Vertical Transmission of the Piry Rhabdovirus by Sigma Virus-Infected Drosophila melanogaster Females

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SUMMARY

Piry rhabdovirus is not transmitted from Drosophila melanogaster females to their progeny. However, in mixed infections with sigma, another rhabdovirus, bearing the g^+ genetic marker, Piry may occasionally be transmitted to offspring. Thus, an endemic Drosophila virus can act as a helper virus enabling vertical transmission of a virus pathogenic to vertebrates.

As viruses are important pathogens in vertebrates, the study of the vertical transmission of these viruses by insect vectors is of great interest. Such transmission provides a mechanism for long term maintenance of viruses in the wild. These viruses although pathogenic to vertebrates are not known to cause diseases of their insect vectors in which they may multiply or not, or may be transmitted to their progeny mainly by the transovarial route (Leake, 1984; Mims, 1981).

In this paper, we have investigated whether vertical transmission of Piry virus can occur in Drosophila melanogaster. Two other rhabdoviruses have already been the subject of such studies. Transovarial transmission of vesicular stomatitis virus (VSV) strain Indiana, a rhabdovirus pathogenic to vertebrates, was found in sandflies (Tesh et al., 1972). The best known and most fully analysed case of regular hereditary transmission of a virus by an insect is that of the sigma rhabdovirus (L'Héritier, 1958), a Drosophila parasite. Sigma does not multiply in vertebrate cell cultures (A. Ohanessian & G. Echalier, unpublished data).

The relationships between sigma and its natural host, D. melanogaster, can be succinctly described as follows (Brun & Plus, 1980; Teninges et al., 1980). In stabilized flies, the virus is transmitted to all progeny by the female parent and to only a fraction of the offspring by the male parent, provided that the virus carries the v^+ genetic marker (as opposed to v^-). There is another category of sigma-transmitting females, designated 'non-stabilized'. This class includes those flies inoculated with a virus carrying the genetic marker g^+ (as opposed to g^-) and the daughters of stabilized males perpetuating a v^+g^+ viral strain. An interesting peculiarity of sigma's hereditary transmission is that when females are inoculated with a mixture of g^- and g^- particles (Duhamel & Plus, 1956; Ohanessian-Guillemain, 1963), the g^- virus may be transmitted to progeny. This does not happen when it is inoculated alone; the g^+ virus therefore acts as a helper virus. This phenomenon probably involves phenotypic mixing, and the external virion protein G is likely to be involved in this mechanism. Piry, another rhabdovirus serologically related to VSV (Frazier & Shope, 1979), induces, like sigma, CO2 sensitivity, which is a symptom specific to Drosophila infected by rhabdoviruses (Bussereau, 1975).

Preliminary experiments on vertical transmission of Piry in Drosophila following inoculation were carried out using two distinct procedures (Table 1). Two different viral strains and three different Drosophila genotypes were used, but the first generation progeny (F1) never became infected.

As exchange of G glycoproteins can take place between rhabdoviruses in mixed infections (Flamand & Bussereau, 1978) and as G may be one of the factors involved in the ability of sigma virus to infect the Drosophila germ line, we investigated whether the presence of a helper virus could promote the transmission of Piry virus.
Table 1. *Lack of Piry virus in the progeny of infected Drosophila females*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Inoculum (DIU)*</th>
<th>Number of inoculated females</th>
<th>Number of F1 flies tested</th>
<th>CO₂ sensitivity†</th>
<th>Titration on CEC‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$10^3$</td>
<td>40</td>
<td>6239</td>
<td>0</td>
<td>NT§</td>
</tr>
<tr>
<td>B</td>
<td>$4 \times 10^3$</td>
<td>20</td>
<td>1463</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>C</td>
<td>$3 \times 10^3$</td>
<td>50</td>
<td>8116</td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>$6 \times 10^2$</td>
<td>55</td>
<td>5933</td>
<td>NT</td>
<td>0</td>
</tr>
</tbody>
</table>

* DIU, Drosophila infectious unit. The relative probability of infection (ratio of the probability of infecting a fly to the probability of forming a plaque in CEC) is high for the viral strains and Drosophila genotypes used in these experiments (Brun, 1984). One p.f.u. corresponds to 10 DIU.
† CO₂ sensitivity tests were performed according to Plus (1954).
‡ F1 fly extracts were assayed on CEC according to Porterfield (1960).
§ NT, Not tested.

Table 2. *Vertical transmission of Piry virus by sigma-infected Drosophila females*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Inoculum (DIU)†</th>
<th>Number of females studied</th>
<th>Uninfected and sigma-injected mothers</th>
<th>Number of crushed F1 adult flies‡</th>
<th>Piry* sample§</th>
<th>Virus yield per sample (p.f.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$1.6 \times 10^4$</td>
<td>61</td>
<td>24 σ⁻</td>
<td>3467</td>
<td>0</td>
<td>$5.0 \times 10^6$ to $1 \times 10^7$</td>
</tr>
<tr>
<td>B</td>
<td>$2.6 \times 10^3$</td>
<td>10</td>
<td>$37 \sigma^*$</td>
<td>4198</td>
<td>4 + (1)</td>
<td>$2.6 \times 10^5$ to $5.5 \times 10^5$</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>30</td>
<td>10 σ⁺</td>
<td>1556</td>
<td>2 + (1)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>$3.5 \times 10^4$</td>
<td>208</td>
<td>$70 \sigma^-$</td>
<td>7888</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* After inoculation, females were caged singly (A and D) or in groups of five (B and C) with two males.
† See Table 1.
‡ When the number of flies was < 100, the volume of saline solution used was 1 ml. For larger numbers, the dilution was proportional to that number. All extracts were divided into two aliquots and frozen at −80°C. The technique of Porterfield (1960) was used to spread one aliquot onto CEC in two Petri dishes, in order to assay for Piry. If the first assay proved positive, the second aliquot was assayed precisely. In both cases, infected CEC were incubated at 30°C for 3 days and the cellular sheet was stained with neutral red.
§ Piry* means that Piry was present in a fly extract.
‖ + (1) indicates that for a given inoculated fly a second sample of F1 fly was Piry*.

Piry virus was injected into the female offspring of v⁺g⁺ sigma virus-infected males. As explained above, these flies were either sigma-infected (σ⁺) or sigma non-infected (σ⁻). As a control, we used the non-infected flies. After inoculation, females were caged singly (Table 2, experiments A and D) or in groups of five (Table 2, experiments B and C) with two males. Preliminary experiments showed that offspring from eggs laid during the first 3 days and after the ninth day post-inoculation (p.i.) were found never to carry Piry virus. Hence, batches laid between 4 and 9 days p.i. were collected. A few days after hatching, each sample of F1 adults was tested with CO₂. Mothers could thus be classified as σ⁺ or σ⁻. Groups of F1 flies were then crushed in a saline solution and their virus content was assayed on chick embryo cells (CEC). A total of 25337 F1 flies from 185 σ⁺ females (324 groups) and 14805 from 124 σ⁻ females (340 groups) were studied (Table 2). Piry virus was detected in 16 F1 groups deriving from 14 σ⁺ mothers. Piry virus is therefore occasionally able to infect the germ cells of Drosophila females in the presence of sigma virus.

The fraction of Piry⁺ (infected) F1 samples is small (16/324), suggesting that only one fly was infected in each group of five. If this is true, the fraction of transmitting females is about 5% (14/309). Each Piry⁺ F1 fly contained less than $10^4$ p.f.u. This titre is rather small compared to
that of *Drosophila* directly inoculated with Piry (>10⁵ p.f.u.). Two factors may lead to underestimation of the titre. First, mixing the virus with an excess of uninfected fly extracts resulted in at least a fivefold decrease of the viral titre, either due to irreversible adsorption of some virions on cell membranes or to the action of proteases or other inhibitory substances. Further, undiluted fly extracts were toxic to CEC. Both effects could have led to an underestimation of the number of Piry+ samples with low virus content and thus of the number of transmitting females.

Most cases of Piry+ transmission (11 out of 14) were observed between the 4th and 5th days p.i. and the rest between 6 and 7 days. These results resemble those obtained with g+ sigma virus which needs a latent period in the females before it infects germ cells. This infection takes place at the oocyte stage, from virus contained in the follicular cells that surround the cysts inside ovarioi lar sheath cells (Bregliano, 1969). The frequency of infected progeny then rises sharply and slowly falls (Plus, 1955). Transmission of Piry seems to follow the same type of process.

Table 2 also shows that Piry is transmitted by σ− females only: 14 Piry+ out of 185 σ− females as opposed to zero Piry+ out of 124 σ−. It may be concluded that sigma acts as a helper virus for Piry, in a similar way to that of sigma g+ in sigma g− transmission by females. Multiplication of sigma and Piry in the same female could lead to the formation of mixed particles bearing Piry nucleocapsids with an envelope containing the sigma G glycoprotein. The mixed Piry–sigma particles could have infected the oocytes and thus resulted in the weak vertical transmission of Piry.

What is the fate of Piry virus in F1 infected offspring? Five percent of doubly infected flies transmit Piry to their progeny. In this case, Piry virus has been introduced at the earliest stage of the life of the fly, i.e. when it is likely to replicate in the egg. This might allow the virus to be included in the germ cells at the moment of their individualization, thus circumventing the problem of penetrating the germ line from the outside. In this case, if the presence of the virus is not deleterious for the cells it would be transmitted to all or part of the progeny cells. The fly would then give rise to a lineage of Piry-transmitting *Drosophila*. If the early presence of the virus is not sufficient to allow the invasion of the germ line, then the fate of each doubly infected fly will reflect that of its mother, with the transmission of Piry by 5% of doubly infected flies to their progeny through the help of sigma g+ virus. Which of those two possibilities is occurring is obviously of great interest for epidemiologists.

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**References**


Short communication


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