(Postlethwait et al., 1980; Belote et al., 1985), and hemolymph and whole body ecdysteroid titers do not differ significantly between D. melanogaster males and females (Handler, 1982; Bownes et al., 1984).

Figure 6. Ecdysteroid radioimmunoassay of whole animal homogenates extracted in 70% methanol. Values represent the mean of two separate assays (each using replicate samples) performed on the same extraction samples. Ecdysteroids are 20-HE equivalents.

As reported above, 20-HE did not stimulate YP synthesis in the Caribfly under our experimental conditions, though it remains of interest whether sex-specific levels of ecdysteroids exist in adult flies. We performed two RIA analyses on a single series of adult male and female whole animal homogenates extracted in 70% methanol. Significant titers were detected in both males and females, with relatively low levels until 5 days after eclosion in both sexes (Fig. 6). At day 6, titers in the females increased to a level of approximately 54 pg/mg fresh weight, compared to 7 pg/mg fresh weight in males. Titers remained at this level for at least 2 succeeding days. The increase in female titer correlates with the time of increasing YP synthesis and the initial appearance of vitellogenic oocytes. While a relationship between YP synthesis and ecdysteroid titer might be deduced, it should be noted that YP synthesis and secretion occurred in 3-day-old ovaries cultured in vitro (Fig. 4), at a time when 20-HE titers were low and comparable to titers in males.

Conclusions

In summary, the YP of Anastrepha suspensa is limited to a single major 48 kDa polypeptide, and possibly another minor polypeptide with a slightly lower molecular weight. Synthesis of the polypeptides is almost exclusively limited to the ovaries, and is first detected at an early stage of yolk deposition. YP derived from the fat body is
minimal, and further immunological studies are necessary to determine if the fat body and hemolymph 48 kDa polypeptide is indeed female-specific and/or deposited into the oocyte. Stimulation of YP synthesis by either 20-HE or a JH analog was not observed in our studies, although females late in vitellogenesis have ecdysteroid titers 6- to 7-fold higher than sibling males. Elucidation of the hormonal regulation of Anastrepha vitellogenesis awaits a more comprehensive analysis.

The prodigious ovarian synthesis of YP has allowed us to isolate mRNA sufficient to produce cDNA libraries from which YP cDNA clones may be isolated (Handler, unpublished). In addition, antigenic homology between Drosophila and Anastrepha YP suggests that Anastrepha YP genomic DNA may be isolated by hybridization to D. melanogaster YP DNA. With cloned YP DNA in hand, we may then be able to define the YP promoter regulatory region by gene-fusion and transformation into Drosophila techniques. Of particular interest will be the ability of the YP promoter region to confer sex-specific expression upon a selectable gene-product such as alcohol dehydrogenase, which has potential use in a genetic-sexing program. In addition, YP gene expression itself may be used as a marker to identify and characterize sex-determining genes in A. suspensa as has been done in Drosophila (Belote et al., 1985). The eventual isolation and manipulation of sex-determining genes will add a new dimension to our ability to genetically sex tephritid populations, and perhaps control their population size.

Acknowledgment

We thank Pat Whitmer and Karen Ogren for technical assistance. This work was supported by grant 86-CRCR-1-2012 from the USDA.

References


Abstract

The distribution of genetic variability in 21 field collected samples of 4 populations of Ceratitis capitata were evaluated examining more than twenty gene-enzyme systems. The samples were obtained from geographically and ecologically distinct localities (Procida Isl., Sardinia, Libya, Kenya). The medfly population of Procida has been repeatedly sampled in the period April 1983 - August 1985. Seasonal fluctuations in the relative frequency of $\text{Hpi}$ and $\text{Hk}_2$ alleles has been observed; in addition, a trend toward increasing genetic variability apparently occurred in this population. Over this three year period, the mean relative frequency of heterozygous Procida flies ($\text{H}$) has been 0.046, a very low value in comparison with those provided by samples from Kenya ($\text{H} = 0.164$) and Sardinia ($\text{H} = 0.115$). So far, the lowest value of $\text{H}$ has been ensured by a sample from a very isolated locality of Libya ($\text{H} = 0.021$), possibly as the result of a "founder effect". The fairly high value of $\text{H}$ provided by Sardinian samples is rather unexpected because it occurs in a peripheral population.

Electrophoretic analyses of a sample of Ceratitis (= Pterandrus) rosa from Kenya, collected together with a sample of C. capitata have revealed a number of diagnostic loci. Special attention was paid to medfly Adh loci because of their relevance for practical and/or theoretical purposes. The two Adh loci codify isoenzymes which show different and integrated developmental pattern and tissue localization. Both isozymes showed low variability levels; generally $\text{Adh}^\text{B}$ and $\text{Adh}^\text{A}$ were the only alleles present or the most common ones; however, some rare $\text{Adh}^\text{a}$ alleles were recently detected in a South-African laboratory strain.

Preliminary observations suggest some degree of variability for larval serum proteins (LSP) in the above mentioned South-African laboratory strain.

Introduction

Allelic variations of proteins - particularly enzyme electrophoretic variants - have provided a much needed tool for studying the basic genetics of the medfly C. capitata and for measuring the variability of its populations. Scattered informations in literature about the genetic make-up of medfly populations (Huettel et al., 1980; Kourt et al., 1985; Milani et al., 1985; Gasperi et al., 1986) have pointed out low variability levels in samples from several mediterranean localities and other peripheral regions in the species' range.
The development in the medfly population genetics, aiming to assess the dynamics of genetic pools in relation to space, time and host species should take into account the possible interspecific competition between C. capitata and other sympatric fruit flies species. On the other hand the assessment of the functional role of the different forms of activity which a single enzyme may display becomes necessary for evaluating the degree of polymorphisms.

The present report gives informations on the work in progress concerning:
- evaluation of variability levels in natural population samples from Kenya, Libya, Sardinia and Procida;
- evidence for biochemical loci which are diagnostic for C. capitata and C. (= Pterandrus) rosa sympatric in Kenya;
- studies on the functional properties of alcohol dehydrogenase (ADH) isoenzymes;
- genetic analysis of larval serum proteins (LSP).

Genetic variability of field populations

The distribution of genetic variability in 21 field-collected samples of C. capitata were evaluated electrophoretically for more than twenty enzymes. These samples which represent different Countries (Kenya, Libya, Sardinia, Procida) differ in number, size, host plant, season and year of collection. The present contribution outlines the more general features of such variability; however the available empirical data seem adequate for a more exacting statistical analysis.

The levels of genetic variability and the differences between samples of the four populations are expressed by the parameters H (heterozygosity, or average number of heterozygous individuals), P (proportion of polymorphic loci), A (allelism, or mean number of alleles per locus) (Tab. I). The values of H, calculated in each sample, range between 0.021 (Libya) and 0.166 (Kenya). Values larger than 0.100 have been provided by Sardinian samples, while among samples from Procida they range between 0.022 and 0.092. The flies from Kenya provided evidence of high genetic variability, while those from Libya, like those from Procida, showed little variability. This findings are in agreement with the general rule of a decreasing trend of genetic variability from the source area of a species towards the periphery of its geographic range. The finding of several low frequency alleles in the Kenya samples supports the hypothesis that this population has maintained a large size (Nei et al., 1975).

The low variability of the Libyan sample might likely be the result of a founder effect in a very isolated locality.

The rather high genetic variability of the two Sardinian samples seems to reflect a peculiar local situation.

In the samples collected in Procida over a three year period, the values of the annual mean heterozygosity suggest a trend toward increasing heterozygosity (Tab. II). The occurrence of alleles never detected before at several loci and changes in relative frequency for the alleles of other loci have been assessed.
<table>
<thead>
<tr>
<th>host name</th>
<th>date of collection</th>
<th>geographic origin</th>
<th>No of loci examined</th>
<th>H</th>
<th>P</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>coffee</td>
<td>Aug. '84</td>
<td>Kenya</td>
<td>25</td>
<td>.161</td>
<td>.48</td>
<td>1.680</td>
</tr>
<tr>
<td>coffee</td>
<td>Oct. '84</td>
<td>&quot;</td>
<td>25</td>
<td>.166</td>
<td>.64</td>
<td>1.880</td>
</tr>
<tr>
<td>orange</td>
<td>Nov. '85</td>
<td>Libya</td>
<td>27</td>
<td>.021</td>
<td>.15</td>
<td>1.185</td>
</tr>
<tr>
<td>fig</td>
<td>Sept. '85</td>
<td>Sardinia</td>
<td>15</td>
<td>.115</td>
<td>.47</td>
<td>1.600</td>
</tr>
<tr>
<td>plum</td>
<td>Oct. '85</td>
<td>&quot;</td>
<td>23</td>
<td>.114</td>
<td>.30</td>
<td>1.391</td>
</tr>
<tr>
<td>bitter orange</td>
<td>Apr. '83</td>
<td>Procida</td>
<td>25</td>
<td>.044</td>
<td>.24</td>
<td>1.240</td>
</tr>
<tr>
<td>apricot</td>
<td>July '83</td>
<td>&quot;</td>
<td>24</td>
<td>.022</td>
<td>.08</td>
<td>1.083</td>
</tr>
<tr>
<td>fig</td>
<td>Sept. '83</td>
<td>&quot;</td>
<td>24</td>
<td>.032</td>
<td>.12</td>
<td>1.167</td>
</tr>
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<td>peach</td>
<td>Sept. '83</td>
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<td>.21</td>
<td>1.250</td>
</tr>
<tr>
<td>peach</td>
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<td>23</td>
<td>.027</td>
<td>.09</td>
<td>1.087</td>
</tr>
<tr>
<td>fig</td>
<td>Sept. '84</td>
<td>&quot;</td>
<td>25</td>
<td>.062</td>
<td>.24</td>
<td>1.240</td>
</tr>
<tr>
<td>prickly-pear</td>
<td>Sept. '84</td>
<td>&quot;</td>
<td>25</td>
<td>.033</td>
<td>.12</td>
<td>1.120</td>
</tr>
<tr>
<td>bitter orange</td>
<td>Dec. '84</td>
<td>&quot;</td>
<td>25</td>
<td>.047</td>
<td>.24</td>
<td>1.240</td>
</tr>
<tr>
<td>bitter orange</td>
<td>Feb. '85</td>
<td>&quot;</td>
<td>25</td>
<td>.074</td>
<td>.32</td>
<td>1.320</td>
</tr>
<tr>
<td>apricot</td>
<td>June '85</td>
<td>&quot;</td>
<td>24</td>
<td>.034</td>
<td>.08</td>
<td>1.083</td>
</tr>
<tr>
<td>peach</td>
<td>July '85</td>
<td>&quot;</td>
<td>25</td>
<td>.076</td>
<td>.24</td>
<td>1.240</td>
</tr>
<tr>
<td>peach</td>
<td>July '85</td>
<td>&quot;</td>
<td>25</td>
<td>.059</td>
<td>.24</td>
<td>1.240</td>
</tr>
<tr>
<td>apricot</td>
<td>July '85</td>
<td>&quot;</td>
<td>23</td>
<td>.063</td>
<td>.26</td>
<td>1.304</td>
</tr>
<tr>
<td>fig</td>
<td>Aug. '85</td>
<td>&quot;</td>
<td>24</td>
<td>.092</td>
<td>.29</td>
<td>1.375</td>
</tr>
<tr>
<td>peach</td>
<td>Aug. '85</td>
<td>&quot;</td>
<td>24</td>
<td>.077</td>
<td>.29</td>
<td>1.375</td>
</tr>
<tr>
<td>peach</td>
<td>Aug. '85</td>
<td>&quot;</td>
<td>25</td>
<td>.052</td>
<td>.20</td>
<td>1.280</td>
</tr>
</tbody>
</table>
TABLE II - Mean values of genetic variability parameters recorded in samples of *C. capitata* from four Countries

<table>
<thead>
<tr>
<th>Locality</th>
<th>Year</th>
<th>Nº of samples analyzed</th>
<th>$\hat{H}$</th>
<th>$\hat{P}$</th>
<th>$\hat{A}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procida</td>
<td>1983</td>
<td>4</td>
<td>0.031</td>
<td>0.16</td>
<td>1.185</td>
</tr>
<tr>
<td></td>
<td>1984</td>
<td>4</td>
<td>0.042</td>
<td>0.17</td>
<td>1.172</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>8</td>
<td>0.066</td>
<td>0.24</td>
<td>1.277</td>
</tr>
<tr>
<td></td>
<td>1983-84-85</td>
<td>16</td>
<td>0.046</td>
<td>0.19</td>
<td>1.211</td>
</tr>
<tr>
<td>Kenya</td>
<td>1984</td>
<td>2</td>
<td>0.164</td>
<td>0.56</td>
<td>1.780</td>
</tr>
<tr>
<td>Libya</td>
<td>1985</td>
<td>1</td>
<td>0.021</td>
<td>0.15</td>
<td>1.185</td>
</tr>
<tr>
<td>Sardinia</td>
<td>1985</td>
<td>2</td>
<td>0.115</td>
<td>0.39</td>
<td>1.391</td>
</tr>
</tbody>
</table>

TABLE III - Allele frequencies at the loci $M_{pl}$ and $H_{k2}$ observed in medfly samples collected in Procida during the years 1983-85

<table>
<thead>
<tr>
<th>host name</th>
<th>date of collection</th>
<th>$M_{pl}$ alleles</th>
<th>heterozygosity</th>
<th>$H_{k2}$ alleles</th>
<th>heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>bitter orange</td>
<td>April '83</td>
<td>0.25</td>
<td>0.75</td>
<td></td>
<td>0.048</td>
</tr>
<tr>
<td>apricot</td>
<td>July '83</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>fig</td>
<td>Sept. '83</td>
<td>0.182</td>
<td>-</td>
<td>0.818</td>
<td>0.297</td>
</tr>
<tr>
<td>peach</td>
<td>Sept. '83</td>
<td>0.061</td>
<td>-</td>
<td>0.939</td>
<td>0.114</td>
</tr>
<tr>
<td>fig</td>
<td>Sept. '84</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>prickly-pear</td>
<td>Sept. '84</td>
<td>-</td>
<td>1.00</td>
<td>0.900</td>
<td>0.180</td>
</tr>
<tr>
<td>bitter orange</td>
<td>Dec. '84</td>
<td>-</td>
<td>0.409</td>
<td>0.591</td>
<td>0.483</td>
</tr>
</tbody>
</table>
| 152
Besides, among Procida samples, seasonal fluctuations in the relative frequencies of \( \text{Mpi} \) and \( \text{Hk}_2 \) alleles have been observed (Tab. III). The relative frequency of \( \text{Mpi}^C \) is high in the summer and low in the winter; early in the year \( \text{Mpi}^B \) is the most frequent allele. \( \text{Hk}_2^B \) has been found only in flies collected late in the summer.

**Diagnostic loci between \text{C. capitata} and \text{C. (= Pterandrus) rosa}**

In Kenya, \text{C. rosa} is one of the economically important pest species; its prevalence on coffee berries is intermediate between those of the other two \text{Ceratitis} species, \text{C. capitata} and \text{C. nigra} (Mukiama, 1985). Electrophoretic assays were performed on individual adults of \text{C. capitata} and \text{C. rosa}, derived from the same batch of pupae, collected in Kenya. The parameters \( H \) (mean per locus heterozygosity), \( P \) (proportion of polymorphic loci), \( A \) (number of alleles per locus) reached fairly similar values between these two species samples, as follows:

<table>
<thead>
<tr>
<th></th>
<th>( H )</th>
<th>( P )</th>
<th>( A )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C. ) capitata</td>
<td>0.166</td>
<td>0.64</td>
<td>1.880</td>
</tr>
<tr>
<td>( C. ) rosa</td>
<td>0.151</td>
<td>0.55</td>
<td>1.800</td>
</tr>
</tbody>
</table>

Out of twenty biochemical loci tested, five resulted fully diagnostic for \text{C. rosa} in comparison with \text{C. capitata}, being represented by alleles causing electrophoretic phenotypes quite distinct from those reported for the medfly (Tab. IV). The high number of \( \alpha_{Gpdh} \) alleles of \text{C. rosa} contrasts with the extremely reduced polymorphism detected in \text{C. capitata} and reported for other insect species (Johnson, 1974). Out of the five diagnostic loci, \( \alpha_{Gpdh} \) is the only one coding for stage specific electrophoretic phenotypes. The identification of suitable diagnostic loci can represent the starting point for constructing an electrophoretic key for identification of the immature stages of \text{C. capitata} and \text{C. rosa}.

The enzyme system alcohol dehydrogenase (ADH)

The two isoenzymes of \text{C. capitata} show different and integrate developmental patterns and tissue localization. ADH-1 reaches high levels in the late larva and pupa, while it falls to low levels in the pharate and full adult stages. ADH-2 activity has been evidenced during the entire developmental cycle.

The two isoenzymes show quite distinct tissue distribution: ADH-1 is found in the larval and adult muscular tissue, while ADH-2 occurs in both stages and is mainly limited to the fat body.

The presence of two isoenzymes undergoing distinct activity changes during development suggest that they may have different functional roles, and be suited to distinct (temporary) life requirements.

ADH-1 is controlled by a structural gene which belongs to Chr. 2 (Zapater and Robinson, in press), while the position of the \( \text{Adh}_2 \) gene has not been established yet. The \( \text{Adh}_2 \) locus is much less polymorphic than \( \text{Adh}_1 \); six \( \text{Adh}_1 \) alleles have been detected in a South African strain (Fig. 1).
TABLE IV - Enzyme loci which permit discrimination (diagnostic loci) between sympatric species Ceratitis (= Pterandrus) rosa and Ceratitis capitata from Kenya

<table>
<thead>
<tr>
<th>locus</th>
<th>alleles*</th>
<th>C. rosa</th>
<th>C. capitata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ak₁</td>
<td>100</td>
<td>-</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>1.000</td>
<td>-</td>
</tr>
<tr>
<td>Idh</td>
<td>125</td>
<td>.045</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>.955</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(A)</td>
<td>-</td>
<td>.072</td>
</tr>
<tr>
<td></td>
<td>100 (=B)</td>
<td>-</td>
<td>.905</td>
</tr>
<tr>
<td></td>
<td>(C)</td>
<td>-</td>
<td>.023</td>
</tr>
<tr>
<td>Adh₂</td>
<td>130</td>
<td>1.000</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>100 (=A)</td>
<td>-</td>
<td>1.000</td>
</tr>
<tr>
<td>Aox</td>
<td>105</td>
<td>1.000</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>100 (=A)</td>
<td>-</td>
<td>1.000</td>
</tr>
<tr>
<td>αGpdh</td>
<td>102</td>
<td>.023</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>100 (=A)</td>
<td>-</td>
<td>1.000</td>
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<tr>
<td></td>
<td>96</td>
<td>.863</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>.023</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>.091</td>
<td>-</td>
</tr>
</tbody>
</table>

*Alleles are labelled according to their relative mobility referred to that of the most common allele of C. capitata taken as 100. (The allele nomenclature used by us for C. capitata population studies is reported in brackets).
Fig. 1 - Polymorphism caused by three alleles at Adh locus, detected by isoelectrofocusing (pH range 4-6.5) in third inst. larvae of a laboratory strain from South Africa.

It is noteworthy that the ADH function is controlled by genes of two loci also in some species of Rhagoletis (Berlocher and Bush, 1982) and Anastrepha (Matioli et al., 1986) of the same taxonomic family of C. capitata.

Larval serum proteins (LSP)

Insect larval serum proteins comprise a small number of molecular species in which the genetic control of developmental patterns deserves special attention. In C. capitata the biochemical features of these molecules have been ascertained and the degree of homologies with the corresponding LSP of Drosophila melanogaster has been assessed (Mintzas and Rina, 1986).

Some degree of genetic variability for medfly larval serum proteins has been detected in a South African strain (Fig. 2). Heterozygous phenotypes occur independently for at least three of these proteins, indicating genetic independence. The molecular form II in Fig. 2 corresponds to the already characterized LSP-4 protein.
Fig. 2 - Isoelectrofocusing patterns of medfly third inst. larvae from a South Africa laboratory strain, showing polymorphism in three out of four proteins of haemolymph (h).

Acknowledgements

We wish to thank Drs. E. Busch-Petersen, E. Boller, U. Cirio and R. Prota for kindly supplying some laboratory strains and the samples of field-collected medfly populations. This work was partially supported by the Purchase Order No PER/5/012/1.018 from International Atomic Energy Agency, Vienna, Austria.

References


Mintzas, A.C., and Rina, M.D. 1986. Isolation and characterization of three major larval serum proteins of the mediterranean fruit fly Ceratitis capitata (Diptera). Insect Biochem. 16: 825-834.


Abstract

A morphological chromosomal polymorphism along with the observation of B chromosomes in a natural population of Ceratitis capitata is reported. A variability affecting the centromere size of chromosome 3 is described. The observed B chromosome is minute, heterochromatic and telocentric. It was found in the male and female germ cells and exhibited, in the males, intra-individual numerical variation with 0B and 1B cells, which suggested a mitotic instability. It was also found, in both sexes, in somatic cells (cerebral ganglia tissue). Only males transmitted the B chromosomes to the progeny. The high rate of transmission suggested a differential utilization of the sperm carrying the B chromosomes or a preferential segregation into secondary spermatocytes.

Introduction

It is of great importance, in monitoring the effectiveness of any genetic control program, to know the degree of genetic variability of field populations. In Ceratitis capitata there is a wealth of reports on biochemical polymorphism of wild populations. Instead, little effort has been devoted to study the magnitude of the chromosomal variability that could be present in populations occupying different ecological niches. The significance of this evaluation is evident if we considered what happened in other diploids with the effectiveness of the control methods employed. A good example are the fluctuations in the efficiency of the genetic control methods used in regulating the dynamics of the screwworm (Cochliomyia hominivorax) wild populations in USA. Cytological analysis revealed that the fluctuations observed were due to the presence of chromosomal polymorphism. In fact, the laboratory population was partially incompatible with some natural ones and several cytological differences among them were observed. This situation was even more complicated because in the field populations also different types of karyotypes existed; their distributions were geographically overlapping, though they were reproductively isolated. Besides it was detected that the dominant type in the field flies could often vary (Richardson et al., 1982). In consequence it was necessary to make a periodical comparison of karyotypes.

A similar situation appears among the fruit fly Rhagoletis cerasi, in which it was observed the existence of incompatibility between geographical races in Europe (Bollet, 1976; Ballocher et al., 1982).

Evidences of the presence of chromosomal polymorphism in a population of Ceratitis capitata were obtained in our laboratory during the cytological analysis related to sex determination studies (Lifschitz, 1980). At least two types of Y-chromosome differing in size have been observed. The Y-chromosome of the laboratory population is acrocentric but a variability affecting the length of the long arm has been found, resulting in a shorter chromosome.
In the medfly, the male sex is determined by the presence of the V-chromosome, and the polymorphism described did not modify the V-chromosome behaviour (Lipschitz, 1980). In some Italian medfly populations a variant in the morphology of the V-chromosome, consisting in the presence of a satellite has also been detected (Gasperi et al., 1983).

Presently an X-chromosome polymorphism is under study in our laboratory.

Abnormal chromosome number is another source of variation. It is of common occurrence in natural populations, as a result of spontaneous errors in cell division; most of them are eliminated by selection but some can survive as polyploids. Polyploidy is the most common condition specially in the higher plants. The accessory or B chromosomes are included in this type of numerical change. They are extra chromosomes that serve no vital function in the organism and are unrelated to the normal complement. They may be present or absent in different members of the population, with little consequences to the organism involved (Stephens et al., 1972). In many species there are special mechanisms leading to a numerical increase of B chromosomes. This is accomplished in several ways in different groups of organisms.

The Bs are widespread and abundant in nature, and up to date, they have been described in more than a thousand of species of plants and more than 260 animal species. In animals 80% of the species with Bs are insects and most of those are from the order Coleoptera, Diptera and Orthoptera. The grasshopper family Acrididae provides a disproportionately large number of examples probably due to popularity of such groups among cytologists.

In spite of the fact that Bs are considered of dispensable nature, in species in which its effects have been studied in detail there is generally a pronounced negative correlation between number of B and fertility. They are generally deleterious in animals when present in high frequency (Melander, 1950; Nur, 1969; Phouenier, 1975; Hewitt et al., 1978). Searching for positive effects of Bs has given conflicting results but in some cases their presence has been correlated with a favorable fitness. There is also fair evidence that, at meiosis, they affect the distribution and frequency of chiasmata, as well as genetic recombination is also influenced and often favored by the presence of Bs.

In this paper we report our findings, from a research which is still in progress, on the observation of B chromosome along with a morphological chromosomal polymorphism in a natural population of medfly.

Materials and methods

Individuals used in these crosses were obtained from a sample of infested fruits harvested from a field population of Caqui (Diospyros sp.) and Chirimoya (Anotia sp.) fruit trees growing in the province of Tucuman (27°S-65°W), Argentina. This region is considered as the North-western limit of the medfly distribution area in which both Ceratitis capitata and Anastrepha sp. geographical ranges overlap; in fact, both species were recovered from infested fruit. The males hatched from the fruits were crossed in single pair matings with wild type females belonging to the Castelar standard laboratory population (Control).

The chromosomal constitution of the offspring of each cross was determined through the cytological analysis of mitosis in the cerebral ganglia cells of a larva sample. Thus in the larvae of one family a B chromosome
was found. A male of this family was crossed with a control female and in the sampled larvae the presence of a B was observed in the ganglia. All the individuals involved in the next crosses were derived from this male.

Meiosis was studied in the primary spermatocytes of males and in the follicular cells of ovaries in females.

Both, ganglia cells and gonads were stained with 2% lacto-propionic orcein during 5 hours and 24 hours at 25°C respectively. The squashes were carried out in a solution of propionic and lactic acid.

Observations and results

The chromosome complement of the medfly population grown at Castellar is 2n=10 autosomes plus XX and XY. Three pairs of autosomes are metacentric and two pairs are submetacentric. The X and Y chromosome are acrocentric (Lifschitz et al. in press).

In the sample of Tucuman population two types of polymorphism were observed: one affecting the morphology of the submetacentric chromosome 3 and other related to an alteration of the chromosome number. Both could be observed in the same individual simultaneously.

Chromosomal polymorphism

During the cytological survey of larvae cerebral ganglia of the progeny produced in the cross of a Tucuman male and a control female, a variation in the morphology of the chromosome 3 was observed. The centromere of the chromosome derived from the Tucuman population was smaller than the one corresponding to the control flies (Fig. 1). In heterozygote condition no abnormality in their pairing was observed. So far no morphological modifications have been observed during the different developmental stages. The fertility of the hybrid is not statistically different from that of the control flies. Further studies on this polymorphism is being carried out.

Morphological characteristics of B chromosomes

In the same squash along with the presence of the polymorphic chromosome 3, a B chromosome was observed. This was a very small, telocentric and heteropynotic chromosome, approximately one-third the size of the X and clearly distinguishable from the regular members of the complement. Frequently it is attached to any of the other chromosomes but sometimes is free (Fig. 2), and when joined end to end to the regular chromosome, the latter looked longer and occasionally a second centromere was observable.

Mitotic behaviour of B chromosome

Preliminary observations of ganglia indicated that the B was pretty stable during the mitosis, because its presence was determined in all
cells of the somatic tissue. Therefore, in the following studies the presence of B was considered positive if it was identified, at least, in ten mitotic figures.

A cross (GI) between a male derived from the Tucuman population (Tuc) and a control female (C) was carried out (Table I). From this cross four larvae were cytologically analyzed and the results for 3 larvae showed 10 autosomes + XY+IB and the other 10 autosomes +XX+1B. From the remaining larvae four males hatched and one of these was mated to a control females (GII). In this case 8 larvae were analyzed: 3 larvae were 10A+XX+1B and 5 larvae were 10A+XY+1B. The rest of individuals were crossed as follows: males GII x females C, males GII x females GII and males C x females GII; the results of the different crosses are shown in Table I. With no exception the classification of adults as B was inferred from the fact that all its analyzed sibs were B. The results that are presented in the upper part of Table I suggest that, although present in the ganglia, the B chromosome are not transmitted by the females to the next generation. In fact, the data obtained indicated that the males transmitted the B chromosome at a high rate. In the lower part of Table I the results obtained in the F2 for the three types of crossings are presented and these confirm the results appeared in the upper part.

**Table I** - Crosses for the Study of B Chromosome Transmission

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of Families</th>
<th>10XX</th>
<th>10XX+8</th>
<th>10XY</th>
<th>10XY+8</th>
<th>Sex ratio</th>
<th>Fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>G Tuc x C</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>G I x C</td>
<td>3</td>
<td>50</td>
<td>0.93</td>
<td>0.56</td>
<td>0.33</td>
<td>0.62</td>
</tr>
<tr>
<td>G II x C</td>
<td>76</td>
<td>50</td>
<td>0.93</td>
<td>0.56</td>
<td>0.33</td>
<td>0.62</td>
<td>0.82</td>
</tr>
<tr>
<td>G C x II</td>
<td>98</td>
<td>67</td>
<td>1.09</td>
<td>0.65</td>
<td>0.33</td>
<td>0.62</td>
<td>0.82</td>
</tr>
<tr>
<td>F2 (II x C)</td>
<td>12</td>
<td>12</td>
<td>0.90</td>
<td>0.56</td>
<td>0.33</td>
<td>0.62</td>
<td>0.82</td>
</tr>
<tr>
<td>F2 (II x II)</td>
<td>5</td>
<td>20</td>
<td>1.09</td>
<td>0.65</td>
<td>0.33</td>
<td>0.62</td>
<td>0.82</td>
</tr>
<tr>
<td>F2 (C x II)</td>
<td>22</td>
<td>16</td>
<td>0.90</td>
<td>0.56</td>
<td>0.33</td>
<td>0.62</td>
<td>0.82</td>
</tr>
</tbody>
</table>

The sex ratios (males to females) calculated on imago stage, for the indicated crosses did not differ statistically from 1:1. Instead, the fertility of each cross calculated as pupa to eggs (adults to pupa is almost 1:1) is statistically different from each other (Table I). Only one family with a very low fertility in the crosses male GII x female C appeared. Only 3 larvae from a total of 48 eggs were produced. The cytological examination of ganglia cells showed that in two larvae two B chromosomes were observed and in the other only one. It is possible that the duplication could affect their fertility.

**Meiotic behaviour of B chromosome in males**

In the medfly it is not easy to study the course of the spermatogenesis in the same individual due to the fact that, in testes, all cells are synchronized in the same developmental stage. Therefore the behaviour of
the B chromosomes in the males have been studied only in some stages of gametogenesis. In primary spermatocytes the B chromosomes appeared as a small telocentric and positively heteropycnotic chromosome sometimes free or attached to any of the regular chromosome of the complement.

It was common to observe in the telophase of the first meiotic division an univalent B. It is characteristic feature of the univalent to lag in their movement onto the metaphase plate at the first division of meiosis (Fig. 3). They may lag to the extent that they probably fail to reach the metaphase plate and thus could be lost as micronuclei. In all testes examined, secondary spermatocytes with six and seven chromosomes have been observed in the same tissue (Fig. 4). Few observations of follicular cells in females ovary have been made and the presence of B chromosome in the primary oocyte was determined.

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**Fig. 1.** - Mitotic neuroblast chromosomes of female. Arrow indicate the chromosome 3 derived from Tucuman population. Note the difference in centromere size.

**Fig. 2.** - Mitotic neuroblast chromosomes. Arrow indicate the acrocentric B chromosome.

**Fig. 3.** - Univalent B chromosome lagging into the metaphase plate at the first division of meiosis.
Discussion

The B chromosomes of Ceratitis capitata were found, in both sexes, in the neuroblasts of cerebral ganglia but also in germ cells. The results of the different crosses (Table I) strongly suggest that the B chromosomes are transmitted to the offspring only by the males. The examination of the testes indicated that there were cells with six and seven bivalents. Taking into account this variability, at least, some larvae with no B chromosome would be expected. However, this is not the case, because the B chromosomes are transmitted by the males to all progenie. If this phenomenon is confirmed it could be due either to a differential utilization of the two types of sperm or to a preferential segregation of B chromosome into the secondary spermatocytes. In the female, the situation is just the opposite because, although, present in the follicular cells of ovarioles, the B chromosomes are not transmitted to the next generation. It is possible that, an elimination mechanism, as for example, preferential segregation into the polar body, could exists.

According to the classification proposed by White (1954) the numerical variation of B chromosome observed in the testes in the medfly represents a mitotic instability. It was suggested that mitotically instable Bs which did not possess an accumulation mechanism would not be maintained in a natural population (Nur, 1963 and 1969). They would be lost during some of the divisions prior to gamete formation. In a considerable number of cases the behaviour of B chromosomes provide them with an accumulation mechanism capable of increasing their frequency in the population from generation to generation (Battaglia, 1964b) even through its presence could be deleterious to his possessors.

Up to date the number of generations analyzed are not enough to ascertain whether an accumulation mechanism exists in the medfly. It is a necessary item to determine the phenomena involved in the maintenance of a given B chromosome.
Acknowledgements

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Abstract

The genetic structure of 12 wild populations of medfly sampled from different geographical areas and different host fruit species have been studied. Each population was analyzed for 25 enzyme polymorphisms detected electrophoretically. All the Mediterranean populations proved to be highly monomorphic ($\bar{H}=0.051$), whereas those from South Africa and Réunion were polymorphic ($\bar{H}=0.234$ and 0.153 respectively). The differences in the genetic structure of these populations may be attributed to founder effects. The pattern of dispersion of the fly from its geographic centre of origin is discussed. Finally, no systematic changes in allele frequencies were observed in populations kept under laboratory conditions.
GENOME ORGANIZATION OF *CERATITIS CAPITATA*: LINKAGE GROUPS AND EVIDENCE FOR SEX RATIO DISTORTERS

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Department of Animal Biology, University of Pavia, Pavia, Italy

Abstract

Seventeen biochemical markers of *Ceratitis capitata* are distributed over four linkage groups. Two of these groups include also one visible marker, as follows:

\[
\begin{align*}
\text{ap, } & \text{Hk}_2, \text{Est}_1, \text{Est}_2, \text{sd, } l, \text{Pgi} \\
\text{w, } & \text{Zw}, \text{Pgd}, \text{Fh}, \text{Had} \\
\text{Mpi, } & \text{Adh}_1, \text{Est}_6, \text{Aox}, \text{Mdh}_2 \text{ (Chr. II)} \\
\text{Pgm, } & \text{Idh}, \text{sd}_2, \text{Got}, \text{Gox}
\end{align*}
\]

The symbols of the \( \text{ap} \ldots \text{Pgi} \) group are listed according to the map order of the loci. Several pairs of enzymes sequentially involved in the energetic flux are coded by linked genes. The loci mentioned above show similar linkage associations also in *Drosophila melanogaster* and *Musca domestica*. The markers \( \text{Zw}, \text{Pgd}, \text{Fh}, \text{Had} \) are X-linked in *D. melanogaster*; they belong to Chr. III in *M. domestica*. No evidence of sex-linkage has been found in *C. capitata*. Linkage group \( \text{ap} \ldots \text{Pgi} \) includes locus \( \text{sd} \) (sex-ratio distorser). Males carrying \( \text{sd} \) generate families affected by excess of males, in the ratio of 5♂:3♀. Factor \( \text{sd} \) is generally inherited through the males, but females also may carry it. The residual genotype does not affect the expression of \( \text{sd} \). Mendelian inheritance of gene markers linked to \( \text{sd} \) suggests that \( \text{sd} \) acts prezygotically. In families of \( \text{sd} \) lineage, excess of females occasionally occurs. Such occurrence suggests the existence of a factor acting as \( \text{F} \) of *M. domestica*. Factor \( \text{sd} \) has been found in flies from many different geographic localities. In medflies from South Africa another sex-ratio distorser which pertains to \( \text{Pgm} \ldots \text{Gox} \) linkage group has been detected.

Introduction

The biochemical approach in *C. capitata* has made available a number of gene-markers and has enabled to extend and integrate the spotty informations, already available, about the basic genetics of this insect (Milani et al., 1982; Malacrida et al., 1985a; Gasperi et al., 1986). The linkage maps of biochemical loci should lead to a better understanding of the genomic functional organization of *C. capitata*; the assessment of the linkage relations of metabolically related enzyme loci is a prerequisite for investigations into the genetic make-up of the different metabolic pathways. The linkage informations made available from enzyme study in *C. capitata* has revealed interspecific chromosome homologies with other Tephritidae flies and with other unrelated dipteran species such as *Drosophila melanogaster* and *Musca domestica*. The comparative analysis between the genome of these
two last species was already carried out by our research team (Malacrida et al., 1985b). Comparative gene mapping besides its intrinsic bearing on phylogenetic problems would provide guide lines for applied purposes.

In natural or laboratory populations, unbalanced allele frequencies of linked genes or differential allele inheritance between sexes, can reveal factors affecting the transmission of linked genes. In different strains of C. capitata, Hk$_2$, Est$_1$, alleles, when linked with the sd gene (sex-ratio distorer) are recovered in a different proportion in the two sexes (Malacrida et al., 1985a). The correlation between the distortion of observed frequencies of both allozyme variants and sexes, provides a significant information for the understanding of the genetic control of sex-ratio in C. capitata. This paper gives the informations provided by biochemical markers on the linkage group arrangement of C. capitata, and on the presence of different sex factors which operate in the populations of C. capitata.

**Linkage groups**

Seventeen biochemical markers are distributed over four linkage groups. Two of these include also one visible marker (Tab. I). The ap ... Pgi group is the only one for which the loci order is known for all markers (Gasperi et al., 1986). Up to date, no evidence of factors blocking recombination in this group has been encountered. It includes a sex-ratio distorer (sd) closely linked to Est$_1$ locus (Malacrida et al., 1985a). Another factor involved in the sex-ratio distortions, has been recently discovered in close association with Got$_1$, a locus of the Pgm ... Gox linkage group.

**TABLE I - Available evidence for entangling the data on linkage derived from the use of biochemical markers and of visible mutants.**

For the last ones the linkage groups' labelling system proposed by Saul and Rössler (1984) has been adopted. Plus sign, hyphens and question marks indicate respectively linkage, free reassortment and combinations not yet tested

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ap)</td>
<td>(w)</td>
<td>(dp)</td>
<td>(ry)</td>
<td>(bro)</td>
</tr>
<tr>
<td>Hk$_2$, Est$_1$, Est$_2$, Pgi</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zw, Pgd, Fh, Had</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mpi, Est$_6$, Adh$_1$, Aox, Mdh$_2$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Pgm, Idh, Got$_1$, Gox</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

The gene cluster Mpi ... Mdh$_2$ includes Adh$_1$ locus, which pertains to chromosome II (Zapater et al., in press); the loci Zw, Pgd, Fh, Had, are linked to...
w (white pupae), currently used for automated genetic sexing.

Each of the four linkage groups includes structural loci which control metabolic related functions such as:

- **Hk**
  - **Pgi**: glycolytic pathway
- **Zw**
  - **Pgd**: pentose shunt
- **Adh**
  - **Aox**: alcohol degradation
- **Pgm**
  - **Gox**: conversion pathway of carbohydrate reserve
- **Idh**
  - **Got**: Krebs cycle

Some of these clusters of functional related loci occur also in other species of the Tephritidae Family so far examined: Rhagoletis pomonella (Berlocher and Smith, 1983), R. completa (Berlocher, 1984) and R. tabellaria (Mc Pherson and Berlocher, 1985). The 17 biochemical loci already mapped of C. capitata show similar linkage associations also in D. melanogaster and M. domestica. The markers Zw, Pgd, Fh, Had, are X linked in D. melanogaster; they belong to Chr. III in M. domestica and, in this species, are often associated with a male factor (M) (Malacrida et al., 1980). The gene cluster Pgm, Idh, Got, Gox is localized in M. domestica on the IV Chr., in association with a female factor (F) (Malacrida et al., 1982).

**Sex ratio distortions**

Sex ratio distortions have been observed in the progenies of single pairs in which flies of the same or of different geographic origin were mated. The distortions were always limited to some families and changed the sex ratio to 5♂ : 3♀ or to 3♂ : 5♀ (Malacrida et al., 1985a). The excess of males (5♂ : 3♀) has been traced to a single genetic factor sd closely linked to Est in linkage group ap ... Pgi (Gasperi et al., 1986). The following features characterize sd:

- Carrier males sire families with excess of sons in the ratio 5♂ : 3♀; gene markers, close to sd and introduced in coupling position with it by heterozygous flies of either sex are found in excess among the male progeny and show a proportional shortage among the females. When sd is introduced by a carrier female, crossingovers between sd and marker loci tend to level the difference between the phenotypic classes, to a degree proportional to the interval between sd and the marker;
- sd is inherited as a simple mendelian factor;
- regular mendelian inheritance (allowance being made for distribution among sexes) of genes linked to sd, indicates that the sex unbalance is not due to loss of female zygotes; it seems therefore likely that sd exerts its action prezygotically;
- sd follows simple mendelian inheritance after outcrossoes to flies of unrelated strains; the expression of sd seems therefore independent from the genetic background.

Reliable repeated evidence indicates that sd can be carried by fertile females, in which presumably its action is inhibited by epistatic factor(s). Females of sd - lines from families showing excess of females (3♂ : 5♀) provide three types of progenies: with 1♂ : 1♀, with 5♂ : 3♀, with 3♂ : 5♀, in the ratio of 1:2:1 (Tab. II, Tab. III). The regular recurrence of these
three types of sex-ratio suggests the existence of another factor reassigning independently from sd. This factor causes sex-ratio distortion complementary to those introduced by sd. Such situation somehow parallels the X-Y, M-F interaction described in M. domestica (Milani, 1971). Sex-ratio distortions of type 5♂ : 3♀ have been observed in crosses involving medflies from South Africa. In these flies, however, the excess of males is inherited in association with Got locus (Tab. IV) which belongs to a different linkage group from sd. Preliminary data suggest that the general features of this new sex distorer are similar to those of sd. The existence of different autosomal sex-factors indicates that the genetic basis of sex determination is less simple than current interpretations imply.

TABLE II - Numbers of families (between brackets) showing opposed distortions or regular sex-ratio in two generations of sib-matings following the cross: __sd-line ♀ x South Africa ♂ ♂

<table>
<thead>
<tr>
<th>Generation</th>
<th>Sex-ratio</th>
<th>Families</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>5♂ : 3♀</td>
<td>(3)</td>
</tr>
<tr>
<td></td>
<td>1♂ : 1♀</td>
<td>(13)</td>
</tr>
<tr>
<td></td>
<td>3♂ : 5♀</td>
<td>(4)</td>
</tr>
<tr>
<td>F₁</td>
<td>5♂ : 3♀</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>1♂ : 1♀</td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td>3♂ : 5♀</td>
<td>(4)</td>
</tr>
<tr>
<td>F₂</td>
<td>5♂ : 3♀</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>1♂ : 1♀</td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td>3♂ : 5♀</td>
<td>(4)</td>
</tr>
</tbody>
</table>

TABLE III - Sex-frequencies and sex-ratios in the two generations shown in Table II

<table>
<thead>
<tr>
<th>Type of cross</th>
<th>Observed N° of families</th>
<th>Sex-frequencies</th>
<th>Total</th>
<th>Sex-ratio Chi²</th>
<th>Chi²</th>
</tr>
</thead>
<tbody>
<tr>
<td>P: __sd-line x South Africa</td>
<td>3</td>
<td>88</td>
<td>134</td>
<td>222</td>
<td>5 : 3</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>472</td>
<td>470</td>
<td>942</td>
<td>1 : 1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>153</td>
<td>97</td>
<td>250</td>
<td>3 : 5</td>
</tr>
<tr>
<td>F₁ x F₁</td>
<td>5</td>
<td>79</td>
<td>129</td>
<td>208</td>
<td>5 : 3</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>286</td>
<td>288</td>
<td>574</td>
<td>1 : 1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>125</td>
<td>80</td>
<td>205</td>
<td>3 : 5</td>
</tr>
</tbody>
</table>
TABLE IV - Distorted sex-ratios associated with the \textit{Idh-Got}_1 chromosomal section in the progeny of a cross involving heterozygous females from South Africa strain

\begin{center}
\begin{tabular}{c}
\textit{Idh}^A \textit{Got}^A_1 \otimes \textit{Idh}^B \textit{Got}^B_1 \\
\textit{Idh}^B \textit{Got}^B_1 \otimes \textit{Idh}^B \textit{Got}^B_1 \\
\end{tabular}
\end{center}

Parental combinations:

\begin{center}
\begin{tabular}{c|c|c|c|c}
& \textbf{SR} & \textbf{Chi}^2 \\
\hline
\textit{♀} & \textit{♂} & \textit{♂} : \textit{♀} & \\
\hline
\textit{IDH-B, GOT}_1^{-}\textit{B} & 25 & 22 & 1 : 1 & 0.19 \\
\textit{IDH-AB, GOT}_1^{-}\textit{AB} & 11 & 37 & 3 : 1 & 0.11 \\
\end{tabular}
\end{center}

Recombinations:

\begin{center}
\begin{tabular}{c|c|c|c|c}
& \textbf{SR} & \textbf{Chi}^2 \\
\hline
\textit{IDH-AB, GOT}_1^{-}\textit{B} & 11 & 14 & 1 : 1 & 0.78 \\
\textit{IDH-B, GOT}_1^{-}\textit{AB} & 9 & 19 & 3 : 1 & 0.76 \\
\hline
\textbf{tot} & 52 & 92 & 5 : 3 & 0.12 \\
\end{tabular}
\end{center}

Sex frequencies in the monofactorial segregation classes of \textit{Idh, Got}_1 loci

\begin{center}
\begin{tabular}{lrrrrrrr}
\multicolumn{1}{c|}{\textbf{Locus}} & \textbf{Heterozygotes} & \textbf{Homozygotes} \\
\cline{2-7}
 & \textbf{♀♀} & \textbf{♂♂} & \textbf{♂ : ♀} & \textbf{SR} & \textbf{♀♀} & \textbf{♂♂} & \textbf{♂ : ♀} & \textbf{SR} & \textbf{Chi}^2 \\
\hline
\textit{Got}_1 & 76 & 20 & 56 & 3 : 1 & 0.07 & 72 & 36 & 36 & 1 : 1 & - \\
\textit{Idh} & 73 & 22 & 51 & 3 : 1 & 1.03 & 75 & 34 & 41 & 1 : 1 & 0.66 \\
\end{tabular}
\end{center}

Acknowledgements

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References


GENETIC VARIATION IN MEDFLY POPULATIONS: IS THERE ANY OVIPPOSITION PREFERENCE PATTERN ASSOCIATED WITH THE TAXONOMIC STATUS OF THE FRUIT OR FRUIT-SIZE OF THE HOST PLANT?

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Abstract

Several hypotheses are proposed to explain the low genetic variation maintained by the introduced populations of medfly. By utilizing the urea denaturation method we did not detect any hidden genetic variability due to the usual electrophoretic conditions. The differences in allele frequencies for the polymorphic loci among these populations do not seem to be correlated either with the taxonomic status of the host fruit or with the size of the fruit. Therefore, our limited data do not suggest any apparent pattern of preference for oviposition sites in medfly populations.
THE SALIVARY GLAND CHROMOSOMES OF THE MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA*

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Abstract

The Mediterranean fruit fly, *Ceratitis capitata*, is known as a major pest of citrus and other soft fruits in many parts of the world. The means of control of the fly is mainly based on chemical insecticides, although there is increasing interest in autocidal and other genetic control techniques. The development of such methods is problematic because of the absence of basic genetic and cytogenetic data for *C. capitata*. Here we describe the polytene chromosomes of the larval salivary glands of *C. capitata* and show them to be suitable for study. Each polytene nucleus has five long chromosomes which correspond to the five autosomes, and were numbered from I to V according to their size. The centromere of each chromosome was recognized as diffuse area, bordered by adjacent heterochromatic regions, since the salivary gland nuclei lack a chromocentre. In all chromosomes the two arms are of unequal length and the longer arm was characterised as the left (L) arm and the shorter as the right (R) arm. Except for the chromosome tips that are very characteristic, there are several distinct regions which constitute important landmarks permitting recognition and identification of the chromosomes.
EXCESS MALE PRODUCTION IN LINES OF THE MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (WIED.) ISOLATED AFTER X-IRRADIATION FOLLOWED BY OUTCROSSING

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Abstract

The descendants of X-irradiated males of *Ceratitis capitata*, after two generations of outcrossing to unirradiated females, were investigated for sex ratio in the progenies of single pair matings. Some progenies were found to show a significant excess of males, others a significant excess of females but most were normal. A programme, of single pair mating within excess male progenies, with selection for the most extreme distortion observed, led to the production of excess male producing lines. Such lines, which appear to be chromosomally normal, consistently show an excess of males without selection. The degree of sex ratio distortion is however variable, the maximum distortion observed in large samples \( n > 750 \) being about 20♀:80♂. Research has been directed towards determining the basis of this variability and whether the phenomenon is postzygotic or prezygotic in origin. Progress in these studies is reported.

Introduction

This area of research arose out of a series of experiments designed to isolate a translocation of an autosomally inherited insecticide resistance gene onto the Y chromosome. The method was based on the pattern established in earlier studies on sheep blowflies (Foster and Whitten 1974) and mosquitoes (Curtis et al. 1976). The principle of the method was to X-irradiate a resistant strain, mass cross the irradiated resistant males to susceptible (SS) females, backcross the \( F_1 \) (RS) males to SS females by single pair mating, test the backcross progeny with a discriminating dose of the appropriate insecticide (to separate RS from SS) and score the survivors for sex (Fig. 1). Any progenies in which the survivors were all male, or mainly male (while the unexposed sibs had a normal sex ratio) would indicate the possibility that a Y-linked translocation of the R gene has taken place (Wood and Busch-Petersen, 1982).

Insecticide resistant strains of *C. capitata* were isolated by Busch-Petersen (1983), by laboratory selection, and in one of these strains (DiR), resistance was shown to segregate as a major gene (Busch-Petersen and Wood 1983).

Materials and Methods

The procedure was to X-irradiate a resistant strain (usually DiR), either as pupae aged 7 days exposed to 1.4 krads X-rays (at 120 rads per minute) or as young adults exposed to 4.5 or 5.0 krads at 120 rads per
minute. Use was made of a PANTAK 300K X-ray generator operating at 300 Kv, 10 mA, located at the Paterson Laboratories, Manchester. The experimental set-up was designed to give a dose rate of 120 rads/min at the sample by placing it 20 cm from the tube. The generator had been modified by the addition of a flattening filter which gave a uniform field of 12 cm diameter. The uniformity was ±0.02.

The mass crosses of irradiated males to DiS females gave plenty of viable progeny. 878 F1 males were backcrossed to DiS females by single pair matings (in replicated experiments of 100-200 pairs) and 620 (70.6%) of these matings were fertile.

The resulting F2 families were screened with insecticide as fourth instar larvae, being immersed for a standard time in an aqueous suspension of the insecticide dissolved in absolute ethanol. Some families were screened with dieldrin, others with malathion and the final series with permethrin. This was because the DiR strain, used in most experiments as the strain for irradiation, was resistant to all three insecticides (Busch-Petersen and Wood 1983). At first dieldrin was the favoured insecticide but problems with delayed mortality led to a switch to malathion or permethrin.

Because of variations in the measured resistance of DiR (and other resistant strains) it was suspected that they might not be homozygous. To allow for this possibility it was the practice to screen the F1 (supposed mainly RS) larvae with a discriminating dose of the appropriate insecticide before backcrossing (Fig. 1). This was to remove any SS males which might be present. The discriminating dose of dieldrin, set initially at 40 mg/l, was lowered to 10 mg/l because of the problem with delayed mortality. The discrimination doses of malathion and permethrin were 20 mg/l and 100 mg/l respectively.

Rearing and insecticide testing were carried out at 26±2°C and 55±5 R.H.

Results

1. Variation in sex ratio after X-irradiation

A number of F2 families produced by this breeding scheme (Fig. 1) showed significantly distorted sex ratios after exposure to insecticide, some with excess males, others with excess females. However, similar distorted sex ratios were also sometimes observed in the unexposed sibs of these families. The frequency distribution of sex ratios in families produced by this breeding scheme differed significantly from a normal distribution whether exposed to insecticide or not. Fig. 2 shows the sex ratio distribution in samples of 353 families not exposed to insecticide. Compared with the distribution in 138 control families (from the same crossing scheme but with the male grandparent not irradiated). The mean sex ratio was normal in both samples. By contrast, the mean sex ratio in malathion and permethrin selected families showed a significant excess of females. All families with excess of males were investigated for sex ratio in succeeding generations but none gave evidence of carrying a translocated R gene. There was, however, evidence of continuing sex ratio distortion in some lines which will be discussed below.
Fig. 1  Procedure designed to isolate a Y-translocated R gene
Fig. 2 Frequency distribution of sex ratio in 353 families from irradiated male grandparents compared with sex ratio in 138 control families (stippled)
2. Sex ratio in strain #425

Family 425 showed only males in a sample of eight survivors from exposure to 20 mg/l malathion. These males were crossed to DiS females and sex ratio investigated in the next and subsequent generations, the strain (#425) being maintained without selection. Sex ratio over 36 generations is recorded in Fig. 3. A significant excess of males was shown in some generations (samples being in the hundreds up to >2000) eg in generation 36 the ratio was 520:621 (\( \chi^2 = 8.94, P<0.01 \)). In two generations there was a significant excess of females but the general trend was towards about 45%.

**Fig. 3** Sex ratio in strain 425 during 36 generations of laboratory culture

*significant deviation from 1:1 (P<0.05)*

The sex ratio distortion in #425 was almost certainly induced by X-irradiation of the male grandparent. Control experiments indicated that persistent sex ratio distortion such as observed in #425 was not observed in families isolated from unirradiated males (Saaid and Wood, unpublished). The breeding programme (outcrossing of irradiated males to non-irradiated females followed by a second generation of outcrossing (Fig. 1)) was designed in the first instance to isolate Y-linked chromosomal translocations. A sample of #425 was checked at F2 to see whether a translocation or other chromosomal anomaly had been induced but the examination was negative. It seemed therefore that the genetic change induced was in the nature of a "point mutation", either Y-linked or autosomal. The breeding scheme excluded the possibility of an X-linked mutation.
3. **Sex ratio in inbred lines derived from strain #425**

An attempt was made to select for increased sex ratio distortion from within line #425 by picking out distorted families. Family 39 gave a sex ratio of 41♀:68♂ in F₁, and was then maintained as a closed line without selection for twenty two further generations (Fig. 4). At first sex ratio reverted towards normal (F₉, F₁₀) and then plummeted to 18% ♀ (χ²₁ = 313.4, P<0.01) at F₇₆ (n = 761). Sex ratio gradually recovered in subsequent generations moving towards sexual parity in an oscillatory pattern suggesting that the effect of a sex ratio distorting gene was being suppressed.

**Fig. 4** **Sex ratio in strain 39 during 23 generations of laboratory culture**

*significant deviation from 1:1 (P<0.05)*

![Graph showing sex ratio fluctuations](image)

Line #39 was clearly heterogeneous, and an attempt was made to produce more extreme sex ratio distortion by further family selection. 35 families (#39♀ x #39♂) were investigated, of which three (#10, #19, #25) had significantly male-distorted sex ratios. These were each maintained as closed lines without selection. Sex ratio fluctuated around 40% ♀ in all three lines for 15 generations and was not more distorted than in line #39.

4. **Environmental influences on sex ratio**

Several factors have been shown to affect sex ratio in these lines, one of which will be reported here: the temperature at which the parental generation is maintained during development. Cultures of line #39 were maintained at a range of temperatures (18°-30°C) in constant temperature cabinets and sex ratio was assessed in their progeny maintained at 25°C. We report the result of an experiment in which #39 F₁₈ was treated during the whole of development (egg-adult), sex ratio being assessed in F₁₉ (Table 1).
Each value of sex ratio in Table 1 is based on combined data from three replicates, and the correlation between temperature and sex ratio (%♀, arcsine transformed) is significant ($r = 0.7453, P<0.05$). The variation is only moderate: 39-43% after treatment at 18°-23°C; 46-50% after treatment at 25°-29°C, the overall sex ratio in this sample of #39 F₁₉ being 43.51%.

Table 1. Sex ratio in strain #39 F₁₉, the parents of which (F) had been reared at a range of temperatures, from egg to adult. Each value is based on combined data from three replicates.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Sex ratio</th>
<th>%♀</th>
<th>$\chi^2_{1:1}$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>342</td>
<td>461</td>
<td>42.6</td>
<td>17.64</td>
</tr>
<tr>
<td>19</td>
<td>405</td>
<td>550</td>
<td>42.4</td>
<td>22.02</td>
</tr>
<tr>
<td>20</td>
<td>591</td>
<td>920</td>
<td>39.1</td>
<td>71.64</td>
</tr>
<tr>
<td>21</td>
<td>444</td>
<td>682</td>
<td>39.4</td>
<td>50.31</td>
</tr>
<tr>
<td>23</td>
<td>437</td>
<td>583</td>
<td>42.8</td>
<td>20.90</td>
</tr>
<tr>
<td>25</td>
<td>654</td>
<td>682</td>
<td>48.9</td>
<td>0.59</td>
</tr>
<tr>
<td>27</td>
<td>507</td>
<td>511</td>
<td>49.8</td>
<td>0.02</td>
</tr>
<tr>
<td>29</td>
<td>6</td>
<td>7</td>
<td>46.2</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3386</strong></td>
<td><strong>4396</strong></td>
<td><strong>43.5</strong></td>
<td><strong>131.08</strong></td>
</tr>
</tbody>
</table>

Discussion

An effect of temperature on sex ratio in the following generation is suggestive of a temperature-sensitive disturbance in male meiosis or spermiogenesis, i.e. a form of segregation distortion (meiotic drive). It is now necessary to confirm this and to identify as precisely as possible the stage in development when the temperature effect takes place. The influence of temperature on sex ratio needs to be understood and controlled if we are to be successful in isolating lines with more strongly and consistently distorted sex ratios. Identifying factors which enhance sex ratio distortion in these lines, is a major priority in our current research programme.

Considering the breeding scheme used to isolate line #425, it is concluded that the sex ratio distortion observed could be due to a dominant genetic mutation either on the Y chromosome or one one of the autosomes. Any change to the X chromosome would not be observed because of two generations of outcrossing to unirradiated females. This breeding scheme also excludes recessive effects. Assuming that the sex ratio distorting gene acts prezygotically, male excess might be due either to an autosomal or Y-linked mutation causing loss of X chromosomes (meiotic drive) or an autosomal spermatid lethal differentially active in X spermatozoa.
It is easy to demonstrate (Saaid 1986) that an autosomal spermatic lethal with full penetrance, limited in its action to X spermatozoa, would quickly be lost (unless there was some advantage to the heterozygote). The proportion of females would rise from a minimum of 33.33% to 49.9% in 9 generations, give or take a degree of stochastic variation. Not only did sex ratio sometimes drop below 33.33% but sex ratio was maintained without regressing completely for 23 generations in #39, and for 35 generations in #425. The hypothesis of a spermatic lethal does not seem therefore to provide a reasonable explanation of what was observed. Even if its action were conditioned by temperature, it would still tend to decline in frequency.

The hypothesis of Y-linked meiotic drive is quite compatible with the maintenance of a distorted sex ratio for many generations as has been demonstrated in studies on the M gene in Aedes aegypti (Hickey and Craig 1966, Wood and Newton 1982) but for this to occur it is necessary to assume that a proportion (at least) of DIS X chromosomes is sensitive to the meiotic drive gene. Given sufficiently sensitive X chromosomes, sex ratios of less than 33.33% would be possible. In A. aegypti, strains with meiotic drive due to the D gene have shown sex ratios consistently less than 6% females (Pearson and Wood 1980). A meiotic drive gene (RD) was isolated in Drosophila melanogaster after chronic irradiation by Novitski and Hanks (1961). This was X1 linked, led to a disruption of the Y chromosome and was temperature sensitive. This, of course, gave a female-distorted sex ratio.

Catcheside and Lea (1945) had earlier found a bias in favour of males in the progeny of irradiated males of D. melanogaster mated to wild type females which they showed was due to extra damage to X spermatozoa which was not sustained by Y spermatozoa. A similar explanation was given for the same observation made in the tsetse fly Glossina morsitans (Curtis et al 1973). However this explanation cannot be applied in the present case of male excess because sex ratio distortion has been observed after two generations of outcrossing and therefore irradiated X chromosomes are excluded from consideration.

As indicated, the sex ratio distortion observed was an unexpected bonus from an experiment designed for another purpose. It must be asked, however, why the experiment produced no family with a Y-translocated R gene after screening 620 F2 families? The following possibilities may be examined:

1. The R gene may not be easily translocated because of an unfavourable location of the autosome, possibly close to the centromere.
2. Resistance may not be as simple genetically as we have supposed.
3. We may have chosen an incorrect dose of insecticide for discriminating genotypes. The correct dose can be difficult to set, particularly in the case of dieldrin because of delayed mortality.
4. We may have used the wrong irradiation dose or exposed the wrong developmental stage.

As far as (1) is concerned, we have no knowledge. As to (2), Busch-Petersen and Wood (1983) found that resistance to dieldrin and resistance to malathion were each inherited as a single major gene. But there was also a strong effect from the genetic background. It is not known whether resistance to the three insecticides (including permethrin) is controlled by the same major gene or closely linked genes. Attempts to separate
dielrin and malathion resistance by several generations of backcrossing to susceptible, applying selection with one or other insecticide in each generation, were partly successful but the result was obscured by a reversion to susceptibility (Saaid and Wood, paper in preparation). Recent tests indicate a less stable resistance to malathion in DiR than had been supposed. Instability of resistance is now thought to be the major problem. Regarding (3), the discriminating dose would not be satisfactory in the case of resistance being unstable. As to (4), we decided, after advice from Robinson and Busch-Petersen to switch irradiation to young adults and expose them at 5 krad. But the final experiment of 200 matings yielding 143 F1 families gave no different results from the earlier experiments.

References


Wood, R.J. and Busch-Petersen, E. 1982. Possible genetic sexing mechanisms for Ceratitis capitata (Wied.). Proceedings of the International Symposium on the Sterile Insect Technique and
BIOTECHNOLOGY
GENETIC SEXING
ABSTRACT

This paper reports on the current state of a project to use recombinant DNA technology, primarily that of P-element mediated transformation to alter the genetic constitution of three species of fruit flies: the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), the melon fly *Dacus cucurbitae* Coquillet, and the oriental fruit fly, *Dacus dorsalis* (Hendel). Two conditional lethal systems are being used to generate male only producing lines for use in sterile insect release (SIRM) programs: 1) a "model" system using neomycin sensitivity; and 2) a potentially "practical" system using xanthine dehydrogenase (XDH) null alleles sensitive to purine. Flies are naturally sensitive to some neomycin type antibiotics, but we have had to develop methods using mutagenesis combined with chemical generation of phenocopies to select for XDH deficient alleles. The basic research plan is to microinject plasmids which incorporate either the bacterially derived phosphotransferase gene which confers neomycin resistance or the wild type XDH allele derived from *Drosophila melanogaster* into very young eggs. Genetically transformed larvae can survive when grown on media containing either neomycin or purine, respectively.

INTRODUCTION—Genetic Control of Fruit Flies

Ideally, genetic control of fruit fly populations would be direct rather than indirectly through the use of genetic sexing to improve the sterile insect release (SIRM) method. Indeed such direct methods involving alterations in sex ratio are being investigated by groups in Italy, Argentina, and Hawaii. These sex ratio distorting systems can be found in nature or induced, or even constructed such as the proposed "contrived" sex ratio meiotic drive method of Lyttie. These direct methods may come to predominate, but, for the present, improvement of SIRM methodology is the main goal.

The rationale for genetic sexing has been discussed numerous times and I will not go over those grounds again. It may be of some interest, however, to briefly mention an hypothetical problem which has generated considerable discussion in Hawaii during a debate over a proposed large scale eradication program and for which genetic sexing could provide a solution. The debate revolved around the creation of "superflies" by improper or incomplete sterilization techniques. The "superfly" hypothesis postulates that genetic variation induced in partially irradiated, partially fertile flies could enter the wild population and produce a new race of flies more dangerous than the original population. However, for the "superfly" threat to materialize would require the occurrence of three events, two of which are highly unlikely: 1) administration of a mutagenizing, but not sterilizing radiation dose during mass rearing; 2) induction of a mutant which increases the virulence of the fly, presumably based on increased host range or
insecticide resistance; and 3) spread of this mutation throughout the wild population.

TRADITIONAL GENETIC SEXING METHODS

Proposed genetic sexing methods fall into two general classes: 1) those based on pupal color polymorphisms; and 2) systems based on conditional lethal genes (we will see that the genetic engineering methods are a special category of the conditional lethal methods). The earliest of the currently available candidates for genetic sexing involve systems based on pupal color dimorphism. At present stocks containing the autosomal recessive white pupa (\(w\)) allele of Rössler (1979) are thought to have the best chance of succeeding. This system involves a translocation between the Y chromosome and the autosome (\(Y^A\)) carrying the wild-type allele at the \(w\) locus. Female pupae are homozygous for the mutant allele and therefore white, while male pupae are brown (wild-type). This system is very successful in distinguishing the sexes by pupal color and is true breeding unless broken down by recombination. Since separation is at the pupal stage, the cost savings from rearing only males are minimal. Moreover, since as many as 100 million flies per day must be sorted mechanically, the effort and cost is high when compared to "automatic" genetic sexing methods.

While it may or may not have potential as a practical method, the pupal color sexing procedure has been used in small field cage trials to demonstrate the general value of genetic sexing procedures (Robinson et al., in press).

Proposed genetic sexing methods using conditional lethal genes have two basic components: 1) a conditional lethal gene, or chromosomal segment, that has good viability when reared under normal conditions, but is lethal when exposed to a particular discriminating environment; 2) a translocation (\(Y^A\)) between the Y chromosome and the autosome which carries the wild-type allele of the conditional lethal gene. The second step (producing the \(Y^A\)) has proven to be relatively easy; suitable conditional lethal genes have proven elusive.

The relative ease with which a genetic sexing system based on alcohol dehydrogenase (ADH) nulls could be constructed in D. melanogaster encouraged persons to attempt similar efforts with the medfly. However, the difficulties encountered in that species suggest that there may be basic genetic differences in the two ADH systems. For example, there may be more than one ADH locus (duplication) in the medfly, a condition found in several species of Drosophila (Mills et al. 1986). Duplicated ADH loci may not be coordinately regulated during development so that mutagenesis techniques that inactivate only one of these two loci will not produce a null phenotype. This may account for Riva and Robinson's (unpublished) finding that EMS point mutations do not give null phenotypes, while the deletions produced by irradiation, can.

There is a mutant allele, rosy eye (\(ry\)), in D. melanogaster which produces a brown eye, deficiency of the enzyme xanthine dehydrogenase, and an egg/larval sensitivity to the compound, purine. It has been shown that the Drosophila rosy locus and the xanthine dehydrogenase (XDH) structural locus are the same. In the medfly, a brown eye mutant, rosy (\(ry\)), has extended larval development times, high mortality, and poor adult emergence when grown on purine medium. Therefore, a series of \(Y^A\)'s involving the wild-type allele at the rosy locus were produced which achieved partially successful genetic sexing (85-90% males)(Saul, 1984). When assayed for XDH the \(ry/ry\) flies are found to be deficient, but there is no direct evidence yet that the rosy mutant in the medfly is at the XDH structural locus as it is in D. melanogaster.
SEXING BASED ON GENETIC ENGINEERING TECHNIQUES

At present, the new techniques of genetic engineering are being used to create sexing systems based on the classical conditional lethal model. There are at least two potential advantages of a genetically engineered system over a classical system even when both are based on the same conditional lethal gene. One of the potential advantages of genetic engineering methods would come from circumventing the problem of stability of chromosomal translocations in mass rearing colonies. The second potential advantage is the possibility of inserting multiple copies of the dominant wild-type gene on the Y chromosome. Theoretically, this could increase the difference in susceptibility to the conditional lethal stress between the heterozygous males and the homozygous females and make it easier to find a discriminating dose for use in mass rearing programs.

Currently proposed methods for using genetic engineering techniques all depend on the transposable P-elements, found in P strains of D. melanogaster. These P-elements are responsible for the hybrid dysgenesis which occurs in certain interstrain crosses. The technique of P-element-mediated transformation has been an efficient means of introducing functional genes into D. melanogaster (Spradling and Rubin, 1982) and has been demonstrated in the distantly related species Drosophila Hawaiiensis (Brennan et al, 1984), suggesting that it may also function in other insects.

Our research plan to demonstrate the possibility of P-element-mediated transformation in fruit flies involves the introduction of a resistance factor, in this case to neomycin, into the Y chromosome to enable males to survive exposure to a discriminating dose while susceptible females will be killed. The neo gene to be introduced onto the medfly Y chromosome is bacterially derived and encodes for a phosphotransferase which inactivates neomycin (in practice G418, or geneticin, an antibiotic related to neomycin is used).

The proposed protocol for producing a genetic sexing strain involves the co-injection of the pUCHsneo transformation vector [incorporating the hs-neo construction (Stellar and Pirrotta, 1985) composed of a neomycin resistance gene (neo) driven by the hsp70 heat-shock promotor], along with the pF25.7wc helper plasmid [which increases the stability of the transposed gene by supplying the enzyme which is necessary for integration(Karess and Rubin, 1984)].

There are several unknowns in our research: 1) is the technique of P-mediated transformation applicable to fruit flies even though it has not yet been shown in any insect outside of the genus Drosophila?; and 2) can transposition to the Y chromosome occur, even though it has not yet been demonstrated in any insect?

Also, intact P-elements in the genome produce the P-cytotype which represses P-mediated transposition in the genome of the recipient organism. Therefore, as a first step it was necessary to screen all three species of fruit flies for the presence of structures homologous to the P-element of D. melanogaster in order to determine the feasibility of transformation. This information on the presence or absence of P-element homologous structures in the DNA of the various species will also be needed to conclusively demonstrate the occurrence of genetic transformation (see below).

We conceive of the neomycin based systems as models for the possibility of transformation to the Y (or other) chromosome in tephritids. There are at least 3 reasons why these systems are not likely to be of practical use in mass rearing programs. Two reasons, the high toxicity of geneticin and its cost (approximately $200.00/gram) are usually cited as the prime objections, but it is possible that new selecting chemicals will be found to overcome these problems. To me the most serious obstacle, in the U. S. A. at the present time, is the need for a bacterially derived phosphotransferase gene in these systems. Environmental controversies
make it unlikely in the near future that a mass release of flies, sterilized or not, will be allowed containing bacterial genes. Our approach has therefore to use a method that has more chance of practical use, i.e., the rosy/purine system. While the XDH gene used for the initial testing is derived from another species, (D. melanogaster), our plan is to identify and clone the XDH structural gene within each tephritid species. This will mean that a genetic sexing method based on the purine/XDH system, will not release flies that contain genes derived from another species. We feel that this will be a critical issue when the time comes for field testing of any genetic sexing method based on genetic engineering techniques.

CURRENT STATE OF GENETIC ENGINEERING RESEARCH

Neomycin based systems, while probably not practical on a mass scale, are logical first candidates for transformation trials since they function in a wide range of species and require no precise knowledge of fruit fly genetics. Systems which use the fly's own genes are more difficult and requires detailed genetic information. In the case of XDH deficient alleles we were able to generate phenocopies using the specific enzyme inhibitor, allopurinol. Under the proper conditions this chemical can produce eye color phenotypes that mimic those of XDH deficient genotypes. We are able to combine this information with our mutagenesis programs to screen for potential XDH null alleles. Those lines derived from flies with eye colors similar to the allopurinol generated phenocopies are then assayed fluorometrically for XDH. This technique has successfully isolated several XDH deficient mutants. It should of course always be borne in mind that this does not prove that these alleles are at the XDH structural locus since regulatory genes could give the same results.

We have successfully overcome almost all of the technical problems associated with egg handling and microinjection for three species of Hawaii's fruit flies. We are able to collect large numbers of eggs less than 1 hour old, dechorionate and microinject with either of the two DNA plasmid systems and obtain high egg to adult survival. We have screened hundreds of individuals of the medfly and the two Dacus species. While there are some potential transformants, definite confirmation will require that two distinct criteria be met:

1) Stable inheritance of the trait. Survival of some individuals on a selecting media is not sufficient proof that transformation has occurred. There is a wide range of responses of insects to standard selection methods and we could be selecting for the small fraction of extremely resistant phenotypes, or we could be observing an introduced gene which is expressed, but not incorporated into the genome.

2) Incorporation of plasmid DNA. It will be necessary to not only show that the trait, neomycin or purine resistance is stably inherited, but also that the fly genome now contains P-element homologous DNA where there had been none before. This re-emphasizes the point made earlier about the need for screening fruit fly genomes for P-element homologous DNA before starting experiments in transformation.

REFERENCES


Abstract

Ongoing USDA genetic sexing research in Hawaii on the Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann), is focused in two areas: (1) the evaluation of pupal colour sexing strains for mass-production and field competitiveness characteristics, and (2) the development of a co-operative genetic engineering project involving gene transformation of a Drosophila P-element. Evidence is presented to suggest that a Hawaiianized backcross hybrid sexing strain has superior lab and field traits compared to a pure foreign strain. On the gene engineering project, microinjection and rearing methods have been developed for the medfly. Several thousand eggs have been injected with, as yet, no evidence for genetic transformation.

Introduction

In the past three years high priority research on genetic sexing of the medfly, Ceratitis capitata (Wiedemann), has progressed in two areas: (1) evaluations of existing pupal colour sexing strains (Robinsou and Van Heemert, 1982) and (2) investigations using recent genetic engineering methodology with the goal of creating a sexing strain based upon P-element transformation (Rubin and Spradling, 1982 and Steller and Pirrotta, 1985).

Pupal Colour Sexing Strains

Almost 3 years ago we imported a medfly pupal colour sexing strain, WP-23, from Dr. Alan Robinson (Research Institute, ITAL, Netherlands) and completed a lengthy series of quarantine tests to ensure safety against a potential added economic threat in Hawaii. In addition, a second pupal colour sexing strain has been created with a Hawaiian medfly genetic background by repeated backcrossing of WP-23 (or hybrid) QQ to Hawaii Lab QQ. The two sexing strains, WP-23 and WP-23 X Hawaii, have been compared with each other and with the standard lab strain regarding the following parameters: rearing (lab), mating competitiveness (field cage), dispersal (field), and longevity (field).

Rearing data were presented in detail at the last International Atomic Energy Agency (IAEA) medfly genetic sexing meeting (Vienna, July 1985, Meeting Report). The pure WP-23 strain, under our laboratory conditions, has had significantly lower rates of fecundity and pupal yield than either the Hawaii Lab or the backcross hybrid strain, WP-23 X Hawaii (Table 1). As expected, the egg fertility rates are significantly lower (60 - 80%) for both translocation-based, sexing strains compared to
the normal Hawaii Lab strain (90+%). With regard to the stability of the sexing strains — males and females emerging from normal brown and mutant white pupae, respectively — both the WP-23 and backcross hybrid lines have shown consistently excellent purity; i.e. less than 1 per 500 of undesired brown pupae females or white pupae males. However, the inherent partial sterilities of the WP-23 and WP-23 X Hawaii strains make them susceptible to contamination by fertile flies originating either from the outside or from inherent, rare genetic recombination which repairs the translocation. Such contamination could pose a serious problem for a mass-rearing operation, especially when the production facility is surrounded by a resident medfly population, as in Hawaii.

Table I: Rearing parameters for pupal colour sexing strains (WP-23 and Hawaiianized WP-23) and a normal strain (Hawaii Lab) in Hawaii (1986).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Purity</th>
<th>Fecundity</th>
<th>Fertility</th>
<th>Pupal Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(eggs/Q/day)</td>
<td>(%)</td>
<td>(% of hatched eggs)</td>
</tr>
<tr>
<td>1. WP-23</td>
<td>99+</td>
<td>15-20</td>
<td>60-80</td>
<td>75-80</td>
</tr>
<tr>
<td>2. Hawaiianized</td>
<td>99+</td>
<td>25-30</td>
<td>60-80</td>
<td>85-90</td>
</tr>
<tr>
<td>WP-23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Hawaii Lab</td>
<td>(-)</td>
<td>25-30</td>
<td>90+</td>
<td>85-90</td>
</tr>
</tbody>
</table>

Recently, we have begun field testing the WP-23 and WP-23 X Hawaii strains in outdoor cages as well as under free-release conditions. The outdoor cages (3m x 3m x 2.5m high) are each placed over a single guava tree in an orchard at a University of Hawaii field experiment station. Observations on fly behaviour inside these cages have been made in a continuing series of studies on oviposition rates (McDonald and McInnis, 1985) and on mating and survival rates of normal and sterile flies under various sex ratios (McInnis et al., 1986). Under such conditions, preliminary experiments suggest that the WP-23 strain mates with other wild (Hawaii) or laboratory strains about one-half as well as do the hybrid WP-23 X Hawaii or Hawaii lab strains (McInnis, unpub. data). We have also begun free-release experiments in an arboretum containing many species of medfly host trees. Pupae of various strains are being dye-marked and set out in the field to allow measurement of emerging adult dispersal and longevity rates. Male and female flies are trapped with trimedlure and protein bait, PIH-7, respectively. In three experiments completed to date, WP-23 males have dispersed and survived at generally lower rates than males of either the hybrid WP-23 X Hawaii, Hawaii, or wild strains (McInnis, unpub. data). So far, female data has been too limited to make a comparison. Future plans call for: (1) releasing and comparing males only, females only, or bisexual populations, and (2) releasing and comparing radiation-sterilized or normal populations. In the latter instance (2), live females will be caught in the field using modified McPhail traps and returned to the laboratory. There they will be allowed to oviposit in order to determine the frequencies of sterile vs. normal matings, and, consequently, to estimate the mating competitiveness of the sterilized, release population.
Research around the world on medfly pupal colour sexing strains is important because such strains remain to date the only viable means to achieve males-only release populations under mass-production conditions. Quantitative assessments of the role of the sterile female are needed under free-release conditions, especially in light of field cage results which demonstrated a significant positive sterile female role (McInnis et al., 1986). Continued efforts to improve the quality of pupal colour sexing strains should continue because of the uncertain outcome of currently exciting genetic engineering approaches to sexing tephritids. Based on a growing body of data, it appears that the backcross hybrid sexing strain is superior to the original, purely European strain (WP-23) for both mass-rearing and field viability parameters, at least in Hawaii. Perhaps results would be reversed if a Hawaii strain were exported to Europe and hybridized there with a native European population. Obviously, much more research is needed to know whether our experience on this matter can be generalized or not.

Genetic Engineering

For over a year now, our laboratory has been working on a genetic engineering approach to genetic sexing under a co-operative agreement with Dr. Krishna Kumaran of Marquette University. A Drosophila P-element, enclosing a bacterial gene which codes for resistance to the antibiotic neomycin, is being microinjected into medfly embryos with hopes of genetically transforming the medfly genome. Specifically, it is hoped that an active neomycin gene will insert itself into the male Y-chromosome directly, in order that only males survive in larval diet containing neomycin. However, since the Y-chromosome represents only ca. 5% of the total medfly genome, and because the Y-chromosome is largely heterochromatic (Southern, 1975), P-element insertion, if any, would likely occur on another chromosome. In such an event, the resistance gene could then be translocated to the Y-chromosome following routine irradiation induction.

The sequence of steps in the entire process is as follows:

1. P-element construction and availability.
2. Development of microinjection procedures for medflies.
3. Development of neomycin (geneticin) bioassay.
4. Assessment of degree of P-element expression, if any.
5. Assessment of degree of genetic sexing breakdown.

The P-element we are using was developed by Steller and Pirrotta (1985) and was provided to us courtesy of Dr. Krishna Kumaran of Marquette University. The P-element is carried by a plasmid vector and contains a bacterial structural gene which codes for the detoxification of the antibiotic geneticin (closely related to neomycin). Activity of this gene has been greatly stimulated by a Drosophila heat shock promoter in successful transformation experiments with Drosophila (Steller and Pirrotta, 1985). Several laboratories world-wide are now utilizing the same heat shock – neomycin P-element – in an attempt to transform embryos of various tephritid species for genetic sexing purposes.
Microinjection procedures, except for slight modifications designed to suit the medfly, are as described for Drosophila by Steller and Pirrotta (1985). Egg dechorionation (when used) and dessication methods for facilitating microinjection have been established, along with the necessary neomycin dose-larval mortality profile (McInnis, unpub. data). To date, treatments of medfly eggs to show 100% kill with neomycin have not been successful. Preliminary embryological studies, following the lead in tephritids established by Anderson (1962), have been conducted to determine optimal embryonic age for injecting P-elements into the polar ends of each egg.

Following microinjection of P-elements into embryos, the stepwise procedure over several successive generations (G₀ to G₃) is as follows below. The designations "neo" and "HI LAB" represent neomycin (geneticin) and the standard, susceptible Hawaii lab strain, respectively.

\[
\begin{align*}
G₀: \text{Injected eggs normal diet} & \rightarrow G₀ \text{ adults} \rightarrow G₀ \text{ eggs} \\
G₁: \text{G₁ eggs neo diet} & \rightarrow G₁ \text{ survivors} \rightarrow \text{Inbreed or X HI LAB} \rightarrow G₂ \text{ eggs} \\
G₂: \text{G₂ eggs neo diet + control} & \rightarrow G₂ \text{ survivors (all } \text{ and } \text{ and } \text{)} \\
G₃: \text{Repeat } G₂ \text{ procedure with control (HI LAB on neom.)}
\end{align*}
\]

To date, several thousand medfly embryos have been injected with P-element DNA without evidence for transformation, i.e. repeated survival on diet containing neomycin. The hatch rate of punctured eggs has risen greatly (5% to 25%) as egg treatment and microinjection methods have improved. Though the probability of success of this project is unknown and possibly low, the world-wide importance of developing efficient genetic sexing systems requires that great effort continues on this and other genetic sexing projects which employ gene engineering methods.

References


ABSTRACT

A genetic sexing strain of *Anopheles quadrinaculatus* Say Species A was synthesized by the induction of a male-linked translocation to create pseudolinkage between malathion resistance (mal<sup>R</sup>), a dominant trait, and sex. The translocation, T(Y;3R)1, breakpoint and the mal<sup>R</sup> locus are within a small, naturally-occurring, paracentric inversion, which effectively prevents genetic recombination in the strain. In other research on pesticide resistance, two dominant DDT-resistance loci, on separate autosomes, were identified in *A. quadrinaculatus* Species A. These two genes are also suitable for genetic sexing of this species.

In research aimed at the identification of transposable elements in *A. quadrinaculatus*, we have observed DNA sequences which show homology with P-element of *Drosophila*. We are actively investigating whether the DNA sequences which show homology with P-element are actually transposable elements.

INTRODUCTION

Currently, we are conducting a research project on the genetics of the species complex, *Anopheles quadrinaculatus* (Say), an important biting nuisance and formerly the principal malaria vector throughout the Southeastern United States. Our research efforts include analysis of variability in natural populations through the use of standard techniques for characterization of chromosomes and isozymes, hybridization of members of the species complex for the identification of potential genetic control mechanisms (e.g., hybrid male sterility), construction of genetic and cytogenetic maps, and use of recombinant DNA techniques for population analysis and in the development of germline transformation. During the course of our studies, we have encountered several pesticide resistance mechanisms, some of which are good markers for genetic crosses and/or recombinant DNA work involving transformation. They also seem to be ideally suitable for genetic sexing. Some of our colleagues have published previously (Roberts et al. 1984) on the presence...
of resistance in field populations. During the course of mapping new morphological and biochemical variants, we employ radiation-induced translocations, inversions, and deletions for the specific assignment of mutants to regions on the polytene chromosomes. In addition to the precise mapping of mutants, the synthesis of Y-linked translocations also yields genetic sexing strains.

We are in the initial stage of our molecular research, and thus far we have completed work to examine *Anopheles quadrimaculatus* in terms of genome organization (i.e., the amounts of highly repetitive, middle repetitive, and unique DNA), construction of genomic libraries for the various members of the species complex, adaptation of existing *in situ* hybridization techniques for the polytene chromosomes of the mosquitoes, and preliminary progress on microinjection of eggs and identification of homologies of DNA sequences with P-element of *Drosophila*.

We have established that *Anopheles quadrimaculatus* is actually a complex and identified three sibling species, tentatively designated as Species A, B, and C. There may be more types, but at this point three species kept separate by both premating and postmating mechanisms have been identified by studies involving hybridization experiments, chromosome analysis, and isozyme analysis.

In this present paper, we have limited our presentation to some of our work on Species A, viz., descriptions of the synthesis of a genetic sexing strain (based on malathion resistance, a radiation-induced translocation, and a naturally-occurring inversion), the identification of two DDT-resistance loci, and the results of our preliminary results in screening natural populations for transposable elements.

**MATERIALS AND METHODS**

**GENETIC SEXING STRAIN:** Two stocks of *A. quadrimaculatus* Species A were used to synthesize a genetic sexing strain: 1) STUTTGART (*mal*<sup>R</sup>) is a malathion resistant strain originally collected near Stuttgart, Arkansas in 1983 - the resistance is due to a dominant gene; 2) ORLANDO (*mal*<sup>S</sup>) strain is susceptible to malathion.

To induce a male-linked translocation adult *mal*<sup>R</sup> males (less than 24 hours old) were exposed to 7 kR gamma rays (at 1,721 R/min) and crossed to *mal*<sup>S</sup> females. The resulting F<sub>1</sub> males were backcrossed to *mal*<sup>S</sup> females. Fourth instar larvae of backcross families were treated with aqueous solutions of 400 ppm of malathion for 10 minutes. Linkage between sex and malathion resistance was taken as an indication that a reciprocal translocation had been induced. Mitotic chromosomes from adult testes and salivary gland polytene chromosomes were prepared as described by Mitchell et al. (1984) and used for cytological confirmation of suspected translocations.
The minimum concentration of malathion that could discriminate between resistant and susceptible genotypes in the egg stage was determined by treating groups of eggs less than 18 hours old with a series of malathion concentrations for 24 hours. Eggs were thoroughly rinsed with deionized water immediately after the 24-hour treatment. Mortality was determined 2 days after the treatment by counting hatched eggs. Larvae that survived the treatment were reared for confirmation of their genotype.

DDT RESISTANCE: The STUTTGART strain also has a high frequency of DDT resistance. After selection of the adults by means of topical application, dosage-response characterization of the resistance strain and the results of preliminary inheritance crosses indicated the possibility of two dominant resistance mechanisms in the selected strain. In an effort to determine the contributions of chromosomes 2 and 3 to the resistance, two new strains were developed. The X chromosome of the resistant strain was replaced by outcrossing resistant males to susceptible females. Mutant markers were employed during a scheme involving five subsequent backcrosses designed to select strains that had either a "resistant" chromosome 2 or 3; except for the chromosomal segments containing the resistance and mutant marker genes, the rest of the genome should have been replaced by the susceptible type. These two strains were assayed by topical application of DDT each generation to acquire data on the inheritance of each mechanism.

P-ELEMENT EXPERIMENTS: Eight natural populations and one laboratory stock of A. quadrmaculatus were used to identify DNA sequences which show homology with P-element of Drosophila. DNA from each population was extracted, digested with restriction endonucleases, electrophoresed with 1% agarose gel, and blotted onto nitrocellulose papers. Two large internal fragments, demarcated by the restriction sites for HindIII and HindIII/SalI, were subcloned into pUC 19 plasmids and used to probe genomic blots.

RESULTS

GENETIC SEXING STRAIN: Twenty nine F2 families showing at least 30% sterility were saved from 95 F1 males. Seven of these families showed a significant sex distortion favoring males, but only one translocation stock, T(Y;3R)1, had no females. Males of T(Y;3R)1 were backcrossed to ORLANDO females for 12 generations (Table 1), and of 10,000 backcross larvae treated with malathion, only 1 adult female was observed. She died soon after emergence so it was impossible to examine whether she was a resistant, recombinant type or a duplication-deficiency type. However, in view of the large difference between
the heterozygous and homozygous susceptible types, this female definitely was resistant.

Table I. Results of rearing \( T(Y;3R)1 \) strain treated as 4th instar larvae with 400 ppm of malathion for 1 hour.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Mean % Sterility</th>
<th>No. female</th>
<th>No. male</th>
<th>% Leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F_2 )</td>
<td>48</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>( F_3 - F_{12} )</td>
<td>36.3 ± 4.9</td>
<td>1</td>
<td>4,327</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Cytological examination of salivary gland chromosomes of \( T(Y;3R)1 \) explained the reason for the lack of genetic leakage of undesirable females in the stock. Three paracentric inversions are present in the \( \text{mal}^R \) stock; \( \text{In}-3Ra \) is located distally near the free end; \( \text{In}-3Rb \) is in the middle of the arm; and \( \text{In}-3Rc \) is proximal and adjacent to the centromere. The \( \text{mal}^S \) stock is homozygous for the standard arrangement. The \( T(Y;3R)1 \) breakpoint is located in region 23C, adjacent to \( \text{In}-3Ra \), which covers Regions 23C-24A of 3R, and this explains the lack of recombinant types that normally cause the breakdown of a genetic sexing strain that is based on the pseudolinkage imposed by a translocation.

Table 2 shows that corrected mortality of eggs of ORLANDO and \( T(Y;3R)1 \) strains were 93.4% and 51.8% at 100 ppm. There was no difference in mortality of the eggs treated with from 200 to 400 ppm of malathion solution, which killed almost all susceptible ORLANDO eggs. The insecticide treatment seemed to stimulate a few susceptible eggs to hatch so that even at 800 ppm the level of unhatched eggs never reached 100%, but none of the hatched susceptibles developed to the 2nd instar. The \( T(Y;3R)1 \) strain when outcrossed to ORLANDO females has a sex ratio slightly favoring females (female:male = 1.2:1) (Table 2). This seems to be partly the reason why the \( T(Y;3R)1 \) strain showed 60.9% - 67.6% corrected mortality when treated with concentrations of malathion higher than 100 ppm. As indicated, it was during the experiments with egg treatment that the female mentioned above was observed. Additional experiments would be required before the egg treatment could be fully implemented for mass production, but the results of our tests indicate that this should be a good method for selectively killing the females.
Table II. Effects of malathion treatment on the corrected mortality of eggs and number of adults produced from the treated eggs.

<table>
<thead>
<tr>
<th>Malathion conc. (ppm)</th>
<th>Egg mortality (%)</th>
<th>No. of adults T(Y;3R)1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ORLANDO</td>
<td>T(Y;3R)1</td>
</tr>
<tr>
<td>control</td>
<td>6.0</td>
<td>36.1</td>
</tr>
<tr>
<td>100</td>
<td>93.4</td>
<td>51.8</td>
</tr>
<tr>
<td>200</td>
<td>99.0</td>
<td>62.4</td>
</tr>
<tr>
<td>400</td>
<td>98.4</td>
<td>60.9</td>
</tr>
<tr>
<td>800</td>
<td>99.9</td>
<td>67.6</td>
</tr>
</tbody>
</table>

DDT RESISTANCE: In each of the DDT-resistant strains obtained from the backcrosses, the frequency of survivors in each generation was about 50%, a frequency which is the expected value for a dominant gene. The X chromosome was not involved in the resistance. There was a clear-cut discrimination of the susceptible and hybrid types; therefore, these resistance mechanisms could be suitable for genetic sexing.

P-ELEMENT EXPERIMENTS: Southern blot profiles showed that sequences homologous with both subclones of P-element are present only in field populations. Genomic libraries of the three sibling species of A. quadrimaculatus have been prepared, and we are currently involved in cloning the DNA sequences homologous to P-element. This will be followed by in situ hybridization experiments and further characterization to determine whether these sequences are indeed transposable elements.

DISCUSSION

Perusal of scientific literature indicates that synthesis of genetic sexing strains of dipteran species via the use of insecticide resistance is relatively easy, but usually in the reports on the systems constructed by using classical genetic and cytogenetic techniques, genetic leakage of undesirable types is a problem. The potential problem of accidentally releasing resistance genes into a natural population is not of great concern when resistance is already widespread, as is the case for A. quadrimaculatus. If leakage is perceived as a problem, the effect of the small, naturally-occurring inversion in suppressing leakage in T(Y;3R)1 can certainly be copied by using radiation-induced inversions. For scientists skilled in classical insect breeding, this task would not be difficult.
In the case of Medfly, the possibility of genetic leakage, which could be accompanied by concomitant release of resistance genes, has precluded interest in using pesticide resistance mechanisms as a conditional mechanism for genetic sexing of this species. This situation, of course, should be of major concern, and the general consensus to avoid pesticide resistance for Medfly is probably justifiable scientifically and most certainly, politically.

Genetic leakage is not limited in its impact to those situations where it is necessary to avoid the accidental release of an undesirable genotype. Controlling leakage is very important in maintaining the contrived arrangement of the genome of a genetic sexing strain during mass production. Recent advances in molecular techniques open new avenues for the synthesis of genetic sexing strains, and indeed for the development of genetic control systems that will be quite different from the sterile insect technique, but the full utilization of recombinant DNA methods for the genetic engineering of operational control methods is still futuristic, and at least for the near future conventional use of the sterile male technique for Medfly control will be continued. Until new systems based on molecular manipulations come on line, it is possible to use a combination of the old and the new, i.e., use germline transformation to insert a conditional mechanism and classical techniques to establish pseudolinkage and to control recombination. In that context, the use of natural or induced paracentric inversions for maintenance of specific gene arrangements will be of value.

REFERENCES CITED

DEVELOPMENT OF INDUCED SEX-SEPARATION MECHANISMS IN Ceratitis capitata (Wied.): EMS TOLERANCE AND SUPPRESSION OF FEMALE RECOMBINATION

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Abstract

Two essential prerequisites for the induction and isolation of recessive mutants, namely mutagen tolerance and suppression of recombination, were investigated in the medfly, Ceratitis capitata. Procedures were investigated for the application of the chemical mutagen, EMS, to developmental stages from eggs to 2-day old adult males. Treatment of adult males with EMS, mixed in a 10% solution of sugar in the drinking water supply, was found to be the optimal procedure. This treatment produced dominant lethality in sperm in direct correlation to the concentration of EMS, without being toxic to the treated adults. None of the other procedures was equally effective.

A series of female recombination suppressors (RS) was induced through gamma irradiation of pupae and isolated through appropriate breeding schemes. Earlier chromosomal analysis had confirmed the presence of reciprocal autosomal translations in all RS lines. It is suggested that recombination between the ap and dc genes is being suppressed in the interstitial segment between the centromere and the translocation breakpoint. The degree of recombination suppression in RS 30/55 was 98.1%. Viability, when measured as mean egg hatch in the heterozygous and homozygous configurations, was 53.4% and 79.0%, respectively.

Introduction

The desirability and advantage of incorporating a genetic sex-separating (GS) mechanism into the sterile insect technique (SIT) for the control or eradication of the medfly, Ceratitis capitata (Wied.), thereby enabling the production and release of only males, has been widely publicised (Boller, 1979; Wood and Busch-Petersen, 1982; Rössler, 1982; Busch-Petersen and Wood, 1983, 1986; FAO/IAEA, 1983, 1985; Robinson et al, 1986). Apart from a consideration of the operational advantages and potential merits of employing such GS strains, attention should equally be given to factors such as the adaptability of existing rearing technology, which may need to be modified to a greater or lesser extent, the financial burden of utilizing, maintaining, and operating such strains, and the ease with which the genetic background of such strains may be altered to suit the local conditions, the potential genetic stability of such strains, and the effect of the sexing procedure on the possibility of recycling waste material. Unfortunately, however, existing knowledge of medfly genetics does not leave much choice in the selection of a suitable GS mechanism. The only presently existing possibilities rely on pupal colour dimorphisms (Rössler, 1979; Robinson and van Heemert, 1982) or on purine sensitivity.
Both mechanisms are potentially useful, but have limiting disadvantages. The former mechanism would not achieve any financial saving in larval rearing, which, in the planned Central American medfly eradication campaign, has been estimated at totalling US $30 million (D.A. Lindquist, personal communication), while the latter would be prohibitively expensive for purine, unless an alternative and cheaper chemical could be found.

As a possible alternative to existing mechanisms the Joint FAO/IAEA Division has initiated a programme aimed at inducing and isolating a series of temperature sensitive lethal (tsl) factors. Suitable induction and isolation procedures were designed to register only tsl factors active during the egg or early larval stages, thus promising a maximum financial return at a minimal operational cost.

tsl mutations are very common in microorganisms (Edgar and Lielausis, 1964), insects (Suzuki et al, 1967; Smith, 1967), and mammals (Daneel, 1941), and have commonly been shown to be the consequence of a single amino acid substitution in a polypeptide (Wittmann et al., 1965), that alters the biological activity of the protein at different temperatures (Jockusch, 1966). Theoretically, it should, therefore, be possible to induce a tsl mutation at any locus involved in the production of a protein, thus rendering such loci potentially very common throughout the length of any one chromosome. Indeed, Suzuki (1970) registered 150 tsl factors on the Drosophila melanogaster X chromosome. tsl mutations are equally common on the autosomes and have been found to account for 10-12% of all ethyl methanesulfonate (EMS) induced lethal mutations (Suzuki, 1970). In a study on the characterization of tsl mutations in Musca domestica, McDonald and Overland (1972) recovered 10 tsl mutations among 900 EMS treated chromosomes. It is thus obvious that, were appropriate EMS treatment procedures and suitable genetic strains available in C. capitata, large numbers of tsl factors could be induced and isolated with relative ease, and subsequently screened for their suitability as GS mechanisms.

The present report deals with the EMS tolerance of the medfly, and describes the isolation of a strain in which female recombination is suppressed.

Materials and methods

EMS treatment

Pure EMS (Merck, 98%) was diluted in a 10% sugar solution of distilled water and fed to adult males in concentrations ranging from 0.02% to 0.12% EMS. Prior to the treatment, flies were separated by sex within 16 hours after emergence to ensure adult virginity. The males were placed in a cage without food and water, while the females were placed in a separate cage with standard nutrients. After one day of starvation, the males were offered for 24 hours appropriate concentrations of EMS in their water supply. After exposure to EMS, the males were supplied with standard food and water, and left for a further 24 hours before being placed in single pairs with untreated females. The effects of the
treatment on parental male mortality, total number of F1 eggs laid, egg hatch, pupal production, and adult sex ratio were recorded.

Recombination suppression

Homozygous "double chaetae" (dc) mutant males were irradiated one day after emergence with 50 Gy of gamma irradiation and mated to virgin "apricot eye" (ap) females. F1 females were subsequently crossed in single pairs to homozygous ap dc males, and the F2 offspring was screened for families containing only the parental phenotypes. Such families were then crossed in single pairs in an attempt to isolate homozygous RS strains, or conditional lethal strains in which the effects of lethal breakpoint homozygosity were eliminated. This was followed by further inbreeding of the dc phenotypes until three consecutive generation had been observed to breed true for the dc allele. The RS families were then isolated and mass reared.

The absence of the non-irradiated ap dc chromosome was confirmed by crossing the RS strains to ap and screening the F1 for the absence of ap phenotypes. The F1 was then further crossed in both directions to the ap dc stock, and recombination frequency between the ap and dc alleles was determined. Only results with the RS 30/55 strain are described here.

Results

EMS treatment

EMS, when supplied to adult 2-day old males in a 10% sugar solution of the drinking water supply, had no toxic effect on the treated males, nor did it influence the proportion of females, mated to treated males, which produced hatching eggs at concentrations of EMS below 0.12% (Table 1). A direct correlation ($\chi^2 = 2.29; P < 0.1$) was observed between the administered dose of EMS and the induced dominant lethality when measured

<table>
<thead>
<tr>
<th>EMS concentration (%)</th>
<th>0.02</th>
<th>0.04</th>
<th>0.05</th>
<th>0.08</th>
<th>0.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>% parental male mortality</td>
<td>1.4a</td>
<td>0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
</tr>
<tr>
<td>% fertile parental pairs</td>
<td>100a</td>
<td>100a</td>
<td>97.8a</td>
<td>97.4a</td>
<td>98.7a</td>
</tr>
<tr>
<td>% corrected F1 survival: hatch</td>
<td>100a</td>
<td>86.5b</td>
<td>63.8c</td>
<td>28.5d</td>
<td>0.0e</td>
</tr>
<tr>
<td>puppies</td>
<td>100a</td>
<td>100a</td>
<td>94.9b</td>
<td>97.1ab</td>
<td>-</td>
</tr>
<tr>
<td>adults</td>
<td>100a</td>
<td>100a</td>
<td>100a</td>
<td>98.7a</td>
<td>-</td>
</tr>
</tbody>
</table>

1) same letter after percentage indicates no significant difference between means of rows (t-test).
Figure 1. Dominant lethality, expressed as F1 egg sterility, induced in medfly males treated as adults with EMS (24 h.) in a 10% sugar/distilled water solution.

as sterility of F1 eggs (Figure 1). There was no correlation of the treatment with subsequent pupation and adult emergence from hatched eggs and from pupae, respectively (Table 1).

Recombination suppression

Strain RS 30/55 was obtained by crossing the isolated heterozygous RS 30 line with the heterozygous RS 55 and continuously selecting the dc phenotype. Selection was done for 4 generations, after which the strain bred true for the dc phenotype. A single non-parental fly was found among the offspring of the cross between RS 30/55 and ap/ap (Table 1). However, as this fly was homozygous both for the ap and dc alleles, and thus could not have originated from the above cross, it must have come from one of the other rearing containers. The parental RS 30/55 was therefore considered to be homozygous for the irradiated dc chromosome. Subsequent cytological analysis revealed the presence in this strain of a reciprocal
Table 2. Summary of results obtained during the isolation of recombination suppressor line RS 30/55 in C. capitata

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ap phenotypes in F1</td>
<td>0.03</td>
</tr>
<tr>
<td>female recombination</td>
<td>0.35</td>
</tr>
<tr>
<td>male recombination</td>
<td>0.17</td>
</tr>
<tr>
<td>degree of recombination suppression</td>
<td>98.1</td>
</tr>
<tr>
<td>homozygous viability 1)</td>
<td>79.0</td>
</tr>
<tr>
<td>heterozygous viability 1)</td>
<td>53.4</td>
</tr>
</tbody>
</table>

1) corrected for control mortality and expressed as a percentage of egg hatch.

The observed viability of RS 30/55 is shown in Table 2. Homozygous viability was significantly reduced, as compared to the standard dc strain, when measured as egg hatch ($t = 8.04; P < 0.01$), whereas no significant difference was observed during subsequent development ($t = 1.00; P > 0.05$). A much more drastic reduction in egg hatch was observed in the heterozygous configuration, both as compared to the control ($t = 11.2; P < 0.001$) and to the RS 30/55 homozygote ($t = 11.1; P < 0.001$). Mean adult emergence in the heterozygous configuration was significantly below that of the control ($t = 4.27; P < 0.01$), but not significantly different from that of the homozygote ($t = 0.77; P > 0.05$).

Discussion

Three criteria were established as requirements of a suitable mutagenic treatment procedure: a) uniform uptake by treated males, to produce a predictable level of fertility in parentally mated females, b) no toxicological effect on treated males with regard to survival and mating efficiency, and c) correlation between EMS concentration and induced F1 lethality. Of 10 procedures tested (Busch-Petersen et al., in press), only adult feeding in 10% sugar-water met all three criteria. This treatment procedure is now being employed in a programme aimed at the induction and isolation of temperature-sensitive lethal factors in C. capitata.

The induction and isolation of recombination suppressors in the medfly resulted in the production of 6 RS strains. Only RS 30/55, being representative of the other strains, is described here. A single reciprocal autosomal translocation was present in each strain (Busch-Petersen and Southern, in preparation). Translocation heterozygotes are known to suppress recombination between marker genes located in the interstitial segment through the production of aneuploid gametes during alternate and adjacent-2 segregation. However, the degree of recombination suppression observed in RS 30/55 is so high that it is unlikely to be caused solely by this mechanism. A more plausible
explanation appears to be that translocation heterozygosity somehow interferes with the initiation or maintenance of cross-over synapsis, thus preventing the occurrence of cross-overs in these regions rather than merely eliminating cross-over chromatids.

The viability of the RS 30/55 heterozygote was significantly lower than that of the homozygote. Such a reduction in viability is inherent in translocation heterozygotes, and is caused by the genetic imbalance of gametes arising through adjacent-1 and adjacent-2 segregation of the translocated chromosomes during cell division. The resultant sterility may vary widely, but commonly averages around 50% (Robinson, 1976; Roberts, 1975). In translocation studies in Anopheles albimanus, Kaiser et al (1983) found the mean egg hatch to range from 54.4% to 64.6%, while Steffens (1983), in isolating heterozygotes in C. capitata through screening for reduced egg hatch, found the viability to range between 26.1% and 54.6%. This compares to a mean egg hatch of 53.4% in RS 30/55. This line is now employed for the suppression of recombination in a breeding scheme designed for our tsl isolation programme.

Acknowledgements

This work forms part of a joint FAO/IAEA programme on the development of genetic sexing mechanisms for the Mediterranean fruit fly, Ceratitis capitata (Wied.). The technical assistance of A. Hafner, A. Pyrek, J. Ripfel, E. Schron and I. Szabo is gratefully acknowledged.

References


Smith, R.H. (1967): Dominant and recessive lethal mutations induced by mitomycin C in Habrobracon oocytes and sperm. Genetics, 56: 591 (abstract only)


The report discusses studies aimed at developing "genetic sexing" systems based on differential sex-limited susceptibility to insecticides, or other chemicals. Selection of potassium-sorbate (PS) resistant medfly strains was carried out, with levels of 0.4% and 0.7% PS in the larval diet. The 0.7% PS selected strain is being reared now for 35 generations. We have isolated a highly inbred line through repeated sib-crosses and exposure to PS in the larval diet. The mode of inheritance of the PS resistant trait was investigated in that line. We also attempted to localize the PS locus on the chromosomes by a scheme of crosses with the available morphological mutant strains. We failed to show any linkage of the trait to any of the chromosomes tested. It seems that the trait is controlled by few loci distributed on more than one chromosome (or that the locus is on the yet unmarked chromosome). We started selection of medfly strains to avermectin, after establishing the discriminating dose for same, (50 ppm solution, for 1 - 2 hours, with freshly laid eggs). That dose resulted in over 95% mortality, and mortality was manifested in reduced egg hatch as well as in later larval stages. We completed 6 selection cycles and increased total survival from 6.4% to 67.5% (using the 50 ppm solution). We started establishing base-line data for selection work with cyromazine (a new s-triazine compound, highly effective as a dipterous larvicide). Levels of less than 4 ppm in the larval diet resulted in total mortality of the exposed flies.
SCREENING FOR A FEMALE-LIMITED TEMPERATURE-SENSITIVE LETHAL MUTATION INDUCED ON A Y-AUTOSOME TRANSLOCATED STRAIN IN *CERATITIS CAPITATA*

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Abstract

The aim of this paper is to describe a screening method for a mutation causing the elimination of females in an early stage of development sought after improvement of the Sterile Insect Technique.

A strain carrying a Y-nig traslocation (all the females are homozygous for the cuticular marker nig and all the males are wild-type) was previously obtained. Such strain shows no evidence of marker severing due to a recombination after more than 55 generations of culture in population cages. The temperature of 35°C was chosen as the restrictive treatment and the most appropriated TSP (Temperature Sensitive Period) was determined to be at the time of cephalic involution during embryogenesis. The permissive temperature was 26°C.

The most convenient mutagenic treatment was found to be provided by 2% EMS acting on eggs during 24 hours. The actual screening involved treating nig/nig females with EMS, crossing them to Y-nig males, selecting males in the progeny and crossing them individually, first to unrelated nig/nig females and later to his daughters. The discriminating treatment was received by a sample of embryos produced in the backcross father x daughter. Families showing a 50% deficit in female progeny after the temperature pulse are candidates to carry the desired mutation.

At present, several families have been already obtained and they are in the process of being further characterized.

Also a number of families probably carrying lethal mutations in the X-chromosome have been obtained.

Introduction

There is a general agreement among the scientists working in the development of a sexing mechanism for the Mediterranean fruit fly about the importance of every effort addressed to the isolation of conditional lethal mutations which, in turn, will be used for the elimination of females (IAEA 1985).

Several approaches have been tried out or are in progress (Saul 1984, Rossler 1979 and Bush-Petersen and Wood 1983). In this report a different one is proposed involving first, the isolation of a traslocation, and then the induction of a temperature sensitive mutation using in both steps a pupal color genetic marker.

A number of advantages have been foreseen in this new approach:

1. The isolation of Y-chromosome traslocation has proved to be quite feasible provide a suitable marker is available in the given autosome (Rossler 1979, Lifschitz et al. 1983) and a lot simpler than the cumbersome isolation of a system of inversions (Bush-Petersen 1985).
2. Temperature sensitive (ts) lethal mutations have proved relatively easy to isolate in other species (Suzuki 1976) and they are widespread through the genome, whereas those mutations sensitive to a particular chemical, (Adh, Purine, Insecticide), will probably show a single or a low number of target for the induction of a point mutation.

3. A further advantage of the use of ts mutations comes from the possibility to induce them having a temperature sensitive period (TSP) or an effective lethal phase (ELP) almost at any time along development. In particular, it would be possible to obtain an embryonic lethal phase with considerable savings in larval food and care.

4. With the ever increasing number of markers and at least one marker available in every chromosome (Saul et al. 1984) the screening protocol proposed in the present communication will be probably useful for other laboratories working in different countries.

Building - up of a strain with sex limited inheritance

The genetic marker niger (Manso and Lifschitz 1979) showed invaluable for the purpose of the present investigation because not only mutant adults and mutant pupae are readily distinguishable from wild-type ones but also mutant larvae can be separated using their dark colored posterior spiracles (unpublished observations). Obviously, the earlier both sexes could be distinguished, the better; that is why this marker was used in order to isolate a Y-autosome traslocation.

For that purpose wild type 5-6 day old pupae were subject to 3 K of x-rays radiation. The males emerging from these treated pupae were crossed in mass to females from a strain homozygous for the recessive pupal color mutant nig (Manso and Lifschitz 1979). Then, the F1 males were singly mated to virgin females from the maternal strain. Those families producing only nig males and nig females were screened for. One particular line, identified as T(Y-nig) 5038 (hereafter T5038) was further studied. Several samples of pupae from this strain were recovered during the first 15 generations and the sex of the imagos were checked upon emergence. Overall 6173 wild type pupae produced only males and 5383 mutant pupae produced only females with no exceptions. A significant (P<0.01) sex ratio deviation (♂:♀=0.81), homogeneous along the generations, was observed. The hatchability of the strain T-5038 was investigated in an experiment in which a number of single mates were set and the percentage of egg hatching was scored individually. The average hatching for the strain was 68.2±1.43% but it is worth to notice that 8 families out of 28 did not produce a fertile progeny which is consistent with an expectancy 50% sterile adults in both sexes or 30% sterile individuals in one sex.

The strain T 5038 showed no evidence of marker severing due to genetic recombination after 55 generations of culture in population cages, so it was deemed as suitable for the next step.

The mutagenic treatment

The previous knowledge in our laboratory indicated that the dispensing of the mutagen to larvae, although it was successful in the production of new morphological mutations, was a procedure with a low repetibility and a very uneven dosimetry. A satisfactory improvement has recen-
tly been achieved by immersion of newly laid eggs in a solution containing the appropriate concentration of the mutagen. In the present experiment EMS [Ethyl Methane Sulfonate] has been used in a water solution kept at room temperature on a shaker for 24 hours. Sample of ± 200 eggs collected from a population cage containing flies of the strain T5038 received growing concentrations of EMS. The rates of survival to larvae (%) hatching and to adult (%) pupal emergence obtained after the treatments are shown in Fig. 1. The hatching level of the control sample in this experience (66%) was close enough to the normal for the strain T5038. The results in Fig. 1 indicate that concentrations of EMS higher than 1.5% are needed to notice any effects on hatching and that only 3% gives an important reduction in percent of hatch and percent of adult survival. These results suggest that a concentration of EMS ranging 1.5-2.0% gives a tolerable level of toxicity but probably it is high enough to yield a good frequency of (preferentially) point mutations. As a means of having an idea of this frequency a pilot small scale experiment was conducted with flies of the strain T5038 aimed to monitor the induction of dominant lethal mutations linked to the X-chromosome. In this experiment newly laid eggs were exposed to two concentrations of EMS and after one generation of mass rearing females were individually mated to T5038 males and the sex ratio was scored in the progeny of each couple; The results pointed out that with 0.2% EMS, 7.3 ± 4.1% pairs produced a progeny significantly biased to the females and the same experiment when the EMS concentrations was raised to 1.5% yielded 20.7 ± 5.6%.

The restrictive temperature and TSP

The limit of tolerance to heat pulses during embryogenesis has recently been investigated and will be explained in detail elsewhere (Cladera an Manso, in prep.). In summary, it is reported there that the three major events during embryogenesis: pole cells migration, cephalic involution and mouth hooks chitinization, could be timed and the observation of the chitinous mouth hooks inside unhatched eggs (Fig. 2) could be used to monitor deleterious effects of early pulses on late embryogenesis. Those results indicated that early embryos are more sensitive than older ones and that by the time of the head involution it was possible to apply a higher temperature pulse with a better survival rate, compared to earlier embryos. The exposure to 35°C during a 15 hours period of 16-hours embryos cultured at 26°C - right before the beginning of the head involution (Fig. 3) - showed, after several experiments, to be close enough but still below the limit of tolerance of this strain (Cladera and Manso, in prep.).

Screening protocol

The actual screening (Fig. 4) involves treatment of vig/vig females with EMS, crossing them to T V-vig*) males, picking up males in the progeny and crossing them individually, first to unrelated vig/vig females and later to his daughters. The discrimination treatment was then received by a sample of embryos produced in the backcrosses father X daughter. Those families showing a 50% deficit in female progeny after the temperature pulse are likely to carry the desired mutation. A second sample of embryos
Fig. 1.- Survival rates of T5038 embryos after a mutagenic treatment with different concentrations of EMS compared to the levels of untreated embryos.

Fig. 2.- Unhatched embryo transparented with the technique of Cladera and Manso (in prep.) in order to show the mouth hooks (m.h.) and pharyngeal bars (p.b.). 125 X.
Fig. 3. - Diagram showing some events during the embryogenesis timed at 26°C and the temperature pulse (thick arrow) used in the screening procedure.

Fig. 4. - Set of crosses involved in the screening procedure described in the text.
served both as a control and a source of parents for the the establishment of the female-limited ts lethal strain. When a family was classified as a putative ts, single pair matings were set with individuals from this second sample. The probability is that 1 out of 4 such families should be 100% ts female lethal. The first results obtained using the above described procedure, and summarized in Table I, were very encouraging. Although a low (ave. 15%) proportion of the families went through the whole scheme a very reasonable 12% positive results were found out (Table 1).

Finally, it must be reported that the temperature test was performed again on the progeny of those six families giving significantly positive results in the previous generation. As a results of this test they were classified as faste positives except in one family where noticeable reduction in the female progeny was observed after the temperature pulse. This line was set aside for multiplication and further studies. Whether this line proves useful for autosexing purposes or not, the fact remains that the screening procedures described in the present paper proved feasible; its success will rely on a substantial increase in both the initial number of couples and the proportion of those couples that successfully complete the screening procedure.

### TABLE I. Initial results of the screening

<table>
<thead>
<tr>
<th>Initial numbers of pairs</th>
<th>Number of families scored</th>
<th>Number of putative ts</th>
</tr>
</thead>
<tbody>
<tr>
<td>108</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>74</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>144</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>326</td>
<td>50</td>
<td>6</td>
</tr>
</tbody>
</table>

Acknowledgement

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References


THE USE OF ALLYL ALCOHOL AS DISCRIMINATING AGENT FOR GENETIC SEXING IN THE MEDITERRANEAN FRUIT FLY 
CERATITIS CAPITATA

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Abstract

Allyl alcohol is being used as a discriminating agent to selectively kill females at an early developmental stage in C. capitata. Several strains with different alcohol dehydrogenase (Adh) electrophoretic patterns have been assayed for their resistance to this alcohol when added to the larval rearing medium. One of this strains, T128 showed survival of only males at certain concentrations. This strain carries a translocation linking an Adh null mutation to the Y chromosome of the males. Data on the fertility, sex ratio and other life parameters of this strain are presented and its suitability for use in mass rearing of only males is discussed.

Introduction

A genetic sexing system for Ceratitis capitata using the alcohol dehydrogenase locus has now been developed. The discriminating agent used in this system is allyl alcohol. This alcohol can be used in a similar way to the secondary unsaturated ones, 1-pentene-3-ol and 1-pentyne-3-ol (Sofer and Hatkoff, 1972 and O’Donnell et al., 1975) which are converted into toxic ketones by the enzyme Adh. This conversion does not take place in individuals lacking Adh activity, which consequently show higher resistance to these type of alcohols than wild type individuals with full Adh activity.

A translocation linking an Adh null mutant to the Y chromosome was isolated (Robinson et al., 1986). In this strain, T128, males are always heterozygous null/positive for Adh (Y-Adh^N/Adh^+ ) and show reduced Adh activity. It was isolated to serve as a marker for identifying new Adh null mutants with relative ease (Robinson and Riva, 1984). However, subsequent experiments showed that it had a great potential as a genetic sexing system (Robinson et al.,1986) but in an inverse way to the previously described method by Robinson and van Heemert (1984) for Drosophila and Rivà and Robinson (1983) for the Medfly.

There are two main aspects with which this report is concerned, first the discriminating property of allyl alcohol to selectively kill females of strain T128; and second the suitability of T128 as a genetic sexing strain in mass rearing.

Materials and Methods

Rearing: As in Robinson and van Heemert (1982).

Strains: +/-; wild type strain originally from the Seibersdorf IAEA laboratory, this strain is homozygous for the S allele at the Adh locus. T128, (Y-Adh^N), a translocation strain linking an alcohol dehydrogenase null allele (N) to the Y chromosome (Robinson et al., 1986). Adh^S/Adh^S and Adh^N/Adh^N, two strains homozygous for the S and F Adh alleles respectively; they were reisolated from the +/- and FF strains from the ITAL laboratory, Wageningen, making reciprocal crosses to homogenize their genetic background for the experiments,
they are highly inbred as they descend from two and six pairs of flies respectively.

Life parameters: Pupal survival and sex ratio were recorded for T128 from the population cages in which it was normally reared. Egg fertility was measured for all the possible type of crosses among +/+ , SS, FF and T128 by mating 4 day old virgin adults (6♂ x 12♀) and collecting eggs three times.

Allyl alcohol resistance: Allyl alcohol was added to the larval rearing medium. The constitution of this medium was as follows: 10.66g. carrot powder, 4g. yeast, and 62.5cc. of liquid, consistent of: 58.06cc. water, 3.55cc. C1H 1N, 0.40cc. 4% formaldehyde and 0.48cc. propionic acid. To this liquid, allyl alcohol was added in different quantities: 0.0μl (control); 20μl (0.032%); 30μl (0.048%); 45μl (0.072%); 50μl (0.080%); and 60μl (0.096%). One hundred eggs (up to 48h. old) per replicate were placed on a strip of black filter paper on a glass Petri dish containing larval medium. The lids were maintained on the Petri dishes for seven days and then removed and egg hatch counted. The dishes were then transferred to a larval rearing box. Larvae were allowed to develop for as long as twenty days, pupae were then collected, allowed to emerge and sex ratio was noted.

Results

Life parameters: Strain T128 is routinely maintained by outcrossing the males (Y:NS or Y:NI) to FF or SS females each generation alternatively, as shown in Figure 1. In this way the strain is characterized by heterozygous females (FS) which show three electrophoretic bands, and males (FN or SN) that only have one band (Riva and Robinson, 1986) and it can be easily checked for contamination, recombination or translocation breakdown. The strain shows some difficulties in the rearing, in some cases with a very low overall survival,

<table>
<thead>
<tr>
<th>Generation</th>
<th>♂♂</th>
<th>♀♀</th>
<th>Progeny Adh genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>T128(Y-N/+ x SS \rightarrow Y-NS</td>
<td>Y-NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>allyl al.</td>
<td>SS</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>T128(Y-N/+ x FF \rightarrow Y-NF*</td>
<td>Y-NF*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>FS</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>T128(Y-NF) x SS \rightarrow Y-NS**</td>
<td>Y-NS**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>FS</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>T128(Y-NS)** x FF \rightarrow Y-NF</td>
<td>Y-NF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>FS</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Mating scheme followed to routinely maintain translocation T128 with electrophoretically distinguishable males and females. The allyl alcohol experiment was carried out on generation 22.
and a consistent excess of females. Table I shows the data on pupal emergence and adult sex ratio for generations 22 to 31 after the translocation was induced and isolated. It can be seen that pupal emergence is higher when the translocation males are crossed to +/+ females. The highest pupal survival (83.6%) is when the males had also been outcrossed to +/+ females the previous generation and were thus, Y-N/+ . The wild type strain, +/+, has very high survival parameters for every life stage (overall survival, egg to adult, 88%). There is always, except in one case, a significant deviation from the 1:1 sex ratio, with an excess of females; in some cases the ratio (d♂:q♀) was as low as 0.5.

Table I. Adult sex ratio and pupal emergence of translocation T128 (Y-Adh^N) maintained in large populations.

<table>
<thead>
<tr>
<th>Parental cross</th>
<th>Progeny genotype</th>
<th>Generation</th>
<th>No. pupae</th>
<th>Pupal emerg. %</th>
<th>No.♂</th>
<th>No.♀</th>
<th>X^2</th>
<th>1:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-N/+</td>
<td>+/+</td>
<td>Y-N/+</td>
<td>22</td>
<td>1713</td>
<td>83.6</td>
<td>579</td>
<td>853</td>
<td>52.4***</td>
</tr>
<tr>
<td>Y-NS</td>
<td>+/+</td>
<td>Y-N/+</td>
<td>28</td>
<td>1420</td>
<td>65.9</td>
<td>358</td>
<td>577</td>
<td>51.3***</td>
</tr>
<tr>
<td>Y-NF</td>
<td>+/+</td>
<td>Y-N/+</td>
<td>28</td>
<td>961</td>
<td>66.5</td>
<td>258</td>
<td>381</td>
<td>23.7***</td>
</tr>
<tr>
<td>Y-N/+</td>
<td>+/+</td>
<td>Y-N/+</td>
<td>31</td>
<td>1392</td>
<td>70.0</td>
<td>309</td>
<td>617</td>
<td>102.4***</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>5486</td>
<td>70.0</td>
<td>1504</td>
<td>2428</td>
<td>217.1***</td>
<td></td>
</tr>
<tr>
<td>Y-NF</td>
<td>SS</td>
<td>Y-NS</td>
<td>26</td>
<td>533</td>
<td>56.3</td>
<td>110</td>
<td>190</td>
<td>68.9***</td>
</tr>
<tr>
<td>Y-NF</td>
<td>SS</td>
<td>Y-NS</td>
<td>27</td>
<td>893</td>
<td>61.8</td>
<td>216</td>
<td>336</td>
<td>26.1***</td>
</tr>
<tr>
<td>T-NF</td>
<td>SS</td>
<td>Y-NS</td>
<td>28</td>
<td>711</td>
<td>65.7</td>
<td>220</td>
<td>247</td>
<td>1.6n.s.</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>2137</td>
<td>60.0</td>
<td>546</td>
<td>773</td>
<td>39.1***</td>
<td></td>
</tr>
<tr>
<td>Y-NS</td>
<td>FF</td>
<td>Y-NF</td>
<td>25</td>
<td>257</td>
<td>51.2</td>
<td>94</td>
<td>140</td>
<td>9.0**</td>
</tr>
<tr>
<td>Y-NS</td>
<td>FF</td>
<td>Y-NF</td>
<td>26</td>
<td>1216</td>
<td>52.7</td>
<td>243</td>
<td>398</td>
<td>37.5***</td>
</tr>
<tr>
<td>Y-NS</td>
<td>FF</td>
<td>Y-NF</td>
<td>27</td>
<td>1096</td>
<td>65.2</td>
<td>230</td>
<td>484</td>
<td>90.9***</td>
</tr>
<tr>
<td>Y-NS</td>
<td>FF</td>
<td>Y-NF</td>
<td>28</td>
<td>531</td>
<td>57.4</td>
<td>131</td>
<td>174</td>
<td>6.1*</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>3100</td>
<td>60.0</td>
<td>698</td>
<td>1196</td>
<td>130.9***</td>
<td></td>
</tr>
</tbody>
</table>

To have an estimation of the effect of male and female genetic background on the egg fertility of T128, all possible types of crosses were set up among +/+, SS, FF and T128. Six virgin males and twelve virgin females, all of them 4 days old, were crossed for each mating type. Eggs were collected four, five and six days later. Results are shown in Table II. It is clear that +/+ females produced a much higher number of eggs and larvae than any other type, and also the +/+ males produce more larvae than any of the other males. T128 behaves very differently depending on its parental type, when this is Y-N+, the egg hatch is almost as high as the control, but fertility drops when the parental type is Y-NS or Y-NF. This agrees with the fact that T128 is easier to rear when outcrossed to +/+ , and the number of pupae reared per generation is higher in routine population keeping (table I). SS and FF have very low fertility, probably due to their inbreeding. When T128 (Y-NF) males are backcrossed to FF females, the egg hatch is as low as 5.2%.
Table II. Egg hatch of strains T128, +/+, SS and FF and their respective crosses. Data from cages with 6 ♂ x 12 ♀, three egg collections per cage.

<table>
<thead>
<tr>
<th>♂♂ (Gen.)</th>
<th>♀♀</th>
<th>+/+</th>
<th>SS</th>
<th>FF</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-N/+ (28)</td>
<td>No. eggs</td>
<td>491</td>
<td>144</td>
<td>452</td>
<td>1087</td>
</tr>
<tr>
<td></td>
<td>No. larvae</td>
<td>387</td>
<td>128</td>
<td>330</td>
<td>845</td>
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<tr>
<td></td>
<td>Egg hatch (%)</td>
<td>78.8</td>
<td>88.9</td>
<td>73.0</td>
<td>77.7</td>
</tr>
<tr>
<td>Y-NS (28)</td>
<td>No. eggs</td>
<td>619</td>
<td>38</td>
<td>221</td>
<td>878</td>
</tr>
<tr>
<td></td>
<td>No. larvae</td>
<td>195</td>
<td>11</td>
<td>157</td>
<td>363</td>
</tr>
<tr>
<td></td>
<td>Egg hatch (%)</td>
<td>31.5</td>
<td>29.0</td>
<td>71.0</td>
<td>41.3</td>
</tr>
<tr>
<td>Y-NF (28)</td>
<td>No. eggs</td>
<td>367</td>
<td>278</td>
<td>248</td>
<td>893</td>
</tr>
<tr>
<td></td>
<td>No. larvae</td>
<td>143</td>
<td>201</td>
<td>13</td>
<td>357</td>
</tr>
<tr>
<td></td>
<td>Egg hatch (%)</td>
<td>39.0</td>
<td>72.3</td>
<td>5.2</td>
<td>40.0</td>
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<tr>
<td>+/+</td>
<td>No. eggs</td>
<td>704</td>
<td>235</td>
<td>525</td>
<td>1464</td>
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<tr>
<td></td>
<td>No. larvae</td>
<td>620</td>
<td>205</td>
<td>311</td>
<td>1136</td>
</tr>
<tr>
<td></td>
<td>Egg hatch (%)</td>
<td>88.1</td>
<td>87.2</td>
<td>59.2</td>
<td>77.6</td>
</tr>
<tr>
<td>SS (8)</td>
<td>No. eggs</td>
<td>612</td>
<td>150</td>
<td>830</td>
<td>1592</td>
</tr>
<tr>
<td></td>
<td>No. larvae</td>
<td>363</td>
<td>76</td>
<td>423</td>
<td>862</td>
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<td></td>
<td>Egg hatch (%)</td>
<td>59.3</td>
<td>50.7</td>
<td>51.0</td>
<td>54.2</td>
</tr>
<tr>
<td>FF (8)</td>
<td>No. eggs</td>
<td>743</td>
<td>82</td>
<td>395</td>
<td>1220</td>
</tr>
<tr>
<td></td>
<td>No. larvae</td>
<td>510</td>
<td>64</td>
<td>50</td>
<td>624</td>
</tr>
<tr>
<td></td>
<td>Egg hatch (%)</td>
<td>68.6</td>
<td>78.1</td>
<td>12.7</td>
<td>51.2</td>
</tr>
<tr>
<td>Totals</td>
<td>No. eggs</td>
<td>3536</td>
<td>927</td>
<td>2671</td>
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</tr>
<tr>
<td></td>
<td>No. larvae</td>
<td>2218</td>
<td>685</td>
<td>1284</td>
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</tr>
<tr>
<td></td>
<td>Egg hatch (%)</td>
<td>62.7</td>
<td>73.9</td>
<td>48.1</td>
<td></td>
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</table>

Resistance to allyl alcohol: This was checked for the different Adh lines available in the laboratory, to find a discriminating dose between males and females with different Adh activity. The results are shown in Table III. The life stage most affected by the allyl alcohol was the larval stage, larval survival decreased with increasing concentration of allyl alcohol for all the strains. Egg hatch seemed hardly affected and pupal survival was slightly increased. The individuals with full Adh activity (FF, FS and SS) were killed by a concentration of 0.072%. Both homozygous strains, SS and FF, showed no survivors at this and higher concentrations. T128 had only male survivors at those concentrations, their Adh genotype being: males Y-NS, females SS from the T128 x SS cross and males Y-NF, females FS from the cross T128 x FF (see figure 1).

No larval development was observed for FF and SS in the higher allyl alcohol concentrations and fully developed larvae were not found dead in the corresponding replicates of both T128 crosses; this probably means that sensitive individuals were killed as newly emerged or very young first instar larvae. Some of such young larvae were indeed found dead when egg hatch was counted.
Table III. Effect of strain and allyl alcohol concentration on survival and adult sex ratio. Two replicates per treatment and strain; each replicate started with 100 eggs.

<table>
<thead>
<tr>
<th>Allyl alc. conc. (%)</th>
<th>Life stage</th>
<th>Strain</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SS (x±SE)</td>
<td>FF (x±SE)</td>
<td>T128 x SS</td>
<td>T128 x FF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>Egg hatch</td>
<td>54.4± 6.9</td>
<td>34.9± 3.9</td>
<td>80.6± 2.2</td>
<td>89.3± 3.5</td>
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</tr>
<tr>
<td></td>
<td>Larval surv.</td>
<td>84.4± 5.6</td>
<td>94.1± 0.6</td>
<td>81.0± 0.6</td>
<td>78.9± 4.4</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Pupal surv.</td>
<td>79.4± 7.1</td>
<td>83.7± 5.9</td>
<td>61.7± 0.4</td>
<td>68.0± 6.6</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Overall surv.</td>
<td>0.365</td>
<td>0.274</td>
<td>0.403</td>
<td>0.479</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>No. ♂</td>
<td>21.5± 0.5</td>
<td>12.0± 1.0</td>
<td>18.0± 3.0</td>
<td>14.5± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. ♀</td>
<td>14.0± 4.0</td>
<td>15.0± 0.0</td>
<td>21.5± 1.5</td>
<td>32.0± 4.0</td>
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<td></td>
</tr>
<tr>
<td>X² 1:1</td>
<td>1.6 n.s.</td>
<td>0.3 n.s.</td>
<td>0.3 n.s.</td>
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<td></td>
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</tr>
<tr>
<td>0.032</td>
<td>Egg hatch</td>
<td>43.1± 1.9</td>
<td>37.2± 6.9</td>
<td>79.4± 3.6</td>
<td>85.5± 3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Larval surv.</td>
<td>73.1± 1.9</td>
<td>94.9± 1.7</td>
<td>62.3± 4.4</td>
<td>52.1± 7.0</td>
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<tr>
<td></td>
<td>Pupal surv.</td>
<td>82.3± 1.0</td>
<td>83.1± 7.2</td>
<td>77.8± 9.8</td>
<td>76.3± 0.6</td>
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<tr>
<td></td>
<td>Overall surv.</td>
<td>0.259</td>
<td>0.293</td>
<td>0.384</td>
<td>0.340</td>
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<tr>
<td></td>
<td>No. ♂</td>
<td>11.5± 2.5</td>
<td>15.5± 2.5</td>
<td>13.5± 0.5</td>
<td>19.5± 1.5</td>
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</tr>
<tr>
<td></td>
<td>No. ♀</td>
<td>14.0± 3.0</td>
<td>14.0± 5.0</td>
<td>24.5± 4.5</td>
<td>14.5± 4.5</td>
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<td>X² 1:1</td>
<td>0.2 n.s.</td>
<td>0.1 n.s.</td>
<td>3.2 n.s.</td>
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<tr>
<td>0.048</td>
<td>Egg hatch</td>
<td>44.5± 4.1</td>
<td>31.2± 7.2</td>
<td>79.0± 1.0</td>
<td>85.0± 3.0</td>
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<tr>
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<td>Larval surv.</td>
<td>48.8±13.8</td>
<td>54.4±37.7</td>
<td>46.7±12.1</td>
<td>41.7±17.5</td>
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<tr>
<td></td>
<td>Pupal surv.</td>
<td>91.4± 1.4</td>
<td>84.6± 9.6</td>
<td>91.0± 1.6</td>
<td>80.0± 5.0</td>
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<td>Overall surv.</td>
<td>0.198</td>
<td>0.144</td>
<td>0.336</td>
<td>0.284</td>
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<td>No. ♂</td>
<td>10.0± 5.0</td>
<td>6.5± 5.5</td>
<td>21.0± 4.0</td>
<td>10.5± 2.5</td>
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<tr>
<td></td>
<td>No. ♀</td>
<td>10.0± 2.0</td>
<td>11.5± 9.5</td>
<td>12.5± 4.5</td>
<td>17.5± 8.5</td>
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</tr>
<tr>
<td>X² 1:1</td>
<td>0.0 n.s.</td>
<td>1.4 n.s.</td>
<td>2.2 n.s.</td>
<td>1.8 n.s.</td>
<td></td>
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<tr>
<td>0.072</td>
<td>Egg hatch</td>
<td>44.1± 3.9</td>
<td>41.0± 8.0</td>
<td>78.9± 3.1</td>
<td>70.8± 1.7</td>
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<tr>
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<td>Larval surv.</td>
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<td>0</td>
<td>11.3± 3.3</td>
<td>10.1± 1.2</td>
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<tr>
<td></td>
<td>Pupal surv.</td>
<td>-</td>
<td>-</td>
<td>95.8± 4.2</td>
<td>100.0± 0.0</td>
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<tr>
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<td>Overall surv.</td>
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<td>0</td>
<td>0.086</td>
<td>0.072</td>
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<td>7.0± 1.0</td>
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<tr>
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<td>No. ♀</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
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<tr>
<td>X² 1:1</td>
<td></td>
<td>8.5**</td>
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<td>7.0**</td>
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<tr>
<td>0.080</td>
<td>Egg hatch</td>
<td>41.9± 7.2</td>
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<td>66.6± 9.0</td>
<td>73.3± 2.5</td>
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<td>Larval surv.</td>
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<td>14.9</td>
<td>16.0</td>
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<td>Pupal surv.</td>
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<td>90.9</td>
<td>91.7</td>
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<tr>
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<td>Overall surv.</td>
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<td>0.090</td>
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<tr>
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<td>10.0</td>
<td>11.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. ♀</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X² 1:1</td>
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<td>10.0**</td>
<td></td>
<td>11.0**</td>
<td></td>
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<tr>
<td>0.096</td>
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<td>34.9± 8.6</td>
<td>74.8± 6.8</td>
<td>74.7± 5.7</td>
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<tr>
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<td>Larval surv.</td>
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<td>0</td>
<td>3.4± 0.4</td>
<td>1.3</td>
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</tr>
<tr>
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<td>Pupal surv.</td>
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<td>-</td>
<td>58.3± 8.3</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overall surv.</td>
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<td>0</td>
<td>0.015</td>
<td>0.010</td>
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</tr>
<tr>
<td></td>
<td>No. ♂</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. ♀</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

231
Larval development was delayed by allyl alcohol, the controls started pupation before the larvae exposed to the alcohol; this difference ranged from two days for the lower concentrations to eight days for the highest one.

Discussion

The genetic sexing system that was being developed in *C. capitata* using the Adh locus involved the translocation of a positive Adh allele to the Y chromosome and the isolation of a strain homozygous for a null allele of this locus (Riva and Robinson, 1983). Combining both strains a genetic sexing system would be produced where the males would be resistant to ethanol and the females susceptible (Robinson and van Heemert, 1981). However although null mutations were induced at the Adh locus after considerable effort, (Riva and Robinson, 1986), none were homozygous viable. As mentioned above (see Introduction), line T128 was induced and isolated for a different purpose (Robinson and Riva, 1984) than to serve as a genetic sexing strain, but the fact that it showed a clear discrimination between male and female survival when exposed to allyl alcohol led to the experiments reported here. This clear discrimination was not expected when extrapolating from the Drosophila data, where strains with 95% reduction of Adh activity showed very small differences in their survival to pentynol when compared to wild type positive strains (O'Donnell et al., 1975). Adh activity has not been measured for the strains used in this work but T128 males are expected to have 50% of the Adh activity of wild type individuals as they are heterozygous null/positive (Oakeshott, 1976). Indeed when they are checked electrophoretically, the active band they have is very faint, especially in the Y-NS individuals.

The discriminating dose of allyl alcohol varies according to the experimental methodology but it has a very narrow range, within which small variations in the experimental conditions become very large ones in the results. In the experiments presented here, male survival (no. males/no. larvae x 100) in 0.08% allyl alcohol is 14%, very similar to the previous data (13%) reported by Robinson et al. (1986). Some males did not survive at this concentration (control male survival for T128 is 19%) but other experiments (unpublished data) indicate that the dose can probably be lowered to 0.060% and still be discriminating.

These results prove that the selective medium with allyl alcohol used by Riva and Robinson (1986) to isolate null mutants was effective, as it positively selects for Adh null heterozygotes. This fact could not be tested prior to those experiments as no Adh null mutants existed for *C. capitata*, but it was expected to work, extrapolating from data from other species (Freeling and Cheng, 1976).

This alcohol shows some of the advantages that are recommended for a chemical suitable to be used in a mass rearing facility (IAEA, 1985); like cost, it wouldn’t be more expensive than ethanol as the quantities required are around sixty times smaller, and the price is certainly not so great. It also acts on an early developmental stage, all the observed mortality was in very young larvae. Its major disadvantage is its high toxicity, but this could be overcome by the use of chemical hoods and restricted areas, especially if the newly emerged larvae can be treated while they are being aerated prior to seeding them on the larval trays. Work is directed now towards this aspect.

As regards fitness of T128, these are only the first results, but the great variability found is both encouraging and deterring. Mortality has been found associated to all the life stages (adult longevity has not been checked) in this strain, this has been reported before for male linked translocations, as well as sex ratio distortion, (Robinson, 1976 and 1984; Rössler, 1979; Robinson and van Heemert, 1982; Riva and Robinson, 1983) but an excess of males
was usually observed. In this case a significant excess of females was consistently found. Cytological observations could help to clarify this aspect. Obviously, for a strain to rear only males, an excess of this sex would be preferable.

However, the fact that the fertility, pupal and overall survival varied depending on the females to which T128 was outcrossed, is promising for the following reason: One of the greatest concerns with laboratory reared insects is the loss of genetic variability and fitness that can even lead to genetic and mating isolation from the target field population (Whitten and Foster, 1975; Bush, 1975; Mackauer, 1976; Huettel, 1976; Bartlett, 1984 and 1985). If the laboratory population is not competitive or doesn’t interact with the feral population at the time of release, then the program can be jeopardized. One solution to the loss of genetic variability is introducing native genes into the laboratory population regularly. This is done in the screwworm program in Mexico, where the laboratory strain is completely replaced regularly by strains derived from the native insects of the geographic area to be treated next with SIT (Mackley, 1986).

With genetic sexing strains, introducing new genetic material is especially difficult as they are carefully manipulated and selected lines, usually highly inbred, where both males and females present certain characteristics, such as translocations and conditional lethal mutations, facts all of them that decrease fitness, but that are precisely what give the strains their genetic sexing properties.

The nice characteristic of a strain like T128 is that this problem can be overcome, as males are in this case the only carriers of mutations and/or chromosomal aberrations. These males can be outcrossed to any type of female, provided that they have full Adh activity, a trait that is wild type and generally spread. New genetic material is thus easily introduced into a laboratory population that at the same time retains all its genetic sexing properties.

References


Oakeshott, J.G. 1976. Selection at the alcohol dehydrogenase locus in Drosa-


INFLUENCE OF DEF ON THE ACTION OF MALATHION AGAINST MALE AND FEMALE *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE)

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*Department of Zoology, University of Manchester, Manchester M13 9PL, U.K.*

Abstract

The effect of different concentrations of malathion on males and females of *Ceratitis capitata* was evaluated, followed by similar treatments with the addition of the synergist DEF (S, S, S-tributyl phosphorothioate). Females were found to be the more tolerant sex in both tests. A strong synergistic influence of DEF in both sexes was observed, the synergistic factors being 6.4 and 5.8 in males and females respectively. The difference in tolerance between the sexes, either to malathion alone or to the malathion/DEF combination, was not in the direction necessary to provide a means of separating the sexes by differentially killing females.

Introduction

The Mediterranean fruitfly (medfly), *Ceratitis capitata* Wiedmann is widely recognised as a serious pest of fruit crops in most tropical and subtropical areas of the world. The wide variety of fruit species affected in countries of the Mediterranean and Central American areas is well documented. In East Africa, the medfly is known to be the main infestant of ripe coffee berries, although no resultant loss in the coffee crop has been established (Abasa, 1972; Waikwa, 1979). Recently, medflies have been observed infesting commercial orange plantations in Kenya (unpublished). In many countries, a strong effort is made to control the medfly, particularly where fruit exports are important (I.A.E.A., 1982). Insecticides are widely used, the most common one currently employed being malathion applied as a bait spray. Alongside chemical treatment it is sometimes the practice to make use of the sterile insect technique (SIT) in which large numbers of the pest are artificially reared, sterilized by irradiation and released over the area of infestation, in order that the sterile males shall mate with the wild females and cause them to lay sterile eggs. The technique has been particularly successful against the medfly in Mexico (I.A.E.A., 1982). In the Mexican programme both sexes are sterilized and released because no means can be found to separate them. It is believed, however, that this control method could be made even more effective if the females could be removed. A considerable effort is therefore being directed towards developing a genetic sexing technique for this species (Anonymous, 1980, 1983, 1985). The present study has been designed to investigate whether separation of the sexes might be achieved by treatment with malathion after pretreatment with DEF (S, S, S-tributyl phosphorothioate) which, as a carboxylesterase blocker, is known to synergise the action of malathion. Professor J. Kroczynski of the Institute of Industrial Organic Chemistry, Warsaw, Poland has informed us (personal communication) that in the housefly *Musca domestica*, DEF acts in a way which increases the difference in tolerance to malathion of the two sexes, the L.D. 50 of males to malathion plus DEF being almost twice that of females. It seemed worth repeating this study with the medfly in case it was the same.
Materials and Methods

The laboratory strains of C. capitata used were reared under standard conditions in the insectaries of the Department of Zoology, University of Manchester. These were SEIB, 19 and SA. Four to five day old flies of each strain were removed from colony cages in paper cups and immobilised by CO2 anaesthesia. The flies were then sexed manually and kept in different cages. 20-25 individuals of either sex were subsequently used for testing at each concentration for each replicated experiment. The malathion test solutions were prepared by successive dilutions of 20 mg/l stock solution of technical malathion in acetone. The standard solution of DEF contained 1g of DEF in 1 litre of acetone. The control was pure acetone. Applications were made as 1 μl of liquid applied topically on single flies, which were then transferred into petri dishes. Treatments (made to each sex at specific concentrations) were as follows: (i) malathion only, (ii) DEF plus malathion and (iii) acetone only (control). In (ii) above the sublethal dose of DEF was applied first, 1 hour before malathion. The application, either of DEF or malathion, was manually made with a repeating dispenser (Microlitre No. 7D5 Hamilton Bonaduz AG) which delivered 1 μl of liquid. Mortality was assessed 24 hours later, and the percent mortality was corrected for control mortality using Abbott's formula (Finney, 1971). The results were plotted on log/probability paper.

Results and Discussion

Table 1 shows the mortalities of males at different concentrations of malathion and malathion with DEF, corrected for control mortality (11/243). The mortalities of females at different concentrations of malathion and malathion plus DEF, corrected for control mortality (21/199), are shown in Table II. Fig. 1. shows the log concentration/probit mortality regression lines of response to the respective treatments. Females demonstrated greater tolerance to malathion than males, confirming an earlier observation by Koren et al. (1984). More tolerance was also exhibited by females than males after pretreatment with DEF followed by malathion. The LC 50's, synergistic factors and the sex susceptibility factor are given in Table III. The influence of DEF is strongly synergistic, resulting in synergistic factors of 6.4 and 5.8 in males and females respectively. The sex susceptibility factor indicates that males are 1.4 times more susceptible to DEF + malathion treatment than females and 1.3 times more susceptible to malathion alone. Kroczynski and Szczesna (unpublished manuscript) reported a sex susceptibility factor of 2.3 with DEF + malathion for M. domestica but in the opposite direction, females being more susceptible. The two species evidently differ in this respect, and there is clearly no value in this particular combination of insecticide synergist for sexing the medfly. It is conceivable, however, that resistance to other insecticide/synergist combinations might be greater in the male sex of the medfly. Koren et al. (1984) showed that selection with malathion on the medfly (applied to adults of both sexes together) enhanced the sexual difference. Selection for 18 generations resulted in 2- or 3-fold increase in tolerance of females while males showed no increase. It could be worth repeating this experiment and testing the selected line with a range of insecticides. The aim would be to find an insecticide resistance which is negatively correlated with malathion resistance. The basis of the sexual difference has been shown by Koren et al. (1984) to come from significantly higher carboxylesterase activity and a significantly reduced inhibition of head acetylcholinesterase. Acetylcholinesterase varia-
Table I. The corrected percentage mortalities of male *C. capitata* treated with different concentrations of malathion only and malathion plus DEF. The percentage mortality at each concentration is based on 3 – 5 replicated experiments.

<table>
<thead>
<tr>
<th>Malathion concentration (mg/l x 10^-6)</th>
<th>Percent (%) mortality (Numbers tested in brackets)</th>
<th>Malathion only</th>
<th>Malathion + DEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0 (109)</td>
<td></td>
<td>33.0 (103)</td>
</tr>
<tr>
<td>2.0</td>
<td>0 (66)</td>
<td></td>
<td>66.3 (60)</td>
</tr>
<tr>
<td>4.0</td>
<td>2.2 (66)</td>
<td></td>
<td>74.7 (62)</td>
</tr>
<tr>
<td>8.0</td>
<td>16.9 (64)</td>
<td></td>
<td>98.2 (63)</td>
</tr>
<tr>
<td>12.0</td>
<td>78.3 (67)</td>
<td></td>
<td>98.3 (67)</td>
</tr>
<tr>
<td>15.0</td>
<td>91.3 (64)</td>
<td></td>
<td>100.0 (64)</td>
</tr>
<tr>
<td>20.0</td>
<td>96.9 (92)</td>
<td></td>
<td>100.0 (68)</td>
</tr>
</tbody>
</table>

Table II. The corrected percentage mortalities of female *C. capitata* treated with different concentrations of malathion only and malathion plus DEF. The percentage mortality at each concentration is based on 3 – 5 replicated experiments.

<table>
<thead>
<tr>
<th>Malathion concentration (mg/l x 10^-6)</th>
<th>Percent (%) mortality (Numbers tested in brackets)</th>
<th>Malathion only</th>
<th>Malathion + DEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.2 (107)</td>
<td></td>
<td>38.5 (104)</td>
</tr>
<tr>
<td>2</td>
<td>11.7 (62)</td>
<td></td>
<td>51.3 (62)</td>
</tr>
<tr>
<td>4</td>
<td>13.5 (62)</td>
<td></td>
<td>73.8 (64)</td>
</tr>
<tr>
<td>8</td>
<td>16.6 (59)</td>
<td></td>
<td>96.3 (64)</td>
</tr>
<tr>
<td>12</td>
<td>40.4 (45)</td>
<td></td>
<td>100.00 (43)</td>
</tr>
<tr>
<td>15</td>
<td>71.4 (43)</td>
<td></td>
<td>97.4 (43)</td>
</tr>
<tr>
<td>20</td>
<td>94.7 (63)</td>
<td></td>
<td>100.00 (47)</td>
</tr>
</tbody>
</table>
Fig. 1. Log conc./probit mortality regression lines for male and female C. capitata treated topically with malathion and DEF + malathion.

- Females treated with malathion only
- Females treated with DEF + malathion
- Males treated with malathion only
- Males treated with DEF + malathion.
nts are known to differ in affinity for various inhibitors and might therefore be associated with negatively correlated resistances.

Table III. LC 50's (mg/l x 10⁻⁶) of malathion and DEF + malathion, synergistic factors and sex susceptibility factors.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Treatments</th>
<th>Malathion only</th>
<th>Malathion + DEF</th>
<th>Synergistic factor¹ (SF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td>9.0</td>
<td>1.4</td>
<td>6.4</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>11.5</td>
<td>2.0</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Sex-susceptibility factor² (SSF)

1. Synergistic Factor (SF) = LC 50 malathion/LC 50 DEF + malathion
2. Sex Susceptibility Factor (SSF) = LC 50 DEF + malathion for females/LC 50 DEF + malathion for males.

Acknowledgements

We are most grateful to Professor Kroczynski for making available to us his unpublished data on Musca domestica and we also are pleased to acknowledge the technical assistance provided by Mr. Graham Proudlove.

References


AUTOSOMAL SEX-DETERMINATION IN THE CABBAGE ROOT FLY, *DELIA BRASSICAE*

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Abstract

In the framework of producing a genetic sexing system in *Delia brassicae*, the cabbage root fly, a series of radiation induced translocations were studied. This species has been described as having an XX♂; XY♂ sex determination system with the sex chromosomes being the smallest chromosome pair, about 3% of the genome.

However, following irradiation a very high frequency of male-linked translocations was isolated. Cytological analysis subsequently showed that in these lines the largest autosome was always involved in the translocation. It was therefore concluded that in this particular population of *D. brassicae*, sex is determined by a dominant male determinant located on chromosome 6.

Introduction

In order to develop genetic sexing systems in pest insects it is essential firstly that the sex-determining mechanism is understood and secondly to assess if there is any variation in the mode of sex-determination. In the majority of insect pests, the details of way in which sex is determined are unclear although generalizations can be made from the classical examples in the literature.

In lower Diptera where there is no karyotypic discrimination between males and females, sex is frequently determined by a discrete dominant male determinant which can occupy different positions in the genome (Bedo 1984, Willis *et al.* 1981 and Martin *et al.* 1980). In most higher Diptera the sexes can be karyotypically distinguished with the Y chromosome functioning as the male determinant. *Drosophila melanogaster* is an exception in that sex is determined by a balance between the X chromosome and the autosomes (Bridges 1925). However, even within a species completely different sex determining mechanisms can be found. For example in the house fly *Musca domestica* as well as the standard XX♂; XY♂ system, populations have been found in which sex is regulated by determinants on one or more autosomes (Franco *et al.* 1982). The interaction between these two systems is dynamic with the autosomal factors appearing to increase in frequency (Denholm *et al.* 1983). In the Mediterranean fruit fly, *Ceratitis capitata* although the major determinant of sex is the Y chromosome (Zapater and Robinson 1986) strong autosomal modifiers have been identified and mapped (Malacrida *et al.* 1987 these proceedings).

In the cabbage root fly *Delia brassicae* (2n=12; XX♂; XY♂) Boyes (1954) described sex-determination as being based on heteromorphism for the smallest chromosome pair. In a closely related species, *Delia antiqua* a similar system was demonstrated (Robinson and van Heemert 1981). However, even here polymorphism for both sex chromosomes does exist (Vosselman 1978). Based on this premise experiments were started to induce and study male-linked translocations in *Delia brassicae*. However, the high frequency with which male-linked translocations were isolated argued very strongly that the smallest chromosome pair were not the sole determinants of sex in this species.
Materials and Methods

The strain of Delia brassicae used in the experiments was derived from a population collected in the vicinity of Leningrad. Males were irradiated with 1500 rad and mated to control females. F1 male progeny were outcrossed individually to normal females and the egg hatch measured. Progeny from matings having more than 20% late embryonic lethality were retained and checked for several generations. Cytology of selected strains was performed using metaphase preparations from testes of late pupae.

Results

From 160 F1 males mated, the progeny of 30 were retained as having reduced fertility and of these 28 could be cytologically confirmed as carrying a translocation. Eleven strains showed inheritance patterns typical of a male linked translocation in that all males showed reduced fertility and all females had normal fertility. In 15 other strains half of the males and females had reduced fertility and the other half had normal fertility indicating that the translocation was segregating independently of sex. In 4 cases all the males had reduced fertility and half of the females, this probably indicates the presence of 2 independent translocations, one male linked and the other autosomal. The segregation of reduced fertility in males and females for a sample of the translocations is given in Table I.

Table I. Segregation of semi-sterility between the sexes in translocation lines of Delia brassicae (SS = semi-sterile; FF = fully fertile).

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Segregation</th>
<th>Type of translocation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. ♀ SS FF</td>
<td>No. ♂ SS FF</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>6 11</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>11 8</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>12 15</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>15 13</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>4 9 3</td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td>7 13 8</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>6 4 5</td>
</tr>
<tr>
<td>26</td>
<td>0</td>
<td>26 26 0</td>
</tr>
<tr>
<td>32</td>
<td>4</td>
<td>6 3 7</td>
</tr>
<tr>
<td>34</td>
<td>0</td>
<td>11 13 0</td>
</tr>
<tr>
<td>37</td>
<td>5</td>
<td>5 2 2</td>
</tr>
<tr>
<td>39</td>
<td>2</td>
<td>5 8 0</td>
</tr>
<tr>
<td>42</td>
<td>23</td>
<td>19 7 7</td>
</tr>
<tr>
<td>52</td>
<td>0</td>
<td>43 31 0</td>
</tr>
<tr>
<td>71</td>
<td>0</td>
<td>68 56 0</td>
</tr>
<tr>
<td>86</td>
<td>0</td>
<td>15 20 0</td>
</tr>
</tbody>
</table>

*M = male-linked; A = autosomal.
Based on the observation that translocations are generally recovered in a frequency relative to chromosome length (Muller and Altenberg, 1930) and as the Y chromosome in Delia brassicae comprises only 3% of the mitotic length, male linked translocations should be exceedingly rare. However, from 30 lines isolated, 15 carried a male-linked translocation (Table II) a significantly higher value than expected. This discrepancy was resolved when the karyotypes of the different translocations were compared.

Table II. Frequency and type of translocations induced following irradiation (1500 rad) of male Delia brassicae.

<table>
<thead>
<tr>
<th>No. F₁ males tested</th>
<th>No. (%)</th>
<th>No. (%) and type of translocation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>semi-sterile lines</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>162</td>
<td>30 (18.5)</td>
<td>15 (50.0)</td>
</tr>
</tbody>
</table>

*M = male-linked; A = autosomal.

Chromosome pairs 2-5 are very similar in length and sub-acrocentric, they are almost impossible to differentiate cytologically. Chromosome 6 is clearly the largest and is sub-metacentric. Cytological analysis was performed on the 11 male-linked translocations and in 10 lines chromosome 6 was involved, in the eleventh line no translocation could be cytologically demonstrated. In three lines with semi-sterility inheritance patterns indicative of autosomal translocations, chromosome 6 was not involved. This chromosome constitutes about 27% of the mitotic chromosome length.

Discussion

These results point strongly to the implication that the largest chromosome is involved in sex-determination in Delia brassicae. It would appear that a dominant male determinant is located on that chromosome. This type of sex determination has already been found in other insect pests, e.g. Musca domestica (Franco et al 1982), Stomoxys calcitrans (Willis et al. 1981), Culex molestus (Gilchrist and Haldane 1947) and Aedes aegypti (McClelland 1962) mosquitoes.

There is, however, a discrepancy in that the frequency of induction of translocations involving chromosome 6 is much higher than would be expected on the basis of chromosome length. However, in studies on Delia antiqua it has been shown (Robinson and van Heemert 1981) that the relationship between chromosome length and translocation involvement does not hold true for all chromosomes i.e. some chromosomes are represented more, and some less, than would be expected on the basis of their length. However, the results are very encouraging for the development of a genetic sexing system in this species as male-linked translocations can be extremely easily induced and isolated.

The fact that the above data represent only a single sample from a single population makes it impossible to speculate as to how widespread this mode of sex determination is. However, if the data from Musca domestica is considered wide geographic variation can be expected.
Acknowledgements

We thank Alan Robinson for commenting on the manuscript.

References


MOLECULAR CLONING OF INSECTICIDE RESISTANCE GENES USEFUL FOR GENETIC IMPROVEMENT OF ARTHROPODS USED AS BIOLOGICAL CONTROL AGENTS

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Abstract

An esterase gene responsible for resistance to organophosphate (O.P.) insecticides was recently cloned from the mosquito Culex quinquefasciatus. This gene is amplified at least 250 fold in the resistant mosquitoes. Moreover, recent advances will allow soon cloning of several other genes encoding insecticide resistance in various insect species.

The esterase gene or such other resistance genes can now be introduced, through genetic engineering, into the germ line of beneficial arthropods used in integrated pest management programs. Such genes can also be transferred as selectable markers for sexing into insect pests used in sterile insect release programs.

Introduction

While more than 400 arthropod species have been reported having developed resistance to insecticides, only about 13 are beneficials. They are mainly predator mites such as the phytoseid Amblyseius fallacis from which several strains have been shown to be resistant to O.P. compounds, DDT, methoxychlor, carbaryl and pyrethroids. Some of these genetically improved strains have been successfully incorporated into integrated pest management programs (WHALON et al., 1982) but, unfortunately, classical genetic selection of other insecticide resistant beneficial arthropods has been achieved only with poor success.

In insects, many insecticide resistant traits involve single dominant genes and thus, it may be possible to use genetic engineering techniques for transfer such genes from pest species to beneficial organisms. In addition, these genes constitute a large group of potentially selectable markers which can be useful for sexing of sterilized male insects released for control of pests: a straightforward strategy for achieving genetic sexing of the medfly and other pest species by recombinant DNA procedures would be to introduce a gene that is selectable and expressed only in male insects.

Recently, in collaboration with laboratories of Dr N. PASTEUR (Montpellier) and G.P. GEROCHIOU (Riverside) we have cloned an esterase gene responsible for resistance in Culex mosquitoes to a large variety of O.P. insecticides. Works are in progress in several other laboratories in the cloning of other genes responsible for insecticide resistance and some of them will briefly be reviewed here.

Results

Cloning of an esterase gene responsible for insecticide resistance in Culex mosquitoes:

In mosquitoes, resistance to O.P. is often correlated with the presence of highly active detoxifying esterases (GERGHIOL and PASTEUR, 1978). One of these highly active esterases, B1, was purified from a temephos resistant strain of Culex quinquefasciatus from California (TEM-R).
A rabbit antiserum raised against the 67 kDa esterase was used to determine that the enzyme is about five hundred times more abundant in resistant mosquitoes than in susceptible ones (MOUCHES et al., 1986a). Thus, the increase of esterase activity in resistant mosquitoes is the result of a large overproduction of the protein itself which can represent between 6 to 12% of the total proteins of the TEM-R strain.

Such an overproduction can be the result of either gene amplification or from a mutation inducing a more efficient transcription of the esterase B1 gene. Indirect evidence that DNA amplification was responsible for esterase overproduction was obtained by using the elegant in-gel renaturation technique of RONINSON (1983). We found that DNA of O.P.-resistant mosquitoes possess additional amplified fragments which are absent in DNA of susceptible ones (MOUCHES et al., 1985b).

Furthermore, screening of a TEM-R cDNA expression library with the specific antiserum yielded a cDNA fragment able to select by hybridization mRNA encoding for esterase B1. This cDNA probe was used to demonstrate that adults of the TEM-R strain contain at least 250 times more copies of the esterase B1 gene than adults of susceptible strains (MOUCHES et al., 1986b). This finding emphasizes, for the first time, the biological importance of gene amplification in the acquisition of resistance to pesticides and open new areas of investigation in pesticide resistance management. Moreover, the first insecticide resistance gene is now available for improvement, by genetic engineering, of arthropods used in biological control.

Cloning of other genes responsible for insecticide resistance:

Using the in-gel renaturation technique, we have shown the presence of specifically amplified DNA sequences in a Culex pipiens strain overproducing esterase A1 and in a strain of the housefly resistant to O.P. insecticides due to a higher level of glutathione-S-transferase (MOUCHES et al., 1985b). Amplification of genes encoding detoxifying enzymes thus appears to be a widespread mechanism in the acquisition of pesticide resistance and the cloning of other such amplified genes will undoubtedly be performed soon.

Major advances have also been made recently in cloning drosophila genes encoding acetylcholinesterase (ACHE), the target of O.P. and carbamates. SPIERER et al. (1983) have used the chromosome walking approach to isolate drosophila DNA sequences coding for the bithorax complex, the rosy gene and the gene for ACHE (Ace+) in one "walk" on the third chromosome. Following this, SOREQ et al. (1985) claimed that a subcloned fragment of the large drosophila Ace+ area was able to hybrid-select a mRNA encoding human ACHE. It turned out recently that the gene encoding the drosophila ACHE lies in fact to some distance of the sequence reported by SOREQ et al. Malathion insensitive ACHE exists in drosophila but the resistance factor is very low, being less than 10. Such genes can not be seriously taken as potentially useful selectable markers, but ACHEs highly insensitive to O.P. and carbamates have been described in a large number of other insect species. We have found that the gene cloned from D. melanogaster is able to hybrid-select the mRNA encoding ACHE from C. pipiens (MOUCHES et al., unpublished results) in accordance with the fact that the two acetylcholinesterases are serologically related (MOUCHES et al., 1985a). The drosophila Ace+ gene can thus be used as a probe to isolate and characterize the gene encoding ACHE from C. pipiens and probably several other insect species.
Probably, the same approach will be possible for the gene coding the Na\(^+\) channel protein which is believed to be the target of pyrethroids. This gene has now been cloned from D. melanogaster (SALKOFF et al, 1986) and its sequence indicates strong conservation with that of vertebrates. It will thus be possible to use it as a probe to isolate the gene from various pyrethroid resistant insects.

In time, other resistance genes will probably be isolated and characterized from arthropods and could then be used in genetic engineering experiments. In the future, insecticide resistance genes can be obtained by altering the insecticide target enzymes by in vitro mutagenesis. Alternatively, insecticide degradative genes can be isolated from naturally occurring insecticide degrading bacteria (SERDAR et al, 1982) and tailored so that they can be expressed in insect cells.

**Conclusion and discussion**

As esterase Bl (MOUCHES et al, 1986 a) and acetylcholinesterase have been conserved to some extent among different insect species, we assume that genes or cDNA encoding them will appropriately be expressed upon their transfer to heterologous insect species. To verify this hypothesis, experiments are being carried out in order to transfer the esterase Bl gene from C. quinquefasciatus to D. melanogaster via P - element transformation.

The successful transfer of resistance genes to the germ line of arthropods used in biological controls will further require suitable vectors which are not yet available. Such vectors need to be constructed by using sequences derived from D. melanogaster P - elements (or baculoviruses) spliced to specific transposable sequences from the insect species to be improved. For genetic sexing, it will be necessary also to splice the gene to an efficient insect promoter sequence that will ensure expression of this gene in only one sex.

Obviously, careful consideration would be necessary before pesticide resistance will be introduced into a beneficial insect (for improvement) or a pest species (as a selectable marker). Cloning of the gene encoding esterase Bl yielded a DNA probe that will help to search for the presence of the amplified gene in various insect pests (AGARWAL et al, 1987). This will allow us to determine if this gene has been accidentally transmitted from one species to another by a so far unknown process. Because they are submitted to very high selection pressures, genes encoding for insecticide resistance are among the best candidates for such environmental analysis. From both the results of these investigations and the knowledge of the genetic modifications involved in acquisition of resistance properties it will be possible in the future to determine safe experimental conditions that need to be used to introduce insecticide resistance genes into a given insect population.

**References**


SYMBIOSIS
NUTRITION
REARING
MICROBIAL LEAF ISOLATES ASSOCIATED WITH
RHAGOLETIS POMONELLA

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Abstract

In limited laboratory trials, the apple maggot, Rhagoletis pomonella
(Walsh), was attracted to some microbial isolates cultured from honeydew.
Enhancement of effective traps by bacterial volatiles is under
investigation. It is theorized that adult flies may be directed to
honeydew for feeding due to an olfactory response elicited by bacterial
volatiles.

Introduction

In studies of the nutrition of the apple maggot fly, Rhagoletis
pomonella (Walsh), Nielsen (1966) showed that adults could not survive and
reproduce on substances usually present on the surface of fruit and
foliage, but can survive when aphid honeydew was present. Fecundity of
females raised on honeydew was equal to that of flies raised on artificial
diets. Inasmuch as a protein source is necessary for the newly emerged
females to reach sexual maturity and lay viable eggs, protein must be
present in the honeydew as free amino acids. Rossiter et al (1982) stated
that most tephritid species have symbiotic bacteria which appear to be
essential for normal development. Two species (Klebsiella oxytoca and
Enterobacter cloacae) were present in 1 bacterial colony type isolated from
the esophageal bulb, and were shown to cause rot in apples. Inasmuch as
the bacterial flora associated with the organ systems originate in the
environment of the fly, and are symbiotic, an olfactory response to the
bacteria may direct the fly to feeding sites. Drew et al (1983) working
with Dacus tyroni reported on bacteria as a natural source of food for
adult flies, while also demonstrating that diets of bacteria, sugar and
water gave equal longevity and increased fecundity compared to a
conventional diet of Brewer's yeast, sugar and water, and that laboratory
flies fed vigorously on the cultures.

Methods

During the summer of 1985, microbial cultures were isolated from
honeydew on which adults of Rhagoletis pomonella were observed feeding.
Attractiveness of these isolates to caged flies was evaluated. Flies were
developed from larvae exiting fruit in the fall of 1985. After pupation,
they were stored at 2.2°C for 90 days, with removal to 25.5°C and high
humidity on 22 January, with emergence starting on 26 March. The number of
flies available limited screening to 75 flies over a period of 5 days (15
flies/day). Interaction of flies upon exposure to 8 washed cultures and a
check over a 5 day period, April 14 to 18, 1986 is shown in Table I.

Results
On the basis of this limited exposure of flies to the cultures, numbers 5, 10, and 32 appeared to have greater attractiveness. One of these cultures, no. 32, was found to be a mixed culture. Ultimately 4 species were identified as follows:

- Acinetobacter calcoaceticus
- Bacillus fastidiosus
- Enterobacter agglomerans
- Micrococcus luteus

Enterobacter agglomerans is similar to the symbiotic bacteria reported by Rossiter et al (1982). These 4 identified isolates from leaf surfaces contaminated with honeydew are not uncommon, and are frequently isolated from air, water and soil. It is theorized that volatile substances produced by these leaf isolates may be an olfactory attractant for feeding adult flies, and could be incorporated in traps for increased efficacy.

Additional studies in 1985 evaluated 4 apple maggot traps, with and without a volatile lure, as described by Ressig 1982. Results of this study are shown in Table II. Traps were replicated twice in each of 2 locations, a commercial block, and an abandoned block, both consisting of McIntosh and Red Delicious cultivars. The Ladd trap consisted of a yellow rectangle 21.6 cm x 27.9 cm x 0.08 cm with four 0.28 cm diameter holes, placed 3.16 cm from center at each 90° of arc. Red hemispheres, 8.25 cm diameter at the base, 3.16 cm high; two pins and holes 0.24 cm in diameter on the base spaced alternately at each 90° of arc. Both the yellow panel and red hemispheres are made of high density UV stable polyethylene. A 9.1 mm x 18.8 mm rubber sleeve stopper impregnated with the apple volatile was attached to each of the traps, and is referred to in the table as odor enhancement (OE).

In the managed block odor enhanced red spheres captured ca. 5 x the number of flies that the red sphere alone captured. In the abandoned block however, the odor enhanced Ladd trap was ca 2 x more efficient than the odor enhanced red sphere, and ca 4 x better than a baited odor enhanced yellow panel.

This evaluation indicates that the red spheres are more efficacious in managed orchards where the majority of flies appear to be sexually mature migrants seeking fruit for mating or oviposition. In the abandoned block, with a high population of indigenous flies, sexually immature on emergence, the odor enhanced Ladd trap gave the highest number of captures.

With a better understanding of fly population dynamics, it would appear that a trap-out strategy is possible when one considers that a single trap can capture 76 flies per season. Studies are continuing to further enhance trap efficacy by the characterization and synthesis of gases associated with feeding stimuli.

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Average Fly Interactions for 1 hr/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>26</td>
<td>10</td>
</tr>
<tr>
<td>32</td>
<td>25</td>
</tr>
<tr>
<td>Check</td>
<td>9</td>
</tr>
</tbody>
</table>

TABLE I. Apple Maggot Fly, Rhaboletis pomonella (Walsh), interactions with six bacterial cultures isolated from honeydew.
TABLE II. Comparative efficacy of 4 apple maggot traps, with and 
without odor enhancement.

<table>
<thead>
<tr>
<th></th>
<th>Managed Block</th>
<th>Abandoned Block</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow Pane 1</td>
<td>0.0D</td>
<td>2.0BC</td>
</tr>
<tr>
<td>Yellow Panel + OE 2</td>
<td>2.5B</td>
<td>1.5C</td>
</tr>
<tr>
<td>Yellow Panel 1</td>
<td>0.5D</td>
<td>12.0AB</td>
</tr>
<tr>
<td>Yellow Panel + OE 4</td>
<td>2.0B</td>
<td>20.5AB</td>
</tr>
<tr>
<td>Red sphere alone</td>
<td>5.0A</td>
<td>17.5A</td>
</tr>
<tr>
<td>Red + OE 5</td>
<td>25.0A</td>
<td>42.0A</td>
</tr>
<tr>
<td>Red hemispheres on yellow panel</td>
<td>1.0CD</td>
<td>31.0A</td>
</tr>
<tr>
<td>Red hemispheres on yellow panel + OE</td>
<td>6.0A</td>
<td>76.0A</td>
</tr>
</tbody>
</table>

1. Pherocon, adult monitoring - AM, mfg by Zoecon, Palo Alto, CA
2. OE, odor enhancement septa by Ladd, (mfg. by Ladd Res. Industries, P.O. 1005, Burlington, VT 05401
3. Pherocon, Apple Maggot Fruit Fly - AM, (mfg. as above)
4. Red spheres from Ladd Trap, (mfg. as above)
5. Ladd Trap (mfg. as above)

Mean separation by the L.S.D. $\times = 0.05$

Acknowledgements

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EFFECT OF CERTAIN NUTRITIONAL AND ALLELOCHEMICAL SUBSTANCES UPON THE OLIVE FRUIT FLY

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Department of Biology, N.R.C. «Demokritos», Athens, Greece

Abstract

Certain nutritional and non-nutritional (allelochemical) substances have been tested with the olive fruit fly, Dacus oleae, Gmel. The effect of amino acids, salts and plant allelochemicals upon larval growth, larval survival, pupation, pupal weight and adult emergence was studied. Methionine, copper, hydroxyquinone and resorcinol depressed larval growth and development at relatively low levels, while glutamic, potassium and tannin at high levels. The effects of all nutrients and allelochemicals tested will be presented and similar work in other insects will be discussed.

Introduction

The insect like any other animal faces in its environment not only the essential nutrients but thousands of other substances which may modify, its dietetics. Some of the non-nutritional substances which are produced by an organism and affect the growth, health, behavior or population biology of members of other species are often called allelochemics (Whittaker, R.H. 1970). On the other hand nutrients are those chemicals which are required by an animal for normal growth, development maintenance and reproduction. In the broader sense both nutrients and allelochemicals may be beneficial or detrimental to the receiving organism depending upon the level present in the diet, as well as upon their interaction.

The olive fruit fly Dacus oleae, Gmel., faces a complex mixture of nutrients and allelochemicals. These may be beneficial or detrimental depending upon their physicochemical properties and their quantity. The effect of certain nutritional and allelochemical substances upon the olive fruit fly larvae will be present and discussed.

Methods and materials

One way to study the effect of chemical substances is by adding them directly to the larval diet. Materials and methods used are described in detail elsewhere (Manoukas 1981, 1982). All common amino acids and essential salts found in the natural and artificial diet of the olive fruit fly were tested. In addition the following allelochemicals were tested; tannin, gallic acid, caffeic acid, resorcinol, hydroxyquinone and phloroglucinol. Many of these compounds have been detected in the olive fruit mesocarp and some of them have been determined in amounts which vary with the olive fruit variety and probably with other factors.

Pupae were counted the 14th day following hatching of the eggs and weight was taken three days thereafter. Larvae were counted and weighted on sample taken the 5th, 6th or 7th day. Ten to forty eggs/g diet were used in the experiments with allelochemicals, while 5 eggs were used in experiments with nutrients. Statistical procedures described by Steel and Torrie (1960) were employed to evaluate the effects of the nutrition-
al substances. Due to the preliminary nature of the results with the allelochemicals no statistical evaluation was made. However standard error of the mean was found to vary between 8 and 23% of the value reported.

Results and discussion

Hatchability of eggs was within normal limits for all experiments. Table I presents the effect of methionine, lysine, glycine and glutamic acid upon pupal yield and adult emergence of the olive fruit fly. It can be seen that the first three amino acids affected pupal yield at relatively low levels while glutamic acid at very high levels. All other amino acids affected pupal yield at intermediate levels (Manoukas, 1981). The value for methionine was in close agreement with the value reported for Drosophila by Wilson, 1946. The level of amino acid required to affect D. oleae larvae, expressed as % of the total amino acids in the basal diet for methionine, lysine and glutamic acid were in close agreement with those reported for Xenopsilla cheopis (Pausch and Fraenkel, 1967). It is not known whether the detrimental effects of these amino acids are caused by toxicities, antagonisms or imbalances (Harper, 1964). It is possible that a toxicity is involved in the case of methionine since this amino acid was proved toxic to the rat (Russel et al, 1952, Benevenga, 1974).

Table II presents the results of the effect of CuSO4 and KCl. It is clear that CuSO4 affected pupal yield at the very low levels while KCl at very high levels. All other salts were toxic at intermediate levels (Manoukas, 1982). It is of interest to note that the olive fruit contains very low level of Cu and very high level of K (Manoukas et al 1978). D. oleae larvae seem to be quite sensitive to copper even at the concentration of 1 mg/100 g diet. Toxicity of copper has been ascribed to interference with enzymatic processes in fatty acid metabolism and it is also known to chelate with amino acids (Fruton and Simmonds, 1961). Excess copper seems to influence the availability of fatty acids in the case of the tea totrix Homona coffearia (Sivapalan and Gnanapragasam, 1980).

Table III presents the results on the effects of tannin, gallic acid, caffeic acid, resorcinol, hydroxyquinole and phloroglucinol. Tannin was detrimental at the level of 0.5% while all other allelochemicals tested were detrimental at much lower concentration. Gallic acid depressed larval weight and pupal weight at the level of 0.2%, while caffeic acid depressed the same parameters at the level of 0.1%. Resorcinol depressed larval weight at the level of 0.05% and was proved lethal at the level of 0.40%. Hydroxyquinole depressed larval weight at the level of 0.02% and number of pupae at the same level. It should be pointed out however that the control diet did not give normal performance of larvae due to high larval density. It is therefore very difficult to make conclusions on this experiment. However the level of 0.08% while it depressed larval survival, larval weight, and larval survival to pupation, it gave pupal weight and adult emergence equivalent to the control. Finally phloroglucinol depressed larval survival at the level of 0.02% and it was lethal at the level of 0.08%. In general it appeared that pupal weight of the survived larvae was not materially affected by the allelochemicals tested. It was however observed that allelochemics made the survived larvae unable to pupate by the 14th day.

The effect of allelochemics upon insects depends upon many factors. It seems also that the efficiency of metabolic mechanisms in neutralizing or counteracting allelochemicals is different in different insects. These differences become larger because of the interactions involved between allelochemicals and nutrients. Tannin was found to be detrimental
TABLE I. Effect of certain amino acids upon pupal yield and adult emergence of the olive fruit fly.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>% in diet</th>
<th>Pupae</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P/g diet</td>
<td>mg/P</td>
<td>% pupae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>0.0</td>
<td>3.0a</td>
<td>5.7a</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.9b</td>
<td>5.5a</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.0</td>
<td>3.1a</td>
<td>6.0a</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>1.3b</td>
<td>5.1ab</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.4d</td>
<td>4.1b</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.1d</td>
<td>4.2b</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.0</td>
<td>2.2a</td>
<td>5.8a</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>2.2a</td>
<td>5.4ab</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.6b</td>
<td>4.6b</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>Glutamic</td>
<td>0.0</td>
<td>3.2a</td>
<td>5.5a</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>2.6ab</td>
<td>3.7b</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>2.4ab</td>
<td>3.7b</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>2.4ab</td>
<td>4.0b</td>
</tr>
<tr>
<td></td>
<td>32.0</td>
<td>1.4b</td>
<td>3.4b</td>
</tr>
</tbody>
</table>

TABLE II. Effect of certain salts upon pupal yield and adult emergence of the olive fruit fly.

<table>
<thead>
<tr>
<th>Salt</th>
<th>% in diet</th>
<th>Pupae</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P/g diet</td>
<td>mg/P</td>
<td>% pupae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CuSO₄·SH₂O</td>
<td>0.000</td>
<td>3.2a</td>
<td>5.6a</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>2.9a</td>
<td>5.0a</td>
</tr>
<tr>
<td></td>
<td>0.010</td>
<td>1.9b</td>
<td>3.7b</td>
</tr>
<tr>
<td></td>
<td>0.020</td>
<td>0.2c</td>
<td>2.3c</td>
</tr>
<tr>
<td></td>
<td>0.040</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>KCl</td>
<td>0.0</td>
<td>3.1a</td>
<td>5.6a</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3.0a</td>
<td>6.6a</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>2.9a</td>
<td>6.5a</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>0.6b</td>
<td>6.1a</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>0.0</td>
<td>-</td>
</tr>
</tbody>
</table>

1Taken from Manoukas, 1981
2Means in the same column followed by the same letter for each amino acid do not differ significantly at the 0.05 level of probability by Duncan's multiple range test.

1Taken from Manoukas, 1982
2Means in the same row followed by the same letter for each salt do not differ significantly at the 0.05 level of probability.
TABLE III. Effect of certain allelochemicals upon the performance of the olive fruit fly larvae.

<table>
<thead>
<tr>
<th>Allelochemical, %</th>
<th>Larvae L/g diet mg/L</th>
<th>Pupae P/g diet mg/P</th>
<th>Adults % pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>17.7 1.0</td>
<td>8.7 4.6</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.5</td>
<td>10.3 0.5</td>
<td>1.3 3.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>1.0</td>
<td>13.7 0.5</td>
<td>2.0 3.4</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.0</td>
<td>12.2 0.4</td>
<td>0.0 -</td>
</tr>
<tr>
<td>&quot;</td>
<td>4.0</td>
<td>15.2 0.1</td>
<td>0.0 -</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>12.8 0.8</td>
<td>8.2 4.9</td>
</tr>
<tr>
<td>Gallic</td>
<td>0.1</td>
<td>15.3 0.6</td>
<td>7.3 4.4</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.2</td>
<td>14.3 0.4</td>
<td>8.1 4.2</td>
</tr>
<tr>
<td>Cafeic</td>
<td>0.1</td>
<td>13.0 0.4</td>
<td>6.0 3.6</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.2</td>
<td>12.8 0.4</td>
<td>7.9 3.9</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>5.3 5.8</td>
<td>5.0 3.9</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>0.050</td>
<td>5.0 4.0</td>
<td>4.0 4.1</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.100</td>
<td>6.0 3.7</td>
<td>3.0 4.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.200</td>
<td>3.3 1.9</td>
<td>0.9 4.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.400</td>
<td>0.0 0.0</td>
<td>- -</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>22.6 1.6</td>
<td>13.6 3.1</td>
</tr>
<tr>
<td>Hydroxyquinoline</td>
<td>0.01</td>
<td>35.0 1.5</td>
<td>7.7 3.4</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.02</td>
<td>20.6 1.1</td>
<td>8.8 3.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.04</td>
<td>26.1 0.7</td>
<td>3.8 3.6</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.08</td>
<td>1.3 0.4</td>
<td>0.6 3.7</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>10.8 0.5</td>
<td>4.3 4.2</td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td>0.01</td>
<td>12.0 0.8</td>
<td>5.1 4.4</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.02</td>
<td>3.0 0.6</td>
<td>2.8 5.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.04</td>
<td>3.7 0.4</td>
<td>1.5 4.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.08</td>
<td>3.8 0.2</td>
<td>0.0 -</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.16</td>
<td>2.8 0.0</td>
<td>- -</td>
</tr>
</tbody>
</table>

To certain Lepidoptera (Feeny, 1968) but not to Orthoptera (Bernays et al. 1978). In addition while the results for gallic acid, phloroglucinol and resorcinol are generally in line with those reported for Agrotis ipsilon larvae (Reese, 1978) are not in agreement with the rest of the allelochemicals tested for the olive fruit fly.

The mechanisms involved in insect metabolism because of the allelochemicals are not known in detail. It is known however from research with mammals that tannins form complexes with amino acids, especially lysine which may not be utilizable by the organism. This may be important for the olive fruit fly which requires free amino acids in the diet. In addition the products of tannin breakdown (phenols) may form complexes with sulfohydryl groups and inorganic elements or may inactivate enzymes.

Acknowledgements

Many thanks to Mrs. A. Kanoussi for typing the manuscript.
References

FURTHER IMPROVEMENTS IN THE MASS REARING OF THE OLIVE FRUIT FLY, *DACUS OLEAE* (DIPTERA, TEPHRITIDAE)

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Abstract

The olive fruit fly, *Dacus oleae* (Gmelin), an important pest of olive fruit in the countries of the Mediterranean Basin, can be reared in the lab in fairly large numbers. Improvements of the system were done in the areas of environmental conditions of adult rearing, the optimization of fly density in the cages, the egg density in the larval diet and design and construction of equipment used in phases of the rearing. A constant temperature of 25°C in the adult rearing gave higher egg production than one at 20°C or fluctuating diurnal one of 17-23°C. Additionally, a density of 5400 insects per cage gave an equally good egg production with the one of 2700 insects per cage. The optimum density of eggs placed on the diet was 15 eggs per gram diet and a large rectangular tray (48x38x2cm) for the larval rearing was better than a small round one (diam. 32.5cm x 0.8cm). Finally, certain improvements in the handling of pupae and the construction of a mechanical sifter of the pupae contributed considerably to labor saving and better space utilization. The above improvements have increased the efficiency of the system.

Introduction

An efficient mass rearing system for any insect is an asset for basic and applied studies. It is, however, a requirement for the application of certain insect control methods such as biocontrol agents, sterile insect releases. Very efficient rearing systems have been developed for certain fruit flies among which are *Ceratitis capitata*, *Dacus dorsalis*, *Dacus cucurbitae*. For the olive fruit fly, *Dacus oleae* (Gmel.), there have been developed methods for the production of insects in more or less small numbers reviewed by Tzankanakis (1971). A more efficient technique has been described by Tsitsipis (1977a). The aim of making a rearing system more efficient involves considerable research on the optimization of its various phases. The optimization procedure is not only a science, but also an art, since certain imaginative innovations could greatly increase the productivity of the system.

It was the aim of the present work to study and introduce improvements in the areas of adult maintenance, larval rearing and substitution of manual by mechanical work. In particular there were studied: the adult holding temperature, the adult density in the cages, the cage hygiene, the optimization of the larval density, the substitution of a round larval tray by a better one, the construction of a mechanical larval diet grinder, of a mechanical sieve for the separation of pupae, of a rack for the larval rearing and the collection of pupae. Utilization of these improvements increased the production capability by at least one third to the one million pupae per week level.
Materials and Methods

Experimental insects derived from Crete and they had been reared in the laboratory for about 30 generations according to a method described earlier (Tsitsipis 1977a). Adults were fed with a solid diet (Tsitsipis 1975) and larvae were reared on diet T (Tsitsipis 1977a). Experiments were done at 25±1°C, unless otherwise specified, 60±5% R.H. and 16 hrs light daily. To study the optimum temperature conditions for the adults, three different regimes were tested: constant 20°C, constant 25°C and a diurnal fluctuating between 17-23°C, the low temperature coinciding with the scotophase. In each regime, 20 pairs of insects were placed, each pair in a small plexiglass cage 12x12x12cm. Eggs were laid through a conical nylon gauze coated in ceresin. The cone penetrated the cages vertically the two ends being outside the cage. The eggs were collected by rinsing water on the inside surface of the cone. Egg production and mortality were recorded daily for a total period of eight weeks. For the adult density experiment, two densities were tested replicated twice: 2700 and 5400 insects per 100x40x30cm cage (Tsitsipis 1977b) at a sex ratio 1:1. Records were taken for five weeks. Egg production, hatchability and adult mortality were recorded five times per week. To improve adult survival, chlorox was tested in the drinking water at 0.05 and 0.1% of the commercial product. The experiment was replicated twice in 30x50x30cm cages with 140 pairs of insects per cage. Records were taken for five weeks and egg production, hatchability and adult mortality for five days per week. To test the optimum larval density in the rearing medium, densities in the range 3-36 eggs/g diet in three egg increments were used. Each density was replicated six times in 12g diet batches. Egg hatchability, pupal recovery and pupal weight were recorded. Furthermore two larval plastic diet trays were compared. One round (Ø 32.5cm x 0.8cm) holding 230g diet and one rectangular (48x38x2cm) holding 1500g diet. A density of 15 eggs/g diet was tested in four replicates. Pupal recovery and pupal weight were recorded. The following equipment was designed and constructed. To give a homogeneous consistency to the granular larval diet a hollow cylindrical mill (Ø 36cm, h=65.5cm) was made. The two bases of the cylinder were closed and the cylindrical surface was made of perforated aluminum sheet with diamond-shaped openings (2.5x7 mm). The diet fed into the lumen of the cylinder was forced through the openings by metal blades rotated by an axis. For the placement of the larvae for development and the collection of pupae an iron rack, 220x62x200cm, was constructed accommodating 115 rectangular plastic trays stacked in five rows. Each tray rests on two horizontal metal guides. The mature larvae leaving the diet drop to the bottom of the rack and through a funnel they are directed into a bucket with moist sawdust for pupation. A mechanical sieve, to separate the pupae from the sawdust, was made. A wire screen, 50x40cm, 1mm² mesh, fixed on aluminum frame, slides on guides horizontally by a reciprocal movement at a frequency of 210 laps/min. The lap distance is 6.6cm. To study the effect of sifting on the pupae, pupae of ages ranging from 0 to 8 days old, at daily increments, were sifted for 0, 0.5, 1, 2, 4, 6, 8, 10, 15 min. Each treatment was replicated five times in batches of 100 pupae each. For the evaluation of data analysis of variance was performed and mean separation was done by the Duncan's multiple range test.

Results

Adults kept continuously at 25°C showed a significantly higher egg
production per initial and/or living female for the first four or eight weeks than those kept at 20°C or 17-23°C (TABLE I). There were no differences found in the egg production between the last temperature regimes. In regard to the adult survival, at 25°C the mean daily number of living females for the first four weeks was 15.7 and for the first eight 12.1. The respective values for 20°C were 15.9 and 12.8 females and for the 17-23°C 18.8 and 14.4 females. Survival in the fluctuating temperature regime was better than the other two regimes, indicating an overall low oviposition rate, especially during the first four weeks.

The results on comparing an adult density of 2700 insects per cage, routinely used in the lab (Tsitsipis 1977a), with a density of 5400 insects per cage showed that the number of eggs per initial female in the low density was by 10% higher than in the high density (TABLE II). Mortality did not differ between the two densities. The highest value was observed during the fourth week. Egg hatchability was also similar in the two densities. The highest egg production was recorded in the third week and then it declined. Egg production during the 5th week was less than 10% of the total. Use of tap water without any preservative in the cages probably affects survival. Addition of clorox (sodium hypochlorite) at 0.1% in the drinking water improved survival by about 20% and consequently egg production, although a concentration of 0.05% was not different from the control. The results on the optimization of larval density (TABLE III) showed that with increasing densities from three to 21, pupal recovery increases from two to 12 per gram diet. Egg hatchability was not affected by density and so was adult emergence. Expressing pupal recovery as percentage of hatched eggs, efficiency decreased with increasing density. Highest efficiency was noted at 3, 6, 18 and 21 eggs, then followed 9, 12, 15, 24, 30, 27, 36 and 33. Examining pupal weight, it decreased with increasing density. At densities above 18 there were no statistical differences in the pupal weight. Comparing the round trays, previously used, at 15 eggs per gram diet, with rectangular ones, pupal recovery per gram diet was 4.0±0.14 and 5.8±0.18, while pupal weight values were 5.5±0.06 and 5.4±0.13 mg respectively. Diet efficiency was significantly better in the rectangular than in the round tray. Mass production of about two million pupae in the lab was accomplished at a diet efficiency of 5.65 pupae per gram diet.

The results of the effect of sifting of pupae on adult emergence is shown in TABLE IV. Adult emergence is adversely affected by the sifting procedure in the first being more sensitive. First day pupae were not influenced by up to two minutes sifting, but four to ten minutes sifting affected adult emergence more than with a shorter sifting duration. Sifting for 15 minutes affected adult emergence the most. Two day old pupae were not affected by sifting for up to four minutes, but longer sifting duration reduced adult emergence. Three day old and older pupae were slightly or not affected in regard to adult emergence.

Discussion

Highest egg production was obtained at 25°C. These results agree with the data of Sacantanis (1953) and Girolami (1979) recommending the range of 23-25°C for highest fecundity. Tzanakakis (1971) reviews the work of many authors most of who suggest daily fluctuating temperatures in the range 15-25°C. Lopez (1965), from preliminary experiments, concludes 23°C as the temperature for better egg production, while Steiner (1964) recommends testing the temperature regimes 22-23 and 27°C for the determination of the one for high and early egg-production. Cage efficiency relates with adult density. Densities higher than the ones reported by
TABLE I. Mean daily egg production per initial and living ♀ in *Dacus oleae* pairs kept at temperature regimes: constant 20°C, constant 25°C, daily fluctuating 17-23°C. Twenty replicates per treatment.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Mean daily egg production</th>
<th>20 °C</th>
<th>17 - 23 °C</th>
<th>25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial♀</td>
<td>Living♀</td>
<td>Initial♀</td>
<td>Living♀</td>
</tr>
<tr>
<td>1</td>
<td>8.2</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
</tr>
<tr>
<td>2</td>
<td>10.5</td>
<td>11.7</td>
<td>11.3</td>
<td>11.3</td>
</tr>
<tr>
<td>3</td>
<td>9.9</td>
<td>13.1</td>
<td>10.3</td>
<td>10.8</td>
</tr>
<tr>
<td>4</td>
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<td>13.4</td>
<td>10.1</td>
<td>10.6</td>
</tr>
<tr>
<td>5</td>
<td>7.5</td>
<td>11.6</td>
<td>8.6</td>
<td>10.1</td>
</tr>
<tr>
<td>6</td>
<td>5.7</td>
<td>11.3</td>
<td>6.9</td>
<td>13.9</td>
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<tr>
<td>7</td>
<td>5.8</td>
<td>10.0</td>
<td>5.8</td>
<td>11.6</td>
</tr>
<tr>
<td>8</td>
<td>0.9</td>
<td>2.6</td>
<td>1.6</td>
<td>3.9</td>
</tr>
<tr>
<td>1-8</td>
<td>7.2a*</td>
<td>10.4c</td>
<td>8.0a</td>
<td>10.2c</td>
</tr>
<tr>
<td>1-4</td>
<td>9.5a</td>
<td>11.8c</td>
<td>10.2a</td>
<td>10.5c</td>
</tr>
</tbody>
</table>

*Comparisons separate for initial and living ♀. Egg production values followed by same letter are not statistically different (P < 0.05) by Duncan’s multiple range test.

TABLE II. Effect of two densities of adult *Dacus oleae* in cages on egg production (two replicates, egg production of 5 days weekly, sex ratio 1:1).

<table>
<thead>
<tr>
<th>Density</th>
<th>Weeks</th>
<th>Eggs/initial♀</th>
<th>% egg hatch</th>
<th>% egg hatch</th>
<th>Mortality ♀</th>
<th>Mortality ♀</th>
<th>Mortality ♀</th>
</tr>
</thead>
<tbody>
<tr>
<td>5400 insects/cage</td>
<td>1*</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>53.2±18</td>
<td>72.0</td>
<td>91</td>
<td>57.9±20</td>
<td>79.5</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>64.7±4</td>
<td>79.6</td>
<td>199</td>
<td>78.7±2</td>
<td>80.0</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>37.4±9</td>
<td>75.9</td>
<td>307</td>
<td>33.8±7</td>
<td>78.1</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>15.7±3</td>
<td>78.7</td>
<td>276</td>
<td>16.9±6</td>
<td>78.5</td>
<td>140</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>171.0±3</td>
<td>896</td>
<td>1148</td>
<td>187.3±9</td>
<td>490</td>
<td>611</td>
</tr>
</tbody>
</table>

* Small egg production not measured.
TABLE III. Effect of density of *Dacus oleae* eggs on diet efficiency (six replicates, 12g diet/rep.).

<table>
<thead>
<tr>
<th>No. eggs/</th>
<th>% egg hatch</th>
<th>No. pupae/</th>
<th>Pupae % hatched eggs</th>
<th>Pupal weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>g diet</td>
<td></td>
<td>g diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>90.6±6.2</td>
<td>2.0±0.5</td>
<td>73.2a</td>
<td>9.1a</td>
</tr>
<tr>
<td>6</td>
<td>83.8±6.0</td>
<td>3.8±0.7</td>
<td>75.5a</td>
<td>7.3b</td>
</tr>
<tr>
<td>9</td>
<td>87.0±4.1</td>
<td>4.7±0.6</td>
<td>59.5bcd</td>
<td>6.6c</td>
</tr>
<tr>
<td>12</td>
<td>86.1±2.5</td>
<td>5.6±0.9</td>
<td>53.7cd</td>
<td>6.1cd</td>
</tr>
<tr>
<td>15</td>
<td>85.8±1.9</td>
<td>6.9±1.9</td>
<td>53.4cd</td>
<td>5.9d</td>
</tr>
<tr>
<td>18</td>
<td>87.8±1.4</td>
<td>11.3±1.6</td>
<td>71.5ab</td>
<td>4.6e</td>
</tr>
<tr>
<td>21</td>
<td>88.2±1.2</td>
<td>12.1±1.2</td>
<td>65.1ac</td>
<td>4.5e</td>
</tr>
<tr>
<td>24</td>
<td>86.8±1.9</td>
<td>10.9±1.7</td>
<td>52.2cd</td>
<td>4.8e</td>
</tr>
<tr>
<td>27</td>
<td>87.8±1.6</td>
<td>11.6±4.2</td>
<td>48.9d</td>
<td>4.4e</td>
</tr>
<tr>
<td>30</td>
<td>87.5±1.3</td>
<td>13.5±1.3</td>
<td>51.6cd</td>
<td>4.2e</td>
</tr>
<tr>
<td>33</td>
<td>85.5±1.7</td>
<td>9.6±2.2</td>
<td>33.7e</td>
<td>4.7e</td>
</tr>
<tr>
<td>36</td>
<td>85.5±1.1</td>
<td>10.6±1.8</td>
<td>34.5e</td>
<td>4.6e</td>
</tr>
</tbody>
</table>

*Values followed by same letter are not statistically different (P < 0.05) by Duncan's multiple range test.

TABLE IV. Adult emergence of *Dacus oleae*, at 25°C, the pupae of which were sifted at different ages for different periods of time (Five replicates of 100 per treatment; sifting frequency 210 movements/min).

<table>
<thead>
<tr>
<th>Sifting duration (min)</th>
<th>Age of pupae in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1</td>
</tr>
<tr>
<td>0.0</td>
<td>82.6a</td>
</tr>
<tr>
<td>0.5</td>
<td>81.4a</td>
</tr>
<tr>
<td>1.0</td>
<td>86.0a</td>
</tr>
<tr>
<td>2.0</td>
<td>83.4a</td>
</tr>
<tr>
<td>4.0</td>
<td>74.4b</td>
</tr>
<tr>
<td>6.0</td>
<td>75.8b</td>
</tr>
<tr>
<td>8.0</td>
<td>72.4b</td>
</tr>
<tr>
<td>10.0</td>
<td>73.0b</td>
</tr>
<tr>
<td>15.0</td>
<td>59.8c</td>
</tr>
</tbody>
</table>

Values followed by same letter are not statistically different (P < 0.05) by Duncan's multiple range test.
many authors in the review of Tzanakakis (1971) were found suitable for mass production. This is considered important for mass rearing conditions where space and labor are important factors. Despite the small reduction in the egg production per female with increased density, still overall egg production is high and therefore use of high densities are justified provided that the insects are given a place to rest and oviposit. In the density of 5400 insects per cage the surface available for each insect is about 4 cm². Small egg production during the fifth week does not justify egg harvest during this period under mass rearing conditions. The results of egg density on larval diet showed a density of 15 eggs per gram diet provides pupae heavier than 5mg. A pupal weight of 5mg is considered found at the lowest permissible limit (Tsiropoulos and Manoukas 1977). A density of 15 eggs, when used routinely for mass rearing, gave occasionally pupal weights slightly lower than 5mg. It was deemed necessary therefore, to reduce density to 12 eggs per gram diet in rectangular trays. At that density in a production of seven million pupae, mean diet efficiency was 4.9 pupae per gram diet with pupal weights ranging from 5.1 to 5.5 mg. Use of the rectangular tray in the mass rearing was justified, since with round trays mean pupal recovery was 2.93 pupae per gram diet (Tsitsipis 1977a). Considering the larval density results it was seen that diet efficiency was lower in the densities from 9 to 15 eggs than at 18 and 21 eggs. Comparing pupal weights, however, it is seen that they were higher in the former than the latter. This indicates that at lower densities fewer larvae grew more antagonizing other larvae that probably could not survive, while at higher densities lower growth resulted in better survival. At densities higher than 21 eggs survival was even lower probably due to the accumulation of excretory material a hypothesis shared by Manoukas (1980). He found increased mortality after the fifth day of larval age and discusses probable causes of death. Pupal recovery at the larval densities tested agree with those of Manoukas and Tsiropoulos (1977) and Manoukas (1980) for the densities they used. The densities these authors used were 5, 10, 20, 40, 60, 80 and 100 eggs per gram diet. Because great intervals exist between consecutive densities, optimization of larval densities cannot be easily made. For larval density optimization proposed densities should be tested under mass rearing conditions that greatly differ from small scale experiments. In the latter case pupal recoveries as high as 10 pupae per gram diet are not unusual, while under mass rearing conditions yields half as high occur. The introduction of mechanical improvements greatly reduced labor and contributed to better space utilization. The sifting procedure did not seem to affect adult emergence when it was performed at ages older than three days. Other parameters were not tested, however, to see whether they are affected, as it was found by Ohinata (1980) who found damage to the flying muscles in the insects Dacus dorsalis, Dacus cucurbitae and Ceratitis capitata when sifting was done during the first 2-3 days of the pupal age. The syndrome of droopy wings observed by Ohinata, denoting muscle damage, between 18 and 43% of pupal development was not observed in our experiments. For safety reasons, however, sifting is suggested to be done after the fifth day of pupal age, where development has exceeded the 43% level (Tsitsipis 1980).

Adult maintenance at 25°C, at a density of 5400 insects per cage with a water supply containing 0.1% chlorox, larval density of 15 eggs per gram diet, use of large rectangular trays for larval rearing and construction of a diet mechanical homogenizer, larval tray rack and a mechanical sieve for the separation of the pupae from the pupation medium are improvements in the mass rearing of the olive fruit fly that made mass rearing of the insect possible, at the one million pupae per week level,
at a much reduced than previously cost.

Acknowledgments

Technical assistance of Mr. S. Koutsoukos, and typing of the manuscript by Mrs. V. Trouposkiadou and A. Kanousi are gratefully acknowledged.

References


Abstract

A comparative study using single pairs of *Ceratitis capitata* Wied. with homogamic and heterogamic crosses between a 20 year old laboratory colony and a field population, collected during April 1984 from grapefruits of Valencia, Spain (11th generation) has been carried out. The laboratory strain was in complete isolation from the field population.

On the other hand, experiments were carried out with the *F*1 and *F*2 hybrids with the same laboratory conditions.

In general, significant differences in the larval development and reproductive patterns of the two considered populations were not observed. It was therefore concluded that a very good and rapid adaptation of the newly collected field population to our laboratory rearing methods was obtained. It was also considered that the composition of the larval diet and the oviposition cages were the most important requirements for this success.

Conditions during the experiments were 26 ± 1°C, 65 ± 5% RH and 12:12 hrs. L:D regime (1.900 lux.).
DIETS FOR ADULTS OF THE MEDITERRANEAN FRUIT-FLY
*CERATITIS CAPITATA* (WIED.)

AIDA M. EL HAKIM, M. M. BASILLY
*Plant Protection Research Institute, Agriculture Research Center, Dokki, Cairo, Egypt*

Abstract

Twenty adult diets were tested for egg production of the mediterranean fruit-fly *Ceratitis capitata* (Wied.). The diets tested were all made as powder or unchangeable pastes as follows:

- Two kinds of dried milk (Similac & Isomil) mixed with sugar (1:1) or treacle (1:1) or honey (1:2) or buminal (1:0.4).
- Cubics of sugar (4.5 gm.) soaked with different amounts of buminal 0.2, 0.4, 0.6, and 0.8 gm.
- Brewer's yeast mixed with buminal at different ratios (2:0.6), (2:1.2), (2:1.5) and (2:1.8) by weight. (With all the diets used cubics of sugar were put in the rearing cages of the adults.
- The results indicated that females fed on enzymatic yeast hydrolyzate and brewer’s yeast (1:1) produced 866.0 eggs, while those fed on enzymatic yeast hydrolyzate alone produced 642.0 eggs.
- Diets composed of enzymatic yeast hydrolyzate + brewer's yeast + buminal are less expensive than enzymatic yeast hydrolyzate alone, of better physical properties and gave higher production of eggs.

Introduction

Early trials have been carried out to maintain permanent colony of the mediterranean fruit-fly in the laboratory for SIT control method. The development of efficient and economical adult diet became of great importance. Our aim of study is to investigate an economic and of good physical properties diet for egg production of the mediterranean fruit-fly *C. capitata*.

Materials and Methods

<table>
<thead>
<tr>
<th>Diet ingredients</th>
<th>Isomil</th>
<th>Similac</th>
<th>Buminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried milk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>27.3 gm</td>
<td>27.6 gm</td>
<td>Dry substance 56.8%</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>5.3 gm</td>
<td>5.6 gm</td>
<td>Ammonia nitrogen 2.4%</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>51.6 gm</td>
<td>55.8 gm</td>
<td>Amino acid nitrogen 3.35%</td>
</tr>
<tr>
<td>Protein</td>
<td>15.2 gm</td>
<td>11.4 gm</td>
<td>Total nitrogen 6.19%</td>
</tr>
<tr>
<td>Choline</td>
<td>53.1 gm</td>
<td>38.0 mg</td>
<td>Crude protein(x6.25)38.69%</td>
</tr>
<tr>
<td>Minerals</td>
<td>4.3 gm</td>
<td>3.0 mg</td>
<td>PH (10%) 4.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nacl 2.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water 43.2%</td>
</tr>
</tbody>
</table>
The diets used were

Two kinds of dried milk (Similac & Isomil) mixed with sugar (1:1) or treacle (1:1) or honey (1:2) or buminal (1:0.4). Cubics of sugar (4.5 gm) soaked with different amounts of buminal 0.2, 0.4, 0.6, and 0.8 gm.

Brewer's yeast mixed with buminal at different ratios (2:0.6), (2:1.2), (2:1.5) and (2:1.8) by weight

Enzymatic yeast hydrolyzate mixed with brewer's yeast (1:1) by weight.

Enzymatic yeast hydrolyzate + brewer's yeast + buminal at ratios of (1:3:1.5) & (1:5:2) by weight.

With all the diets used cubics of sugar were put in the rearing cages of the adults as a source of carbohydrate.

Experimental procedure

Experiments for determining fecundity, fertility and longevity of adults were done as follow. Sixty pairs of newly emerged flies were transferred to rearing cages in three replicates, each cage contained 20 pairs. The flies were supplied with the diets used, a source of water and sugar. Eggs of each cage were daily collected and transferred on a dark cloth moistened with water and kept in a petri-dish. Total number of eggs, hatchability and life of the flies were daily recorded. Enzymatic yeast hydrolyzate was used as a standard for every group of diets.

Results & Discussion

Table 1 indicates that females fed on similac mixed with sugar or treacle or honey laid the same number of eggs. The mixture of similac andbuminal significantly increased, the number of eggs laid per female (308.4). The standard diet is significantly efficient than any of the diets used.

Hatchability is different among the diets, percentage hatching of eggs the standard (93.9%) is better than the diet composed of similac and buminal (87.7%), the previous two diets are better than similac mixed with sugar or treacle or honey.

It seems that components of the diet affects fertility of the male. Adults fed on similac and buminal or similac and honey significantly lived longer period than adults fed on other diets or the standard.

The second group of diets show the same trend of the first group, isomil and buminal is significantly better than isomil and sugar or treacle or honey in producing eggs, but not efficient as the standard diet enzymatic yeast hydrolyzate. Fertility of males fed on the standard diet is significantly higher than males fed on diets composed of dried milk isomil. Longevity of males and females fed on isomil and buminal or isomil and honey is longer than the standard and other diets.

The same table clarifies that the use of sugar soaked with different amounts of buminal is failure for producing eggs of the mediterranean fruit-fly. Females laid an average number of eggs 30, 38, 31.3 and 29.3 during their lives when fed on sugar and different amounts of buminal. Adults lived few days as they stuck at the sticky cubics of sugar and died.
Diets composed of brewer's yeast and buminal are generally efficient in producing eggs than the previous diets (no 1 to 12). The addition of different amounts of buminal 0.6 gm or 1.2 or 1.5 or 1.8 differentiate the texture of the diet and gradually increase the number of eggs laid per female except the last one (no 16). Two grams of brewer's yeast mixed with 1.5gm buminal gave higher production of eggs than the other ones, and is of good physical properties. Percent hatching of eggs of all the diets used are similar to the standard diet. There is no significant difference between longevities of all males and females among all the treatments.

The table also indicates that nutrition of the mediterranean fruit fly on different sources of protein increase the number of eggs laid per female. A mixture of enzymatic yeast hydrolyzate and brewer's yeast at a ratio of 1:1 gave the highest production of eggs and improved the physical properties of the enzymatic yeast hydrolyzate. Other mixtures composed of enzymatic yeast hydrolyzate + brewer's yeast + buminal are efficient in producing eggs as the standard diet. Percentage hatching of eggs, longevities of males and females of all the diets used are equal.

The economics of producing eggs in table II clearly indicate that the cost of producing cm. by the use of brewer's yeast and buminal is 0.2 P. while it is 14.0P by the use of enzymatic yeast hydrolyzate. By other means 14.0P is the cost of producing 1 and 70 cm of eggs by using enzymatic yeast hydrolyzate alone and the mixture of brewer's yeast and buminal respectively.

Table I: Biological characters of adult mediterranean fruit-fly fed on different composed diets

<table>
<thead>
<tr>
<th>No.</th>
<th>Diets components</th>
<th>Total no of eggs per female</th>
<th>Hatching %</th>
<th>Mean longevity/days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Similac+Sugar (1 gm : 1gm)</td>
<td>42.5</td>
<td>83.2</td>
<td>40.8 35.0</td>
</tr>
<tr>
<td>2</td>
<td>Similac+treacle (1 gm : 1gm)</td>
<td>61.2</td>
<td>86.9</td>
<td>37.5 34.4</td>
</tr>
<tr>
<td>3</td>
<td>Similac+honey (1 gm : 1gm)</td>
<td>74.8</td>
<td>84.2</td>
<td>45.3 39.0</td>
</tr>
<tr>
<td>4</td>
<td>Similac+buminal (1 gm : 0.4gm)</td>
<td>308.4</td>
<td>87.7</td>
<td>45.5 47.1</td>
</tr>
<tr>
<td>St.</td>
<td>Enzymatic yeast hydro-540.1 lyzate LSD (0.05)</td>
<td>203.1</td>
<td>6.8</td>
<td>10.2 11.6</td>
</tr>
<tr>
<td>5</td>
<td>Isomil+Sugar (1gm : 1gm)</td>
<td>122.0</td>
<td>88.6</td>
<td>39.0 42.7</td>
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<tr>
<td>6</td>
<td>Isomil+treacle (1gm : 1gm)</td>
<td>127.5</td>
<td>88.9</td>
<td>36.8 38.0</td>
</tr>
<tr>
<td>7</td>
<td>Isomil+Honey (1gm + 2gm)</td>
<td>95.7</td>
<td>87.9</td>
<td>54.3 41.8</td>
</tr>
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</table>

273
Continued. Table 1

<p>| | | | | | |</p>
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<tr>
<th></th>
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<tr>
<td></td>
<td>8</td>
<td>194.5</td>
<td>89.5</td>
<td>42.8</td>
<td>39.0</td>
</tr>
<tr>
<td></td>
<td>(1gm + 0.4gm)</td>
<td>540.1</td>
<td>93.9</td>
<td>36.8</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>St. Enzymatic yeast hydro-lyzate</td>
<td>59.5</td>
<td>5.1</td>
<td>8.4</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>91.0</td>
<td>7.3</td>
<td>2.8</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>30.0</td>
<td>89.9</td>
<td>17.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Sugar+buminal (4.5gm : 0.2gm)</td>
<td>38.0</td>
<td>88.3</td>
<td>16.7</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>31.3</td>
<td>87.8</td>
<td>11.9</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>Sugar+buminal (4.5gm : 0.4gm)</td>
<td>29.3</td>
<td>85.9</td>
<td>13.3</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>31.3</td>
<td>87.8</td>
<td>11.9</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>Sugar+buminal (4.5gm : 0.6gm)</td>
<td>29.3</td>
<td>85.9</td>
<td>13.3</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>31.3</td>
<td>87.8</td>
<td>11.9</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>Sugar+buminal (4.5gm : 0.8gm)</td>
<td>29.3</td>
<td>85.9</td>
<td>13.3</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>337.8</td>
<td>95.1</td>
<td>24.0</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td>Brewer's yeast+buminal (2gm : 0.6 gm)</td>
<td>437.3</td>
<td>95.9</td>
<td>24.2</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>490.1</td>
<td>91.3</td>
<td>26.1</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>Brewer's yeast+buminal (2gm : 1.5gm)</td>
<td>562.4</td>
<td>95.7</td>
<td>22.7</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>562.4</td>
<td>95.7</td>
<td>22.7</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>Brewer's yeast+buminal (2gm : 1.8gm)</td>
<td>562.4</td>
<td>95.7</td>
<td>22.7</td>
<td>25.0</td>
</tr>
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<td></td>
<td>16</td>
<td>562.4</td>
<td>95.7</td>
<td>22.7</td>
<td>25.0</td>
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<td>643.1</td>
<td>96.5</td>
<td>45.5</td>
<td>41.1</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>771.4</td>
<td>97.6</td>
<td>45.6</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>771.4</td>
<td>97.6</td>
<td>45.6</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>Enzymatic yeast hydrolyza-+brewer's yeast (1gm : 1gm)</td>
<td>649.2</td>
<td>95.6</td>
<td>46.6</td>
<td>44.2</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>562.4</td>
<td>95.7</td>
<td>22.7</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>Enzymatic yeast hydrolyza-+brewer's yeast (1gm : 3gm : 1.5gm)</td>
<td>649.2</td>
<td>95.6</td>
<td>46.6</td>
<td>44.2</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>562.4</td>
<td>95.7</td>
<td>22.7</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>Enzymatic yeast hydrolyza-+brewer's yeast (1gm : 5gm : 2gm)</td>
<td>649.2</td>
<td>95.6</td>
<td>46.6</td>
<td>44.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>562.4</td>
<td>95.7</td>
<td>22.7</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>Enzymatic yeast hydrolyza-</td>
<td>656.3</td>
<td>94.4</td>
<td>45.0</td>
<td>42.6</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>656.3</td>
<td>94.4</td>
<td>45.0</td>
<td>42.6</td>
</tr>
</tbody>
</table>
Table II: Economics of producing eggs of the Mediterranean fruit-fly on large scale (2000 cm pupae in a cage 2x1x2 m).

<table>
<thead>
<tr>
<th>No.</th>
<th>Diet components</th>
<th>Eggs produced per cage (piaster)</th>
<th>Cost of Producing cm of eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Brewer's yeast + buminal (200 gm + 150 gm)</td>
<td>300</td>
<td>60</td>
</tr>
<tr>
<td>18</td>
<td>Enzymatic yeast hydrolyzate + brewer's yeast (100 gm + 100 gm)</td>
<td>508</td>
<td>2815</td>
</tr>
<tr>
<td>19</td>
<td>Enzymatic yeast hydrolyzate + brewer's yeast + buminal (50 gm + 150 gm + 75 gm)</td>
<td>462</td>
<td>1438</td>
</tr>
<tr>
<td>20</td>
<td>Enzymatic yeast hydrolyzate + brewer's yeast + buminal (33 gm + 166 gm + 75 gm)</td>
<td>389</td>
<td>962</td>
</tr>
<tr>
<td>St.</td>
<td>Enzymatic yeast hydrolyzate</td>
<td>393</td>
<td>5670</td>
</tr>
</tbody>
</table>
RECYCLING LARVAL MEDIA FOR MASS-REARING THE MEDITERRANEAN FRUIT FLY

N. D. BRUZZONE
Entomology Unit, FAO/IAEA Laboratories, A-2444 Seibersdorf, Austria

Abstract

Large scale SIT Programmes involve aerial release of millions of sterile flies. Improving and developing technologies to reduce costs of mass-rearing of the Mediterranean fruit fly Ceratitis capitata (Wied.) is, therefore, considered a major target by both research scientists and project managers.

Following an exploratory test carried out at the Medfly mass-rearing facilities of Metapa de Dominguez (Mexico), the potential of reusing the larval medium was further investigated. Spent medium containing viable insects was treated by microwave radiation before reseeding with larvae developing in a starter medium.

Results showed that in most of the treatments, the quality of the flies mass-reared on recycled media was comparable to that of flies produced on freshly made standard media, in terms of pupal weight, pupal recovery, larval duration, adult emergence and flightability.

Introduction

Due to its adaptability to laboratory rearing conditions and to different dietary ingredients, the medfly can easily be reared without the use of complex techniques and exotic nutrients. Thus, the larval diets currently utilized were empirically developed without knowledge of the basic nutritional requirements of the insect (Steiner, L.F. & Mitchell, S., 1966). On the other hand, the rearing media have often been modified in attempts to improve their efficiency and economy: changes were made mainly because of different suitabilities and financial advantages of local components, with consideration to the physiological and nutritional needs of the insect.

A test to recycle the standard larval finishing medium used for mass rearing the medfly is described. The goal of recycling is to reduce costs of mass-producing medflies.

The test follows one previously carried out at the Medfly mass production facilities of Metapa de Dominquez, Mexico, (Bruzzone N.D. and A.J. Schwarz, in press). The test described here examines the effects of using a larval starter medium (Fay, H.A.C., in press) and adding nutritive supplements to the spent medium before reusing.

Materials and Methods

a) First cycle

The spent medium to be tested was obtained from the standard Seibersdorf wheat bran based diet (Nadel, D.J., 1970). The starter medium was similarly prepared in amounts of 250 g that received 5 ml of eggs suspended in 25 ml of water (Fay, H.A.C., cit.). One 250 g aliquot was eventually combined with 4.75 kg of finishing medium. Eggs had been previously incubated for 48 hours before being seeded onto the starter media at the concentration of about 500 eggs/g medium. This concentration
resulted in about 25 eggs/g of finishing medium. The pH of the larval starter diet was adjusted to 4-4.3.

Starter media containing larvae were held in small plastic trays for 48 hours at 29 ± 1°C and 90% RH and then manually transferred in four equal pieces to the finishing medium with a metal spatula. The finishing medium was further maintained in the above environmental conditions for 24 hours before being moved to 20 ± 1°C and 90% RH where larval maturation was completed. Mature larvae were collected over three days by the free popping system into dry wheat bran in large metal trays.

Pupation occurred in a dark room (red radiation) at 20°C and 60% RH and was completed in 48 hours. After pneumatic separation from the bran, pupae completed their maturation in shallow layers in screen-bottomed trays, at 22°C and 70% RH.

Under the above conditions, the embryonic, larval and pupal stages completed their life cycle in about 20 days.

b) Second cycle

The bran medium for recycling was taken immediately after the 3rd larval collection and 24 hours before the fourth, which was eliminated.

The spent medium was treated in batches of 400 to 450 g for 3 minutes each (20.8 sec/g) in a microwave radiation source (500 W, 2450 MHz radiation). Diet temperature, checked by thermocouples after the microwave administration, showed variations of 50 to 80°C, depending on the distribution of the medium within the oven. It was noticed that these values tended to increase slightly by overheating of the oven after repeated use.

After radiation treatment, the spent medium was mixed in batches of about 3.6 Kg in a small industrial mixer with water added progressively until the appropriate estimated texture was achieved. The mixing lasted about 20 minutes. The optimum quantities of spent dry medium and free water had been previously found to be between 63 to 65% and 35 to 37%, respectively.

The experiment consisted of 7 treatments taken from a single batch of spent medium, and one control of fresh standard medium. Most treatments were formed for recycling by adding 50 g of starter to 960 g of medium, containing the 64% of spent recycled medium and the 36% of additional free water. For two treatments the recycled medium was seeded directly with eggs without using starter diets. In these cases the recycled medium was used in batches of 1 Kg for each treatment.

During larval development and maturation, both the recycled diets and the controls were held at the standard environmental conditions, previously described. Also, pupae were collected and held for maturation as before.

The quality of pupae and adult flies was assessed by the RAPID Quality Control Method. Values were obtained for the mean pupal size classes, the percentage of pupae that were recovered from each dietary treatment, the mean larval durations (length of larval stage in days), the percentage of adult flies emerging from the pupae and their flightability (Boller et al., 1981).

The mean pupal size class was obtained from 3 samples of 20 ml of pupae (or less when this amount was not available), randomly obtained from each of the three daily collections and mechanically sorted by size with a machine (Zelger and Russ, 1976).

The percentage of pupae recovered was derived by integrating data on the mean pupal size classes, the total volume of pupae provided by each treatment and the empirical egg hatchability index calculated as a mean from 2 samples of 100 eggs each.
The mean larval durations were extrapolated from the relative proportion of the number of pupae that were collected on each of three days, with the total number of pupae obtained from each treatment. Both the mean percentages of emergence and the mean flightability index of adults were obtained by integrating values obtained from groups of 4 samples, each of 100 pupae, taken from the second larval collections. These tests were performed at 25°C and 60-70% RH.

c) Experimental design

The recycled medium was tested with and without starter media and either without additional antimicrobials (treatment R 1 and 3) or with addition of 0.125% w/w of antimicrobial (Na benzoate) in water solution (treatment R 2 and 4). In treatments R 1 and R 2 the eggs were directly seeded onto the medium surface.

The R 4 medium was also used to test for effects of supplemental nutrients. These consisted of additions of carbohydrate (Sucrose 4% w/w) or protein (whole brewer's yeast 2% w/w) or a combination of carbohydrate and protein (Sucrose 2% w/w plus whole brewer's yeast 1% w/w).

When added, the nutrients were suspended in a water solution for about 15 minutes, then mixed with the solid phase (spent medium). Diet formulations are shown in Tables 1–3.

Table 1: Formulations of the standard wheat bran finishing medium and of the larval starter medium used in the first cycle and in the experiment (percentages w/w).

<table>
<thead>
<tr>
<th></th>
<th>Larval starter wheat bran medium</th>
<th>Finishing standard wheat bran medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bran</td>
<td>24.0</td>
<td>24.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>16.0</td>
<td>16.2</td>
</tr>
<tr>
<td>Whole Brewer's yeast</td>
<td>8.0</td>
<td>8.1</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1.12</td>
<td>0.56</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Water</td>
<td>50.4</td>
<td>50.5</td>
</tr>
</tbody>
</table>

Table 2: Formulations of the recycled media not supplemented (percentages w/w).

<table>
<thead>
<tr>
<th></th>
<th>R 1</th>
<th>R 2</th>
<th>R 3</th>
<th>R 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spent, microwave treated recycled bran medium</td>
<td>64</td>
<td>64</td>
<td>61.5</td>
<td>61.5</td>
</tr>
<tr>
<td>Free water</td>
<td>36</td>
<td>35.875</td>
<td>33.5</td>
<td>33.375</td>
</tr>
<tr>
<td>Na benzoate</td>
<td>0</td>
<td>0.125</td>
<td>0</td>
<td>0.125</td>
</tr>
<tr>
<td>Egg seeding system</td>
<td>Direct</td>
<td>Direct</td>
<td>Starter</td>
<td>Starter</td>
</tr>
<tr>
<td>(5% w/w)</td>
<td></td>
<td></td>
<td>(5% w/w)</td>
<td></td>
</tr>
<tr>
<td>Bulk pH</td>
<td>4.9–5.0</td>
<td>4.9–5.0</td>
<td>4.9–5.0</td>
<td>4.9–5.0</td>
</tr>
</tbody>
</table>

279
Table 3 Formulations of the recycled media supplemented (percentages w/w).

<table>
<thead>
<tr>
<th></th>
<th>R 5</th>
<th>R 6</th>
<th>R 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spent, microwave treated, recycled bran medium</td>
<td>59.5</td>
<td>58.5</td>
<td>57.5</td>
</tr>
<tr>
<td>Free water</td>
<td>33.375</td>
<td>33.375</td>
<td>33.375</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Whole Brewer's yeast</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Na benzoate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg Seeding system</td>
<td>Starter (5% w/w)</td>
<td>Starter (5% w/w)</td>
<td>Starter (5% w/w)</td>
</tr>
<tr>
<td>Bulk pH</td>
<td>4.9-5.0</td>
<td>4.9-5.0</td>
<td>4.9-5.0</td>
</tr>
</tbody>
</table>

Results and discussion

As shown in Table 4, results achieved with recycled media were in many cases comparable to those of the freshly made controls.

Table 4 Comparison of development and performance of medflies reared on various types of recycled media. 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean pupal weight 2)</th>
<th>% of pupal recovery</th>
<th>Larval duration</th>
<th>% of adult emergence</th>
<th>Flightability index</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 1</td>
<td>7.5 ± 0.4</td>
<td>35.97 ± 11.79</td>
<td>6.8</td>
<td>69.13 ± 6.42</td>
<td>86.58 ± 1.75</td>
</tr>
<tr>
<td>R 2</td>
<td>7.5 ± 0.3</td>
<td>48.61 ± 11.40</td>
<td>6.9</td>
<td>73.28 ± 3.70</td>
<td>86.20 ± 2.65</td>
</tr>
<tr>
<td>R 3</td>
<td>8.7 ± 0.1</td>
<td>51.19 ± 1.62</td>
<td>6.1</td>
<td>90.69 ± 2.63</td>
<td>84.82 ± 6.26</td>
</tr>
<tr>
<td>R 4</td>
<td>8.8 ± 0.0</td>
<td>48.62 ± 7.92</td>
<td>6.0</td>
<td>90.05 ± 2.87</td>
<td>87.46 ± 3.90</td>
</tr>
<tr>
<td>R 5</td>
<td>8.8 ± 0.1</td>
<td>57.16 ± 4.44</td>
<td>6.0</td>
<td>93.71 ± 2.49</td>
<td>84.65 ± 7.04</td>
</tr>
<tr>
<td>R 6</td>
<td>8.9 ± 0.1</td>
<td>60.89 ± 6.45</td>
<td>5.9</td>
<td>93.84 ± 3.50</td>
<td>83.94 ± 6.84</td>
</tr>
<tr>
<td>R 7</td>
<td>8.9 ± 0.2</td>
<td>63.03 ± 9.19</td>
<td>6.0</td>
<td>95.00 ± 1.69</td>
<td>85.74 ± 4.79</td>
</tr>
<tr>
<td>Control</td>
<td>9.2 ± 0.0</td>
<td>68.61 ± 5.58</td>
<td>5.5</td>
<td>96.39 ± 0.73</td>
<td>89.32 ± 5.31</td>
</tr>
</tbody>
</table>

1) Values are means ± SD of 5 samples for each treatment.
2) Calculated from mean pupal size classes by Zegler and Russ (1976, cit.)

The media of treatments R 5, R 6, and R 7, which were supplemented, were particularly good as regards pupal size, pupal recovery, and adult capacity to emerge and fly. It also appeared that the larval duration might be improved by supplemental protein (e.g. treatment R 6), while carbohydrate seemed to promote a heavier pupa and a higher rate of pupal recovery (e.g. treatment R 7). There were little or no differences between the values for adult emergence and flightability indexes.

Without supplements it appeared that both the use of antimicrobials and the starter medium technique improved the pupal size and pupal recovery. Also the larval duration seemed to be shortened by the same combination and the starter medium technique alone seems to improve the percentage of adult emergence. The flightability indexes did not appear to be affected by any test factors.
Acknowledgements

This work forms part of a joint FAO/IAEA research programme, and was funded in part by the "Dipartimento per la Cooperazione allo Sviluppo" of the Italian Foreign Office.

I am indebted to A.P. Economopoulos (FAO/IAEA) and R.E. Gingrich (FAO/IAEA) for critical review of the manuscript. D. Lindquist (FAO/IAEA) and A. Schwarz (SARH-DGSV) are thanked for criticism.

V. Wornoayporn is acknowledged for technical assistance, P.S. Judt for carrying out the Quality Control tests.

References


Fay, H.A.C. Reduction in the dietary space required for initial larval development of Mediterranean Fruit Fly, Ceratitis capitata (Dip: Tephritidae), under mass-rearing conditions. In press.


A MODULAR APPROACH TO FRUIT FLY PRODUCTION
FACILITIES FOR THE MEDITERRANEAN FRUIT FLY
CENTRAL AMERICAN PROGRAM

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U.S. Department of Agriculture/APHIS/PPQ/Mexico, P.O. Box 991 Brownsville, TX 78520, U.S.A.

Introduction

A system for producing sterile flies that relies on a modular design will produce a better-quality product while maximizing available resources, spreading risk and minimizing the costs of production slow-downs or failures.

The Basic Concept

The designing of a modular fly production facility is achieved by constructing a central service core which contains most, if not all, support services that are common to the entire facility (Figure 1).

The central service core provides the necessary support for the rearing modules, which are constructed as separate buildings surrounding the service core. The central service core is designed, constructed and equipped to provide for electricity, purified water, steam, compressed air, and pupae irradiation services. These services and systems include back-up or "fail-safe" equipment for emergencies.

Several alternatives are possible in configuring a modular facility, depending on local needs and the resources available. (Figure 2).

The modular fly production units are self-contained unitized buildings capable of producing from 150 to 200 million flies per week. Several of these units can be incorporated into a fly-production facility until the desired or optimum facility size has been reached (Figure 3 and key). The advantages of this approach to insect-rearing can be expressed in biological, administrative and economic terms.

Biological Advantages

Some of the more obvious biological advantages of utilizing the modular system are the following:
FIG. 1. Modular Production Facility

FIG. 2. Modular Production Facility II
Each fly production unit is isolated from the other production units and would not normally be impacted by a biological problem affecting the others. (Diet materials are the only real biological link between the modules, with one central warehouse/diet supply used for the entire facility.)

Biological problems affecting one module can serve as an early warning that would enable management to search for a solution before the problem impacted other modules.

Overall facility fly production is not adversely affected if one unit suffers a catastrophic drop in fly production. The remaining units can increase production to minimize the drop in production.

The development and application of new rearing techniques can be verified in one module before applying them to all the remaining modules.

In an emergency situation, one or more modules can provide a new "start-up" colony for a module shut down and/or sanitized because of a problem.

Individual modules will allow the facility to rear different species of fruit flies and/or insects at the same time.
Financial Advantages

Construction and equipment costs can vary considerably, but actual experience and bids have shown the modular system to be considerably more cost effective.

The buildings in a modular system are constructed as like units and can be operational within 6-8 months. This helps limit cost over-runs and other problems associated with long-term construction. Large units require a greater period of time to construct and cannot be used until they are totally finished.

A back-up capability is provided for each module by the use of multiple generators, radiators, water purification plants, boilers, compressors and other key equipment needed to maintain the operation. This allows timely maintenance and prevents module close-down due to equipment failure.

Smaller units of equipment can cost proportionally less than single larger pieces. In some cases small equipment can cost more but the low installation and maintenance costs for this equipment make it a more desirable and cost effective option. Modular facilities thus provide economies of scale in equipment expenditures.

The same economies of scale can apply to construction costs. Building four smaller buildings can cost less than building one large unit having the same amount of floor space. Modular construction can be achieved with smaller construction crews, pre-fabricated components and take advantage of the cost savings available when buying multiple components and/or equipment.

A current estimate of equipment and construction costs for modular and single large production facilities follows:

<table>
<thead>
<tr>
<th>Item</th>
<th>Modular</th>
<th>Single Large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factory Construction, including all services</td>
<td>$925,000</td>
<td>$3,000,000</td>
</tr>
<tr>
<td>Administration &amp; support bldgs.</td>
<td>$125,000</td>
<td>$230,000</td>
</tr>
<tr>
<td>All equipment/furniture</td>
<td>$2,200,000</td>
<td>$3,633,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$3,250,000</strong></td>
<td><strong>$6,864,000</strong></td>
</tr>
</tbody>
</table>

Production/Quality

A fly quality comparison between the single large plant and modular design is difficult, since they are not totally "like" systems. However, experience has demonstrated that large plants present a greater problem when controlling quality. These quality problems have been due to the inability to control plant environments, isolate microbe problems and other factors.
Although these problems can affect any plant, regardless of size, the one large plant design presents more difficulties because the area to be controlled is so much more extensive.

In a large plant, when air conditioning and humidity systems must be modified, corrected or serviced, it can disrupt the total fly production and quality. This disruption can be extensive, depending on the time necessary for the modifications or maintenance. Any structural changes such as construction of walls or partitions will also have a negative impact on production.

Production in Mexico's single-unit Medfly laboratory has been affected by a series of production problems due to air-conditioning and humidity management. Post-emergence percentages for pupae averaged 65-70 percent throughout 1985. This problem has reduced the efficiency of the plant and fly quality.

An analysis and proposal for modifying the facility's air handling system has been prepared to try to control temperatures and excessive humidity problems. The time needed for completing the modifications could be several months. In the interim, production throughout the facility will continue to be affected.

In contrast, the modular design facility can be modified and maintained module by module and not greatly impact the overall facilities production or quality of the flies.

To avoid many of these problems the Medfly production facility in Guatemala was built on a modular plan.

In this modular facility, we have found that corrections to the production environment can be made more readily and that the required regimes for temperature, humidity, etc., can be maintained with less difficulty.

Facility Maintenance

Facility maintenance can also be approached from a modular or unitized concept. This can best be expressed by reviewing the individual basic elements involved in the maintenance operation.

The central service core can be maintained by a general plant maintenance staff. This staff also takes care of all "outside" maintenance required at the facility.

The general maintenance staff also maintains an overall parts and equipment inventory for the facility.

Facility maintenance management determines the preventive maintenance and equipment replacement schedules for the central services core and each module.

All equipment and parts can be standardized and interchangeable between modules.
All routine module maintenance can be done by an internal maintenance staff to avoid employee traffic between modules. Each module maintains a small inventory of replacement parts. Module maintenance is limited to the replacement of defective components.

Facility Management

The overall management of a modular factory complex offers some advantages not found in a single large facility.

Personnel: The individual rearing modules can provide management the opportunity to compare performance between units doing exactly the same task, under the same conditions. Performance, in this case, could be measured by production levels, fly quality and management of resources.

The multiple rearing modules can also promote healthy competition between the individual module managers and their employees.

The inherent back-up capability factored into a modular operation offers a flexibility and reliability not available in a single large factory. Managers can cover programmed or unscheduled vacancies with the personnel pool available from other modules.

Training is also easier since the tasks are alike in each module, and on-the-job training can take place before the facility is fully completed. One completed module can function as a training site while the remaining modules are under construction.

Employee rotation can also be implemented within and between modules, which would provide for a variation in duties and environment.

Each plant would be a self contained unit having its own plant manager, personnel to perform routine maintenance, fly-rearing and quality control activities.

Perhaps most important, a modular structure can enhance management's capacity to monitor both product and process specifications, thus facilitating rapid identification of problems, timely response, and minimization of costs due to production slow downs. In order to fully utilize the increased control and coordination capacities inherent in a modular structure, traditional management techniques must be modified in order to facilitate both vertical and lateral movement of information.

The organizational chart in Figure 4 suggests a communication and coordination mechanism capable of linking management vertically and horizontally. The facility has traditional vertically-organized units; however, the bottom-up communication paths are supplemented by lateral communication channels. Lateral communication paths tap different information sources and help to protect upper managers from the universal propensity of lower-level employees to filter, analyze and pre-package information.
Each facility has vertically-organized units, such as Central Services, Irradiation, Shipping, Warehousing and Rearing, which in the modular concept is composed of multiple identical units. Within the rearing module, all functional groupings, such as quality control, production, packing and maintenance also have leaders. In addition to the traditional quality control unit monitoring fly quality, every unit within the facility has an internal quality control group that monitors processes and procedures to determine if tasks are being carried out according to specifications. Communication is vertical within these groupings.

The facility is also cross-cut laterally by three in-house operations review teams that separately address management, technical and quality control issues, and identify inter- and intra-unit problems of coordination, communication, task performance and technology (Figure 5). These teams are composed of a rotating membership of one lower-level employee from each of the modules, and within each module from the functional units. The mission of the operations review teams is to identify problems they and their colleagues personally encounter. All three operations review teams meet regularly and communicate directly with the Module Manager and the Facility Manager. Communication is lateral.

A second review group, composed of advisory panels of specialists from outside the facility, is a necessity both to review program operations and to enhance communication with industry and the general public.

FIG. 4. Modular Production Facility

![Diagram of Modular Production Facility]

(Figure 4 shows a hierarchical diagram of the modular production facility, with the Facility Manager at the top, followed by Managers for Modular I, Modular II, Modular III, and Modular IV, each with Management and Technical levels.)
FIG. 5. Quality Control Review Team

- Management Supervisor Modular I
- Quality Control Supervisor Modular II
- Maintenance Supervisor Modular III
- Rearing Supervisor Modular IV
- Operations Level Worker (Rotating)

public. Panels addressing technical, medical and policy and environmental issues are recommended. Access to them and by them at all project levels is required.

In Mexico and Central America, several production facilities already exist or are planned for the near future. The outside technical review teams would provide an additional link among these plants, by monitoring performance and facilitating communications among the facilities.

Information Flow: One of the most important problems the facility manager would face is that of information flows and evaluation. To reduce the problems of information overload at management levels, organizational memory should be developed through formal written records of meetings and through participation of individuals from all project levels. Although individuals within organizations constantly learn and can recall solutions to their problems, organizations themselves have no memory other than written records.

Conclusions

The modular concept, as outlined, offers many advantages not present in the single large plant design. Its flexibility and risk-spreading
considerations justify its use and -- as an added bonus -- it costs considerably less. The quality problems and long-term maintenance problems of a single large facility confirm the feasibility of a modular approach.

The ideas presented here are a reflection of our experiences in Mexico, Guatemala, the United States, and other areas. As the process of Medfly control continues throughout Central America, we hope to learn from those activities and continue to refine the techniques and technologies to meet the ever-changing needs and demands of sterile insect production.

Acknowledgements

The following people participated in a recent U.S. Department of Agriculture workshop on modular laboratories. Their contributions helped define and develop the ideas presented in this paper: Mr. James Bennett, Maya Construction Company, Houston, TX., USA.; Dr. Derrell Chambers, USDA/APHIS/Guatemala City, Guatemala; Dr. Ed Gersabeck, USDA/APHIS/Tuxtla Gutierrez, Mexico; Dr. H.C. Hofmann, USDA/APHIS/Mexico City, Guatemala; Mr. Felipe Jeronimo, Medfly, Guatemala City, Guatemala; Mr. Richard Kobayashi, USDA/APHIS, Honolulu, Hawaii; Dr. Harold Mabry, USDA/APHIS/Mission, TX, USA.; Mr. Gerardo Ortiz, Medfly, Tapachula, Mexico; Dr. William Schultz, USDA/ARS/Albany, California; Mr. Arturo Schwarz, Medfly, Tapachula, Mexico; Mr. Lynn Stevens, USDA/APHIS, Tapachula, Mexico; and Dr. Robert I. Vargas, USDA/APHIS, Honolulu, Hawaii, USA. I would also like to thank Dr. Hilary Lorraine Chambers, Mr. Ed Stubbs and Mr. Patrick Gomes for their contributions to this paper.
REPRODUCTION
CONSIDERATIONS ON THE REPRODUCTIVE BEHAVIOR OF ANASTREPHA PSEUDOPARALLELA, LOEW 1873 (DIPTERA, TEPHRITIDAE)

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Abstract

Reproductive behavior of Anastrepha pseudoparallela was studied under natural conditions. Hourly surveys were made in two Passiflora alata plantations in three different years. By using adhesive traps temporally different frequency peaks for the capture of females (11AM-12) and males (5-6 PM) was determined. By direct observation, it has verified that the largest number of females ovipositing occurred between 11AM-1PM and the mating always took place at the end of the afternoon or beginning of night (5-7PM). The females laid their eggs in green fruits and there was a strong sign of the existence of a deterring-pheromone.

The matings lasted for about 2-3 hours, on the lower surface of the leaves where the females were found, but attempts of mating of fruits can occur.

Introduction

In Brazil, the most economically important tephritids belong to the genus Ceratitis, represented by the single species Ceratitis capitata, and to the genus Anastrepha, which consists of more than 70 species. The latter genus, which is widely distributed, comprises the species Anastrepha pseudoparallela, also known as "passion fruit fly" because it infests some species of Passiflora, whose fruits have important nutritive and pharmacological properties. According to Zucchi (1978), A. pseudoparallela is a tropical species distributed throughout Brazil, Peru and Argentina which has been found to infest also mangoes (Mangifera indica) and guava (Psidium guava).

Few studies have been published on the basic biology and on the extent of damage caused by A. pseudoparallela. Studies have been carried out on the salivary glands of males (Silva, 1978), on the behavior of this species in the laboratory (Polloni, 1981, Silva et al, 1985), and preliminary observations have been published on the extent of infestation and on the duration of pupal development (Polloni, 1984). In view of the apparent lack of studies on the biology of this fly under natural conditions, the present study was undertaken as a contribution to the understanding of the biology of A. pseudoparallela by providing information on the reproductive behavior observed in natural populations.

Material and Methods

Two Passiflora alata plantations belonging to University farms were used: the first (Area 1 - 312m²), located in Uberlândia, and the second (Area 2 - 64m²), in Ribeirão Preto. Both were rectangular and subdivided into blocks with numbered stakes.
One hundred and twenty four surveys carried out for 13 different days were made in Area 1 from May to September 1984, 153 (for 16 days) from January to July, 1986, and 108 surveys were made in Area 2 (for 10 days) from April to June, 1985.

The surveys were made by walking through all the blocks of the plantation and recording the sex, location and behavior of each fly observed. These observations were made hourly from early morning to sunset and temperature, relative humidity and light intensity were recorded.

The frequency of flight activity was determined in Area 1 (1984) by using adhesive traps distributed in different positions throughout the plantation. These positions were changed daily during the period of observation (11 days). Captured flies were removed from the traps every hour and fixed.

Results and Discussion

A - Direct observation

With respect to the daily cycle of activity of *A. pseudoparallela*, we noted wide variation in the frequency of flies from day to day, place to place and year to year, probably owing to environmental variation and to the level of infestation. However, temperature was one of the main factors affecting fly distribution on the host plant, as shown by the data summarized in Figure 1.

There seemed to be a slight positive correlation between temperature and number of flies. Furthermore, when the temperature interval from 24 to 29°C (most frequently recorded temperatures) is considered, the percentage of flies observed was 77% in A, 54% in B and 68% in C.

B - Observations using traps

When the number of individuals under the host plant was determined, males and females carrying out some type of behavior (feeding, aggressiveness, oviposition, resting, cleaning) were recorded. It was not always possible to observe directly individuals performing flight activity. Thus, the purpose of the adhesive traps was to obtain a sample of the population which was performing flight activity at each hour. The results are presented in Table I.

Initially, the analysis of the table shows that a larger number of males (241) than females (163) was captured, indicating that males move more frequently. On the basis of the total number of insects collected, two peaks of capture were found to occur during daytime hours: one between 11 A.M. and 12 (n = 57), and the other between 5 and 6 P.M. (n = 84). It can be seen that the number of days of observation was not identical during all periods, but, since we noted that the insects do not move in the absence of light, we decided that it was not necessary to repeat the observations every day. The table also shows that temperature seems to play an important role in starting insect flying activity, because even though the amount of light appeared to be sufficient between 7 and 8 A.M., very few insects were captured in the traps at that time. We thought it convenient to separate a main body of data in the table (from 8 A.M. to 7 P.M.) to standardize the observations that were repeated for a relatively similar number of times. These results are shown in Figure 2.

The highest percentage of the total number of females captured was collected between 11 A.M. and 12, whereas most males were captured between 5 and 6 P.M. These two temporally different frequency peaks may be related.
Figure 1 - Number of flies vs temperature scatterplot. Each numbered point represents the number of superimposed hourly observations.

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to the times of reproductive behavior recorded by direct observation and described below.


<table>
<thead>
<tr>
<th>Observation</th>
<th>Number of</th>
<th>Mean</th>
<th>Number of flies collected</th>
</tr>
</thead>
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<tr>
<td>days (hrs)</td>
<td>observed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>days</td>
<td>Temp</td>
<td>RH</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------</td>
<td>------</td>
<td>----</td>
</tr>
<tr>
<td>5 — 6</td>
<td>3</td>
<td>13.3</td>
<td>92</td>
</tr>
<tr>
<td>6 — 7</td>
<td>4</td>
<td>15.5</td>
<td>91</td>
</tr>
<tr>
<td>7 — 8</td>
<td>5</td>
<td>18.2</td>
<td>80.3</td>
</tr>
<tr>
<td>8 — 9</td>
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<td>8</td>
<td>22.0</td>
<td>63.7</td>
</tr>
<tr>
<td>19 — 20</td>
<td>2</td>
<td>20.5</td>
<td>76</td>
</tr>
<tr>
<td>TOTAL</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

C - Behavior

a - Oviposition

Our observations showed that the female can lay her eggs on fruits at different stages of development starting from very young fruit, but always on green fruits. After finding a fruit, the female lands on it, walks around it, inserts her ovipositor and may or may not lay eggs. The duration of oviposition is extremely variable, ranging from 5 seconds to 15 minutes. We observed females that, after selecting a fruit, performed from 1 to 16 insertions at different points on its surface. From each punctured site, some fluid exudes which the female sometimes utilizes as food. Immediately after removing the ovipositor from the skin of the fruit, the female walks around the fruit dragging her ovipositor throughout its surface. This behavior is sometimes interchanged with ovipositor cleaning, stopping to suck the exudate, a short flight and a return to the fruit, where the ovipositor is again protruded and dragged about. This ovipositor-dragging behavior is a strong sign that also in this species the female may have a oviposition-deter­ring pheromone as demonstrated for other tephritids such as several species of Rhagoletis and two species of Anastrepha itself, i.e. A. suspensa (Prokopy
Females (n=240) vs. Males (n=162) in terms of Hour of the day and Percentage of flies collected with adhesive traps.

Figure 2 - Frequency distribution of the number of A. pseudoparallela flies collected with adhesive traps.

Figure 3 - Distribution of the time points of reproductive behavior of A. pseudoparallela.
(a) Number of females with the ovipositor inserted into the fruit (N=90).
(b) Number of mating pairs (N=17).
et al., 1971) and A. fraterculus (Prokopy et al., 1982). This sign of oviposition control has also been observed in the laboratory by Simões et al. (1978) for A. obliqua and A. sororcula, as in order to obtain more ovipositions in artificial containers it was necessary to change or wash them up at two days' intervals. In laboratory observations under way, we noted that A. pseudoparalella females may lay one or several eggs during a single oviposition.

Ovipositing behavior tended to occur preferentially between 11 A.M. and 1 P.M. although it could also occur throughout the day (Figure 3). The tendency towards greater ovipositing activity between 11 A.M. and 1 P.M. as shown in figure 3A led us to associate these data with those presented in Figure 2, which shows a larger number of females captured in the traps during this time interval. The captured females were probably moving about when looking for appropriate egg-laying sites. Analysis of the ovarian development of these females may confirm this hypothesis.

b - Mating

The mating behavior observed in nature was limited to the end of the afternoon, and the main stimulus for the starting of this activity seems to be the decrease in light intensity. Figure 3B shows the time distribution of the 17 matings observed in nature. It can be seen that this time corresponds to that of greatest capture of males in the traps (Figure 2) and also to a slight increase in the number of females captured. However, males definitely moved about more frequently during this period. Direct observation also showed that males fly very intensely during this time. A case was observed in which the male flew to the leaf where the female had alighted, stood facing her and jumped over her, which then lifted the ovipositor. This occurrence should be emphasized because it contrasts with what occurs with most tephritids, whose males exhibit calling behavior and it is the female that flies to the leaf where the male is (Burk, 1983, Morgante et al, 1983).

Mating behavior was similar to that described by Silva et al. (1985) under laboratory conditions.

The meeting site and the site where mating started was the lower surface of the leaves, mainly those closer to the ground.

We would like to point out two cases in which the male approached the female on the fruit. On one occasion, the female was laying eggs and the male walked around the fruit feeding on the liquid exuding from the punctures made by the female. The female expelled the male; he jumped over her and she expelled him again, continuing to walk rapidly around the fruit dragging her ovipositor. On the second occasion, there actually was an attempt at mating with a female standing on a fruit at 5:20 P.M. The male landed on the fruit, faced the female and jumped over her, which freed herself from him by taking a short flight and then returning to the fruit. The male vibrated his wings and made a new unsuccessful attempt at mating. These facts should be recorded because it should be investigated whether, this species as well, two strategies for the initiation of matings probably exist, as discussed by Burk (1983) for A. suspensa.

Of the matings observed here, only two were followed from the start and lasted 2:30 and 2:43 hours, respectively. Of the matings whose initiation were not observed, 9 lasted less than 1 hour (range: 15 to 54 minutes), 3 more than 2 hours (2:07-2:36), 1 lasted 3:20 hours and 2 were observed for almost 5 consecutive hours (4:37-4:53) after which the observation was discontinued. On the following morning, we observed that in one of these cases the couple was still on the lower surface of the leaf, although apart, but it was not possible to establish the time when the activity ended. On the basis of laboratory observations (unpublished data), we
can state that the median value for the frequency distribution of 60 matings observed was 2 hours and 40 minutes.

Conclusions

1. Males of A. pseudoparallela seem to show more intense flight activity than females.
2. Females were captured in larger numbers between 11 A.M. - 12 and males between 5-6 P.M.
3. The females of A. pseudoparallela lay their eggs in green fruit since the early phase of fruit development.
4. The largest number of female ovipositing was observed between 11 A.M. and 1 P.M.
5. Mating in nature occurred always at the end of the afternoon after 5 P.M.
6. The insects met and started mating on the lower surface of leaves, as is the case for a large number of tephritid species.

Acknowledgements

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References


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REPRODUCTIVE BEHAVIOUR AND POST-MATING FEMALE GUARDING IN THE MONOPHAGOUS MULTIVOLTINE Dacus Longistylus (Wied.) (DIPTERA: TEPHRITIDAE) IN SOUTHERN EGYPT

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Abstract

Systematic observations on the behavioral ecology of mating in Dacus longistylus (Wied.) were made in Qena, Egypt, during the summer of 1986. This tephritid, has been reported from India to Senegal, where it is known to occur on Calotropis procera Ait., an asclepidaceous Milky weed of a woody perennial character with large inflated fruits.

D. longistylus is a monophagous multivoltine tephritid with a resource defense polygyny mating system lacking the lekking, puffing, and accoustical sigalling behaviors, which are typical of many tropical frugivorous tephritids. As in monophagous temperate species, males monopolize and defend the female oviposition site, mating females attempting to oviposit. But unlike the univoltine temperate species, where the early season matings occur on the foliage, matings of D. longistylus take place nearly exclusively on the host fruit. Females are raped an average of 3-4 times before they manage to oviposit and the last male to mate a female aggressively tries to guard her from other patrolling males during her following oviposition attempt. Males apparently do not recognize the female they are defending after a mating, and do so only for a fixed period after which they attempt again to mate females on the fruit.

Introduction

According to Wiedmann (1830), Becker (1903), Efflatoun (1924), and Ali (1957), Dacus (Leptoxyda) longistylus (Wied.) = Trypeta longistylus Wiedmann (Steyskal and El-Bialy, 1967), occurs in India and Burma, extending to Arabia, Ethiopia, Sudan, Egypt and through French Equatorial Africa to Senegal. In these places it is found on Calotropis procera Ait., an asclepidaceous woody perennial Milky weed, that grows to 1-5m. In Egypt this host is distributed south of latitude 30°, including Southern Sinai, oases in the Western Desert, the Red Sea coast, and is very common in the southern governorates of Qena and Aswan, where it is known as Oshar (Tackholm, 1974).

El-Zoheiry (1948), reported that D. longistylus, feeding primarily on C. procera, had adapted to the economically important cucurbits in Egypt. He recommended that C. procera plants be destroyed as a measure of control. Azab and Kira (1954) confirmed that this fly is a serious cucurbit pest. Ali (1957), however, proved that D. longistylus is specialized on C. procera, while another species D. ciliatus (=brevistylus) Loew, is confined to cucurbits. Ali (1957) also described some aspects of the biology of D. longistylus.
In the present study we report on our systematic field observations of the mating behavior of wild *D. longistylus* flies in Upper Egypt. Our specific objectives were: 1) to census the distribution of males and females on fruits, leaves and flowers every hour throughout the fly activity period (0500-1900h); 2) to observe interactions between flies equally on fruits, leaves and flowers throughout the activity period; 3) to determine if the female-guarding behavior of males is based on mate recognition.

Material and Methods.

Observations were made in the Nile Valley, on the edge of the Eastern Desert, approx. 15km South of Luxor, in July - August 1986. Temperatures and relative humidities were recorded using a thermohydrograph. Two isolated *C. proccera* plants, separated 4m from each other were selected for the first part of this study. Both were nearly 2m high, and after removing some stems for convenience of the observers, each plant had five green stems, with clusters of terminal flowers and fruits in all stages of development. Fruits were classified into three categories: "small fruits", which were still not inflated, with a diameter from 2 to 5cm; "inflated fruits" of diverse ages and measuring 8-15cm long by 5-10cm wide; and "old" fruits, which were in diverse stages of shrinking, and yellowing. For three consecutive days, July 3-5, flies were observed from 0500 to 1900h, taking a census of location every hour, and the time between census periods we spent equally observing encounters on fruits, leaves and flowers. Additionally other vegetation in the area was regularly inspected for fly presence. Mating pairs were carefully marked with oil colors on the thorax or abdomen, to follow up on their individual history and to detect arrival and dispersal movements. Unfortunately our plants were destroyed the night of the third observation day, so that the marked and observed fly population was lost. Some of the males still patrolled for some time the fruit on the ground and other individuals, mainly females were found on the nearest plants respectively 8, 40 and approximately 100m away.

Further observations were carried out on five days (from 0600 to 1200h) between July 16 and August 18, in which the postmating behavior of 30 marked pairs was recorded.

Results

Location and Time of Activities

The average number of *D. longistylus* flies observed in hourly census counts together with the mean of temperature and relative humidity is shown in TABLE I. There were always considerably more males than females present on the host plant. The male to female ratio reached a peak of 3.88 at 0900h, decreased again to a low of 1.92 at 1500h, and from there increased again towards the end of the day to 3.57.

Activity of *D. longistylus* is mainly affected by light intensity and temperature. With the first light before sunrise flies become active, both males and females visit the flowers.
TABLE I. Average numbers of D. longistylus flies /hourly census counts observed on C. procera, with average temperature and relative humidity. Qena, Egypt, summer 1986.

<table>
<thead>
<tr>
<th>Time</th>
<th>Av. Males /Census</th>
<th>Av. Females /Census</th>
<th>M/F Ratio</th>
<th>Av. Temp. °C</th>
<th>Av. R.H. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0500</td>
<td>18.0</td>
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<td>2.25</td>
<td>23.2</td>
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<td>7.0</td>
<td>3.57</td>
<td>33.0</td>
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of the host, and males move to the fruit. By the time direct sunrays reach C. procera, more males move to the fruit and also female visits to the fruit increase (FIG. 1A). With higher temperatures, flies move to positions protected from the sun, males on the underside of the fruit and females under foliage. From around 1200h onwards more and more males and females move to the lower foliage, reaching a peak around 1500-1700h (FIG. 1B). Once temperatures begin to decline, fly presence on fruits and flowers (FIG. 1C) increases shortly again, before males and females congregate on the highest foliage facing the last sunlight. Overall males spent 54.9% of their time on host fruit (50.7% on inflated fruit, 1.8% on small fruit, and 0.4% on old yellow fruit); 41.4% under host leaves (29.0% on the upper half of the foliage and 14.4% on the lower half); and 3.7% on host flowers. Females spent only 12.5% of their time on host fruit (10.2% on inflated fruit, 2.0% on small fruit, and 0.3% on old green-yellow fruit); 82.9% on host leaves (44.4% on the upper half of the foliage and 38.5% on the lower half); and 4.6% on host flowers. On some C. procera plants, which had only flowers at that time rarely were flies seen, and these were mostly females. Additionally, in a mixed citrus, guava and mango orchard, approx. 50m away, regularly some D. longistylus flies, mainly females, were observed resting in the inner canopy.

Activities on Fruit and Foliage

Most of the interactions and encounters between males and males and females occur on the inflated host fruits. Throughout the mornings, males patrol these fruits, which often occur in pairs or clusters of 2-4 fruits of varying age, by actively walking around their circumference in constantly changing angles. Encounters of relative low intensity between patrolling males occur continuously. Dominant males either jump successively on top of other males to reconfirm their sex and apparently to control them whenever they start to patrol actively, or they chase them from the fruit. They are success-
FIG. 1. Location of observed *D. longistylus* flies on *C. procera*. A) Percentage observed on host fruit. B) Percentage observed on host leaves. C) Percentage observed on host flowers.
ful only temporarily, as the diameter of the fruit does not allow for the immediate detection of returning males or visiting females. As shown in TABLE II, the number of males patrolling varied depending on the time of the day and the degree of development of the individual fruits, optimal fruit

<table>
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<th>Time</th>
<th>A (3)</th>
<th>B (4)</th>
<th>C (3)</th>
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<td>1730</td>
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</tr>
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<td>Total</td>
<td>58</td>
<td>12</td>
<td>38</td>
<td>27</td>
<td>26</td>
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</table>

Females on the other hand, remain throughout most of the day on the host foliage, carrying out only short visits to fruits, inspecting them with the probosis and on occasions probing with the ovipositor. These visits are generally interrupted by patrolling males. In face to face encounters, which females evade with relative ease, males generally retreat to avoid scaring the female from that fruit, and then try to follow her during her inspection. Whenever females initiate puncturing, males attempt to jump on top of them from the back, being regularly succesful in grasping her with their front legs and pulling up the ovipositor with the hind legs.

Rarely did a male grasping a female lose her again, including the common cases in which the struggling pair would fall from the fruit to lower foliage or the ground. Even in situations the male lost the hold of the female with his frontlegs, mostly due to the ensuing scramble with approaching rivals, and was being dragged around only united with the resisting female by the aedeagus, he generally did not let her go and often recovered his position on top of the female. On numerous occasions the patrolling male managed to pull the female's puncturing ovipositor from the fruit and to insert his aedeagus into the ovipositor still bend downward.

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The observed male-female encounters on the fruit during the hours of the day are shown in FIG. 2B. An additional 5.1% of male-female encounters took place on foliage, of which no matings resulted. These occurred when a male pursued a female to the foliage, or when he detected a female near the fruit.

A total of 46 matings were observed during the first three observation days (FIG. 2C): all took place on inflated green fruit, and all resulted from rapes of females inspecting fruit or attempting to oviposit. Mating time averaged 53 min. + 15 min and mating pairs were nearly always disturbed considerably by other males. Most mating pairs moved soon to more protected positions on the host foliage, (isolated mating pairs generally remained on the fruit, the female walking around inspecting the fruit). A mating pair was eaten by a mantis sitting on a leaf above a fruit cluster. During uncoupling the thin aedeagus was stretched between the mounted male and the female's ovipositor over more than 1cm before separating in a snapping action.

In a majority of the observed matings a post-mating guarding behavior was detected, in which males returning from a mating to the fruit, actively chased other males from the fruit and circled "nervously" around the returning female, aggressively trying to protect her from rival males.

Females attempting to oviposit were generally subject to several rapes in a day or even consecutive days until they were able to oviposit. After matings they generally returned again to the same fruit. Marked females, that remained on the observation plants, were mated an average of 3 to 4 times per female, reaching extreme cases of respectively five and ten matings on one day. Only in cases where the puncture in the fruit had caused a significant latex flow, did males refrain from attempting to mount her.

Ovipositions reached a peak around midday (FIG. 2C), a time when many males were involved in matings, or were confined to the shaded part of the fruit. They lasted an average of 154 + 35min. During this relatively long period females are apparently subject to considerable predation as two abdomen with ovipositor were found still puncturing fruits. When they are ovipositing females extend an ovipositor with a movable angle (larger than all the rest of their body), which apparently permits them to bridge the 2 to 3cm wide inflated air space and to find the center of the fruit on which the eggs are deposited.

After terminating oviposition, females spent an average of 14 + 7min. walking around the oviposition site and moving the probosis up and down, possibly marking the site. An analysis of 36 fruits indicated that there were rarely more than three ovipositions in one fruit, with normally only one or two successfully reaching the seed-center. The amount of larvae varied considerably (Ali, 1957), with a majority between 20 and 40, that turn the elongated approx. 2 by 3cm, lcm-narrow fruit centers into a dark powdery mass with faeces. Younger clutch larvae were observed two times to be subject to strong predation by ants that penetrate the shrunkken fruit through the exit hole of the first clutch. After ovipositing, marked females were seen mainly feeding and dispersing. On one occasion a female was seen ovipositing five days after her previous oviposition.
FIG. 2. Sexual behavior of D. longistylus on C. procera.
A) Average number of male-male encounters/observation period.
B) Timing of male-female encounters. C) Timing of matings 
(N=46), and ovipositions (N=18).
Additional Post-Mating Observations

In one of the additional 30 matings observed in the second part of this study, a male did not return to the fruit of the mating after mating, and in two cases females did not return. Another two matings occurred away from the fruit, when patrolling males detected a female on the foliage.

It took returning males after separation of genitalia an average of 78 + 49 sec. to reach the fruit, (part of which the male continued to hold the female, while retracting his aedeagus), and females 133 + 82 sec. In 18.5% of cases the returning male was defeated while trying to chase away other males from the fruit, and in another 14.8% he was overcome, sometimes by as much as three or four, while attempting to protect the returned or another female initiating oviposition.

In the most common outcome, 80.8% of returning females, unable to differentiate the male attempting to guard her from other patrolling males, were scared away one or several times, while encountering the male that had just mated her (16.7%), or other males (83.3%). They returned an average of 3.6 times from the foliage, before they were either force-mated again, managed to oviposit, or did not return. The average time after a mating, that returning males were observed aggressively chasing other patrolling males, was 10.2 + 2.7 min.; additionally they attempted to defend an arriving female initiating oviposition for 6.3 + 2.1 min. Females scared off several times, and continuing to return after this postmating guarding behavior had apparently elapsed, were mated again by the same male on three occasions, and on seventeen by other males.

Males succesfully defending their returning female until oviposition was sufficiently initiated, was observed five times; in one of these a different female was protected at the same time, and in another case the "wrong" female was guarded, and the returning female was chased away. On two occasions, females returning again after having been scared off one or several times, managed to oviposit unprotected, by either finding hidden positions or by ovipositing during noon-time on the side of the fruit exposed to the sun, without being detected by males congregated on the shady side of that fruit. Also on three occasions a female was mated again at the end of her two and a half hour long oviposition, once by the same male that protected her and two times by other males.

Discussion

This study reports on the mating system of a monophagous multivoltine Dacus species and describes for the first time in Tephritids observations on mate guarding behavior.

The relationship between the mating behavior and aspects of the ecology of frugivorous fruit flies has been established (Prokopy, 1980; Burk, 1981). In species with restricted host ranges, often temperate in distribution, fruit resources are predictable in space and time, allowing males to monopolize this encounter site. In polyphagous fruit flies on the other hand, which are mostly tropical, the greater variability and less predictability of the fruit resources result in fewer encounters for males defending territories there. Matings occur mainly on the foliage determined by female choice.
A feature found regularly in both types of mating systems in frugivorous tephritids is the existence of dual male mating modes, that maximize on a seasonal basis in temperate species, and on a daily basis in more tropical species, the frequency of encounters with receptive and unresponsive females (Parker, 1978). In temperate, univoltine Rhagoletis species, due to the relative synchronization of female emergence, males pursue an early season strategy attracting receptive females on the host foliage (Smith and Prokopy, 1979). With the onset of oviposition males shift for the rest of the season to the host fruit, to intercept ovipositing females (Boyce, 1934; Prokopy and Roitberg, 1984).

In more tropical, polyphagous species, such as Dacus, Ceratitis, and Anastrepha, the relative importance of the two male mating modes is reversed and the majority of matings take place in male aggregations away from the host fruit, mediated by male sexual signals and female mate choice (Tychsen, 1977; Prokopy and Hendrichs, 1979; Aluja et al., 1982; Hendrichs, 1986; Iwashashi and Majima, 1986).

The mating system of the papaya fruit fly, which is tropical, but at the same time has a restricted host range, is intermediate between these two mating systems in the importance of both male mating modes (Landolt and Hendrichs, 1983).

D. longistylus, being monophagous, has predictably a resource defense polygyny mating system (Emlen and Oring 1977), similar to the univoltine temperate Rhagoletis species. However, unlike these species, no secondary male mating mode on the foliage seems to occur. Throughout their activity periods during the day males remain on the host fruit competing to intercept arriving females. The only matings observed on host foliage resulted from males on the fruit detecting females nearby. Probably due to the year-round availability of the fruit of their sole host, and their multivoltinism, no marked synchronization of female emergence occurs. Additionally it might not be economical for males to attract receptive females on the foliage when these probably will be intercepted afterwards on the fruit by other males.

D. longistylus males appear to have lost all signalling behaviors known from other Dacus species. Also morphologically (wings are nearly all transparent), males seem not selected for visual signalling as in tephritids with elaborate courtship and female choice (Burk, 1981). Multivoltine monophags represent apparently an extreme in the differential importance of the two male mating modes in frugivorous tephritids. Instead they have evolved other adaptations to their mating mode at the sole encounter site, such as the observed male guarding in D. longistylus.

This guarding behavior could be described as a post-insemination non contact preoviposition female guarding (Thornhill and Alcock, 1983), closer to the one reported by Waage (1979) in the odonatan Calopteryx maculata, than the one observed in the dipteran dung fly (Parker, 1974) or cactus fly (Mangan, 1979). It represents an adaptation to a resource defense polygyny mating system in which possibly due to sperm precedence, a high operational sex ratio, and the fact that the monopolized resource is the oviposition site, males have to compete intercepting females, and also have to assure that the female initiates oviposition successfully afterwards.
In spite of the relatively low rate of success in the guarding behavior, males apparently increase their fitness by hyperactively chasing for a limited period other patrolling males from the fruit and by defending and allowing during this period incoming females to oviposit. The relatively short post-mating period, however, after which males patrol again the fruit attempting to mate incoming females, probably marks the limit where the net costs, of further fighting and waiting for the returning female, become too high in terms of energy and the possibility of defending a "wrong" female and not mating her. This period is apparently sufficient to cover the critical time required by a female to return after a mating to the same fruit, to find a suitable site and to initiate puncturing until enough latex from the fruit surrounds the female's ovipositor. From this moment on, patrolling males no longer place their abdomen near the female's ovipositor, apparently to avoid getting their fine aedeagus into contact with the rapidly hardening latex. The fact that non-contact guarding takes place, in spite of the short postmating effort required, possibly indicates that it allows a more successful defense of the ovipositing female, than a contact guarding as in the dung fly Scatophaga stercoraria (Parker, 1974).

It remains to be determined whether males remove the sperm of the previous male as in C. maculata. The extremely long male aedeagus supports speculations by Thornhill and Alcock (1983), that possession of a long intromittent organ is characteristic of insects with multiple mating females of lengthy internal reproductive tracts. By positioning his sperm in the narrow spermathecal duct close to the exit of the storage organ, the competitor sperm is pushed distally and sperm precedence is assured. The fact that males force repetitively truly nonreceptive females to expend considerable energy and time in their competition with other females for oviposition sites, receiving apparently only unneeded sperm in return, does not indicate a sperm replenishment polyandry.

The sex ratio of emerging adults was found to be about 1:1. The fact that the operational sex ratio on the host plant was skewed in favour of males, might indicate that females avoid between ovipositions the host plant due to disturbance by males and predation pressure, feeding and resting in more protective vegetation, and dispersing to find new fruit.

Acknowledgements

We would like to express our special thanks to Dr. D. A. Lindquist, for his support during our work for IAEA in Egypt and the opportunity to carry out these observations parallel to our studies on the Mediterranean fruit fly.

References


Abstract

This paper presents the result of an experiment carried out in two different olives groves of Central Italy during a year of high infestation (1983) in order to study the relationship between the number of ovipositions and some physical parameters of the olive drupe.

The results show that among the variables considered for each drupe such as weight, length of the longer and shorter axes, the cultivar, the locality, and the date of sampling, weight best explains statistically the variability of the number of ovipositions per drupe. This link nevertheless decreases in time not only because of the increase in infestation, but above all because of the attractive and repellent substances produced by the olives during the ripening.

Introduction

In the field of bioetological research on the olive fly, different contributions have been made dealing with the relationship between the variable of infestation and the size of drupes as well as between infestation and the weight of drupes.

Recent studies have shown that if the average infestation is modest, the D. oleae prefers to lay eggs in larger size drupes, but if the level of infestation is high, drupes of smaller weight and size are also affected (PUCCI and AMBROSI 1982; CHESI 1982; CHESI and SANDI 1982; CHESI et al. 1983). The comparison between the sizes of drupes infested by first stage larvae and eggs with the sizes of healthy olives (PUCCI and AMBROSI 1982), and also the comparison between the weight of the infested olives and the healthy ones (CHESI and SANDI 1982; CHESI et al. 1983), should account for this behaviour. In this study are reported the results of an experiment conducted to point out some of the relationships which link the number of ovipositions per drupe to the weight and size the drupe.

1 CIRIO (1971) and CIRIO and GHERARDINI (1981) report that the biggest olives can bear several ovipositions and that the effect of the marking lasts no more than 5 days.
Material and methods

The research was conducted in two olive groves in Central Italy, one situated in the locality of Bivio Castel Rigone in the Comune of Passignano on Lake Trasimeno (Province of Perugia) and the other on the "Marsiliana Farm" in the Comune of Massa Marittima (Province of Grosseto).

In both fields 5 plants of the cv. Moraiolo were selected for our study. Also five additional cv. Dolce Agogia trees were selected in the first grove. The trials consisted of three samplings made respectively September 23rd, October 11th and October 24th. In each sampling 2 kg of drupes per plant were picked at random; later on, samples consisting of 200 large olives, 200 medium-size olives and 200 small ones were taken from the total of the olives belonging to the same cultivar and locality. The classification of the olives into large, medium and small was based on weight.

Afterwards, the olive samples were divided into two groups of 100 units each. The olives in the first group were placed in a cage to verify how many D. oleae would subsequently emerge from their cocoons; the following data was collected for each drupe of the second group: the number of oviposition, the weight, the length (long axis); the width (short axis), the cultivar, the locality, the data of sampling.

Data analysis

Methods of statistics were used to analyse the data collected from the above sampling. The study was carried out in two phases, the first one being preliminary to the second in which a statistical model was formulated to illustrate the dynamics of the oviposition. In both phases the number of ovipositions in each drupe was considered a Y-dependent variable in relation to the other variables available such as weight, length, width, the cultivar, the locality and also the data of sampling of each drupe; we also considered the resulting variables by multiplying the length of the drupe by the width, the first by the square of the second, in order to examine size correlated to the volume of drupes.

In the preliminary phase using log-linear type models, the most relevant conclusion was that the drupe weight variable, taken either alone or in relation to the different factors, was the one that most explained the variability of the oviposition number.

According to this first result we carried out the frequency distribution of the oviposition number in the drupes belonging to specific weight classes. As we can see in fig. 1 the dependence of the oviposition number on the drupe weight decreases progressively from the first to the third sampling. As infestation increases, the insect seems to turn more and more towards lighter weight olives.

In each distribution the average \( \mathbb{E}(Y) \)-and variance-\( \text{var}(Y) \)-values were calculated in order to study their functional link: the best fitted curves were \( \text{Var}(Y) = 0.384 \times \mathbb{E}(Y) \) in the first sampling, \( \text{Var}(Y) = 0.399 \times \mathbb{E}(Y) \) in the second, and \( \text{var}(Y) = 0.435 \times \mathbb{E}(Y) \) in the third. We observe
Fig. 1
Average number of ovipositions per drupe as weight and sampling data vary

Fig. 2
Value trend estimate based on the model of the number of ovipositions in relation to the variation of the weight in the first sampling.

Fig. 3
Value trend estimate based on the model of the number of ovipositions in relation to the variation of the weight in the second sampling.

Fig. 4
Value trend estimate based on the model of the number of ovipositions in relation to the variation of the weight in the third sampling.
that if the average is equal, the distribution variance increases in time, that is the choice of the drupe in which eggs are laid appears more at random in the second and third samplings compared to the first.

Using the specified relationship between expectation and variance we formulated more appropriate statistical models to explain the link between the Y-dependent variables and variables and factors considered, using maximum quasi-likelihood methods (McCULLAGH, 1983) and the analysis of deviance techniques.

Figs. 2, 3 and 4 show the trend of the expected number of ovipositions depending on the drupe weight variation, the cultivar, the locality and the sampling: the dependence of the ovipositions number on weight decreases from the first to the third sampling but at the same time no further explanatory elements emerged. In the fitted models the explained deviance went from 51% for the first sampling to 14% for the last.

The reduction of the influence of weight on the oviposition number is only partially justified by the general increase of infestation because the D.oleae seems to give greater attention to the lighter drupes. In figs. 2, 3 and 4 we note that as the weight increases, the slopes of the curves decrease progressively in the same manner, - even though the levels of infestation are not the same in the different cultivars and localities.

The progressive reduction of the influence of weight may therefore be attributed not only to the increase of the infestation but above all to the intervention of other factors which affect the olive during the ripening and which inhibit the power of attraction of the heavier drupes in favor of the lighter ones.

As far as the statistical model concerned, see DUMINICI et al. 1986 original paper.

Conclusion

The results point out that the probability of having one or more ovipositions in the same drupe depends on its weight. This relationship is more marked when the olives begin to ripen (51% of explained deviance) and tends gradually to attenuate (14% of the explained deviance) in the last sampling (fully ripe).

It is hard to think that the insect can evaluate the weight of the drupe and it is more likely to assume that some factor correlated with weight, exerts an attraction on the fly. Whatever this attractive element may be it fades with time, perhaps just because the fruit begins to ripen and the largest olives are primarily involved. The progressive decline of weight influence on the oviposition number is only partially justified by the general increase in infestation which induces the fly to lay eggs in the smaller drupes, but it depends especially on the biochemical changes in the fruit which produce attractive and repellent compound (FIESTAS RUS DE URSINOS et al. 1972; GIRULAMI et al. 1975; VITA and BARBERA 1978; GIRULAMI et al. 1975; GIROLAMI et al. 1979; GIRULAMI et al. 1981; CIRIU and GERARDINI, 1981; VIANELLO et al. 1982). Furthermore, we have to consider the importance of other physical parameters in relation to the ovi-
position as extensively documented by SACHANTANIS (1953), URPHANIDIS et al. (1958, 1959), MARTELLI (1965) and CHESI et al. (1983).

Finally, a further result is that the heavier drupes, though subjected on the average to more than one oviposition, are capable of guaranteeing more than the other the complete development of the progeny.

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Abstract

Laboratory experiments with single pairs have been carried out in order to study the parental age effects on the adult progeny's longevity and reproduction of Ceratitis capitata Wied.

The results obtained with adults whose parents were 5, 15 and 21 days old showed that, in general, the highest fecundity and fertility levels were observed in females originated from old parents. Males lived significantly more than females and the life span of males originated from old parents was higher than the one of males offspring from young parents. The considered populations did not differ significantly in the fecundity and fertility periods.

Conditions during the experiments were 26°C ± 1°C; 65% ± 5% RH and 12:12 hrs. L:D regime.

Introduction

Studies on the relationship between reproductive activity and aging in fruit flies species, usually conclude that the fecundity and fertility rates decrease as the adult age increases, and point out that the peak in eggs and larvae production is the most important time to study control measures. Nevertheless, a lack of extensive works is observed about the influence of parental age on the parameters defining the adult offspring reproduction along their life.

In a former study with Ceratitis capitata Wied., which was presented to the XVII International Congress of Entomology (CEC/IOBC "ad-hoc meeting": Fruit Flies of Economic Importance) in Hamburg (FRG), 1984, it was concluded that the weights of pupae obtained from crosses between old males and females were significantly higher than the ones estimated from the younger ones, irrespective of the pupal age and the larval density, except when the quantity of protein, Hansenula anomala, in the larval diet was reduced from 7% to 2% by weight. This result was obtained at a series of experimental conditions ranging from 19°C to 28°C and from 57% to 78% RH, with a fixed light-dark regime of 12:12 hours. (Muñiz and Gil, 1986).

Our proposal in this paper has been to obtain parameters of the reproductive activity of Ceratitis capitata Wied. for the three different parents' ages.
Material and methods

Isolated couples from a well adapted population of the Mediterranean fruit fly to the laboratory conditions, reared with a new larval diet that includes *Hansenula anomala* as protein source (Andrés and Muñíz, 1984; Muñíz and Andrés, 1983) were introduced in specially designed oviposition cages to study their reproductive activity (Muñíz, 1986).

Eggs were daily collected and percentages of hatching were observed in order to obtain fecundity and fertility data. Longevity of males and females were also checked. A Student's t-test was carried out with data of couples proceeding from three groups of parents (5, 15 and 21 days old), in order to study the possible differences between two averages; 18 isolated couples were used for each parental age group.

Results and discussion

The fecundity patterns are presented in TABLE I. The three populations did not differ significantly in the preoviposition period, oviposition period, total eggs per female and daily eggs per female. However, a tendency to increase the total fecundity of females originated from old parents was observed, as well as a higher daily fecundity in females from 21 days old parents.

Similar effects were obtained for the fertility, whose results are showed in TABLE II.

On the other hand, life span was longer in males obtained from old than younger parents, and in all cases longer than in females (TABLE III).

These results contrast with the ones obtained by Tsiropoulos with *Dacus oleae* Gmel., who found a significant decreasing in the adult progeny's fecundity rate (Tsiropoulos, 1984).

We consider that the results presented in this paper show the necessity

**TABLE I. Fecundity of the Mediterranean fruit fly, *Ceratitis capitata* Wied.**

<table>
<thead>
<tr>
<th>Population</th>
<th>Preoviposition period (days)</th>
<th>Oviposition period (days)</th>
<th>Total eggs per female</th>
<th>Daily eggs per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3,22 ± 0,55 (n=18)</td>
<td>25,78 ± 1,67 (n=18)</td>
<td>1622 ± 130 (n=18)</td>
<td>44,90 ± 4,50 (n=39)</td>
</tr>
<tr>
<td>B</td>
<td>3,00 ± 0,00 (n=17)</td>
<td>26,71 ± 1,50 (n=17)</td>
<td>1701 ± 95 (n=17)</td>
<td>43,87 ± 6,09 (n=41)</td>
</tr>
<tr>
<td>C</td>
<td>3,06 ± 0,24 (n=18)</td>
<td>25,39 ± 1,91 (n=18)</td>
<td>1731 ± 143 (n=18)</td>
<td>54,68 ± 4,69 (n=37)</td>
</tr>
</tbody>
</table>

T = 26°C ± 1°C; RH = 65% ± 5%; 12:12 hrs. L:D regime
TABLE II. Fertility of the Mediterranean fruit fly, *Ceratitis capitata* Wied.  
A: Adults originated from 5 days old parents. B: Adults originated from 15 days old parents. C: Adults originated from 21 days old parents. (x ± S.E.)  
T = 26°C ± 1°C; RH = 65% ± 5%; 12:12 L:D regime

<table>
<thead>
<tr>
<th>Population</th>
<th>Fertility period (days)</th>
<th>Total larvae per female</th>
<th>Egg hatch ( % )</th>
<th>Daily larvae per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23.17 ± 1.63 (n=18)</td>
<td>981 ± 95 (n=18)</td>
<td>61.94 ± 4.82 (n=18)</td>
<td>26.54 ± 3.73 (n=39)</td>
</tr>
<tr>
<td>B</td>
<td>23.71 ± 1.59 (n=17)</td>
<td>1193 ± 84 (n=17)</td>
<td>71.30 ± 4.33 (n=17)</td>
<td>29.28 ± 4.26 (n=41)</td>
</tr>
<tr>
<td>C</td>
<td>22.94 ± 1.81 (n=18)</td>
<td>1083 ± 103 (n=18)</td>
<td>62.93 ± 3.12 (n=18)</td>
<td>33.06 ± 4.07 (n=37)</td>
</tr>
</tbody>
</table>

TABLE III. Longevity of the Mediterranean fruit fly, *Ceratitis capitata* Wied. 
A: Adults originated from 5 days old parents. B: Adults originated from 15 days old parents. C: Adults originated from 21 days old parents. (x ± S.E.)  
T = 26°C ± 1°C; RH = 65% ± 5%; 12:12 hrs. L:D regime

<table>
<thead>
<tr>
<th>Population</th>
<th>Adult longevity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>A</td>
<td>49.28 ± 6.23 (n=18)</td>
</tr>
<tr>
<td>B</td>
<td>60.82 ± 6.32 (n=17)</td>
</tr>
<tr>
<td>C</td>
<td>52.11 ± 3.90 (n=18)</td>
</tr>
</tbody>
</table>

To increase this kind of research in order to acquire a better knowledge of the populations tails, to get reliable data on their reproductive activity and evaluate their incidence in the fruit damage. In this way, it will possible to apply control measures with a higher degree of efficiency.

References


EFFECT OF OVIPOSITION NET HOLE SIZE AND TREATMENT OF NET WITH SUGAR SOLUTION OR LUBRICANT-RELEASE AGENT ON MEDFLY EGG PRODUCTION AND COLLECTION

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Abstract

In laboratory rearing of Ceratitis capitata (Wiedemann), an oviposition net with hole dimensions similar to ovipositor's, resulted in egg production significantly higher than that of nets with holes several times the dimensions of an ovipositor. If net combined small and large holes, females clearly preferred the small holes for oviposition. Although small-hole net resulted in increased egg sticking, the overall number of eggs collected (eggs which dropped) was still much larger than in large-hole net cages. Insect mortality was not affected by the small-hole dense net, even under crowding conditions. Application of sugar-water solution to the oviposition net was found to increase egg laying. A similar increase was observed when a dry lubricant-release agent was sprayed on the net, and egg sticking was reduced considerably.

Introduction

In the Seibersdorf Entomology Laboratory, mass-reared adult Medflies are kept in 200 x 180 x 20 cm aluminium cages provided with adult food (3 parts sugar mixed with 1 part protein hydrolyzed) and water. The two wide sides of the cage are covered with nylon net which serves as oviposition surface. Eggs are laid through the net holes and drop into water. They are collected daily. Cages are loaded with ca. 4.5 litres of pupae which yield ca. 3.5 flies per cm² oviposition surface. Cages are maintained for two weeks only, since survival and egg production declines rapidly because of crowding conditions. Similar systems are used in other Medfly mass rearing facilities (Schwarz et al. 1985).

When the present experiment was started, the ovipositor net used in the laboratory had 2.2 mm² holes (1.44 x 1.50 mm each). It had been observed that during egg laying the female's ovipositor was squeezed in the corners of the rather large hole (it could accommodate several ovipositors). In addition, in certain instances, when nets with smaller holes than the above were used on one cage side only, it was observed that females preferred the smaller net hole side for oviposition. These observations initiated the present study of different hole size nets. It was also decided to investigate the effect of the sugar solution as well as the lubricant-release agent when applied to the oviposition net.
Materials and Methods

Adult insects of the "Sohag" Seibersdorf colony (originated from Egypt) were used. All pupae were of the second collection day and size class 5 (ca. 8.2 mg). Small plastic cages, 11 x 11 x 15.3 cm, provided with water and adult protein food and kept at 25±1°C, 55-65% R.H. and a 12 hour light/12 hour dark regime, were used. Thirty insects at a 1:1 sex ratio were introduced into each cage. A 9 cm diameter hole cut out in the box (cage) base was covered with oviposition net for egg laying.

In the first experiment (Table I), five different oviposition nets were compared. Their hole dimensions were as follows: 0.1 mm² (0.32 x 0.31), 2.0.3 mm² + 1.0 mm² (0.29 x 1.10 + 0.93 x 1.10) double-hole net; 0.5 mm² + 0.6 mm² (0.52 x 0.95 + 0.63 x 0.95) double-hole net, 1.3 mm² (1.13 x 1.14) and 2.2 mm² (1.44 x 1.50). In the double-hole nets, small and large holes were alternating. During the experiment, eggs laid during a 24 hour period (12 light/12 dark) were collected and counted twice a week. Surviving insects were recorded at the beginning of each collection period (start of light).

In the second experiment (Table II), two different oviposition nets were used: 0.1 mm² (0.32 x 0.31) and 0.3 mm² + 1.0 mm² (0.29 x 1.10 + 0.93 x 1.10). In each net, two different insect densities were compared: 30 and 300 insects per cage at a 1:1 sex ratio. Insect survival was studied in separate cages containing insects of the same production date and size as in the egg collection test. This was found necessary because in the high density cages it was impossible to prevent losses of live insects whenever dead insects were removed. Because of the very large number of eggs produced in the high density cages, eggs produced only during the first 4 hours of the photophase were collected and counted in 3 out of the 5 collection dates.

In the third experiment (Table III), only small-hole oviposition net (0.1 mm²) was used. The net was brushed with sugar-water solution or sprayed with a lubricant-release agent (poly-tetra-fluor-ethylene Tygadure spray, Fothergill and Harvey Ltd., Lancashire, England). The above treatments were applied before the insects were placed into the cages. In only one treatment (sugar Sw) application of sugar solution was repeated weekly. All eggs laid during the first 66 hours of the experiment (3 nights and 2½ days) were collected and counted separately to study the immediate effect of the various treatments.

Results and Discussion

a. Oviposition net hole size.

As Table I shows, the number of eggs laid through the net decreased as the hole size increased. Thus, the smallest hole (dimensions similar to ovipositor's) produced 38 eggs per living female per day as compared to 18.2 eggs in the largest hole, i.e. more than double. Intermediate hole sizes gave intermediate production rates. In the double-hole nets used,
the smaller of the two holes appeared to be the important one. An observation made in the 0.3 + 1.0 double-hole net confirmed the above: in 17 out of 20 egg layings observed, the ovipositor was in the small hole. In the few instances when the ovipositor was found in a large hole, it was squeezed in a corner or at a side of the hole.

When the female abdominal tip and ovipositor were measured, their dimensions were found as follows: base of abdominal tip 0.6 mm² (diam. 0.88), top of abdominal tip 0.35 mm² (diam. 0.67), first ovipositor segment 0.06 mm² (diam. 0.29), second segment 0.03 mm² (diam. 0.21). The average egg section at mid-length was 0.05 mm² (diam. 0.26). Thus, the dimensions of the ovipositor were similar to those of the smallest net hole, while the largest net hole could accommodate about 25 ovipositors.

Table I also shows that the highest percentages of eggs found stuck on the net were observed in the smallest hole net and the lowest in the largest hole net. Double-hole nets gave results different from those expected (according to the size of their small holes). Thus, although the small holes (preferred for egg laying) were 7 and 4.5 times smaller than the hole in the largest hole net, egg sticking was not significantly different. For some reason, the combination of small and large holes in the same net resulted in lower percentages of eggs stuck as compared to those expected from the size of the small hole. Egg sticking could be affected by several factors, among others, sticky substances deposited on the net during egg laying, insect excreta or food ingredients, and the texture and hole geometry of oviposition net.

In conclusion, it appears that mechanical stimulation during egg laying, similar to that of natural egg laying when the female punctures the fruit to deposit its eggs, is necessary for high egg production rates under laboratory conditions. In spite of the increased sticking observed in the small hole nets, the number of eggs that dropped into the water was still much larger than that of the large hole nets (e.g. 22 vs. 14.5 eggs per female when the smallest and largest oviposition net holes are compared).

b. Insect density and oviposition net hole size.

When two different insect densities were tested in the smallest hole net (0.1 mm²) and the double-hole net combining 0.3 + 1.0 mm² holes (which had indicated low egg sticking), no difference in female survival was observed under same insect densities. On the other hand, increased insect density resulted in similarly decreased survival in both oviposition nets. Thus, it appears that no increased mortality occurs in the small hole net cages because of decreased aeration or other possible toxic condition, even under crowding conditions. Egg production decreased under overcrowding. The decrease was more than expected according to survival, the phenomenon being more intense in the small hole net. Also, egg sticking increased considerably under overcrowding in the small hole net, while, interestingly enough, it decreased in the double-hole net. Thus, it seems that overcrowding decreases the overall egg collection in the small hole net, while in the double-hole net not much effect was observed. This resulted in similar egg collections in the two nets under crowding conditions (Table II).
**TABLE I.** Egg production and % eggs that stuck on the oviposition net when Medfly was kept in cages with different hole-size oviposition nets for 15 days. Five replications per treatment, means followed by same letter in the columns are not significantly different (P = 0.05, Duncan's test).

<table>
<thead>
<tr>
<th>Oviposition net hole area (mm²)</th>
<th>Eggs per living female per day laid through the net*</th>
<th>% eggs stuck on the oviposition net</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>38.0 a</td>
<td>41.5 c</td>
</tr>
<tr>
<td>0.3 + 1.0**</td>
<td>27.9 b</td>
<td>18.9 a</td>
</tr>
<tr>
<td>0.5 + 0.6**</td>
<td>26.2 b</td>
<td>26.2 ab</td>
</tr>
<tr>
<td>1.3</td>
<td>20.3 c</td>
<td>33.4 bc</td>
</tr>
<tr>
<td>2.2</td>
<td>18.2 c</td>
<td>18.2 a</td>
</tr>
</tbody>
</table>

* At the end of the 15-day period 72 - 88.5% of females were surviving in the five replicates.

** Double-hole nets: small and large holes in alternating order.

**TABLE II.** Female survival, egg production and % eggs that stuck on the oviposition net resulting from two Medfly cage-densities and hole size nets for 14 days. Five replications per treatment, means followed by same letter in the columns are not significantly different (P = 0.05, Duncan's test).

<table>
<thead>
<tr>
<th>Oviposition net hole area (mm²)</th>
<th>Insects/cage at start</th>
<th>% females surviving at the end of the 14-day period*</th>
<th>Eggs per initial female laid during checking periods**</th>
<th>% eggs stuck on the oviposition net</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>30</td>
<td>98.7 a</td>
<td>100.9 a</td>
<td>27.2 b</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td></td>
<td>63.1 b</td>
<td>40.2 c</td>
</tr>
<tr>
<td>0.3 + 1.0</td>
<td>30</td>
<td>98.7 a</td>
<td>58.7 bc</td>
<td>23.9 b</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td></td>
<td>48.2 c</td>
<td>10.3 a</td>
</tr>
</tbody>
</table>

* Because it was very difficult to prevent some insects from escaping, survival was studied in different cages held under exactly the same procedures and conditions as those for egg production study.

** Eggs laid through the net during a 24-hour period (12 light and 12 dark, egg collections 1 and 2) or during a 4-hour period only (hours 0-4 of light period, collections 3 to 5) were collected and counted five times during the experiment.
c. Oviposition net treatment with sugar solution or lubricant-release agent.

As Table III indicates, a substantial increase in egg laying, especially during the first days following treatment, was achieved by treating the net (0.1 mm²) with 5% sugar-water solution. A similar or even higher increase was observed when the net was treated with a lubricant-release agent. In addition, the latter resulted in a substantial decrease of egg sticking on the net. Survival data indicated no substantial effect of both sugar and lubricant-release treatments.

Increased egg laying was also observed in Dacus tryoni when fructose was incorporated in the oviposition substrate, while sucrose and glucose did not stimulate increased egg laying (Eisemann and Rice, 1985). In the same study it was found that fructose was effective only when it was accessible to tarsal and/or labellar gustatory sensilla. Thus, it appears that sugars, found in fruits in nature, stimulate egg laying of fruit flies under artificial rearing conditions. The increased egg laying observed in the lubricant-release treatment of this study could be due to favourable mechanical condition and stimulation of egg laying process.

TABLE III. Egg production and % eggs that stuck on the oviposition net when Medfly was kept in cages with small hole (0.1 mm²) net treated with sugar water solution or lubricant-release agent spray (lub-rel). Five replications per treatment, means followed by same letter in the columns are not significantly different (P = 0.05, Duncan's test).

<table>
<thead>
<tr>
<th>Oviposition net treated with*</th>
<th>66-hour period</th>
<th>14-day period (following the 66 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eggs per living female per day laid through the net</td>
<td>% eggs stuck on the oviposition net</td>
</tr>
<tr>
<td>Control</td>
<td>25.9 cd</td>
<td>20.2 ab</td>
</tr>
<tr>
<td>Water</td>
<td>20.4 d</td>
<td>30.5 cd</td>
</tr>
<tr>
<td>Sugar 5</td>
<td>33.8 ab</td>
<td>30.7 cd</td>
</tr>
<tr>
<td>Sugar 5w</td>
<td>37.3 a</td>
<td>27.7 bc</td>
</tr>
<tr>
<td>Sugar 25</td>
<td>28.2 c</td>
<td>37.8 d</td>
</tr>
<tr>
<td>Lub-rel</td>
<td>32.8 b</td>
<td>14.1 a</td>
</tr>
</tbody>
</table>

* Control = no treatment, water = net brushed with tap water, sugar 5 = net brushed with 5% sugar water solution, sugar 5w = as before but weekly (all other treatments only at start), sugar 25 = 25% sugar solution, lub-rel = spray.
References


QUALITY
USE OF HARSH LABORATORY SELECTION IN IMPROVING MASS-REARED STRAINS

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Department of Entomology, University of California, Berkeley, California, U.S.A.

Abstract

"Harsh selection" is imprecisely defined here as a survival of the order of 1% when a genetically-heterogeneous population is exposed to some environmental condition. "Selection" implies that some genotypes have a lower and other genotypes a higher probability of survival. High-survival types will therefore be present at a higher frequency both in the survivors and their progeny. Randomly-acting factors, no matter how great a reduction in survival they cause, have only a destructive, not a selective effect.

Although everyone knows that such natural selection is continuously acting, we often are unaware that it is also acting on the laboratory populations on which we are a major, non-random, selective force. The force we exert must be harsh, since many lab-reared species are capable of more-than-100-fold numerical increase in each generation. More than 99% of the potential next generation must be in some way destroyed, preferably in some purposeful constructive way. The problem is that we seldom give thought to the possible selective effects. So we are surprised when an insect strain developed over many years to the mass-rearing stage proves to be ineffective when released in the field (Bush et al. 1976).

My purpose in this note is to examine (1) how thinking about the modes and the effects of the selection we inevitably exert could improve the insect strains we develop, and (2) how some data from diverse sources may be useful in work on this problem. In California, both state and federal governments expend large sums to keep tephritid flies out and to eradicate them whenever they are introduced; so California scientists who work on tephritids must do so elsewhere. My own experience was with the olive fly during a stay in Greece many years ago; one thing that struck me then was that the highly-trained scientists who were studying the flies were not deeply involved with the rearing, that was done by technicians. In the work I have done at Berkeley since 1967 on the hemipteran, Oncopeltus fasciatus, most of the strain development, cage design, and rearing has been done solely by me. It is this that has made me so keenly aware of the crucial role of selection by man in all insect-rearing. The problems I encountered, though with a very different insect, were almost identical to those I observed in the rearing of the olive fly.

How should the surplus population be destroyed? Like tephritids, young female Oncopeltus can produce hundreds of eggs over a period of weeks. The surplus problem can be easily solved at this stage, by collecting and rearing only the early egg production, and discarding the producing cages. I have used this method (that minimizes work) occasionally, and have searched the literature for studies evaluating the effect of colony-
maintenance through early, middle, or late egg-production stages, but found no data. However, there are strong reasons why this method should not be routinely used. Early egg production is in part from females who will die early, mated to males who also will die early, even under protected laboratory conditions. Such short-lived insects will occur in any strain, but it is hard to see why we should select for short adult life. I have therefore usually discarded the eggs produced during the first third of the egg-production phase. Very little more work is required, and since egg production is high in the mid-phase one can collect enough eggs in a few days to start the next generation with only a 10-14 day delay compared to the early-phase collection. However, this method will at best maintain the quality of the population, without improving it.

We have now arrived at the complex problem of what "improvement" of a strain means and— if that can be decided—what kind of selection is likely to cause improvement. The 1980 conference (King and Leppla, 1984) sponsored by the USDA-ARS is of great historical interest. It represents the reaction of one of the world's most powerful and intelligent bureaucratic organizations to its recognition that insect mass-rearing is far more complex than anyone had hitherto believed. The introductory statements, by R.F. Moore and E.F. Knipping, include recognition (only by Moore) that there are "problem areas in insect-rearing programs." Three geneticists discuss the problem of "genetic deterioration" in laboratory colonies; they propose a variety of methods to maintain "quality," but do not indicate which ones ought to be given priority. All would require a significant increase in the complexity and cost of rearing. Although this is highly instructive, one is left with the impression that these authors have not personally dealt with the problems of rearing: how to make the best use of limited resources, what can (if necessary) be temporarily neglected and what cannot, what minor changes in method seem to be worth trying, etc.

The most important element in the selection I use at Berkeley is that in the early stages I was unable to maintain constant rearing conditions, and now I am unwilling to do so. Perfect constancy and clockwork regularity are obviously major departures from natural conditions, that will aggravate the abnormal selective effect of the rearing environment. This is one reason why I am opposed to the "cookbook" concept used by Singh and Moore (1985), whose collection of "recipes" for rearing many insect species is designed for technicians. The other reason, of course, is that I believe rearing is too important to be left solely to technicians. As a historical note, anyone who consults this work will see that it has no recipe for rearing Oncopeltus. I was asked to contribute one, but could not meet the standards set by the editors. In the next volume, someone else will; however, as rearers become aware of the great tolerance of this species to deviations from any standard, few will adhere to it, not even technicians.

At this moment, my insects at Berkeley have been unattended for several weeks and will get no attention for several weeks longer. Many are early-instar larvae, growing slowly in the cool (20°C) summer of my laboratory, with diurnal fluctuations of both temperature and light. The previous generation went through a completely different experience; the larval stage grew rapidly at 29°C in an unlighted incubator. The adults were separated into male or female groups in the final (5th) instar, and about half of them are simply being held at room temperature so that a progeny of long-lived parents can be obtained from them. The
other half were exposed to 12° for about 10 days, with over 50% mortality, and then restored to 29° as mixed males/females to produce the eggs that are now cool-reared larvae. Survivors of such a capricious regimen (never twice the same) are likely to retain heterozygosity and any other characteristics that confer adaptability to sudden, unpredictable changes.

I now anticipate several reasonable criticisms. First, there is no assurance that such erratic shifts in rearing method will confer adaptability to field conditions or competitiveness with wild types. But neither can any other approach so far proposed make such a guarantee. Rearing cannot mimic precisely the complexities of a vast ecosystem, or the immensity of the natural populations. Second, the vagaries of rearing methods intended to maintain high adaptability are incompatible with high efficiency of "factory-scale" mass-rearing, in which the goal is to produce the maximum number of insects in minimum time at minimum cost. This is alas true as things now are. But I wish to remind my audience that we are still in a very primitive stage.

Should the adult stage be the major focus of harsh selection? Having dismissed the egg stage—perhaps unwisely—as a desirable one for surplus population reduction, and digressed into the areas of flexible vs. rigid rearing methods, I return to the main problem of when and how to destroy the surplus. Since doing this at any stage later than egg production entails more work, justification—theoretical since there is hardly any experimental—is required. It is easy to cause any desired level of mortality at any stage, but difficult to analyse the pros and cons of such selection in terms of the ultimate quality of the population. No one knows how specific the selective effect of harsh selection at any stage will be, i.e., whether larvae selected to survive cold become adults with enhanced cold-resistance. A few trials of 4th-instar larvae held at 12° for a week were discouraging, with very high mortality, many survivors never becoming adults on restoration to 25°, the few that did being abnormally small and producing few progeny (that are being reared for testing). Even if it proves that exopterygotes like Oncopeltus, where the larva has much in common with the adult, show correlated selection, this might not apply to selection in either the larval or the pupal stage of Diptera. The experiment might well be worth trying, especially with very small larvae, since population reduction at this stage would minimize the work of rearing large numbers to the adult stage. So far, I have only varied larval rearing conditions within the range where selection (as measured by mortality) is mild. It must be emphasized that selection can act on traits such as fecundity or mating drive without any obvious high-mortality effect; in time, all the population will die, but it is possible that the earliest to die or the least fecund will be different individuals when environmental conditions are different. This is why biochemical geneticists are so concerned with detecting changes in the frequency of allozymes; even though they do not know what such changes mean, the very occurrence of change is a danger sign of deviation from the wild type, that alas we do not know how to prevent or reverse.

Although the adult stage is costly in time and resources (especially space) most of the planned selection I have used has been on adults. They have proved they can attain this stage under the given larval conditions, and many are likely to prove able to reproduce as their parents did. They will gradually die even if nothing is done, and doing
nothing has in fact been one selection method, usually after separating 
by sex to eliminate production of fertile eggs (females kept virgin will 
eventually begin to lay eggs, but these can be neglected). Adult longev-
ity is being selected for, and can be more intense on males since they 
are longer-lived. To keep the ultimate population from shrinking to a 
dangerously low level, cages are observed every few days, and selection 
ends when roughly 250-300 females are still alive. These are then mixed 
with males in new cages and the next generation initiated. Since aged 
insects have a lower egg production level for a shorter period, no eggs 
are discarded. There has been no selection for low fecundity, however, 
since the observed effects are only caused by aging of the phenotype; 
progeny can grow rapidly and prove very fecund if tested at an early age. 
Intensity of selection can be adjusted at will, and has rarely been at 
less than the 3% survival level for females because the population size 
in any generation is usually in the 10,000-15,000 range for all adults. 

From this beginning, originally intended to make rearing exert less 
time-pressure on me, I moved to the intentional exertion of selection 
pressure on my strain, by holding the male or female adults at 15° 
instead of the fluctuating room temperature (20-25°). This was continued 
for 3 generations, holding period being roughly 4 months (since not all 
adults in one generation appear simultaneously). To test what had 
occurred, I set 87 male-female pairs from the survivors for measurement 
of individual female egg-production at 29-30°. Such measurements simul-
taneously test the mating drive of the males, and (since most eggs 
hatched) their sperm production. Individual tests, though laborious, 
allow an estimate of variance. 

The results are summarized in Table 1, as ext. B. For comparison, 4 
similar egg-production tests are included: Ext. A was done earlier on 
the same strain prior to the 3 generations of cold-survival selection; 
ext. AM was on the same strain, but milkweed seed was used as the food 
instead of the sunflower seed on which my strain has been reared for more 
than 10 years; ext. C was done on the progeny of B (not held at 15° but 
reared under conditions similar to A); and ext. CM on the same progeny 
of B but fed milkweed seed.

Table 1. Fecundity changes in *Oncopeatus fasciatus* caused by harsh 
selection for adult survival at 15°.

<table>
<thead>
<tr>
<th>Experiment:</th>
<th>AM</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>CM</th>
</tr>
</thead>
<tbody>
<tr>
<td># of M/F pairs:</td>
<td>28</td>
<td>34</td>
<td>87</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td># of egg-layers:</td>
<td>26</td>
<td>28</td>
<td>23</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Eggs/day, range:</td>
<td>10-36</td>
<td>2-26</td>
<td>2-26</td>
<td>2-38</td>
<td>10-48</td>
</tr>
<tr>
<td>Eggs/day, mean</td>
<td>23.1</td>
<td>15.1</td>
<td>14.1</td>
<td>20.6</td>
<td>32.2</td>
</tr>
<tr>
<td>Eggs/day, C.V.</td>
<td>22%</td>
<td>29%</td>
<td>34%</td>
<td>43%</td>
<td>27%</td>
</tr>
<tr>
<td>Oviposition period, mean days</td>
<td>28.0</td>
<td>25.5</td>
<td>16.1</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>C.V.</td>
<td>31%</td>
<td>51%</td>
<td>51%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Total eggs/female, mean</td>
<td>647</td>
<td>384</td>
<td>256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.V.</td>
<td>41%</td>
<td>50%</td>
<td>60%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Of the survivors of long exposure to 15° (C), most soon died and produced few or no eggs. The mean rate for the 26% of the group that had measurable production was only slightly below that for the parent strain, but since oviposition period was shorter lifetime egg production per female was only 67% of that of the unselected parent strain. But the survivors had undergone truly harsh selection. Although roughly 5% of the females had survived the prolonged cold-storage ordeal, only 1.25% produced significant numbers of eggs and 0.5% produced a disproportionate share of the eggs. I cite these laborious experiments, that produced enormous numbers of dead adults at great cost, primarily because of the data for the progeny of the survivors of expt. B. Expt. C, based on normal rearing, yielded the highest egg-production rates (on sunflower seed) I have ever observed; 6 of the 23 females had rates higher than the highest for the original strain (A), and (on milkweed seed as food, CM) 8 of the 28 females likewise outdid the best rate for AM. I interpreted this as confirmation—with a very different species, under very different conditions—of what I call the “Dubinin effect,” that will be the topic of the following section.

The “Dubinin effect” and the concept of correlated selection.

In 1951, a reference in Dobzhansky’s revised edition of his classic Genetics and the Origin of Species led me to a remarkable paper by Dubinin and Tiniakov (1946) on the effect of harsh overwintering selection on Drosophila funebris. It is remarkable not only for its full comprehension of a new phenomenon, but because it was done under such harsh human conditions, soon after the devastation of World War II. Dubinin had been one of the earliest discoverers of the cyclic, seasonal changes in the frequency of several chromosome inversions in Drosophila populations, and the purpose of his overwintering study was to measure the selective effects on inversion frequencies. The very low percentage of survivors did show frequency changes, but not of a simple and consistent kind. The measurement of fecundity may have been incidental, but revealed that the cold-survivors had a daily rate of egg production 32% higher than the unselected controls; the effect persisted in the progeny of cold-survivors, although only 18% higher than the controls. One reason this work has been neglected is that fecundity is a variable parameter, very sensitive to many environmental factors, so that statistical proof involves large, complex experiments. Another is of course the disappearance of Dubinin himself. Interest in the ecological significance of chromosomal polymorphism persists and is reviewed for Drosophila pseudoobscura by Crumpacker et al. (1977), but fecundity has not been studied.

To me, the significance of the Dubinin effect is not merely that a trait (high fecundity) that was not selected for is nevertheless selected simultaneously with the trait (retention of reproductive ability during prolonged exposure to cold) that was. Such co-selection could be a chance effect due to temporary linkages of other genes on the same chromosome as those that enhance cold-survival. But the very existence of a genetic mechanism for cold-survival indicates that the population has in previous generations frequently undergone selection for it. It is therefore advantageous for the survivors to be highly fecund, since this makes rapid population increase possible. Natural selection will tend to favor association not only of genes that work cooperatively at the same time but also of genes that will enhance the survival of the genotype later. That is how I define “correlated selection.” Note that
the Dubinin effect has as a corollary the perhaps surprising association of lower fecundity with adaptation to warm weather conditions; it is at least conceivable that a surplus of eggs may overcrowd limited food resources and so lower survival probability. However, this emphasis on fecundity surely oversimplifies the effect, that may extend to many other traits not so far studied. We do not even know at what selection level the effect appears or is maximal, nor how impermanent it is when selection is relaxed.

The gain I foresee in reared insect populations is that the human-directed selection will go beyond simple varying of rearing conditions within the range where survival is high, and further emulate natural selection by occasional shifts to conditions where a large fraction of the population cannot survive. Extreme exposure to cold is not the only easy selection technique, since starvation or dehydration (risks that are present in nature even in mild-weather conditions) are even simpler options. A population subjected to pressures that are not only varied but sometimes intense is likely to be nearer the wild-type than one reared under constant favorable conditions.

Physiological mechanisms underlying adaptation. My purpose in this concluding section is to attempt a crude reconstruction of what selection is acting on at the phenoypic level (where the genotype interacts with the external world). The allozyme patterns that electrophoresis reveals represent only a minute fraction of the enzymes within an insect. Geneticists like them because they are the expressions of single genes, and from results for a sample of 100-200 individuals the frequencies of each allele within different populations is calculable, as well as genetic diversity (cf. the general article by Bartlett in vol. 1 of Singh and Moore, 1985).

However, these enzymes are the building-blocks of much more complex systems. Within each system, many enzymes (and their allelic variants) are integrated into a functional entity, e.g., the mechanism that produces energy by oxidation of various substrates. Biochemists have reconstructed the many interactions between enzymes of this system in vitro, but precisely how it functions in the intact organism is so far unanalyzable, at least in complete detail. What can be easily measured is the environmental interaction, usually as the rate of oxygen consumption. Even at this level (and much less at the level of its components) we have no idea how and where selection acts. It is possible that relatively few enzymes control the whole system, so that genetic variation at these loci can cause rapid response to environmental change.

Another major system (perhaps an interacting group of subsystems) involves biosynthesis. This is expressed as embryonic growth during the egg stage, larval growth, pupal metamorphosis when this stage occurs, and adult production of sperm or ova. All are coupled to the energy-producing system. The larval growth stage has been studied in most detail, and two recent reviews (Gordon, 1984 and David et al. 1983) present many basic principles that are relevant to all insect species. The work of David sums up a lifetime of thought, that (unlike that of Dubinin) was recognized and rewarded by his country; I cite it because it is within a larger work on the genus Drosophila, and for that reason its general significance may not be known to all. In my own review, I also tried to highlight the key concept of G.A. Sacher, so obscured by its locus of publication that even Jean David (who was clearly trying to recreate the concept in his review) was unaware of it.
The Sacher concept is brilliantly simple, and illustrated schematically in Fig. 1, that represents the well-known relations between metabolic rate (upper straight line) and growth rate (lower bell-shaped curve) when plotted vs. the reciprocal of the absolute temperature (°K). The upper line represents the cost of fastest growth; highest efficiency + quality; fastest growth

1/temperature (°K)

Rates of Growth (G = 1/development-time) and Metabolic Energy Production (M)

REFERENCES CITED

THE ALCOHOL DEHYDROGENASE LOCUS (ADH) OF DACUS OLEAE: FURTHER EVIDENCE FOR SELECTION UNDER ARTIFICIAL REARING

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Abstract

Most natural populations of D. oleae contain three alleles at the ADH locus: S (in frequencies about 0.6), F (0.4) and I (in less than 0.01). In colonies maintained on artificial food the frequency of I rises to 0.3 within the first five generations. The selection pressure associated with this increase and the subsequent maintenance of the resulting stable polymorphism is very large: the fitness of some genotypes is less than 5% of that of the fittest genotype (Zouros et al., 1982). The theory of population genetics suggests that with time the intensity of selection on such a costly polymorphism will decline, possibly through the selective accumulation of modifiers. We have examined this possibility and found no evidence for it. 170 pupae from colony "Aghia Triada 80" that was 30 generations old and contained the three alleles F, I, S in frequencies 0.50, 0.28 and 0.22 respectively, were mixed with 830 pupae from colony "Aghia Triada 82" that was 6 generations old and contained the three alleles in frequencies 0.81, 0 and 0.19. This provided a gene frequency input of 0.75, 0.05 and 0.20. Gene frequencies in the new colony were measured in each of the first ten generations and also at generations 15 and 20. The frequency of allele I increased to 0.4 within the first nine generations and remained around this value for the remaining ten generations. Thus, selective pressure acting on the ADH polymorphism did not diminish after 30 generations of "coexistence" in the colony of the three alleles in about equal frequencies. This reinforces the view that the three ADH polypeptides interact differently with some factor in the larval food, and provides further impetus for search for the identification of this factor.

Introduction

Most control programs of harmful insects involve the rearing of the insect under artificial conditions. This is particularly true for biological programs of control or programs that combine biological and traditional techniques. The conditions that prevail in such colonies are often quite different from those experienced by natural populations of the species. Food, oviposition site, temperature and photoperiod regimes, population density, presence or absence of predators and parasites are some of the most important factors that can be radically different in the artificial and natural environment. The effect of these differences on the insect can be quite dramatic. Laboratory or factory conditions may alter the developmental, physiological or behavioral responses of the insect in such a way that artificially reared colonies may soon become unsuitable for the purposes they were established (e.g. Bush et al., 1976). This raises
In the last few years we became interested in the degree of genetic
cchanges occurring in colonies of the olive fruit fly Dacus oleae under
conditions of artificial rearing, a problem first investigated by Bush and
Kitto (1979). Dacus oleae is a monophagous insect totally dependent on the
olive fruit. Most likely because of its monophagy, it proved very difficult
to culture in large numbers under artificial conditions. Yet, a method for
its culture has been developed and is in operation in the Department of
Biology of "Demokritos". A basic description of the rearing technique can
be found in Tsitsipis (1982). An outline of the main findings from
comparative genetic studies of artificially reared and wild flies is
presented in Zouros and Loukas (in press).

The purpose of this communication is to provide a summary of our
research on the alcohol dehydrogenase locus (ADH) of the olive fruit fly,
to present new evidence in support of the notion that natural and
laboratory habitats differ in some factor (or factors) that exerts a direct
and powerful selection pressure on the naturally occurring allozymes
of this locus, and to comment on the implications of these observations in the
control of this harmful insect.

The response of the ADH polymorphism to larval substrate

The ADH locus has been extensively studied in Drosophila from the
molecular, physiological and population-evolutionary points of view
(Kreitman, 1983; Mills et al., 1986; van Delden, 1982). In D. melanogaster
there are two naturally occurring allozymes, F and S, which differ from
each other by a single amino acid substitution (a third allozyme occurs in
only few local populations in low frequencies). Many more variants
(mutations) occur at the DNA level, but they are "silent", i.e. they are
confined to the untranscribed or untranslated parts of the locus or the
third position of the codon. The F/S polymorphism is universal and exhibits
a remarkable macrogeographical pattern (Oakeshott et al., 1982). S is more
common in the tropics, but with increasing latidude (either north or south)
its frequency declines and the frequency of F increases. This broad
geographical pattern (blurred, as expected, on a microgeographical scale
because of local conditions or because of stochastic noise) coupled with
the observation that only two allozymes can establish themselves in natural
populations inspite the ample opportunity for mutation at the molecular
level, strongly suggest that selection is the principal factor for the
ADH variation. There must be two types of selection acting simultaneously. A
purifying selection that does not allow the establishment of other
allozymes except F and S, and a balancing selection that, on a large spatial
and temporal scale, prevents the extinction of one or the other allozyme.

Dacus oleae has a much more limited geographical distribution than D.
melanogaster, but it is interesting that in this species too there are two
major allozymes, F and S, in frequencies 0.3 and 0.7, respectively. A third
allele, I, also occurs in natural populations in low frequencies, usually
less than 0.01. Only Greek populations were studied in detail, so it is
premature to conclude that this distribution of allele frequencies is
common to all populations of the fly. Even if this turned out to be the
case, knowledge of the distribution pattern alone cannot resolve the issue
of whether the variation is due to some form (or combination of forms) of
natural selection or it is due to random drift of gene frequencies within
local populations coupled with gene flow among populations. An answer to
this question is suggested from the study of the response of the ADH polymorphism under conditions of artificial rearing of the fly.

In contrast with natural populations where the I allele is practically absent, a colony of *Dacus oleae* that was maintained under artificial rearing for about 180 generations contained this allele in frequency 0.12. This prompted Zouros et al. (1982) to establish new laboratory colonies from pupae collected from infested olive fruits and follow the change in the frequencies of the ADH allozymes through time. They established four such colonies and observed that in all of them the frequency of the I allele increased within the first five generations from less than 0.01 to approximately 0.3. Later experiments of similar nature produced identical results (Economopoulos and Loukas, 1986). The unfailing repeatability of this result leaves no doubt that it is a real phenomenon that cannot be attributed to a random process. Selection is the only alternative explanation. Making use of the trajectories of the gene frequency change during the first 12 generations in two newly established laboratory colonies Zouros et al. (1982) estimated the degree of selection that is responsible for these genetic changes. They found that all three heterozygotes (FS, FI, SI) had a much higher fitness than the three homozygotes (FF, SS, II), that one or the other heterozygote for the I allele had the highest fitness, and that the II homozygote had the lowest fitness. Relative to IF or IS the fitness of the II homozygote was about 0.02, meaning that for every one progeny produced by an II parent, an IS or IF parent produced 50 progeny. This is one of the strongest cases of selection shown to be associated with an enzyme polymorphism and leads to two obvious questions: (1) which of the many differences that exist between the artificial rearing regime and the natural habitat are responsible for this effect on the ADH allozymes? (2) are the ADH allozymes the direct target of selection or they serve as markers of selection acting at closely linked but undetected sites of the genome?

In regard to the first question the search for the selective agent has been narrowed to the larval food substrate. Economopoulos and Loukas (1986) transferred a colony maintained on artificial medium for 24 generations back on olive fruits. They observed that in one generation the frequency of the I allele dropped from 0.28 among the artificially reared parents to 0.08 in the flies that emerged from olive fruits. This response demonstrates that selection on the ADH variation is reversible and identifies larval food (olive fruit versus artificial substrate) as the factor responsible for it. Economopoulos and Loukas (1986) also observed a mild but significant effect of temperature on the rate of I frequency change upon introduction of artificial rearing, suggesting that a chemical constituent in the artificial larval food whose concentration is temperature-dependent is responsible for the effect. Finally, presence or absence of symbiotic bacteria - artificially reared flies lack the symbiotic flora that is always found in olive fruit flies collected from the wild- appears to have no effect on the ADH response (Economopoulos and Loukas, unpublished).

The experiment described here was designed in part to answer the second question and in part to investigate the theoretical possibility that the selection pressure on the ADH polymorphism in laboratory colonies will abate with time.
The perturbation experiment

Let us assume that a single mutation event was responsible for the appearance of allele I in natural populations of D. oleae. At the time of its first appearance each mutation is in linkage disequilibrium in the population in the sense that it occurs only within one genetic background among the many that comprise the population. If with time the mutation increases its frequency in the population, recombination will randomize its occurrence with respect to the variety of genomic backgrounds existing in the population. If, however, the mutation is kept in low frequency (either accidentally or because of selection against it or against a neighboring gene) this randomization may take very long periods of time, the actual length of time depending on the recombination distance between the mutation and the selected locus and the size of the population. Theoretically, this could be the case with the I allele of D. oleae. When a laboratory colony is established from a random sample of animals from a natural population, the very few chromosomes that carry the I allele will multiply with a much higher rate than others. For the sake of the argument we will assume that this increase is caused by selection acting not on I itself, but on a gene with which I is in linkage association. Because selection is very strong and the population size is small, in the first few generations selection will overcome the effect of recombination and appear as acting on the I allele itself. After a few generations the population will reach equilibrium with all three ADH alleles in approximately equal frequencies. At this stage recombination will prevail and given enough time a state of random association between ADH alleles and the undetected selected genes in the background genotype will be established. After this stage the ADH polymorphism will be neutral to the forces of natural selection and its frequencies will be subject only to random drift, a process whose results will be apparent only after long periods of time.

To test this possibility we established a new colony by mixing individuals from two laboratory colonies with different histories and different ADH allozyme frequency distributions. Colony "Aghia Triada 80" was thirty generations old and its frequencies for F, I and S were 0.497, 0.282 and 0.221, respectively. In this colony the three allele frequencies were at approximate equilibrium for about 20 generations, and non-random associations between ADH alleles and undetected variants in the background genotype were expected to have ceased to exist or to be greatly reduced. If selection was not acting on the ADH locus itself, chromosomes extracted from this colony ought to be of equal fitness regardless of the specific ADH allele they happened to carry. Colony "Aghia Triada 82" was 6 generations old and was established from a sample that did not contain the I allele. The two alleles, F and S, in this colony were in frequencies 0.812 and 0.188, respectively.

The new colony (to which we refer as the "perturbation colony") was established by mixing 170 purae from "Aghia Triada 80" and 830 pupae from "Aghia Triada 82". Thus, the expected gene frequency input was 0.75, 0.05 and 0.20 for alleles F, I and S. After mixing, a random sample of 150 pupae were removed and scored for ADH to provide an estimate of the actual gene frequency input (generation 0 in Table 1). The remaining pupae were used to establish the colony which was maintained on artificial medium. The colony was sampled every generation for the first ten generations and, also, at generations 15 and 20. The method for the electrophoretic separation of the three ADH allozymes was the same as in Zouros et al. (1982). The results from this analysis are shown in Table 1.
It can be seen from Table 1 that the frequency of allele I rebounded and quickly returned to the levels present in colony "Aghia Triada 80". The increase was as dramatic as in colonies established afresh from animals collected from the wild. It would appear from these observations that no genetic changes have occurred in colony "Aghia Triada 80" that would have "neutralized" the selection forces acting on the ADH locus. Thus, the possibility that selection acts on a hidden genetic polymorphism in linkage disequilibrium with the ADH alleles appears unlikely. Either selection acts directly on the ADH alleles or else the linkages with the selected genes are permanent (not subject to decoupling through crossing over). In either case the value of the ADH polymorphism as a sensitive indicator of selection forces acting on the colony is in no doubt.

Departing from the assumption that selection acts directly on the ADH locus, we may make several additional inferences. Zouros et al. (1982) estimated that the segregational load in the colony "Aghia Triada 80" because of the ADH polymorphism was 0.47, thus costing the population about half of its reproductive potential. They suggested that "given enough time the population will acquire modifier genes whose effect will be to ease the selection pressure at the ADH locus". McDonald and Ayala (1978) provided strong evidence for the existence of such modifiers for (or regulatory genes of) the ADH locus in Drosophila melanogaster. It appears from this experiment that no reduction of selection pressure on the ADH locus had occurred. Several possibilities may account for this observation. It is possible that the theoretical load does not translate into genetic deaths. This will happen if at equilibrium the population size of the colony is density-dependent (e.g. because of some limiting factor in the environment). In density-regulated populations a certain amount of deaths will occur under any genetic milieu, and these deaths may absorb a large part of the fitness differentials among ADH genotypes. In this case there will be no selection for the elimination of these differentials, or selection will be only moderate and its results will be seen only after long periods of time. Another possibility is that modifiers for the ADH locus do not exist in Dacus oleae, or variation for them (on which selection may act) does not exist in natural populations or laboratory colonies of the species. The experiment cannot distinguish between these alternative explanations.

TABLE I

<table>
<thead>
<tr>
<th>Generation</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
</tr>
<tr>
<td>0</td>
<td>0.736 (0.026)</td>
</tr>
<tr>
<td>1</td>
<td>0.731 (0.025)</td>
</tr>
<tr>
<td>2</td>
<td>0.681 (0.028)</td>
</tr>
<tr>
<td>3</td>
<td>0.643 (0.030)</td>
</tr>
<tr>
<td>4</td>
<td>0.629 (0.027)</td>
</tr>
<tr>
<td>5</td>
<td>0.569 (0.028)</td>
</tr>
<tr>
<td>6</td>
<td>0.556 (0.028)</td>
</tr>
<tr>
<td>7</td>
<td>0.535 (0.028)</td>
</tr>
<tr>
<td>8</td>
<td>0.506 (0.028)</td>
</tr>
<tr>
<td>9</td>
<td>0.451 (0.028)</td>
</tr>
<tr>
<td>10</td>
<td>0.465 (0.028)</td>
</tr>
<tr>
<td>15</td>
<td>0.314 (0.026)</td>
</tr>
<tr>
<td>20</td>
<td>0.335 (0.026)</td>
</tr>
</tbody>
</table>
Implications for the insect's control.

The most important result of the experiment described here is the reinforcement of the hypothesis that selection acts on the ADH locus itself. This provides valuable clues for search for the factor or factors that are responsible for this response. Identification of these factors would suggest modifications in the rearing protocol that may improve the yield and quality of laboratory colonies. As noted, these factors are most likely chemical compounds in the larval food that act as substrates, inhibitors or activators of alcohol dehydrogenase. These chemicals may be ingredients of the food medium or may be produced secondarily during larval growth. It also appears desirable to establish a laboratory colony that will be homozygous for the I allele of ADH. Such a colony may have a number of interesting properties. Because the I allele is favored under laboratory conditions, one may expect that colonies containing only this allele will have higher yields under the method of artificial rearing currently in use. Also, because the I allele is apparently selected against in the natural habitat, a colony homozygous for this allele may be most appropriate to provide insects for sterilization and release. Low survival of larvae carrying the I allele on olive fruit might mean that, in the event a small fraction of the released insects are fertile, the offspring of these flies will die before eclosion. This in turn may allow for lower radiation levels for sterilization and, thus, for an increase of longevity and mating competitiveness of released flies. Clearly, a long series of laboratory and field experiments is needed before the profound response of ADH allozymes to the shift from natural to artificial larval substrate can be of use for the insect's control.

References

BEHAVIOUR OF A GENETIC SEXING STRAIN OF MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA*, DURING LARGE SCALE REARING

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Abstract

The availability of strains of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) which enable sexing of mass-reared populations to be carried out has great significance for suppression/eradication programmes for this pest. Using a white pupal colour mutant and a male-linked translocation a line was produced in which males emerged from brown pupae and females from white pupae. The line appeared stable and it was decided to use the line for field releases on Procida Island, Italy, the mass rearing being carried out at the IAEA laboratories at Seibersdorf.

The line was reared for 10 generations over a period of 8 months and ca 2.2 million flies were produced. Early in the mass rearing it was apparent that something adverse was occurring as by the 5th generation, 14% males were emerging from white pupae and 28% females from brown pupae. By generation 9 the values were 18% and 44% respectively. Scrupulous attention was paid to ensure that contamination did not occur. During mass rearing the fertility rose from 66% to 76%.

A computer model was developed to try to understand the course of events occurring in the population. It was concluded that neither contamination alone nor recombination alone was responsible for the observed breakdown. However, if viability differences were combined with a small degree of recombination then the course of events in the mass reared population could be accurately simulated.

It is concluded that viability differences under mass rearing conditions have to be taken into account when choosing a gene system for the development of a genetic sexing line.

Introduction

The development of a strain which would permit the sexing of mass-produced Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), would be of great significance to eradication/suppression programs, since it would reduce the cost of producing the desired number of males, would eliminate the blind stinging of fruit by the released females, and might enhance the suppressive effect of the sterile males. Rössler (1979a) reported the development of a strain in which the wild type allele of a dark pupa mutant, *dp* was translocated onto the Y chromosome. Hence male pupae were brown and black pupae yielded females. Rössler (1979b) described a mutant giving rise to white pupae (*wp*) and Robinson and van Heemert (1982) reported the synthesis of several strains in which the wild type allele was translocated...
onto the Y chromosome so that male pupae were brown and female pupae were white.

In 1983 a Research Co-ordination Meeting on genetic sexing systems for C. capitata (IAEA 1983) recommended that field experiments to evaluate a male-only sterile insect release be undertaken. The Joint FAO/IAEA Division of IAEA carried out field work in 1983 on Procida Island, Italy in cooperation with ENEA (Robinson et al. 1986). This was to be accomplished with one of the white/brown pupal dimorphism strains developed by one of us (Robinson and van Heemert 1982, Robinson and Riva 1983) viz. T101. This strain was established at the Seibersdorf Laboratory of IAEA and rearing commenced with two objectives: (1) to provide irradiated males for field studies on Procida, and (2) to monitor the biological characteristics and integrity of the strain under conditions of large scale rearing. While it is generally stated that crossing over does not occur in C. capitata males, Rössler (1980, 1982a, 1982b) reported that crossing over was found in translocation strains, but he concluded that the level of genetic recombination was "too low to cause leakage in "genetic sexing" strains". Robinson and van Heemert (1982) came to a similar conclusion.

This paper reports the behaviour of the Y-autosome translocation strain T101 and discusses the possible causes for the breakdown based on the results of computer simulation.

Materials and methods

1. Rearing

In late April 1983, 175 ml (ca 10,000 pupae) were received by the IAEA Seibersdorf Laboratory from ITAL, placed directly in oviposition cages, and emergence commenced 3 d later. Adults were maintained in 30 x 30 x 50 cm cages and females laid eggs through a fine mesh screen over the front of the cage. Eggs were aerated for 2 d before being added to a carrot medium similar to that described by Finney (1956). Since experience at Wageningen was that discrimination between the white and brown pupae declined if the larvae were reared under crowded conditions, the Seibersdorf conditions were such that 6.5 pupae developed per gram of medium. Each larval rearing unit comprised 600 g of medium in a 20 x 10 cm shallow plastic tray. Mature larvae left the medium voluntarily and pupated in bran. After 2 d the pupae were gently separated from the bran and held in a shallow layer until required. All rearing was carried out in a controlled environment of 25 °C and 70% RH and a 12 h photoperiod. To avoid contamination with normal medflies, access to the rearing room was strictly controlled, and any pupae removed from the room for tests were not returned.

In each generation the following parameters were measured; percentage egg hatch, pupal size with a grading machine described by Zelger and Russ (1976), the percentages of brown and white pupae, and percentage adult emergence and flight ability (FAI) by a procedure similar to that described by Boller et al. (1981). These tests were carried 10 to 22 times in each generation and so $\overline{x} \pm SD$ values could be determined. In addition, samples of 100 brown and 100 white pupae were placed in covered containers for emergence of adult flies. Once emergence was complete the number of males and females emerging from the white and brown pupae was recorded. These tests were also made 10 to 22 times per generation.

When pupae of the G4 generation were obtained a further line designated SG1 was established from males that emerged from brown pupae and females that emerged from white pupae. Approximately 200 pairs of adults were used to initiate this line. The offspring were inbred to obtain a SG2 generation.
2. The Model

The model entails a recurrent computation of the different male and female karyotypes by random combination of the gamete types produced by the preceding generation (Curtis and Hill 1971). Such a deterministic model simulates the behaviour of a very large, panmictic population with discrete generations. All simulations start at t = 0 with the pure genetic sexing line i.e. all males emerge from brown pupae and females from white. A recombination percentage between the translocation breakpoint and the wp locus is chosen and this then operates through all consecutive generations. To simulate contamination, a single addition (at t = 1) of certain karyotype(s) is included, expressed as a percentage of the existing population. Fitness reduction of certain karyotypes is simulated by multiplying the karyotype frequency with a certain factor, between 1 and 0, each generation before the gamete frequencies are calculated.

Results

1. Mass rearing

Over an 8-month period ca 2.22 million flies were produced. Once a large colony was obtained in the G-2, production was decreased because it was still several months until the proposed field experiments. Flies (2.216 million) of the G-5, G-6, SG-1 and SG-2 generations were shipped to Procida weekly for 2 months in September and October 1983. Thereafter production was decreased and the strain was discarded after G-10.

Initially egg hatch was 66% which agrees with the data of Robinson and Riva (1983). However, it gradually increased to reach 76% in the G-10. When received at Seibersdorf brown pupae (putative males) constituted 60% of pupae but by the 9th generation brown pupae comprised 87% of all pupae. Pupal size was good indicating that the rearing conditions were appropriate (Table I). A mean pupal size of 5 corresponds to a mean pupal weight of 8.2 mg on day 4 of pupal life at 25 °C (Hooper 1986). Flight ability was acceptable in the early generations but declined after G-7 and the reason for this is not known.

To conserve material, the proportion of females in brown pupae and of males in white pupae in the P stock was not measured. Initially 0.5% males were detected in the flies emerging from white pupae and this percentage increased in successive generations from G-3 to reach 18.4% in G-9. Similarly with brown pupae, females constituted 4.4% of the emerging flies of G-1 and this percentage increased to 44% in G-9 (Table II). While initially adult eclosion was 71-77%, this parameter varied from generation to generation and there was a marked increase in emergence from brown pupae by G-9. How much of this variability and increase can be attributed to the rearing conditions and how much, if any, to the changing genetic composition of the strain is unknown.

During the procedure of size sorting pupae in the G-3, a large number of black pupae (which were found to be dead pupae) was noted for the first time. Indeed black pupae comprised 14.8% in G-3 and 9.1% in G-4. Thereafter black pupae comprised 0.5-1.9% of all pupae. Again, whether this phenomenon was due to the rearing conditions (which were not changed during this program) or to the genetic events occurring in the strain is not known.

The presence of 20% females from brown pupae in the G-4 threatened the viability of the male-only release experimental program due to commence one month later. Hence a new line, SG-1 was established by combining in mass females emerging from white pupae and males emerging from brown pupae. It is clear (Table II) that while this "clean-up" procedure improved the genetic sexing characteristics inbreeding produced a SG-2 generation which
Table I. Percent egg hatch, pupal size, percent brown pupae, and flight ability (FAI) of adults in successive generations of the T101 strain of *C. capitata* (mean values ± SD).

<table>
<thead>
<tr>
<th></th>
<th>% egg hatch</th>
<th>% brown pupae</th>
<th>Pupal size</th>
<th>FAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>-</td>
<td>59.8</td>
<td>5.2</td>
<td>-</td>
</tr>
<tr>
<td>G-1</td>
<td>66.0 ± 9.1</td>
<td>53.7 ± 3.7</td>
<td>5.0 ± 0.3</td>
<td>75.1 ± 9.8</td>
</tr>
<tr>
<td>G-2</td>
<td>65.5 ± 9.9</td>
<td>62.2 ± 4.5</td>
<td>4.9 ± 0.6</td>
<td>82.4 ± 3.5</td>
</tr>
<tr>
<td>G-3</td>
<td>64.9 ± 8.8</td>
<td>61.8 ± 3.8</td>
<td>4.5 ± 0.4</td>
<td>77.4 ± 6.6</td>
</tr>
<tr>
<td>G-4</td>
<td>62.9 ± 13.6</td>
<td>63.2 ± 4.3</td>
<td>4.4 ± 0.4</td>
<td>66.9 ± 11.3</td>
</tr>
<tr>
<td>G-5</td>
<td>73.7 ± 6.7</td>
<td>67.5 ± 5.0</td>
<td>4.8 ± 0.3</td>
<td>77.4 ± 3.2</td>
</tr>
<tr>
<td>G-6</td>
<td>78.4 ± 6.2</td>
<td>73.3 ± 3.4</td>
<td>4.8 ± 0.5</td>
<td>63.3 ± 9.3</td>
</tr>
<tr>
<td>G-7</td>
<td>74.9 ± 8.8</td>
<td>76.5 ± 2.5</td>
<td>5.0 ± 0.5</td>
<td>55.8 ± 11.5</td>
</tr>
<tr>
<td>G-8</td>
<td>76.5 ± 7.0</td>
<td>77.7 ± 5.4</td>
<td>5.5 ± 0.2</td>
<td>38.0 ± 18.1</td>
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<tr>
<td>G-9</td>
<td>73.8 ± 10.8</td>
<td>86.8 ± 2.3</td>
<td>5.5 ± 0.2</td>
<td>48.2 ± 7.8</td>
</tr>
<tr>
<td>G-10</td>
<td>75.8 ± 8.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SG-1*</td>
<td>73.8 ± 4.8</td>
<td>63.1 ± 9.3</td>
<td>5.5</td>
<td>-</td>
</tr>
<tr>
<td>SG-2*</td>
<td>74.8 ± 8.0</td>
<td>63.5 ± 4.5</td>
<td>5.2 ± 0.1</td>
<td>-</td>
</tr>
</tbody>
</table>

*See text for details.

Table II. Percent emergence and sex from brown (males expected) and white (females expected) pupae of the T101 strain of *C. capitata* (mean values ± SD).

<table>
<thead>
<tr>
<th></th>
<th>White pupae</th>
<th>Brown pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% E</td>
<td>% ♂♂</td>
</tr>
<tr>
<td>G-1</td>
<td>71.0 ± 15.6</td>
<td>0.5</td>
</tr>
<tr>
<td>G-2</td>
<td>61.1 ± 26.2</td>
<td>0.2</td>
</tr>
<tr>
<td>G-3</td>
<td>66.2 ± 12.9</td>
<td>2.0</td>
</tr>
<tr>
<td>G-4</td>
<td>71.1 ± 16.4</td>
<td>8.0</td>
</tr>
<tr>
<td>G-5</td>
<td>80.0 ± 11.9</td>
<td>14.2</td>
</tr>
<tr>
<td>G-6</td>
<td>87.3 ± 8.0</td>
<td>15.2</td>
</tr>
<tr>
<td>G-7</td>
<td>75.7 ± 13.1</td>
<td>11.6</td>
</tr>
<tr>
<td>G-8</td>
<td>72.9 ± 11.5</td>
<td>18.7</td>
</tr>
<tr>
<td>G-9</td>
<td>68.6 ± 15.0</td>
<td>18.4</td>
</tr>
<tr>
<td>SG-1*</td>
<td>58.7 ± 26.1</td>
<td>4.3</td>
</tr>
<tr>
<td>SG-2*</td>
<td>74.9 ± 10.1</td>
<td>10.3</td>
</tr>
</tbody>
</table>

*See text for details.
was not different from G-6 (see further under 2.1).

Two possible causes for breakdown of the genetic sexing characteristics of the strain could be either contamination by normal flies, or recombination. We were aware of the necessity of preventing contamination by normal flies bred elsewhere at the laboratory. For that reason the maximum isolation that could be achieved was implemented. No one involved with the normal medfly colony entered the locked mutant rearing room, one technician was wholly responsible for rearing the T101 strain, all activities involved with the rearing e.g. egg and larval production, medium preparation etc., were carried out in the one room, and no material taken from the room for tests was ever returned to the room. Hence it is difficult to see how contamination of the T101 strain by normal flies could have occurred. The parental pupae were placed directly upon arrival into oviposition cages and emergence did not occur until 3 d later. Contamination by normal males was therefore impossible, and contamination could only have occurred by normal females, and only by them ovipositing eggs into the egg collection troughs. A daily search of the room was made and if any loose flies were found they were destroyed.

2. Simulation

During rearing of the line in Wageningen none of the aforementioned problems had been observed and the line had been maintained for about 40 generations without selection. In an attempt to explain the course of events a series of simulations was performed in which the effects of recombination, contamination and differential viability were assessed.

2.1 Recombination

The effect of recombination in the translocation males is to generate nearly equivalent frequencies of brown pupae females and white pupae males. The rate at which this occurs is dependent on the recombination frequency between the translocation breakpoint and the wp locus. Fig. 1 shows this effect for increasing recombination values. Recombination has little effect on the total proportion of brown and white pupae i.e. the population still comprises almost equal numbers of brown and white pupae. Low levels of recombination (> 0.20%) have very little effect on the genetic sexing characteristics of the line and even after 50 generations the recombinant types only amount to around 5% of the population. Rössler (1985) arrived at a similar conclusion. Using this type of graph and knowing the recombination frequency, predictions can be made as to how long a particular genetic sexing strain can be effectively used if for example a 5% contamination rate is acceptable. Re-selection of the genetic sexing strain from a population containing recombinant and non-recombinant types can be achieved by mating in single pairs males emerging from brown pupae with females emerging from white pupae followed by progeny analysis in the single families. Two types of families are produced depending on the genotype of the male parent. Families showing segregation of brown pupae (males) and white pupae (females) are retained as they are descended from matings between +/-wp translocation males and wp/wp females. The rest of the matings generate wild-type pupae as they are descended from +/- translocation males and wp/wp females and they are discarded. Recombination of the type described here does not lead to any loss of the translocation by the males. The population therefore remains semi-sterile with all males being translocation heterozygotes and females having the normal karyotype.

The data for the experimental population presented in Table II does not fit with a predicted pattern assuming a low frequency of recombination for two reasons. Firstly, in the experimental population there was not an equivalent increase in recombination types in the brown and white pupae; by
G-9 there were 18% males in the white pupae but 44% females in the brown pupae (Table II). Secondly in the experimental population there was an increase in the overall frequency of brown pupae in the population (Table I), this does not happen as a consequence of recombination. Recombination, in isolation, was therefore discounted as the origin of the observed events.

![Graph showing percentage of females in brown pupae over generations for different levels of recombination.]

Fig. 1. Effect of various levels of recombination in male Ceratitis capitata on the percentage of females in brown pupae of line TlO1.

2.2 Contamination

As indicated under 1. strenuous efforts were made to exclude contamination of the genetic sexing strain by other strains. Nevertheless the possibility could not be excluded and several contamination strategies were simulated.

2.2.1 Wild type virgin female contamination

This type of contamination is fairly unlikely, however, it does remain a possibility. Fig. 2 shows simulations based on two different levels of contamination. The populations have been simulated for 20 generations. In general, this type of contamination has very little effect on the genetic sexing characteristics of a population even at the relatively high level of 5%. In all cases a stable situation is reached. There is a fixed proportion of female emergence from the brown pupae with this proportion being dependent on the level of initial contamination. However, even with 5% contamination there are only 11% of females in the brown pupae. White pupae produce exclusively females. As in the case of recombination all the males in the population continue to carry the translocation. This pattern of segregation for pupal colour and sex in no way simulates the observations made in the experimental population.
Fig. 2. Effect of contamination of line T101 by virgin female Ceratitis capitata on the segregation of pupal colour and sex; a) 1% and b) 5% contamination, (single addition at t = 1).
2.2.2 Wild-type male contamination

Contamination of the population by a single male remains a distinct possibility and mating of such a male with females of the genetic sexing line has disastrous consequences. All the male progeny from such a mating will be chromosomally normal and have normal fertility and thus be at a selective advantage as regards the genetic sexing translocation males. The result of two simulations are shown in Fig. 3. Several events take place following this type of contamination, the most significant of which is the disappearance of brown pupae. Seeding the genetic sexing line with a single wild type male would lead to fixation of the wp allele. As this process of fixation is taking place there is increasing disintegration of the genetic sexing line. What is also interesting is the apparent latent period following low levels of contamination during which no effects of contamination can be seen. However, once noticed contamination causes destabilization in a few generations. Following this type of contamination males appear in the white pupae much earlier than females in the brown pupae. This is the complete opposite of what was found in the experimental population (see Table II) and it can therefore not be the explanation of the course of events. Paralleling the decreasing frequency of brown pupae there is a decrease in the frequency of the translocation in the population.

2.2.3 Wild type male and female contamination

This type of contamination is equivalent to the introduction of fertilized wild-type females which take part in oviposition. Fig. 4 simulates two levels of contamination and in all cases a stable polymorphism is formed. Examining firstly the population of brown pupae in the population, low frequencies of contamination (Fig. 4a) lead to almost complete disappearance of the brown pupal phenotype. However, if there is a higher degree of contamination a considerable proportion of brown pupae remain in the population (Fig. 4b). Males and females are equally divided within the two pupal phenotypes although the rate at which this equilibrium is reached is different. As with 2.2.2 the translocation disappears rapidly from the population and a latent period is also present. The fact that a stable polymorphism is attained together with the equivalence in the distribution of the sexes within the pupal phenotypes indicated that this type of contamination had not occurred during mass rearing.

None of the above simulations either alone or in combination produced a population model which bore any resemblance to the course of events in the experimental population. Differential viability was then included even though under relaxed laboratory rearing there had been no evidence for such an effect (Robinson 1984) although Rössler (1979b) had demonstrated some fitness reductions of the wp/wp line.

3. Recombination plus Reduced Viability of the wp/wp Genotype

Fig. 5 gives the results of simulations with varying degrees of viability reduction for wp/wp females and a fixed low level of recombination (0.05%) between the translocation breakpoint and the wp locus. Decreasing the fitness of the wp/wp genotype leads to a selection for the wild type pupal colour and the population eventually becomes monomorphic for the wild type allele. The frequency of females in the brown pupae increases at a faster rate than the build up of males in the white pupae. Both these observations were made in the experimental population and they are presented graphically in Fig. 6 in the same way as the simulation. Comparison of Fig. 5 with Fig. 6 indicates that if we assume a reduced viability of the wp/wp females of between 0.4 and 0.3 together with a low
Fig. 3. Effect of contamination of line T101 by virgin male Ceratitis capitata on the segregation of pupal colour and sex; a) 0.01%, b) 1.0% contamination.
Fig. 4. Effect of contamination of line T101 by virgin male and female *Ceratitis capitata* on the segregation of pupal colour and sex; a) 0.01% and b) 1.0% contamination.
Fig. 5. Effect of reduced viability of the wp/wp females together with 0.05 recombination in line T101 on the segregation of pupal colour and sex; a) 0.3 and b) 0.4 reduced viability.
Fig. 6. The observed data for line T101 of Ceratitis capitata under mass rearing conditions.

frequency of recombination an comparable picture of the observed trends in the experimental population emerges.

Discussion

Unexpected difficulties arose during the mass-rearing of the genetic sexing line in that there was a rapid increase in the proportion of females in the brown pupae with a corresponding loss of the white pupal phenotype. Neither of these observations was made during approximately 36 generations of maintenance under small scale laboratory rearing (Robinson 1984, Robinson unpublished results). During that period no selection was performed and the line was continuously inbred. Using a simple model several strategies were examined in an attempt to simulate the course of events in the experimental population. Neither recombination nor contamination or indeed a combination of both produced population profiles that in any way simulated the experimental populations. What was clear was that very low levels of recombination do not have disastrous effects on the genetic sexing efficiency of the line as the build-up of recombinants occurs slowly. Rössler (1985) came to a similar conclusion. The white pupae mutant wp was reported by Rössler (1979) to have reduced egg hatch and larval survival as compared to wild type. This parameter was then included in the model together with a low frequency of recombination with the result that the experimental results could be satisfactorily simulated. It was therefore concluded that these two factors may have acted in combination and caused the observed instability of the line under conditions of mass rearing. The lack of this "instability" under small scale rearing where relaxed rearing conditions apply is due to the much less severe selection pressures present. The model did not assume any changes in the viability of the different segregational products. It remains therefore unexplained why there was an increase, albeit slight, in the fertility of the population.
This study illustrates that, together with the technical problems associated with the up-scaling of a genetic sexing strain to the mass rearing level, attention must be paid to ensure that mass rearing conditions are adapted to the biological and genetical requirements of the strain.

The use of male-linked translocations to synthesize genetic sexing systems is based on the close linkage of a particular gene with the male determining chromosome, any loosening of this linkage by recombination results in time in a loss of effectiveness of the genetic sexing system. Low levels of recombination are however, tolerable. The presumed absence of recombination in males of higher Diptera make this type of genetic sexing construction extremely attractive. However, in Drosophila melanogaster Meigen (Hiria zumi 1971), Lucilia cuprini (Wiedemann) (Foster et al. 1980), Musca domestica L. (Lester et al. 1979) and C. capitata (Rössler 1982a, b) recombination in males has been demonstrated albeit at a lower level than in females. These observations necessitate a much more precise selection of male-linked translocations so that breakpoints are located in close proximity to the gene being used. This has already been achieved in 6 mosquito species where recombination occurs in both sexes and efficient genetic sexing systems have been selected (Curtis et al. 1976, Baker et al. 1981, Curtis 1978, Seawright et al. 1981, McDonald and Asman 1982 and Robinson 1986).

The fact that viability reductions are so critical for the stability of a line under conditions of mass rearing imposes restraints on the choice of the gene in that systems which show any indication of viability reduction are unsuitable for further development.

Acknowledgements

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References


Hooper, G.H.S. 1986. Application of quality control procedures to large


ENVIRONMENTAL ASSESSMENT OF THE QUALITY OF MASS-READED STERILE INSECTS

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Abstract

The overall quality of mass-reared sterile insects can be measured by extending the rationale of the standard ratio test to field conditions. If competitiveness is calculated using the ratio of sterile to wild insects that exists at release (rather than at mating) the resulting value is a measure of the potential of the sterile insects to survive to mating age, to distribute themselves in the same way as target insects and to mate in competition with the target insects. This value is especially pertinent to a situation such as a puparial release of tephritid fruit flies when there is a delay between release and mating. It is the value needed to calculate for such circumstances the required ratio of sterile to wild insects to achieve the desired reduction of fertility (and hence rate of decrease) in the target population. Determination of both overall and mating competitiveness during field releases requires procedures and precautions that have hitherto been overlooked.

Introduction

Mass rearing for the 'sterile insect release method' (SIRM) requires vigilance in 'quality control' through the application of various tests (e.g. see Boller et al, 1981). The ultimate test of quality, however, is the field trial where we have the opportunity of measuring the overall performance of our mass-reared product when in competition with target insects in their natural habitat. It is the purpose of this paper to show how the rationale of the standard competitiveness or ratio test (which is done in the laboratory or in small field cages) can be extended to trials in large field cages or to full-scale field trials so that an overall measure of quality can be estimated.

The meaning of competitiveness in SIRM

The object of the sterile insect release method (SIRM) is to reduce the mean fertility of the target females so that the target population goes into a rapid decline. To effect a decrease in numbers, the sterile/wild ratio (S : W) must reduce to below one the extent to which the average mature target female gives rise to another mature target female. Assuming the released insects are completely sterile, the ratio required to reduce the rate of target female reproduction per female (R₀) to any desired level (R₀') is found by

\[
S/W = [(R₀/R₀') - 1]/C
\]

where C is the mating competitiveness of the sterile males relative to that
of the target males. For instance, if $S : W$ was 9 : 1 we would expect a 10-fold reduction in $R_0$ so long as the average sterile male fertilized target females at the same rate as the average target male (i.e. $C = 1$). If the average sterile male was only half as successful ($C = 0.5$) then we would only expect a 5-fold reduction in $R_0$ and would require a $S : W$ ratio of 18 : 1 to achieve a 10-fold reduction. Competitiveness therefore dictates the effective $S : W$ ratio and therefore affects the number of sterile insects required and hence the cost of SIRM. The above equation requires a correction factor if released flies are not completely sterile, but the conclusions are analogous.

Competitiveness, as defined above, is governed by several factors, viz: (a) the degree to which sterile males associate in 'leks' which are attractive to target females; (b) the degree to which the target female prefers one male type over another within a lek; (c) the degree to which a female is likely to mate again after mating with a sterile male; (d) the degree to which the sperm of one male type displaces that of another if females mate more than once (Hooper, 1978).

Of the above factors (b) is the most easy to quantify in laboratory or field cages (e.g. see Meats and Fay, 1976) but the resulting measure will not be a true estimate of total competitiveness. The best procedure is to use the 'ratio test' (Haisch, 1970; Fried, 1971) where sterile (S) males are caged in various ratios with normal (W) males and females and the hatch rate of any eggs laid is recorded. Competitiveness is then found by

$$C = \frac{W}{S} \cdot \frac{H_n - H_C}{H_C - H_S}$$

where $H_n$, $H_C$ and $H_S$ are the hatch rates arising respectively from crosses of $W \varpi x W \sigma$, $W \varpi x (W \sigma + S \sigma)$ and $W \varpi x S \sigma$. The test has been used extensively to establish the optimum irradiation dose for sterility since there can be a 'trade off' between degree of sterility, physiological vigour and hence competitiveness (see Hooper and Katiyar, 1971). When $H_S$ is zero, the equation reduces to

$$C = \frac{W}{S} \cdot \left( \frac{H_n}{H_C} - 1 \right)$$

The formula is valid only when the fecundity of target females is unaffected by mating with a sterile male and if sterile females do not lay eggs if they are released along with the sterile males.

**Mating competitiveness vs overall quality of sterile males**

A competitiveness value can be used to calculate the desirable $S : W$ ratio of mature insects at mating. It is not however an adequate guide to the numbers of insects which must be released to achieve that ratio, particularly if sterile insects are distributed when in an immature state, as in a puparial release with tephritid fruit flies. In the latter case, not only is an estimate of the numbers of wild flies at the equivalent stage required but also a knowledge of (a) how the survival rates of the two types compare; (b) the extent to which the sterile flies distribute
themselves within the habitat in the same way as the target flies. The complete measure of the effectiveness of a sterile fly can be defined as $p_x/p_y$ where $p_x$ is the probability at release of a sterile male surviving to maturity and mating with a target female and $p_y$ is the analogous probability for a target male that is at a stage from which it can be expected to mature at the same time as the released sterile males.

The value of $p_x/p_y$ can be obtained by solving the equation for $C$ (given earlier) but using the $S:W$ ratio of equivalent stages at release rather than the ratios prevailing at the time of mating. We designate this quantity as $C_T$ to indicate that it is competitiveness calculated from the release ratio.

Estimating $C_T$ in field cages

Fay and Meats (1987) give details of trials conducted in large (tree-containing) field cages ($3 \times 3 \times 3$ m) with the Queensland fruit fly, Dacus tryoni (Froggatt). Flies were released inside the cages in a teneral state so that the trials were analogous to puparial releases, with a delay between release and mating. The aim was to quantify both $C$ and $C_T$ for differently acclimated types of sterile fly since both $C$ and survival rate to mating (hence $C_T$) were expected to vary between types according to environmental conditions.

Table I shows what can be done with data from such an experiment. It contrasts 4 hypothetical types of sterile fly, viz: (1) a type with a survival rate inferior to the wild type but with equal mating competitiveness - its overall competitiveness ($C_T$) of 0.44 can be rated as 'poor'; (2) a type with both survival rate and mating competitiveness inferior to the wild type - its overall competitiveness of 0.22 can be rated 'very

Table I. Calculation of total competitiveness ($C_T$) and mating competitiveness ($C$) using $S/W$ values at release and mating respectively. 4 Hypothetical examples of cage trials contrasting 4 types of sterile fly.

<table>
<thead>
<tr>
<th>Type of sterile fly</th>
<th>$S/W$ release</th>
<th>$S/W$ mating</th>
<th>Hatch rate as % control</th>
<th>Expected hatch rate</th>
<th>$C$</th>
<th>$C_T$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>4</td>
<td>20</td>
<td>20</td>
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<td>0.22</td>
</tr>
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<td>3</td>
<td>9</td>
<td>9</td>
<td>20</td>
<td>10</td>
<td>0.44</td>
<td>0.44</td>
</tr>
<tr>
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a. Type 1 has lower survival rate to mating than wild flies but has equal mating competitiveness; Type 2 has lower survival rate to mating than wild flies and lower mating competitiveness; Types 3 and 4 survive as well as wild flies but only Type 4 has equal mating competitiveness.

b. Value of $100 H_c/H_n$, see text.

c. Based on $S/W$ at mating, i.e. $100/((S/W)+1)$. 

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poor'; (3) a type with a survival rate equal to the wild type but with an inferior mating competitiveness - its overall competitiveness of 0.44 can be rated as 'poor'; (4) a type with both survival rate and mating competitiveness equal to the wild type - its overall competitiveness is 1.0 indicating a quality equal to wild flies.

The advantage of cage studies is that the ratio \( S : W \) of sterile to wild insects at release is known without error, the time to mating can be observed directly and the survival rates to mating and \( S : W \) at mating can be established with very little error. The disadvantage is that (no matter how large the cage) the experimental environment is not exactly equivalent to truly natural conditions. In particular, the restricted space does not allow us to measure the ability of sterile flies to distribute themselves effectively within the habitat.

**Estimating \( C_t \) in open field trials**

There are formidable problems in estimating \( C \) and \( C_t \) with open field trials: there will be many different age-classes of wild fly present; a proportion of the mature female population may have been mated for some time so that the mean fertility of wild females cannot be directly related to the current \( S : W \) ratio; population numbers will be changing so that the proportions and numbers in the various age-classes will change with time; there may be no way of estimating the density of immature flies and hence the survival rate between release and mating of either the sterile flies or of their co-evals in the wild population; wild flies may be distributed in a patchy way and sterile flies may not distribute themselves in an identical pattern. Some of these problems require that a series of cage trials is run in parallel.

In a cage trial, where single cohorts of wild and sterile flies of identical age are compared, we can establish the weekly survival rates for wild and sterile flies respectively as \( P_w \) and \( P_s \). If it takes \( x \) weeks for them to mature from release (note that \( x \) could be a fraction) then

\[
\frac{S}{W}_{\text{release}} = \left( \frac{P_w^x}{P_s^x} \right) \frac{S}{W}_{\text{mating}}
\]

This formula cannot be used for the field flies by simply substituting \( S/W \) for mature flies caught in traps, since numbers of wild flies will be changing, many age-classes will be present, some of which could have become mature weeks ago. The solution to this problem requires that we calculate \( S/W \) from population estimates and not from \( S/W \) ratios as they occur in traps. Traps must therefore be calibrated for all relevant kinds of weather and habitat (e.g. see Kapatos and Fletcher, 1983).

**Estimating \( S/W \) at mating**

What we want is a measure of \( S/W \) as it applies to numbers of flies that are becoming mature in the current interval of time. This can be found for either type of fly. If \( N_{m,t} \) is the number of maturing adults in week \( t \) and \( N_t, N_{t-1} \) are the numbers of adults of all ages in weeks \( t \) and \( t-1 \) respectively, then

\[
N_{m,t} = N_t - QN_{t-1}
\]
where \( Q \) is the weekly survival rate of mature adults established in a field cage trial. \( S/W \) at maturity is therefore \( N_{m,t}(\text{sterile})/N_{m,t}(\text{wild}) \).

This value may or may not be the same as that for mature flies of all ages, i.e. \( N_t(\text{sterile})/N_t(\text{wild}) \). We still have to estimate the separate contributions to mating of the different age-classes of males. To do this we require a knowledge of how mating propensity of males changes with age (e.g. see Fay and Meats, 1983) and the numbers in each age-class \( (N_x) \) of mature flies in the total mature population.

For both wild and sterile flies, the numbers now present in an age class that matured (and was estimated) \( x \) weeks ago can be found by \( N_{m,t-x}Q^x = N_x \). If \( Z_x \) is the mating propensity of mature age class \( x \) divided by the mating propensity of newly matured flies (mature age class zero) then the effective number of flies in a given age class in terms of number of newly matured flies (i.e. the number of 'newly emerged fly equivalents') is \( N_xZ_x \). Thus the \( S/W \) value to be used in calculation of \( C \) for newly emerged flies is found by

\[
S/W_{\text{calc}} = \left[ \sum (N_x, \text{sterile}Z_x, \text{sterile}) \right]/\left[ \sum (N_x, \text{wild}Z_x, \text{wild}) \right]
\]

**Estimating \( S/W \) at release**

The ratio of sterile flies at release to their co-evals in the wild population, i.e. \( S/W_{\text{release}} \) hence \( S/W_{\text{release}} \) requires a knowledge of the rate of increase in numbers of maturing flies. For either type of fly, \( \lambda_t \) is the rate of increase of \( N_m \) per week and it takes \( x \) weeks from release to maturity, then the number at release \( (N_R) \) is

\[
N_R = \lambda^xP^{-x}(N_t-QN_{t-1})
\]

where \( P \) and \( Q \) are, as before, estimated from a cage experiment. \( S/W \) at release is therefore \( N_R(\text{sterile})/N_R(\text{wild}) \).

Pulse labelling (i.e. giving sterile flies released in different weeks a different mark) will enable us to obtain independent estimates for sterile flies of \( N_x \) values, \( N_R \), \( N_m \), \( x \) and \( Q \) and can thus enable many of the results from the simultaneous cage studies to be calibrated.

**Estimating fertility rate of \( W \) females at mating**

Fertility of trapped but submature wild females can be assessed by caging them with fertile males. The extent to which fertility is reduced by current \( S/W \) values cannot be assessed by simply catching mature females since many of the latter would have mated at an earlier date when \( S/W \) may have been different. If \( S/W \) rises (as expected) during the trial, \( C \) and therefore \( C_w \) will appear to decline due to an inappropriate \( S/W \) being used. The solution to this problem is laborious and requires the discarding from the fertility data results from any female that has laid more than one round of eggs; this can only be done by checking the number of corpora lutea per ovariole (e.g. see Fletcher, 1975).
The problem of patchy distribution

If a significant proportion of sterile flies are 'misguided' in habitat selection they will not be sampled in proportion to their numbers unless an appropriate system of stratified sampling is used to cover in the right proportions all possible parts of the habitat that they may find themselves in (e.g. see Snedecor and Cochran, 1967); the patterns of dispersion and hence strata are first found by systematic sampling (e.g. see Debouzie and Thiolouse, 1986). If sterile flies are not sampled adequately, S/W estimates will be biased downwards leading to overestimation of fly quality in terms of both C and C_r. Sterile fruit flies certainly appear to distribute themselves differently to wild ones (e.g. see Teruya, 1986) but the full extent of this phenomenon in a large scale field trial has yet to be realised.

Competitiveness at high S:W ratios

A final problem comes when very high S/W values are reached as wild flies become rare. Even if sterile flies form active leks in all the appropriate places, there will come a time when the number of wild flies is less than the number of leks and leks without wild flies will be ineffective in reducing the fertility of wild females for reasons due to the shortage of the latter and not due to lack of competitiveness. C and C_r will artifically become low at this stage - a misleading result which we can expect as the value of S/W climbs to a value in the vicinity of the average number of flies (I) in a lek. Experiments with different release rates could determine the value of S/W (as a proportion of I) at which this is likely to happen.

As wild flies become extinct in whole patches of the environment towards the end of a campaign, sterile flies distributed in these areas are obviously wasted, again leading to a misleading estimate of their quality (Meats, 1983). Appropriate sampling (see earlier) will identify these areas in terms of S/W values which are unacceptably high for use in calculations of competitiveness.

There is another potential source of error (which becomes exaggerated at high values of S/W) if we neglect to take the precaution of only measuring wild fly fertility in recently matured flies (see earlier). If we fail to do this, then towards the end of a trial, some areas of the range will cease to 'produce' new wild flies and the only wild females caught will be ones which mated when S/W was much lower; their fertility will be found to be depressed to a much lesser degree than expected from the current S/W value, so if the latter is used a misleadingly low competitiveness estimate will result.

Conclusion

Estimation of C (and hence C_r) through experiments in large field cages is relatively accurate for such environments but these results may not be appropriate to the general environment. Estimation of C and C_r with open field trials appears to be a better alternative but it presents us with a formidable set of obstacles, many of which have hitherto been overlooked. We must use a habitat-based sampling design and calibrated methods; we must work from data on distribution and abundance to derive S/W values and not rely on S/W values obtained directly from traps; we must have data on age structure and rate of increase; we must only use fertility data from
recently matured females; we must recognize when S/W values are unsuitably high for use in calculations. Even with all these precautions we still need data from contemporaneous 'single cohort' trials in field cages for information on survival rates.

Each obstacle can be overcome by using supporting data, but each datum brings its own error term and it may be found that cage trials alone can give results which are sufficiently accurate to predict the progress and outcome of a SIRM campaign.

References


ARS-MOSCAMED QUALITY CONTROL PILOT PROJECT

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Abstract

The ARS-Moscamed Quality Control Pilot Project, located in Guatemala, Central America, is under the supervision of the Director of the Insect Attractants, Behavior and Basic Biology Laboratory (USDA-ARS) in Gainesville, Florida, in conjunction with the Guatemalan Mediterranean Fruit Fly Commission (Moscamed). The pilot project is operating under a three year contract (June 1984 to September 1987) specifically for the transfer of quality control research technology taken directly from ARS into an ongoing Mediterranean fruit fly mass-rearing and sterilization laboratory. The specific objective of this project is to install an organized system of production measurements and assays and test the ability of this quality control system to detect product deviations based upon production levels, abnormalities or fluctuations. The quality assessment tests of weight, sex ratio, eclosion, flight ability, flight ability index (FAI), flight propensity (startle or irritability index), male mortality, and mating propensity and mean mating index (speed) are standardized in equipment, technique, conditions and methodologies. Base-lines of predictable quality limits are established and monitored and control limits are set. All data is entered onto standardized computerized forms and finally the integration process between the pilot project and the existing quality control laboratory personnel takes place. The ARS technology, investigated and finalized by the research scientists, is transferred directly into the mass-rearing facility and into all medfly liberation locations within Guatemala. Field tests in the dry and rainy seasons are made in several coffee fincas to correlate factory quality control tests to field impact. The quality control field specifications are then related to field performance in terms of survival, dispersal patterns and finally the impact of released medflies on wild populations. Weekly investigations into fluctuating wild medfly population levels and behaviour are also made.
ECOLOGY
THE OVERWINTERING STRATEGY OF THE QUEENSLAND FRUIT FLY, *DACUS TRYONI*

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Abstract

In the southern part of its range in eastern Australia the adult is the only life-stage of *Dacus tryoni* (Froggatt) that can successfully overwinter. From late autumn onwards the adults aggregate in sheltered refuges where they remain throughout the winter months (June-September). When temperatures rise again in spring the flies disperse from the overwintering refuges and seek out hosts.

During the winter months the flies become sexually inactive and the nulliparous females resorb their ovarian follicles sequentially. Parous females are able to resorb the contents of their eggs and the sperm also disappears from the spermathecae of mated females when they no longer have viable eggs in their ovaries. Experiments in which samples of flies were taken from an overwintering site and maintained in the laboratory at 25°C indicated that the females do not retain or accumulate sufficient bodily reserves of protein during the winter months to enable them to mature eggs when conditions become favourable again. At the end of winter, females need to feed on proteinaceous material, mature their eggs and mate before they can start infesting the spring fruit.

Introduction

The Queensland fruit fly *Dacus tryoni* (Froggatt) is a serious pest of cultivated fruits in eastern Australia, and has a current distribution which extends from Cape York in tropical North Queensland to temperate coastal areas in southern New South Wales.

*D. tryoni* is multivoltine and in the northern part of its range breeds more or less continuously and may pass through up to seven generations each year (Meats, 1981). In the more southern areas it has fewer generations, and in the Sydney region, where this study was carried out, there are around four per year, because breeding is curtailed by the low temperatures experienced during the winter months.

Studies carried out in Camden and Wollongong which are located 65km and 80km south of Sydney respectively, indicated the adults are the only stage that can successfully overwinter. They show major physiological and behavioural adaptations that increase their ability to survive during this period.

Survival is enhanced by acclimation to the lower temperatures the flies experience as winter approaches (Meats, 1976a,b). This enables them to reduce their cold-torpeor level to a low as 2°C in winter, compared with 7°C in summer, and also prolong their survival time at sub-zero temperatures.

This paper describes some of the changes that occur in the behaviour and reproductive physiology of *D. tryoni* during the winter months and discusses their significance in relation to control procedures.
Overwintering behaviour

Studies carried out in Camden (Fletcher, 1975, 1979) indicate that as winter approaches the adults move into sheltered locations (or refuges) which in urban areas are often gardens with leafy evergreen trees and shrubs. In non-urban areas tree-lined creeks and gulleys are favoured overwintering sites.

Within the overwintering refuges the flies spend most of their time resting on the undersurface of leaves, particularly in the central parts of the trees. Frequently, groups of flies congregate in a small area and up to five or six are sometimes observed sitting on a single leaf without any indication of aggression towards each other. This situation is in direct contrast to their behaviour during the warmer months when individuals are fairly aggressive to each other and a fight usually ensues when two flies land on the same leaf. In the middle of the day when temperatures rise high enough (>15°C) the flies take short flights and explore the leaves of various plants within the overwintering site, presumably foraging for food and water.

![Graph showing the relationship between survival and mean temperature for males and females during the winter of 1973 in Camden.](image)

**FIGURE 1.** The relationship between 'survival' and mean temperature for males and females during the winter of 1973 in Camden. Weekly numbers of marked males caught in 15 cue-lure traps located 0.5-1.0km from the overwintering site are also shown.

Estimates of 'survival' (i.e. losses due to mortality and emigration combined), based on mark-recapture studies using Jolly's stochastic model (Fletcher, 1979) indicate that as long as minimum temperatures do not fall much below 0°C mortality rates are low and overall survival remains between 0.8 and 1.0 for both sexes (Fig.1). 'Survival' rates drop markedly at the end of the winter period when mean temperatures rise above the maturation threshold (i.e. 13-13.5°C). This decrease in 'survival', however, is not due to increased mortality but results from a high rate of emigration of...
adults from the refuge. This was confirmed by trapping studies in which cue-lure baited traps were placed around the overwintering site at distances of between 0.5 and 1km and checked at weekly intervals. During the week when the 'survival rate' of males in the overwintering site decreased most markedly (5-12 Sept), large numbers of males that had been marked in the week or two previously at the overwintering site (as part of the mark-recapture studies) were caught in the traps (Fig.1). Although no females were trapped because they rarely respond to cue-lure, it seems reasonable to assume that the decrease in their survival which occurred at the same time as the males, was also due to emigration. The survival data suggest that males migrated from the overwintering refuge at a higher rate than females, but the general trend was the same for both sexes.

Changes in the ovaries of females

At emergence, the paired ovaries of female D. tryoni each contain around 40-45 ovarioles comprised of a germarium and ovariole stalk (Fig.2). When conditions are favourable each germarium gives rise to a string of follicles which sequentially pass through a pre-vitelligenic phase, followed by vitelligenesis when yolk is deposited in the developing oocyte. In the final stage the nurse cells are extruded and a chorion is laid down around the elongated primary oocyte to form an egg.

FIGURE 2. Schematic representation of the changes which occur in individual ovarioles of female D. tryoni during the winter months. Solid arrows indicate successive stages of maturation, broken arrows the effects of resorption.
Examination of nulliparous females collected from overwintering sites at regular intervals revealed that during the winter months follicles continued to be differentiated from the germarium but were later resorbed (Fig. 2). The terminal follicle was resorbed first and when unfavourable conditions persisted the penultimate and subsequent follicles were also resorbed, so that towards the end of winter females typically had two or more resorbed follicles in each ovariole (Table 1).

<table>
<thead>
<tr>
<th>Stage</th>
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<th>23</th>
<th>6</th>
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<td>13.7</td>
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<td>17.4</td>
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A similar situation occurred in those females that were parous at the start of winter. Some egg-laying occurred on warm days during early winter (even though the eggs do not survive), and this resulted in a general decrease in gravid females with eggs in their ovaries. As the winter progressed the contents of any eggs remaining in the ovaries were resorbed, so that eventually only the flattened egg shells remained. Mature eggs appear to be less readily resorbed than follicles at an earlier stage of development and as a result the penultimate follicle is often resorbed before there is any obvious signs of resorption of an egg in the same ovariole (Fig. 2). A further interesting aspect of resorption in gravid
flies is that once the eggs have been fully resorbed, leaving only the flattened shell, or disappeared completely from the ovaries (it remains to be determined if flattened eggs are laid into fruit or simply ejected from the ovaries) the stored sperm disappears from the spermathecae, in the majority of such females (Table 2). Presumably it is broken down and the constituents resorbed, although its fate remains to be ascertained.

<table>
<thead>
<tr>
<th>No. of eggs</th>
<th>No. of females</th>
<th>Percentage with sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>15+</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>11-15</td>
<td>6</td>
<td>83</td>
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<td>6-10</td>
<td>9</td>
<td>100</td>
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<tr>
<td>3-5</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>88\textsuperscript{A}</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>74\textsuperscript{A}</td>
</tr>
<tr>
<td>0</td>
<td>33</td>
<td>30</td>
</tr>
</tbody>
</table>

\textsuperscript{A} In most individuals without sperm the remaining eggs showed obvious signs of resorption.

**Protein requirements**

After emergence, females of *D. tryoni* need a dietary source of proteinaceous material before they can develop eggs, a situation that has been exploited for control purpose by the use of protein hydrolysate bait sprays to suppress adult populations (Bateman, 1982).

The response of flies to protein hydrolysate baits is very low during the winter months (Bateman, personal comm.), due presumably to the reduced protein requirements as a result of the cessation of ovarian development. The question arises, however, as to whether the flies slowly accumulate and store protein in their fat bodies during the winter months so that they are able to mature eggs when ovarian maturation is promoted by the warm weather in early spring.

To investigate this situation, during the winter of 1984 samples of flies were taken at monthly intervals from overwintering sites in the Camden district. Twenty individuals from each sample were killed and dissected immediately to determine the condition of their ovaries. The other flies were divided into two batches and placed in cages held at 25\(^\circ\)C in the laboratory. One batch was provided with sugar and water only and the other batch was provided with protein hydrolysate as well as sugar and water.

Samples of females from the two batches of flies placed at 25\(^\circ\)C on the 1st June were removed and dissected on the 14th June and 1st July. Samples from the two batches collected in the field on the 1st July were dissected on 1st August and samples from those collected on 1st August were dissected on 14th August. The stage of the ovaries and the number of resorptions were determined for all nulliparous females. In the case of gravid females with eggs in their ovaries, it was not always possible to determine how many resorption bodies were present, so no attempt was made to estimate the