POLYMORPHISM OF THE HEREDITARY SIGMA VIRUS IN NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER

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ABSTRACT

Previous studies have shown that, in natural French populations of Drosophila melanogaster, 10 to 20% of the flies are infected by the noncontagious, hereditary rhabdovirus sigma responsible for CO₂ sensitivity. These populations are also polymorphic for two alleles [ref(2)Po and ref(2)Pp] of a gene for resistance to the sigma virus. Evidence is given here that two viral genetic types, differing in their response to the ref(2)Pp allele, are present in these populations of flies; the most common type is only slightly sensitive to the ref(2)Pp allele.

IT is well known that the agent responsible for CO₂ sensitivity in Drosophila melanogaster is a noncontagious rhabdovirus called sigma, which is transmitted only through gametes (BERKALOFF, BREGLIANO and OHANESSIAN 1965; L'HERITIER 1970; TENINGES 1972; BERNARD and PETITJEAN 1975). Many investigations permitted by the very convenient symptom of CO₂ sensitivity have been conducted since 1969 upon natural populations of flies; they provided evidence for the presence of about 10 to 20% infected flies (σ+ flies) in many French populations (FLEURIET 1976). There has been renewed interest in this situation since the discovery that some pathogenic viruses of vertebrates, such as VSV, yellow fever virus, Japanese encephalitis virus, and LaCrosse virus, are transmitted transovarially (via females gametes) in their insect vectors (TESH, CHANIOTIS and JOHNSON 1972; WATTS et al. 1973; FINE 1975; ROSEN et al. 1978; AITKEN et al. 1979). This mechanism would account for their overwintering and their maintenance in insect populations.

Another feature quite common in natural populations of D. melanogaster (FLEURIET 1976) is a polymorphism for the two alleles of a gene for resistance to sigma virus: ref(2)Po and ref(2)Pp (Po and Pp in this paper). Since genetically different strains of sigma virus, differing in their response to the Pp allele, have been obtained in the laboratory (OHANESSIAN-GUILLEMAIN 1963; GAY 1968, 1972), it was of interest to investigate what types of response to the Pp allele are found in virus strains collected from the wild. Analysis of the rules of transmission of the sigma virus has shown that two conditions are possible for an infected fly: “stabilized” and “nonstabilized” (L'HERITIER 1970). When a male is in the stabilized condition, it will transmit sigma virus to some of its offspring.

The percentage of infected flies in the offspring of a $\sigma^+$ stabilized male is called its “valence,” which can range from 0 to 100%. It is known from laboratory strains that multiplication of the virus carried by the spermatozoon may be impaired in an egg laid by a $P^o/P^o$ female. As a rule, the valence of a male is lower when crossed with a homozygous $P^o/P^o$ female than with a homozygous $P^o/P^o$ female, but this effect of the $P^o$ allele depends upon the genetic type of the virus. Comparing the valences of a stabilized male with mates of different genotypes may therefore be used to discriminate different genetic viral types.

**MATERIALS AND METHODS**

The operational test used in the experiments reported in this paper consists of crossing individual stabilized males with females of the $P^o/P^o$ and $P^o/P^o$ genotypes. For this purpose, each male is kept for 2 days in a vial with a $P^o/P^o$ female; then it is transferred, without etherization, to a new vial in which it again remains 2 days with a $P^o/P^o$ female. Since it is known that the valence of a male decreases with age (Sigot 1953), the same male is further transferred to a third vial containing a new $P^o/P^o$ female. The valence of the male with each female is determined by measuring the percentage of CO$_2$ sensitive flies among the offspring of each cross.

To ensure clarity of the method, Figure 1 presents the results obtained with 18 males of one sample (Ménétriol 1975 I). Each rectangle represents 1 male and each male is represented in the 3 diagrams by a number: the first diagram (I) gives the distribution of the values obtained with the first $P^o/P^o$ females; the second one (II) shows the distribution given by $P^o/P^o$ females and the third one (III) shows the distribution observed with the second $P^o/P^o$

![Figure 1](image-url)
females. It can be easily observed that the valences are lower in diagram II than in I and III and that this effect must be due to the $P^p/P^p$ allele. The comparison of I with III shows the slight effect of aging.

The males originated in samples of wild flies collected each autumn in different regions of France. In nearly every sample, about 10 to 20% of the flies are infected with sigma virus. Some of the samples consisted of individuals trapped as adult flies, but generally flies were collected on a bait as larvae, making it impossible to determine from how many females they have arisen. Collected females were then isolated, each in a separate vial, allowed to lay eggs and then submitted to CO$_2$ treatment. Only the lines that came from $\sigma^+$ stabilized females were kept. For each female, a few (at least 5) young $F_1$ males, 2 or 3 days old, were tested by 3 successive crosses as previously described; the distribution of the valences allows classification of the viral strain carried in the corresponding line.

The $P^p/P^p$ and $P^o/P^o$ homozygous lines providing the females used in these crosses were derived by brother-sister matings from flies collected in the wild (at Ménétrœl in 1972).

RESULTS

Since 1973, the viral strains carried by the flies collected have been tested for their response to the $P^o$ allele. The results obtained with each sample of flies (flies collected in the same place, at the same time) are presented in a diagram giving the valence of a male with the $P^o/P^o$ female, as a proportion of a value that is its average valence with both $P^o/P^o$ females. If the $P^o$ allele had no effect upon the sigma virus present in a line, the points would be aligned on the bisector. No such case has been observed. In a very few cases (less than 1% of the males) points are found on or above the bisector. They usually represent males whose valence decreases very steeply with aging, their valence with the second $P^o/P^o$ female is very low, so that their valence with the $P^o/P^o$ female is higher to the average valence with both $P^o/P^o$ females. Such males might carry a viral type that is not, or at least only very slightly, sensitive to the $P^p$ allele. A more likely explanation is that they carry a particular viral type that differs from the others, but not necessarily in its response to the $P^o$ allele. It has been shown that laboratory virus strains sharing different incubation times can differ in their response to aging. Such results could also be obtained with "mosaic stabilized" males carrying different spermatozoa clones that are produced at different times. No precise experiment has been conducted to analyze the viral type carried by these males. Whatever the explanation, males of this type, and their virus strains, cannot be very frequent in the wild. No example of a line carrying such a type of viral strain in all its individuals has ever been observed.

The 22 strains tested (Table I) can, without ambiguity, be distributed into two classes. As examples, Figures 2 and 3 show the seven diagrams obtained with the seven samples collected in 1973. In the first class (Figure 2), the valences with $P^o/P^o$ females are zero or very close to zero. In the second class (Figure 3), valences with $P^o/P^o$ females remain high, although on the whole distinctly lower than valences with $P^o/P^o$ females. On the basis of the test, therefore, two genetically different viral strains, which differ in their response to the $P^o$ allele, are found in natural populations of flies. A possible correspondence between class I and the $P^-$ viral genetic type, on the one hand, and class II and...
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**TABLE 1**

*Distribution of the samples*

<table>
<thead>
<tr>
<th>Year of collection</th>
<th>Viral type I</th>
<th>Viral type II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biziat (2)</td>
<td>Saint Martin (2)</td>
</tr>
<tr>
<td></td>
<td>Beaune (4)</td>
<td>Montluçon (2)</td>
</tr>
<tr>
<td></td>
<td>Ménétréol (3)</td>
<td>Nevers (5)</td>
</tr>
<tr>
<td></td>
<td>Toulon (2)</td>
<td></td>
</tr>
<tr>
<td>1973</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1974</td>
<td></td>
<td>Guérande (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ménétréol I (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ménétréol IV (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ménétréol VI (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Charentes (5)</td>
</tr>
<tr>
<td>1975</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cordoba (Spain) (1)</td>
<td>Guérande (8)</td>
</tr>
<tr>
<td></td>
<td>Ménétréol (9)</td>
<td>Bizi (17)</td>
</tr>
<tr>
<td>1976</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1977</td>
<td></td>
<td>Ménétréol (2)</td>
</tr>
<tr>
<td>1978</td>
<td>Providence (U.S.A.) (1)</td>
<td>Ménétréol (3)</td>
</tr>
<tr>
<td></td>
<td>Honolulu (U.S.A.) (1)</td>
<td>Chantelle (2)</td>
</tr>
<tr>
<td>1979</td>
<td>Providence (U.S.A.) (8)</td>
<td></td>
</tr>
</tbody>
</table>

For each sample, the number of lines tested is given in parentheses. In 1975, Ménétréol I, IV and VI are successive collections at the same place made during September and October.

the $P^\pm$ viral type, on the other, should be noted. These two genetic types were previously defined by their ability to multiply after being injected in a $P^p/P^p$ reference strain (OHANESSIAN-GUILLEMAIN 1963).

It should be noted that, so far, four viral strains collected in the United States have been analyzed: those given in Table 1 and one collected 20 years ago (Gay, personal communication). All four were of class I. More collections would be necessary to detect the possible presence in North America of class II viral strains.

It should also be noted that the viral genetic type perpetuated within inbred lines of flies kept in the laboratory seems to be very stable. For example, a second series of tests carried out in 1975 on lines derived from four samples collected and tested in 1973 showed that the viral types, after two years in the laboratory, were the same as in 1973: Biziat and Ménétréol were still of the class I, Saint Martin and Montluçon of the class II. Many other series of successive tests, conducted over several years upon viral strains kept in the laboratory, lead to the same conclusion.

Only one viral type has been found in each collection of flies tested in the present investigation. This is easily explained when flies are collected as larvae since they most probably all come from eggs laid by a single female, and the stabilized lines derived from the sample carried the same viral strain. All samples sharing class I strains have been collected as larvae and quite normally
have been found to carry only one viral class. However, in 1976 at Ménétréol, for example, a large number of adult females (287) were collected, from which 12 different stabilized lines were derived and nine tested: all carried a class II virus. Therefore, from the limited data available, one can conclude that each local population of flies tends to perpetuate a single genetic type of virus. Nevertheless, Ménétréol samples show one instance of a change occurring with time; in 1973, class I virus was initially detected, but in the following years only class II virus could be detected.

In conclusion, the genetic type of the sigma virus whose transmission by stabilized males is completely insensitive to the \( P^p \) allele has not been detected, at least with a notable frequency, in natural populations of flies. Two main virus types seem to be present in these populations, with the most frequent one being only slightly sensitive to the \( P^p \) allele. These findings now need to be analyzed, together with those obtained previously, dealing with the poly-
Figure 3.—Second viral genetic type: comparison of the valence of an individual male with a $P^o/P^o$ female with its value with a $P^o/P^o$ female (see text). Results observed with 3 1973 samples.

morphism of natural populations of flies for both alleles of ref(2)P (Fleuriet 1978). Taken together, these features could be partially responsible for the frequency of $\sigma^+$ flies in natural populations.

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Literature Cited


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