Short Communication

Detection of Clones in the Nervous System of Drosophila melanogaster

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Summary. Mitotic recombination was induced, by X-irradiation at the blastoderm stage, in flies heterozygous for one of the temperature-sensitive paralytic mutations shibire and tp-2. The results show that these mutations can be used to detect the presence of clones in the central nervous system through the temperature-sensitive paralysis of individual legs. Mitotic recombination can also be used to examine the effects of these mutations in the peripheral nervous system; shibire is thus shown to affect the function of sensory neurons.

Key words: Clones -- Nervous system -- Shibire -- Drosophila melanogaster.

Although clonal analysis using the technique of mitotic recombination has yielded considerable information about the development of the imaginal discs in Drosophila (see, for example, Garcia-Bellido, 1975), it has not been applied to the nervous system. Such analysis could provide information about the number of cells contributing to the nervous system at any stage of development, about the time at which these cells divide, and about the clonal relationships between different cells. In this report it is shown that mutations affecting behaviour can be used as markers to demonstrate the presence of clones in the nervous system.

The temperature-sensitive (ts) mutation shibire\textsuperscript{ts} (Grigliatti et al., 1973; Poodry et al., 1973) (allele shi\textsuperscript{S7139}, Siddiqi and Benzer, 1976; 1–52.2) was used for these experiments. At 18°C shi\textsuperscript{ts} flies behave normally, whereas after 1–2 min exposure to 29°C they are completely paralysed. On returning to 18°C they recover rapidly. Gynandromorph analyses have shown that this paralysis is due to action of the gene in the nervous system, and that the paralysis of each leg is independent (Hall et al., 1973; Deak, 1976). Thus use of X-ray-induced mitotic recombination (Stern, 1936) should permit one to obtain homozygous shi\textsuperscript{ts} clones in the nervous system, and the presence of such clones in the thoracic ganglion should be recognizable through the reversible paralysis of individual legs.
Table 1. Temperature-sensitive paralysis of individual legs

<table>
<thead>
<tr>
<th>Genotype b</th>
<th>X-ray treatment (rads)</th>
<th>Number of flies examined</th>
<th>Flies with ts leg paralysisa</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ore-R</td>
<td>1000</td>
<td>1319</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
| y cho shi
  + + +    | 0                      | 723                      | 0                           | 0      |   |
| y cho shi
  + + +    | 1000                   | 784                      | 11 (1)a                     | 1.4    |   |
| y cho shi
  M(1) o  | 0                      | 613                      | 0                           | 0      |   |
| y cho shi
  M(1) o  | 1000                   | 2974                     | 42 (3)                      | 1.4    |   |
| y cho shi
  M(1) o  | 1000c                   | 1009                     | 2                           | 0.2    |   |
| y w       | 1000                   | 443                      | 0                           | 0      |   |
| y w tp-2  | 1000                   | 732                      | 24 (1)                      | 3.2    |   |

Only those flies are recorded that showed reversible behaviour after 4 trials at each temperature. The number of flies with two paralysed legs is shown in parentheses.

The various mutations are described in Lindsley and Grell (1968).

These flies were maintained at 27°C throughout development then kept at 17°C after eclosion.

Eggs were collected at 25°C and X-irradiated at the blastoderm stage (3 ± 1/2 h after eggaying; Wieschaus and Gehring, 1976) with a dose of 1000 rads (50 kV, 25 mA, 0.3 mm Al filter, 215 r/min). The animals were kept at 17 ± 1°C. Adult females of appropriate genotypes (Table 1) were collected and examined for leg paralysis at 30 ± 1°C after 20–30 min exposure to this temperature. Any flies with immobile legs were returned to 18°C for 30 min and re-examined. If the defect observed at 30°C was no longer detectable at 18°C, the fly was returned to 30°C and rechecked. This process was repeated several times.

The results of these experiments are shown in Table 1. Temperature-dependent leg paralysis was not observed in irradiated Oregon-R (wild-type) flies, nor in unirradiated flies heterozygous for shi1. When, however, eggs heterozygous for shibire were irradiated, 1.4% of the resulting adult flies showed temperature-sensitive leg paralysis. Such paralysis of individual legs is indicative of shibire clones in the corresponding neuromeres.