Methods for the Linkage-Group Determination of Insecticide-Resistance Factors in the Housefly

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RIGHT:
planes for malathion in acetone and for that in acetone plus soy bean oil were

\[ Y = -12.780 + 13.733 x_1 + 3.091 x_2 \] and

\[ Y = -13.721 + 13.733 x_1 + 3.091 x_2 \] respectively. The results of tests for heterogeneity and for parallelism have shown no significant discrepancy. The mean probit difference was calculated to compare the mortality produced by two types of deposit. The result \( d = 0.941 + 0.149 \) obtained means that the toxicity of malathion deposit for the house fly is decreased when the soy bean oil was added to acetone as solvent for impregnating the filter paper. Under the condition of this experiment deposit is a far more important factor than exposure time in determining the mortality. A doubling of deposit was here as effective as an increase in log time by \( (b_1 \log 2)/b_2 = 1.327 \) which corresponds to a multiplication of the time by 21.7.


11. イエバエにおける殺虫剤抵抗性遺伝子の連鎖群決定法

There are two major approaches for investigating the genetics of insecticide-resistance in insects: one is the toxicological examination of dosage-response data either at a single discriminating dose or at multiple scalar doses. The information to be derived from such a toxicological test in morphologically unmarked progeny of crossing experiments is rather indirect and inferential; Tsukamoto\(^{19}\) has discussed the reliability of such log dosage-probit mortality \((\log p)\) relation in a previous paper. The other is the use of visible markers in crossing experiments and the examinations for segregants of these mutants. By such a method, the data available are more precise and the investigators can get rather direct information on the genetics of insecticide-resistance.

Recently several visible mutants have been reported in various insect pests of medical or agricultural importance, such as Musca domestica (by Milani\(^{19}\), Sullivan and Hiroyoshi\(^{12}\), Hiroyoshi\(^{2}\), Tsukamoto et al.\(^{11}\)); Cchliomyia hominivorax (by LaChance and Hopkins\(^{5}\)); Culex pipiens (by Laven\(^{8}\) and Kitzmiller\(^{6}\)); Aedes aegypti (Craig and VandeHey\(^{7}\) and VandeHey and Craig\(^{16}\)); Latheticus oryzae, Tribolium castaneum, T. confusum (by Sokoloff\(^{11}\)); Blattella germanica (by Cochran and Ross\(^{19}\)); etc.; hence the genetic analysis of insecticide-resistance by means of visible mutants and statistical analysis now become possible to apply to these insect pests.

Most of the genetic analyses of insecticide-resistance by means of visible markers have been limited to Drosophile because of the extensive background of the formal genetics of species in this Genus. For determining the linkage groups of the genetic factor or factors investigated, certain statistical methods such as factorial analysis and subsequent analysis of variance have been employed by various geneticists (Crow\(^{3}\); King and Sömmne\(^{6}\); Oshima and Hiroyoshi\(^{12}\); and Tsukamoto et al.\(^{11}\)), but without any description of the actual procedure of factorial analysis which is less familiar to insect toxicologists.

The purpose of the present paper is, therefore, to describe the practical procedures which have been employed by the present author and his co-workers in genetic analysis of the housefly Musca domestica L. Although actual results...
obtained with these analytical methods will be reported in detail in separate papers, preliminary data on BHC-resistance have been used as an example for the explanation of calculations. This is the second paper of the series of genetic studies on insecticide-resistance carried out in the laboratory at Osaka University.

Crossing Procedure

Unlike in *Drosophila*, no sex-linked visible mutant has yet been found out in the housefly while numbers of visible mutants have been located on all five autosomes. This evidence suggests that the X chromosome of the housefly is genetically almost or completely inert, and hence no sex-linked resistance factor may be expected. Furthermore, since preliminary experiments indicated a negligible maternal effect on the resistance level, no reciprocal crosses are attempted in subsequent experiments. The crossing procedures described here, therefore, are designed to determine the linkage group of autosomal resistance factors.

For determining the quantitative influence of particular chromosomes on the level of insecticide resistance, it is necessary to use several multichromosomal mutant strains in which each autosome is marked with a visible mutant. Multichromosomally marked resistant strains are synthesized by crossing the unmarked resistant strain with susceptible mutant strains, and from the hybrid by making repeated backcrosses accompanied by selection both for visible mutant markers and for the resistance. In these special multichromosomal mutant strains, the mutant symbols are arranged in order of the linkage group and each linkage group is separated by the semicolons in parentheses as in *Drosophila*. For example, the notation R(a;b;c;d;e) stands for an insecticide-resistant strain in which the 2nd, 3rd, 4th, 5th and 6th chromosomes are marked with visible markers, a, b, c, d and e, respectively.

A schematic representation of typical crossing procedures to detect both dominant and recessive resistance factor(s) is given in the next page. Figure 1 also illustrates the chromosomal constitutions in the F1 male backcross systems to detect both dominant and recessive resistance factor(s).

I. Analysis of "dominant effect" of resistance factor

Males of the F1 hybrid offspring of the S×R cross are backcrossed to females of the susceptible marker strain used. Adult flies of the resultant backcross progeny are then tested for their resistance levels by topical application of the insecticide or other appropriate methods. This procedure should detect any "dominant effect" of resistance factor(s) in the heterozygotes of the backcross offspring, viz. a comparison can

\[
\begin{array}{c}
\text{Resistant} \\
X; R; R; R; R; R \varphi \\
X; R ; R; R; R; R \varphi \\
\text{Susceptible} \\
X; S; S; S; S; S \varphi \\
X; S; S; S; S; S \varphi \\
\end{array}
\]

without the backcross strains, viz. a comparison can

\[
\begin{array}{c}
\text{Resistant} \\
S; S; S; S; R \varphi \\
S; S; S; S; R \varphi \\
\text{Susceptible} \\
R; R; R; R; S \\
R; R; R; R; S \\
\end{array}
\]

Figure 1. Schematic illustration of examples for backcrossing systems to detect both dominant and recessive resistant factors. R and S symbolize chromosomes derived from resistant and susceptible strains, respectively.

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be made between the resistant heterozygote
$R/+ \,$ and the susceptible genotype $+/+$ among
the siblings of either sex.

When the number of different marked chromo­
somes is $n$, segregation of $2^n$ different kinds of
phenotypes is expected in the back­
cross progeny with a theoretical ratio of $1:1:1:1:
1:......:1$. For example, if all 5 autosomes are
marked with mutant characters, the following
32 kinds of combinations of different phenotypes
may be expected in the backcross offspring:

1) $+;+;+;+;+$ 2) $a;+;+;+;+$
3) $++;b;++;+$ 4) $a;b;++;+$
5) $+;++;c;++$ 6) $a;+;c;++$
7) $++;b;c;+;+$ 8) $a;b;c;++$
9) $+;++;d++;$ 10) $a;+;d++;$
11) $++;b;d++;$ 12) $a;b;d++;$
13) $+;++;c;d++;$ 14) $a;++;c;d+$
15) $++;b;c;d++;$ 16) $a;b;c;d++$
17) $+;++;e++;+$ 18) $a;++;++;e$
19) $+;++;e++;$ 20) $a;b;++;e$
21) $++;b;c++;+$ 22) $a++;e;c++$
23) $+;++;c;e++;$ 24) $a;b;e;c++$
25) $+;++;d++;$ 26) $a;++;d;+$
27) $++;b;+;d;+$ 28) $a;b;+;d;+$
29) $+;++;c;e++;$ 30) $a;++;c;e++$
31) $++;b;c;d++;$ 32) $a;b;c;d++;$

Among these phenotypes, the symbol $+$ indicates
heterozygosity for the marker gene concerned
(e.g. $+/b$) as well as heterozygosity with regard
to $R$ chromosome ($+/R$). Therefore, when a
resistance factor is located on a particular
chromosome, for example in the same linkage
group as the marker $b$, the particular flies marked
with the homozygous recessive mutant $b/b$ should
be susceptible to the insecticide concerned.

Thus the linkage group to which the dominant
resistance factor belongs may be directly detected
by examining the visible marker characteristics
of the survivors and the victims of a discriminat­
ing dose of the insecticide.

Although the case where $n=3$ or 4 is the most
convenient for practical examinations of visible
phenotypes of flies, all the possible combinations
with five markers have been shown above for the
schematic explanation. The arrangement of
phenotypes listed above corresponds to that used
in the factorial analysis which will be described
later.

Other types of crossing system are also possible
in cases where a multichromosomally marked
resistant strain is available (Crosses 2 and 2').

In these crosses, however, it is the phenotypes
for the mutant in the particular linkage group
concerned (e.g. $b/b$) that would be resistant ($RI+
$) and the phenotypes for its wild-type allele ($b/+$)
that would be susceptible ($+/+$) to the insecticide.

2. Analysis of “recessive effect” of resistance
factor

When the resistance factor is recessive or
incompletely dominant, a backcross of the $F_1$
males to a resistant marker strain should detect
any “recessive effect” of the resistance factor(s),
since it produces offspring containing resistant
homozygotes for comparison with the hetero­
zogotes. A schematic representation of such a
backcross system is shown as Crosses 3 and 3'.

The following combinations in interstrain
crosses are also possible, if necessary (Crosses 4
and 4').

In these crossing systems, the mutant pheno­
types in the progeny of crosses 3 and 3' would
be homozygous both for the resistance factor
and for the mutant marker with which it is linked,
while the wild-type phenotypes are heterozygous
both for the resistance factor and for the marker
for the chromosome in which it is located. Thus
if a recessive resistance factor $r$ is linked with
marker gene $a$, then $a/a$ genotypes in the
Table 1. Schematic arrangement of arc-sine transformed survival rates for statistical analysis.

<table>
<thead>
<tr>
<th>Phenotype (i=1, 2, ..., k)</th>
<th>Dose or Replication (j=1, 2, 3, ..., I)</th>
<th>Sum</th>
<th>Mean ( \theta )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>( \theta_{11} )</td>
<td>( \theta_{12} )</td>
<td>( \theta_{13} )</td>
</tr>
<tr>
<td>2</td>
<td>( \theta_{21} )</td>
<td>( \theta_{22} )</td>
<td>( \theta_{23} )</td>
</tr>
<tr>
<td>3</td>
<td>( \theta_{31} )</td>
<td>( \theta_{32} )</td>
<td>( \theta_{33} )</td>
</tr>
<tr>
<td></td>
<td>...</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Sum</td>
<td>( \theta_{1} )</td>
<td>( \theta_{2} )</td>
<td>( \theta_{3} )</td>
</tr>
</tbody>
</table>

Effect of the A chromosome

\( \bar{(A-a)(B+b)(C+c)(D+d)(E+e)} \)

\( = \ ABCDE - ABCDE + AbCDE - abCDE \)

\( + AbcDE - abCDE + AbCDE - abcDE \)

\( + ABCDE - abCDE + AbcDE - abcDE \)

\( + ABCDE - abCDE + AbCDE - abcDE \)

\( + ABCDE - abCDE + AbcDE - abcDE \)

where each capital letter corresponds to each R chromosome in heterozygous (Crosses 1, 1', 2, and 2') or homozygous (Crosses 3, 3', 4, and 4') condition, and each small letter corresponds to the S chromosome in homozygous (Crosses 1, 1', 2, and 2') or heterozygous (Crosses 3, 3', 4, and 4') condition. The relation between these symbols for the mean survival rate (\( \bar{\theta} \)) and visible phenotypes is summarized in table 2.

In a similar way, interactions between two or more resistance factors belonging to different linkage groups can also be calculated. For example, the interaction between resistance factors on the linkage groups B and C is as follows:

Interaction between the B and C chromosomes

\( = (A+a)(B+b)(C-c)(D+d)(E+e) \)

\( = \ ABCDE + AbCDE - AbCDE - abCDE \)

\( - AbcDE - abCDE + AbcDE + abcDE \)

\( + ABCDE + abCDE - AbCDE - abcDE \)

\( - AbcDE - abCDE + AbcDE + abcDE \)

\( + ABcDE - AbCDE - abCDE - abcDE \)

\( - AbCDE - abCDE + AbcDE + abcDE \)

\( + ABCDE + AbCDE - AbCDE - abcDE \)

\( - AbcDE - abCDE + AbcDE + abcDE \)

\( - AbCDE - abCDE + AbcDE + abcDE \)
Table 2. Schematic comparison between the symbols for the mean survival rate and phenotypes of the progeny.

| Symbol   | Mean survival rate | Phenotypes in backcross progeny
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCDE</td>
<td>$\bar{a}$</td>
<td>$++;++;++;++$</td>
</tr>
<tr>
<td>aBCDE</td>
<td>$\bar{b}$</td>
<td>$a;++;++;++$</td>
</tr>
<tr>
<td>AbCDE</td>
<td>$\bar{c}$</td>
<td>$a;++;++;++$</td>
</tr>
<tr>
<td>abCDE</td>
<td>$\bar{d}$</td>
<td>$a;++;++;++$</td>
</tr>
<tr>
<td>abcdE</td>
<td>$\bar{e}$</td>
<td>$a;++;++;++$</td>
</tr>
<tr>
<td>ABCDe</td>
<td>$\bar{f}$</td>
<td>$a;b;c;d;e$</td>
</tr>
<tr>
<td>abcde</td>
<td>$\bar{g}$</td>
<td>$a;b;c;d;e$</td>
</tr>
</tbody>
</table>

Table 3. Schematic representation of analysis of variance.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>$SS_T = \Sigma 0_i^2 - \frac{\bar{0}_i^2}{kI}$</td>
<td>$kI-1$</td>
<td>$\frac{SS_T}{kI-1}$</td>
<td></td>
</tr>
<tr>
<td>Phenotype</td>
<td>$SS_P = \frac{\Sigma 0_i^2}{I} - \frac{\bar{0}_i^2}{kI}$</td>
<td>$k-1$</td>
<td>$\frac{SS_P}{k-1}$</td>
<td>$\frac{MS_P}{MS_E}$</td>
</tr>
<tr>
<td>Dose or Replication</td>
<td>$SS_D = \frac{\Sigma 0_i^2}{k} - \frac{\bar{0}_i^2}{kI}$</td>
<td>$I-1$</td>
<td>$\frac{SS_D}{I-1}$</td>
<td>$\frac{MS_D}{MS_E}$</td>
</tr>
<tr>
<td>Error</td>
<td>$SS_E = SS_T - SS_P - SS_D$</td>
<td>$(k-1)(I-1)$</td>
<td>$\frac{SS_E}{(k-1)(I-1)}$</td>
<td></td>
</tr>
</tbody>
</table>

Thus the effect of each R chromosome and the interaction between R chromosomes are actual quantitative function calculated in arc-sine units. Simultaneous calculations of these effects or interactions are performed practice with the convenient plus-minus method described by Yates. Test for significance of these effects are based on analysis of variance, and when the effect of a particular R chromosome is statistically significant (at 5\% level) or highly significant (at 1\% level), it may be inferred that a resistance factor is associated with this chromosome.

The analysis of variance is performed by dividing the mean square for the phenotypes ($MS_P$) by the mean square for error ($MS_E$) to get the variance ratio ($F$-value) as shown schematically in table 3. When the calculated $F$ for the phenotypes is greater than that expected at the 5\% level, the sum of squares for the phenotypes ($SS_P$) are further subdivided into $kI$ kinds of sum of squares for each chromosomal effect or interaction, where $k (=2^n)$ is the number of phenotypes as already shown in table 1. The degree of freedom for each sum of squares is 1, and hence the sum of squares for each effect equals that of the mean square in this instance. The mean square for the effect of each R chromosome (or for interaction between combinations of R chromosomes) can be calculated from the value for effect already obtained in arc-sine units, as follows:

$$\text{Sum of squares} = \frac{SS_E}{(k-1)(I-1)}$$

The variance ratio $F$ for each effect or interaction can be obtained by dividing each mean square by that for the error ($MS_E$) in the usual manner. When the resistance to an insecticide is completely due to a single factor, only one chromosomal effect will be statistically significant in high degree. When two or more resistance factors act additively, two or more chromosomal effects will show significance. In cases where some factor acts synergistically with the major factor, an interaction between these chromosomal
Table 4. An example of genetic analysis for recessive BHC-resistance factors in the following backcross: HR(bwb;ocra;ar;ac)'f × F₁(HR(bwb;ocra;ar;ac)'f × Labci;dci;ci"ci"

<table>
<thead>
<tr>
<th>Phenotype (2; 3; 5; 6)</th>
<th>10-30 μg BHC/fly</th>
<th>50 μg BHC/fly</th>
<th>100-300 μg BHC/fly</th>
<th>Pooled</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Survival rate %</td>
<td>No. Survival rate %</td>
<td>No. Survival rate %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bwb; ocra; ar; ac</td>
<td>29 51.72 45.98</td>
<td>15 46.67 43.09</td>
<td>26 38.46 38.32</td>
<td>127.39</td>
<td>42.46</td>
</tr>
<tr>
<td>+; ocra; ar; ac</td>
<td>30 10.00 18.44</td>
<td>35 11.43 19.76</td>
<td>18 5.56 13.63</td>
<td>51.83</td>
<td>17.28</td>
</tr>
<tr>
<td>bwb; +; ar; ac</td>
<td>33 39.70 39.04</td>
<td>15 33.33 35.26</td>
<td>20 35.00 36.27</td>
<td>110.57</td>
<td>36.86</td>
</tr>
<tr>
<td>+; +; ar; ac</td>
<td>34 2.94 5.74</td>
<td>34 8.82 17.28</td>
<td>19 5.26 13.26</td>
<td>36.28</td>
<td>12.09</td>
</tr>
<tr>
<td>bwb; ocra; +; ac</td>
<td>54 38.89 38.58</td>
<td>35 34.29 35.84</td>
<td>28 23.87 32.31</td>
<td>106.73</td>
<td>35.58</td>
</tr>
<tr>
<td>+; ocra; +; ac</td>
<td>44 6.82 15.14</td>
<td>31 3.19 10.27</td>
<td>18 0.00 0.00</td>
<td>25.41</td>
<td>8.47</td>
</tr>
<tr>
<td>bwb; +; +; ac</td>
<td>51 21.57 27.67</td>
<td>48 27.08 31.36</td>
<td>25 24.00 29.33</td>
<td>88.36</td>
<td>29.45</td>
</tr>
<tr>
<td>+; +; +; ac</td>
<td>52 5.77 13.90</td>
<td>44 6.82 15.14</td>
<td>23 8.70 17.16</td>
<td>46.20</td>
<td>15.40</td>
</tr>
<tr>
<td>bwb; ocra; ar; +</td>
<td>26 38.46 38.29</td>
<td>18 44.44 41.80</td>
<td>23 56.52 48.74</td>
<td>128.83</td>
<td>42.94</td>
</tr>
<tr>
<td>+; ocra; ar; +</td>
<td>26 26.92 31.25</td>
<td>34 14.71 22.56</td>
<td>15 15.38 23.09</td>
<td>76.90</td>
<td>25.63</td>
</tr>
<tr>
<td>bwb; +; ar; +</td>
<td>28 28.57 32.31</td>
<td>31 25.81 30.54</td>
<td>18 27.78 31.81</td>
<td>94.66</td>
<td>31.56</td>
</tr>
<tr>
<td>+; +; ar; +</td>
<td>46 65.22 53.86</td>
<td>44 6.82 15.14</td>
<td>11 0.00 0.00</td>
<td>69.00</td>
<td>23.00</td>
</tr>
<tr>
<td>bwb; ocra; +; +</td>
<td>43 51.16 45.67</td>
<td>52 44.23 41.69</td>
<td>12 33.33 35.26</td>
<td>122.62</td>
<td>40.87</td>
</tr>
<tr>
<td>+; ocra; +; +</td>
<td>28 35.71 36.70</td>
<td>36 0.00 0.00</td>
<td>22 9.09 17.55</td>
<td>54.25</td>
<td>18.08</td>
</tr>
<tr>
<td>bwb; +; +; +</td>
<td>51 19.61 26.29</td>
<td>62 16.11 23.67</td>
<td>10 20.00 26.56</td>
<td>76.52</td>
<td>25.51</td>
</tr>
<tr>
<td>+; +; +; +</td>
<td>49 2.04 8.21</td>
<td>67 1.64 7.35</td>
<td>15 0.00 0.00</td>
<td>15.56</td>
<td>5.19</td>
</tr>
<tr>
<td>Total</td>
<td>624 477.07 601</td>
<td>390.75 301</td>
<td>363.29 1231.11</td>
<td>410.37</td>
<td></td>
</tr>
</tbody>
</table>

Factors will be significant.

(Example)

For a convenience of explanation, an actual example of an analysis for the recessive effect of BHC-resistance in a Japanese resistant strain is employed below. Table 4 gives the 24-hours mortality data after topical application with various doses of gamma-BHC in the following backcross progeny (this backcross corresponds to Cross 3 in the previous section):

HR(bwb;ocra;ar;ac)'f × F₁(HR(bwb;ocra;ar;ac)'f × Labci;dci;ci"

Where the resistant strain used is multichromosomally marked with the visible mutants, bwb (brown-body color, the 2nd chromosome), ocra (ocra eyes, the 3rd chromosome), ar (aristapedia, the 5th chromosome), and ac (ali curve, the 6th chromosome), respectively. Therefore, the analysis is effective to recessive resistance factors on all the autosomes but the 4th chromosome. From this crossing system, segregation of 16 kinds of phenotypes is expected (k=2⁴=16), and the table contains data from three dose ranges (l=3).

The recessive effects of R chromosomes or chromosomal interactions have been calculated from the mean survival rate shown in the last column of table 4. The Yates’ calculating procedure is given in table 5.

The analysis of variance has been done by calculating sum of squares as follows:

\[ \text{Total:} \]  
\[ \text{SS}_T=45.98^2+18.44^2+39.01^2+\ldots+26.56^2 \]
\[ =1231.11^2=9374.48 \]

Phenotypes:
\[ \text{SS}_P=127.39^2+51.83^2+\ldots+15.56^2 \]
\[ =1231.11^2=6672.29 \]

Doses:
\[ \text{SS}_D=477.07^2+390.75^2+363.29^2-1231.11^2 \]
\[ =440.65 \]

Error:
\[ \text{SS}_E=9374.48-6672.29-440.65=261.54 \]

Then the mean square for each effect or interaction has been calculated. For example, the mean square for the 2nd chromosomal effect is
\[ \frac{3\times4805.40}{16}=480.540. \] (See table 5)
Table 5. Calculation of chromosomal effects and interactions on the resistance.

<table>
<thead>
<tr>
<th>Mean(O)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4 (Effect)</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.46</td>
<td>+59.74</td>
<td>103.69</td>
<td>197.59</td>
<td>410.37</td>
<td>(Total)</td>
</tr>
<tr>
<td>17.28</td>
<td>48.95</td>
<td>88.90</td>
<td>212.78</td>
<td>160.09</td>
<td>2</td>
</tr>
<tr>
<td>36.86</td>
<td>44.05</td>
<td>123.13</td>
<td>91.11</td>
<td>52.25</td>
<td>3</td>
</tr>
<tr>
<td>12.09</td>
<td>44.85</td>
<td>89.65</td>
<td>68.98</td>
<td>24.69</td>
<td>2x3</td>
</tr>
<tr>
<td>35.58</td>
<td>68.57</td>
<td>19.95</td>
<td>9.99</td>
<td>53.27</td>
<td>5</td>
</tr>
<tr>
<td>8.47</td>
<td>54.56</td>
<td>41.16</td>
<td>42.26</td>
<td>-8.45</td>
<td>2x5</td>
</tr>
<tr>
<td>29.45</td>
<td>58.95</td>
<td>25.87</td>
<td>13.47</td>
<td>-2.65</td>
<td>3x5</td>
</tr>
<tr>
<td>15.40</td>
<td>30.70</td>
<td>43.11</td>
<td>11.22</td>
<td>-6.37</td>
<td>2x3x5</td>
</tr>
</tbody>
</table>

Table 6. Analysis of variance for the data given in tables 4 and 5.

<table>
<thead>
<tr>
<th>S.V.</th>
<th>S.S.</th>
<th>D.F.</th>
<th>M.S.</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>SS_T=9374.48</td>
<td>47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenotypes</td>
<td>SS_P=6672.29</td>
<td>15</td>
<td>444.82</td>
<td>5.90**</td>
</tr>
<tr>
<td>2</td>
<td>4805.40</td>
<td>1</td>
<td>4805.40</td>
<td>63.75**</td>
</tr>
<tr>
<td>3</td>
<td>511.89</td>
<td>1</td>
<td>511.89</td>
<td>6.79*</td>
</tr>
<tr>
<td>2x3</td>
<td>114.30</td>
<td>1</td>
<td>114.30</td>
<td>1.52</td>
</tr>
<tr>
<td>5</td>
<td>532.07</td>
<td>1</td>
<td>532.07</td>
<td>7.06*</td>
</tr>
<tr>
<td>2x5</td>
<td>13.39</td>
<td>1</td>
<td>13.39</td>
<td>0.18</td>
</tr>
<tr>
<td>3x5</td>
<td>1.32</td>
<td>1</td>
<td>1.32</td>
<td>0.02</td>
</tr>
<tr>
<td>2x3x5</td>
<td>7.61</td>
<td>1</td>
<td>7.61</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>43.26</td>
<td>1</td>
<td>43.26</td>
<td>0.57</td>
</tr>
<tr>
<td>2x6</td>
<td>91.83</td>
<td>1</td>
<td>91.83</td>
<td>1.22</td>
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<tr>
<td>3x6</td>
<td>195.25</td>
<td>1</td>
<td>195.25</td>
<td>2.59</td>
</tr>
<tr>
<td>2x3x6</td>
<td>0.95</td>
<td>1</td>
<td>0.95</td>
<td>0.01</td>
</tr>
<tr>
<td>5x6</td>
<td>35.14</td>
<td>1</td>
<td>35.14</td>
<td>0.47</td>
</tr>
<tr>
<td>2x5x6</td>
<td>127.04</td>
<td>1</td>
<td>127.04</td>
<td>1.69</td>
</tr>
<tr>
<td>3x5x6</td>
<td>120.78</td>
<td>1</td>
<td>120.78</td>
<td>1.60</td>
</tr>
<tr>
<td>2x3x5x6</td>
<td>67.19</td>
<td>1</td>
<td>67.19</td>
<td>0.89</td>
</tr>
<tr>
<td>Doses</td>
<td>SS_P=440.65</td>
<td>2</td>
<td>220.32</td>
<td>2.92</td>
</tr>
<tr>
<td>Error</td>
<td>SS_E=2261.54</td>
<td>30</td>
<td>75.38</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at 5% level
** Significant at 1% level

The analysis of variance summarized in table 6 indicates that the 2nd chromosomal effect on the resistance is the most significant one at least among the chromosomes analyzed although the 3rd and the 5th chromosomal effects are also significant at 5% level. In other words, BHC-resistance is multifactorial and at least three recessive factors are responsible for the resistance in this
highly resistant strain. However, it is not a principal purpose to report or to discuss on the mode of inheritance of BHC-resistance in this section, but these unpublished data have been employed merely as an example for explaining the calculating procedures in the factorial analysis. Detailed results of the genetic analysis of BHC-resistance in the housefly will be described in a more complete form elsewhere.

Consideration

In the present paper, the symbols $R$ and $r$ have generally been used for dominant and recessive resistance factors respectively, and the symbol $+$ for either recessive or dominant susceptible alleles. Some investigators used heedlessly the symbol $r$ for the susceptible allele of the dominant resistant factor $R$. Such a symbolization is, however, unsuitable to express a recessive resistant factor. In addition to these symbols, $R$ and $S$ are also used as general terms for resistant and susceptible strains or for the chromosome derived from the resistant strain ($R$ chromosome) and from the susceptible strain ($S$ chromosome), regardless of their dominance.

Since no crossing-over is observed in males of the housefly like Drosophila, determination of the linkage group of resistance factor(s) is based on backcrossing the $F_1$ male, so that each chromosome behaves as a unit in the crossing systems described above. Therefore, these factorial analyses are highly effective not only to determine the linkage group for the resistance factor qualitatively, but also to compare the relative effectiveness of each $R$ chromosome or chromosomal interaction quantitatively. In some insects such as mosquitoes, however, the $F_1$ male backcross is not fully effective to detect the linkage group because the crossing-over occurs in both sexes of these insects. Thus genes located on different arms of a chromosome sometimes segregate independently of each other.

Together with the careful examination of dosage-mortality line described in the previous paper\(^{10}\), these genetical and statistical analyses described in the present paper may bring more reliable informations on the genetics of insecticide-resistance.

Methods for the determination of locus of the resistance factor on the chromosome will be described in a separate paper.

Acknowledgements: The author wishes to express his thanks to Professor H. Kikkawa for his direction; to Professor T. Okamoto for his valuable suggestions on the statistical methods; to Dr. T. Hiroyoshi and to Miss R. Suzuki for their critical discussions and useful suggestions. Thanks are also due to Professor A. W. A. Brown, University of Western Ontario, Canada, for his kindly reading of the original manuscript.

Summary

Methods and procedures of crossing experiments for determining the linkage group of both dominant and recessive effects of insecticide-resistance factor or factors by using visible multichromosomal mutant marker strains have schematically been described. Statistical treatment of the results obtained is then performed by the factorial analysis, to reveal those chromosomes which had a significant influence on the resistance level.

References Cited


(with English summary, 60.)

12. DDTで淘汰された高機系イエバにみられる交叉抵抗性

昆虫の殺虫剤抵抗性に関する研究（第7報** 波本 忌（イバラ農芸研究所） 39. 7. 31 受理）

DDTで淘汰された高機系イエバの交叉抵抗性は欧米産イエバのそれと違わなかった。

材料および実験方法

いずれの個体群も抵抗性の発達が平衡状態にたってから7・8代目のものを使用した。抵抗性の発達が平衡にたってから更に10数代にわたって淘汰が試みられたが、抵抗性はそれ以上に淘汰しなかった。

使用した殺虫剤の大部分は technical grade のものを再結（点まで）精製し、若干のものは精製したものである。すなわち、DDT (108-108.5), DDD 2,2-bis (p-chlorophenyl) 1,1 dichloroethanesb (109-109.5), DDT 2,2-bis (p-tolyphenyl) 1,1,1 tri-chloroethanesb (86-87), DDT 2,2-bis (p-fluoro- pheny1) 1,1,1 trichloroethanesb (44.5-45.0), DDDT 2,2-bis (p-bromophenyl) 1,1,1 trichloroethanesb (141-142), Methoxychlor (88-89), DMC di (p-chlorophenyl) methylcarbinob (68-69), Lindane (112.5-113.5), Aldrin (104-105), Dieldrin (176-177), Isodrin (240-242), Methylparathion (36-36.5), Malathion (156/7mm), Dimethoate (51-52), Dipterex (79-81), DDVP (120/14mm), EPN (37), AC 5727 m-isopropyl-phenyl N-methylcarbamate (73-74), Sevin (141-142), a-dl-trans-allethrin (50-50.5) である。カルコ内は各殺虫剤の箇点（または箇点）のCをもとめ、各殺虫剤の側につけた数字はそれぞれの箇点の相対的な係数によって合成したものを示す。なお、各殺虫剤のLD95 はさきに報告した方法によってもめた。

実験結果および考察

第1表に示すように塔素系殺虫剤とにくに近縁の化合物にいちじるしい交叉抵抗性を示す。DBrDTにたいしては DDT よりやわい感受性をしめすが、DFDT, DTDT および Methoxychlor にたいしては DDT よりも、つよい感受性をしめす。DDT 抵抗性の高機系イエバも抵抗性の原因もともに DDT 塔塩塩醇醇のたき活性にもとづくと考えられるが、DFDT, DTDT および Methoxychlor が DDT よりもつよい殺虫力をしめすのは、これらの殺虫剤は DDT よりも DDT-塩醇塩醇醇の働きをうけにくいためとおもわれる。有機磷系殺虫剤およびピチリン系殺虫剤に対する感受性の程度はことなる。なお、カーペイミート系殺虫剤 AC 5727 に対しても感受性の程度はあまりわからない。

欧米に分布しているイエバ Musca domestica domestica でもすでに DDT 抵抗性系は塡素系殺虫剤とにくに近縁の化合物につよい交叉抵抗性をしめすが、DFDT, DTDT, Methoxychlor などは DDT よりもつよい殺虫力をしめすことから、有機磷系殺虫剤およびピチリン系殺虫剤に対しては交叉抵抗性をしめさないことから，Sevin につよい交叉抵抗性をし