas et al., 1973; Rajagopalan et al., 1973, 1975). Therefore, translocations causing a high level of sterility would probably be required for successful genetic control.

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A translocation complex, designated 10A3l, was induced by irradiation and selection at two successive generations in Delhi stocks (Krishnamurthy & Laven, 1974). In the generations immediately following its induction this translocation caused a mean sterility of 70%. Males carrying this translocation complex, and also wild type males, were irradiated and several lines with about 80% sterility were selected from the progeny of the irradiated males. A preliminary account of three of these translocation lines, with indications that the sterility level declined if they were bred without selection, was given by Krishnamurthy (1974). The present paper describes all the lines and gives more detailed information about the phenomenon of decline in sterility level. Further data are also given on the IS31B line described by Krishnamurthy & Laven (1974).

Materials and methods

Males carrying the translocation complex 10A3l and the genome and cytoplasm of the Delhi strain of C. p. fatigans were irradiated. Males collected from the wild population in Delhi area were also irradiated.

At the time of irradiation the males were aged 24 hours from eclosion. The dose was 3500 rads of gamma rays from a cobalt 60 source at a dose rate of 130 rads/second. Laboratory reared material of the Delhi strain maintained in this Unit was used for the initial outcrosses of the irradiated material. Subsequently males carrying the translocations were outcrossed to a strain with cytoplasm of Bangkok origin and genome of the Delhi strain (Krishnamurthy & Laven, 1974). Most of the studies of sterility of the translocations were made in the products of these crosses but in three cases the translocations were further transferred into a strain with Paris cytoplasm and Delhi genome (Krishnamurthy & Laven, 1974).

Sterility was measured by examining the egg rafts 48-72 hours after they were laid and counting the hatched and unhatched eggs. Unhatched eggs were further classified as embryonated or unembryonated. In the data used for analysis these two types have been pooled, because it seems likely that unembryonated eggs result from early deaths due to the action of the translocations: the sex ratios in the mating cages were approximately 1:1 and the production of unembryonated eggs due to sperm exhaustion is unlikely because of the known ability C. fatigans males to inseminate several females (Sharma et al., 1972; Krishnamurthy, 1974).

Results

After irradiation, the 10A3l and wild type males were mated to virgin females of the Delhi strain. The rafts were examined and an average of 69% dominant lethality in the wild type males was recorded. The sterility of the irradiated 10A3l males was even higher (85%) because of the additional contribution of the translocation.

Surviving larvae of both the strains were reared and the F1 males were mated to untreated Delhi strain females. The percentage sterility of each of the F2 rafts was determined. In the progeny of the irradiated 10A3l males only egg rafts with a sterility rate above 75% were retained. These were tentatively identified as those involving newly induced translocations in addition to the pre-existing translocation complex of 10A3l, as their hatch was outside the range of the 10A3l strain. Twenty out of 173 F2 rafts showed this high level of sterility. But no translocation with above 80% sterility was recovered.

Among the F2 rafts derived from the irradiated wild type males, 27 out of 134 showed partial sterility, but only one of these had a high level of sterility (70%). After a further generation of outcrossing of males, the F3 rafts were examined and cases where all the F2 had not inherited male linked partial sterility (i.e. non-male linked translocations) were discarded. Further evidence of male linkage was obtained in the F4 rafts from outcrossed F3 males. On this basis, nine translocations from the irradiated 10A3l and one from the irradiated Delhi strain were selected. However, the latter translocation subsequently proved not to be effectively male linked and was discarded. From the nine selected lines the translocations were transferred into the strain with Bangkok cytoplasm and Delhi genome (Krishnamurthy & Laven, 1974). Three of the translocations were also transferred from the Bangkok cytoplasm to the stock with Paris cytoplasm and Delhi genome and the resulting stocks are referred to as "integrated strains" and given the prefix "IS". Subsequently the lines were inbred and the sterility studied for 28-30 generations.
Until the F1 generation the translocations were maintained by selecting a few rafts
with high sterility to become the parents for the next generation. Subsequently, for each
of the translocations, one line was maintained without selection (i.e. all rafts were pooled
to provide the next generation’s parents) in addition to continuing the selected line. The
mean sterility in each generation of all the selected and unselected lines is plotted in Fig. 1
and the graph indicates stability of sterility level in the selected lines and decline in all
the unselected lines.

A more detailed presentation of the data for three of the translocations is given in
Fig. 2. This indicates the mean sterility of the rafts counted at each generation, the
standard deviation between egg rafts and the selection differential applied in the selected
lines. There was some tendency for the standard deviation to increase in the later generations
of the unselected lines and the graph also suggests a steeper decline in sterility in line 335
compared with 324.

To study the apparent variation between the lines further, the regression equations of
sterility on generation were calculated for each of the unselected lines for the first 11
generations after selection was suspended and the equations are shown in Table 1. In all
cases the regression coefficients were significantly negative, varying from 0.91 to 3.38%
per generation. The apparent heterogeneity between these slopes was tested by analysis of
variance (Sokal and Rohlf, 1969) and found to be significant.

However, as indicated in Fig. 1, eventually all the lines converged to a level of about
50% sterility after which there was no further decline in sterility.

The IS-31B strain with a male linked translocation described by Krishnamurthy & Laven (1974)
was submitted to similar monitoring of sterility and a similar decline in sterility was noted.
The data from monthly samples of 100 rafts is presented as histograms in Fig. 3. There was
a slow shift in favour of rafts with higher fertility from May 1973 to February 1974 and over
this period the mean sterility declined from 72.0% to 49.6%. Similarly a strain, designated
D3/71, was found to have declined in mean sterility level over a two year period from 86.8%
to 75.7%.

Effect of outcrossing on sterility level.

To test the hypothesis that the declines in sterility were due to changes in the genetic
background leading to an enhanced frequency of chromosomally balanced segregation at meiosis,
males of the IS-31B strain were outcrossed to females of the (Pa)De/De strain, which has no
translocation, for three successive generations and then inbred, unselected, for two genera­
tions. The mean sterility of the outcrossed line showed a slight increase over the five
generations from 58.5% to 63.5%, whereas a steady decline from 58.5% to 48.3% was simultaneously
recorded in the normal IS-31B colony. The difference between the fifth generation of the
outcrossed line and the contemporary sterility level in the normal colony was significant
(t = 9.3, d.f. = 130, p < 0.001). The result suggests that changes in the genetic background
are at least part of the explanation of the decline in sterility.

Males of the D3/71 line were outcrossed to (Pa)De/De similarly. The mean sterility of
D3/71 before outcross was 75.7% and after one outcross the sterility level rose to 81.5%.
The increase was statistically significant (t = 3.79, d.f. = 72, P < 0.01) and in this stock
also it appears that at least part of the change in sterility is attributable to changes in
the genetic background.

Chromosomes involved in the IS-31B, and 328 translocations

Experiments were carried out to determine which of the chromosomes were involved in the
translocations, using the ruby eye marker which is on chromosome 2 (Ilitis et al., 1965). Two
of the translocation lines, IS-31B and 328, were investigated. In each case males from
unselected translocation lines which had declined to a level of about 50% sterility were used.
In the case of IS-31B, females of a stock with Paris cytoplasm and homozygous for ruby eye
were used for crossing and the F1 was inbred. The results (Table 2) show very similar ratios
of ruby and black (wild type) eye in both sexes i.e. there was no linkage of the male linked
translocation to ruby eye. The ratios in each sex deviated somewhat from the Mendelian 3:1 ratio because of reduced viability of ruby eye larvae.

Males with translocations 328 were mated to ruby eye females and the male progeny back-crossed to ruby eye females and the progeny showed complete linkage of the male linked translocation to the ruby eye locus.

Thus the translocation break points were not the same in the two lines. Chromosome 2 is certainly involved in translocation 328 and is probably not involved in IS-31B. If chromosome 2 is involved in the IS-31B translocations, the breakpoints must be remote from the ruby eye locus, while in translocation 328 they must be close to it.

Male linkage of translocations

The accumulated data on the egg rafts produced by males of each of the nine translocation lines provided a stringent test of the tightness of the male linkage of the translocations in each strain because cross-overs between the M locus and the translocation would generate males without translocations and hence, in the next generation, egg rafts with full fertility. Translocation line No. 335 gave 33 fully fertile rafts from 7430 scored. This is probably attributable to a cross over between the M locus and the translocation at an earlier generation. However, the other eight lines gave no fully fertile rafts: the number of rafts examined over 26 to 28 generations in seven of the lines totalled 11,774 and in line No. 325 a total of 21,288 rafts were examined without finding a single fully fertile raft.

Competitiveness of integrated strains 325 and 335 male in field cage tests

The genome of the integrated strains originated from the indigenous Delhi population but the translocations in the integrated strains might cause them to differ from the indigenous strain in characteristics such as mating competitiveness. Therefore tests of mating competitiveness were carried out with IS-325 and IS-335 males versus Delhi males for Delhi females. Males of the integrated strain were released into the field cages with equal numbers of males and virgin females from the laboratory colony of Delhi origin. The ages of the competing males were varied as follows:

1. Males of both types aged 21-36 hours.
2. Integrated strain males aged 36-60 hours, Delhi males aged 12-36 hours.
3. Both types aged 36-60 hours.

On the second day after release into the field cage the females were offered a blood feed. The blood fed females were collected and egg rafts were obtained in the laboratory. The rafts were scored for normal hatch, incompatibility or partial hatch, 48 hours after the laying of the rafts. The results of these tests are given in Table 3.

The results indicate that males of both the lines had reduced competitiveness when they were young (12-36 hours after eclosion). However when IS-325 males were given a 24 hour age advantage over Delhi males they competed equally. When both the IS-325 and Delhi males were 36-60 hours old the IS-325 males showed only slightly reduced competitiveness. However males of IS-335 exhibited markedly lowered competitiveness, even when they had an age advantage of 24 hours over the competing Delhi males.

Discussion and conclusions

The irradiation of translocation line De 10A31 produced several lines with high sterility and this is assumed to be due to the induction of additional translocations, i.e. translocation complexes were produced.

The observations in respect of the sterility level of the lines with translocation complexes indicated that when high sterility rafts were selected to provide the parents of each generation the level sterility was more or less stable, but when the lines were reared without selection
A steady decline over a period of time was noticed (Fig. 1). All the unselected lines converged towards a level of 50% sterility between the 16th and 18th generation. A more rapid decline in sterility level of two initially highly sterile translocation in C. fatigans was observed by Bhalla et al. (1974).

The cause of the decline in sterility in the unselected lines must be that natural selection favoured the less sterile rafts over the high sterility rafts, but the nature of the genetic difference between high and the low sterility rafts is not certain. Two alternative hypothesis are as follows:

(i) Disintegration of the translocation complexes as a result of crossing over.

(ii) Selection of modifying genes which cause directed segregation of the translocation complexes.

The fact that the fertility of all the unselected lines converged on 50% might suggest that in each case the newly induced translocations were lost by crossing over, leaving only a single male linked reciprocal translocation. However, the data showing that one of the lines with 50% sterility showed close linkage of the translocations with \( ru \), while another did not, are hard to reconcile with hypothesis (i) since it is clear that all the 50% sterile lines do not carry the same male linked translocation of the 10A31 line from which they all originated.

Dennhöfer (1974, 1975) has presented evidence that, in various species including Culex pipiens, genes exist which control the segregation of single reciprocal translocations and that different alleles cause the same translocation to be associated different and very specific levels of sterility. It may be that a similar situation exists in C. fatigans and that the original translocation complexes have remained intact but, in the unselected lines, alleles have been selected which have shifted the sterility level from the original 75% to 50%.

An attempt was made to test for genes which influence the sterility levels of the IS-31B and D3/71 strains by outcrossing the translocations into a genetic background which had not previously contained a translocation. The results were not conclusive because although some increase in sterility occurred the original levels were not restored. The most satisfactory way to distinguish between hypothesis (i) and (ii) would be cytological comparison of the selected (75% sterile) and unselected (50% sterile) lines. Such comparisons have not yet been made. With the stocks used in the present experiment the distinction between the two hypotheses may not be very important because, in either case, the only way that these translocation lines could be retained with high sterility levels would be by fairly frequent selection and this would be inconvenient during mass rearing for a large field release operation. If hypothesis (i) is true there would be some possibility of obtaining new translocation complexes with more complete linkage of the components, e.g. rotational complexes in which a complex exchange is induced as one radiation induced event. Conversely, if hypothesis (ii) is correct, it might be possible to select a genetic background devoid of alleles which can cause sterility decline. It is noteworthy, in this connexion, that in the D3/71 line with genome of Paris and Freetown origin the sterility level did not decline below 75% during two years of laboratory breeding without selection.

The mating competition experiments showed that IS-335 had unconditionally reduced competitiveness. However, IS-325 males showed markedly reduced competitiveness when young but were more nearly normal when older. A similar situation was found in the IS-31B stock (Brooks et al., 1975). This might be a consequence of chromosome damage at the breakpoint of the 10A31 translocation from which both IS-31B and IS-325 were derived. Alternatively males of the laboratory Delhi strain, which were used to compete with the integrated strains, may mature unusually early. This situation may have evolved as a result of selection pressure under prolonged laboratory mass rearing for those males that can inseminate at an early age. At the time of the tests the integrated strains had either not been subjected to similar selection pressures or had been subjected to them only for a few generations.
The following main conclusions have been drawn from the observations:

It has been possible to produce fully male linked translocation complexes in C. fatigans with up to 80% sterility but the problem has not yet been solved of obtaining stocks whose sterility level remains stable without selection and which have mating behaviour fully comparable with the laboratory Delhi strain.

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REFERENCES


TABLE 1. STERILITY DECLINE IN THE MALE LINKED TRANSLOCATION LINES OVER THE FIRST ELEVEN GENERATIONS AFTER SELECTION WAS SUSPENDED

<table>
<thead>
<tr>
<th>Translocation line</th>
<th>Cytoplasm</th>
<th>Regression equation of sterility (S) on generation (g)</th>
<th>Variance about regression line</th>
<th>tests of significance of regressions</th>
<th>pair-wise comparison between slopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>304</td>
<td>Ba</td>
<td>S=83.20-2.44g</td>
<td>10.34</td>
<td>8.39***</td>
<td>-</td>
</tr>
<tr>
<td>319</td>
<td>Ba</td>
<td>S=73.49-2.69g</td>
<td>18.26</td>
<td>5.71***</td>
<td>-</td>
</tr>
<tr>
<td>324</td>
<td>Ba</td>
<td>S=78.39-0.91g</td>
<td>14.52</td>
<td>2.51*</td>
<td>-</td>
</tr>
<tr>
<td>325</td>
<td>Ba</td>
<td>S=81.82-2.15g</td>
<td>24.92</td>
<td>5.15***</td>
<td>0.15 n.s.</td>
</tr>
<tr>
<td>IS-325</td>
<td>Pa</td>
<td>S=72.93-1.80g</td>
<td>15.66</td>
<td>6.13***</td>
<td></td>
</tr>
<tr>
<td>326</td>
<td>Ba</td>
<td>S=80.79-2.57g</td>
<td>10.20</td>
<td>9.62***</td>
<td>-</td>
</tr>
<tr>
<td>327</td>
<td>Ba</td>
<td>S=82.82-3.38g</td>
<td>25.23</td>
<td>9.08***</td>
<td>-</td>
</tr>
<tr>
<td>328</td>
<td>Ba</td>
<td>S=79.44-2.33g</td>
<td>27.83</td>
<td>5.95***</td>
<td>0.24 n.s.</td>
</tr>
<tr>
<td>IS-328</td>
<td>Pa</td>
<td>S=77.47-1.65g</td>
<td>35.74</td>
<td>3.60**</td>
<td></td>
</tr>
<tr>
<td>329</td>
<td>Ba</td>
<td>S=75.93-1.54g</td>
<td>6.39</td>
<td>9.19***</td>
<td>-</td>
</tr>
<tr>
<td>335</td>
<td>Ba</td>
<td>S=82.54-3.19g</td>
<td>24.35</td>
<td>7.74***</td>
<td>-</td>
</tr>
<tr>
<td>IS-335</td>
<td>Pa</td>
<td>S=78.24-0.94g</td>
<td>12.68</td>
<td>3.98**</td>
<td>4.97***</td>
</tr>
</tbody>
</table>

Analysis of variance of slopes of regression lines:

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between slopes</td>
<td>11</td>
<td>1140.86</td>
<td>103.71</td>
<td>5.59***</td>
</tr>
<tr>
<td>Deviations from regression</td>
<td>125</td>
<td>2317.67</td>
<td>18.54</td>
<td></td>
</tr>
</tbody>
</table>

Notes:
*P < 0.05     **P < 0.01     ***P < 0.001; n.s. = not significant

Ba = Bangkok;     Pa = Paris
TABLE 2. RESULTS OF TEST CROSSES TO DETERMINE WHETHER CHROMOSOME 2 IS INVOLVED IN TWO OF THE TRANSLOCATIONS. MALES OF THE TRANSLOCATION LINES WERE CROSSED TO RUBY HOMOZYGOTES AND THE TABLE RECORDS THE PROGENY OF INBREEDING OF BACKCROSSING THE F1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Type of crossing</th>
<th>F2 or backcross progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black eye</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black eye</td>
</tr>
<tr>
<td>IS-31B</td>
<td>F1 inbred</td>
<td>289</td>
</tr>
<tr>
<td>328</td>
<td>F1 crossed to ru ♀♂</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>238</td>
</tr>
<tr>
<td></td>
<td></td>
<td>202</td>
</tr>
<tr>
<td>S. No.</td>
<td>Integrated strain tested</td>
<td>Age of integrated strain males</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>IS-325</td>
<td>12-36</td>
</tr>
<tr>
<td>2</td>
<td>IS-325</td>
<td>36-60</td>
</tr>
<tr>
<td>3</td>
<td>IS-325</td>
<td>36-60</td>
</tr>
<tr>
<td>4</td>
<td>IS-335</td>
<td>12-36</td>
</tr>
<tr>
<td>5</td>
<td>IS-335</td>
<td>36-60</td>
</tr>
</tbody>
</table>

The partially hatching rafts were either due to misclassified females inadvertently released with the integrated strain males or to double insemination.
FIG. 1. THE MEAN STERILITY OF EGG RAFTS MONITORED AT EACH GENERATION OF THE TRANSLOCATION LINES; DATA ARE GIVEN BOTH FOR THE LINES IN WHICH THERE WAS CONTINUED SELECTION FOR EGG RAFTS WITH HIGH LEVELS OF STERILITY AND ALSO FOR THE SUB-LINES IN WHICH THERE WAS NO SELECTION.
FIG. 2. DETAILED DATA ON THE STERILITY LEVELS OF EGG RAFTS IN THREE OF THE TRANSLOCATION LINES

- - - - - SELECTED LINES
----------- UNSSELECTED LINES

STANDARD DEVIATION (SELECTED LINES)
STANDARD DEVIATION (UNSSELECTED LINES)

MEAN STERILITY OF SELECTED RAFTS USED AS PARENTS FOR NEXT GENERATION

NUMBER OF GENERATIONS OF SELECTED LINES

TRANSLOCATION NUMBER
327
329
324
FIG. 3. HISTOGRAMS TO INDICATE THE DISTRIBUTION OF STERILITY IN SAMPLES OF 100 EGG RAFTS MONITORED EACH MONTH FROM THE IS-31B LINE