An Abnormal Staining Capacity of the Sixth Salivary Gland Chromosome of a Strain of Drosophila virilis

By

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Introduction

In the course of my studies on the salivary gland chromosomes of Drosophila virilis, I found a certain abnormality in their staining capacities. The small sixth chromosome of the New Orleans strain has this peculiarity; it takes a definitely faint stain than the same chromosomes of other strains.

Two wild American strains of D. virilis, New Orleans and New York, sent some years ago by Doctor M. Demerec of the Carnegie Institution of Washington, Cold Spring Harbour, have been inbred in our Laboratory. From their external appearance flies of this type can hardly be distinguished from those of the Japanese wild strains. Detailed comparison shows, however, that the wing length of the former is a little shorter than that of the latter; but such a difference may be found even among wild strains from different localities in Japan—the Keijyo strain, for instance, has the longest wings, Hiroshima the next, and Kyoto the shortest. Both the New Orleans and New York strains have shorter wings than the Kyoto strain. Furthermore the wings of the Kyoto and Hiroshima strains are more pointed at the end of the third longitudinal vein than those of the New Orleans and New York strains. The Keijyo strain has wings as rounded as those of the American. Thus the seeming phenotypical differences between the American and Japanese strains are hardly definite distinctive characters.

The two American wild strains were mated with some Japanese wild strains and the salivary chromosomes of their F1 larvae were studied. Although nothing peculiar was found in the chromosomes of the hybrid of the New York and Japanese strains, abnormality was found in the sixth chromosome of the hybrid resulting from the cross between the New Orleans and Japanese strains (Figs. 3 and 4).

Characteristics of the sixth chromosome of the New Orleans D. virilis

Maps of the normal salivary gland chromosome have been published by Heitz (1934), Hughes (1936) and this writer (1936).
They agree in that the seventh band from the proximal end is lightly stained, the eighth rather deeply and the ninth lightly, much like the seventh (Bands 100C2–100D3 in Hughes' map and VI1E–VI1G in the writer's). All other bands take a deep stain more or less in the same degree. There is no $\beta$-heterochromatin at the proximal end which is attached by its euchromatic region to the $\alpha$-heterochromatin. The total length of this chromosome is about 10 $\mu$ (Fig. 1).

As shown in figs. 3 and 4, the sixth chromosome of the hybrid larvae of the New Orleans and Japanese strains consists of two components, one stained as deeply as other chromosomes and the other distinctly lighter. Moreover the component parts are of different lengths, so that by casual observation they appear not to be in synaptic conjugation. Minute studies of the individual bands, however, reveal that the number and arrangement of the bands of the components agree precisely and they are fused band by band. These observations make it certain that the two conjugate completely at somatic synopsis, the abnormality in question thus not belonging to any known chromosomal aberration such as deficiency, inversion or translocation.

Careful comparison of the sixth chromosome of the New Orleans with those of other stocks shows that the New Orleans chromosome is broader and longer, so that the bands, which look continuous in other strains, appear wavy or broken into dots. Thus the chromosome of the New Orleans strain seems to contain the same quantity of chromatic substance as that of other strains.

The sixth salivary chromosomes of 24 strains from various localities of Japan and China, as well as of America, were examined. All of them have normally staining sixth chromosomes except one American stock, Gap$^2$ hump/lethal 6a, which stained lightly. The original strain of this mutant stock is unknown to us, but it is possibly also New Orleans or else another American strain having a sixth salivary chromosome with the same peculiarity.

**Treatment of the New Orleans sixth chromosome with various fixatives**

According to Metz (1935), and Doyle & Metz (1935), preliminary fixation with certain chemicals may give the bands of the salivary chromosomes of *Sciara* a different appearance than when directly stained with aceto-carmine. The salivary chromosome of the New Orleans strain was treated with bichromic acid, formalin, picric acid and mercuric chloride and then stained by aceto-carmine as usual.
Fig. 1. The normal sixth salivary chromosome in incompleted somatic synapsis. The vacuolar structure, which is attached to the proximal end of the sixth chromosome, is α-heterochromatin. Fig. 2. The sixth salivary chromosome taken from a fly of the New Orleans stock. This is wider and longer than the normal chromosome. Figs. 3 and 4. The sixth chromosome heterozygous for normal and the New Orleans chromosomes. The difference in staining capacity and length between the two chromosomes can be seen. Fig. 5. The sixth chromosome derived from crossing over between the normal and the New Orleans chromosomes. The left side of the chromosome is the New Orleans and the right side is the new chromosomes whose proximal half is made up of the normal chromosome. Fig. 6. A sixth chromosome of which the left side is the normal and the right side is made up of two kinds of chromosomes—the proximal half of the normal and distal half of the New Orleans chromosome.
**Bichromic acid.** When treated with a solution of 15 grams bichromic acid in 100 cc. of distilled water, the affinity of the chromosome to aceto-carmine is greatly reduced. If the concentration of bichromic acid is reduced to a quarter of the above, the bands become visible but the \( \beta \)-heterochromatin becomes amorphous and hard to observe; the nucleolus is stained clearly, showing a meshwork. The characteristic double appearance of the sixth salivary chromosomes of the hybrids of the New Orleans and other strains is brought out by the differential staining reactions of the components.

**Formalin.** In the salivary chromosomes fixed with fifty percent formalin before staining with aceto-carmine, the distinction between the chromatic and achromatic substances diminishes. The vacuolate nucleolus, however, appears distinctly. Treatment with ten percent formalin does not diminish the distinction between the New Orleans and the normal sixth chromosome.

**Picric acid.** In the material treated with a saturated solution of picric acid diluted with the same volume of distilled water, the salivary chromosomes appear like those in formalin. The New Orleans chromosome can easily be recognized.

**Mixture of formalin and picric acid.** When treated with a mixture of formalin and picric acid, the nucleolus becomes obscure though vacuoles within can be seen. The bands of the salivary chromosomes look similar to those in the material treated with picric acid; the New Orleans chromosome is more lightly stained than the normal chromosome.

**Mercuric chloride.** Mercuric chloride fixation is peculiar in its effect, in that the bands become more granular and the difference between the thick and thin bands becomes more conspicuous in comparison to the case of simple staining with aceto-carmine. The nucleolus becomes obscure and its inner structure invisible.

As stated above, the sixth salivary chromosome of the New Orleans strain appears different, in all the fixatives tried, from the other wild strains. It is thus clear that the abnormal staining capacity of New Orleans chromosome is in no way correlated with any peculiar effect of acetic acid. The chromosomes and their bands can be seen in a living nucleus of the salivary gland mounted in body fluid surrounded by mineral oil. Because of its small size, however, the sixth chromosome can hardly be recognized among the tangled mass of chromosomes. It is therefore not known whether the abnormal staining capacity exists in the living cell or not; but it is likely that even in the living state the density of the chromatin of the New Orleans chromosome is different from those of other strains.
Crossing over between the normal and abnormal chromosomes

The crossing over in the dot-like sixth chromosome of D. virilis was first discovered by Chino and Kikkawa (1933) in their studies of the Japanese stock. They have shown that the crossing over in this chromosome rarely occurs in room temperature, but its frequency can be increased by high temperature, the optimum being 30°C.

Some New Orleans females were mated with males heterozygous for the mutant gene Gap and homozygous for glossy, which belong to the sixth linkage group, and F₁ females were backcrossed with glossy males. The number of F₂ flies and recombination values are summarized in Table 1. The recombination value obtained from the female heterozygous for the normal chromosome is 0.10 ± 0.037 and that from the New Orleans chromosome is 0.04 ± 0.028. The former value is more than twice the latter, but the difference is not statistically significant because of the largeness of the standard error.

The crossover Gap female obtained from the mother, heterozygous for New Orleans chromosomes, died after its emergence. The glossy female was successfully mated with her brother and a stock of this strain was established. This stock has a markedly low fertility.

<table>
<thead>
<tr>
<th>Matings</th>
<th>Non-crossovers</th>
<th>Crossovers</th>
<th>Recombination values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirosima × gl.</td>
<td>929</td>
<td>1</td>
<td>0.11</td>
</tr>
<tr>
<td>Gp. gl. × gl.</td>
<td>949</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Keiyo × gl.</td>
<td>601</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>Gp. gl. × gl.</td>
<td>619</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Kyoto × gl.</td>
<td>904</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>Gp. gl. × gl.</td>
<td>924</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Kyoto ac. Gp. gl. × gl.</td>
<td>1007</td>
<td>1</td>
<td>0.10</td>
</tr>
<tr>
<td>Gp. gl. × gl.</td>
<td>1042</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3441</td>
<td>3</td>
<td>0.10 ± 0.037</td>
</tr>
<tr>
<td>New Orleans × gl.</td>
<td>657</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gp. gl. × gl.</td>
<td>696</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>New Orleans × gl.</td>
<td>931</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>Gp. gl. × gl.</td>
<td>938</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>New Orleans × gl.</td>
<td>913</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>ac. Gp. gl. × gl.</td>
<td>899</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2501</td>
<td>1</td>
<td>0.04 ± 0.028</td>
</tr>
</tbody>
</table>
and viability. It is likely that the New Orleans strain gives the same
crossover value as the normal strain, but the number of crossovers
actually recovered is small on account of their viability. At any rate
there is no decisive evidence that the abnormality in the sixth chromo-
some of the New Orleans stock makes the crossing over in the
hybrid strain more difficult than in ordinary cases.

Cytological demonstration of crossing over
in the salivary chromosome

The glossy female, which resulted from a crossing over between
a normal and a New Orleans chromosome, was mated with a male
having the normal sixth chromosome. The F1 larvae were examined
for their salivary chromosomes. About half of the larvae had the
normal sixth chromosomes and the other half had chromosomes con-
sisting of a normal and an abnormal haploid (Fig. 6). The abnormal
haploid chromosome is made up of a lightly staining New Orleans
chromosome in its distal half (VI1A-VI1G) and a deeply staining
normal chromosome in its proximal half (VI1F-proximal end). Since
the father of these flies was a homozygote for the normal sixth
chromosome, it is certain that the abnormal sixth chromosome is
derived from the mother which was a crossover between a normal
and a New Orleans strain. Thus the genetic result collaborates per-
fectly with the observation of the salivary chromosomes.

So far cytological demonstrations of crossing over have been
made by Creighton & McClintock (1931) and Brink & Cooper (1935)
in Zea mays, by Stern (1931) with D. melanogaster and by Chino &
Kikkawa (1933) with D. virilis. The observation stated above is
another demonstration of this phenomenon which is perhaps even
clear than any one by those previous authors.

Gap mutant originated from the New Orleans strain

The mutants so far known in the sixth chromosome of D. virilis
are acute, abdomen rotatum, Gap, hump, oily, stubby, glossy and
lethal 6a (Chino 1936–1937). Of these, Gap was obtained more
often than any other mutant and their allelomorphs now amount to
ten; they are all dominants and lethal in homo except in certain
strains. Another Gap mutant, Gap11, appeared in a stock from New
Orleans. The salivary chromosome has the expected peculiarity.

Further remarks

The sixth salivary chromosome of the New Orleans strain differs
from that in the other strains in its lower staining capacity, probably
on account of the smaller quantity of chromatic material contained. Otherwise it has no peculiarity—either in the arrangement of bands or in the crossingover value, though the data are somewhat inconclusive. Moreover GapⅪ, which is an allelomorph of the Gap mutants, was discovered in the same stock. Only the flies having the chromosomes of the new composition, resulting from crossing over between the New Orleans and normal chromosomes, show markedly lower fertility and viability. This cannot be due to any peculiar genic action of the New Orleans because this stock has normal fertility. Detailed investigations give no evidence of deficiency or duplication of the bands in the chromosome of the new composition. Even if a change had occurred in the achromatic region between the chromatic bands, it is unlikely that such a change would affect the fertility, because the chromatic bands, which represent the loci of the genes, are apparently normal. The last possibility is the position effect of some gene or genes brought into a different chromosome by crossing over. This remains to be shown by more adequate material.

**Summary**

1. The sixth salivary gland chromosome of the New Orleans strain of *D. virilis* has a markedly lower staining capacity than that of other strains.

2. This chromosome is also wider and longer than those in other strains. The arrangements of the bands, however, show no difference when crossed, a complete somatic synapsis occurs between this chromosome and the corresponding chromosome of any other strains in the heterozygous condition.

3. Among 23 strains from various localities of Japan and China, as well as of America examined, none had a sixth chromosome like that of the New Orleans. Only a mutant stock, Gap² hump/lethal 6a derived from the American Gap² stock, contained such a chromosome.

4. No difference was found in the frequency of crossing over between the sixth chromosomes of the New Orleans and that of other strains.

5. In a new composite chromosome derived from such crossing over, the distal half from VI1A to VI1G is made up of the New Orleans chromosome and the other half of a section, VI1F to the proximal end, of a normal chromosome. This gives another case of cytological demonstration of the phenomenon of crossing over.

6. A new allelomorph of GapⅪ was discovered in the New Orleans stock.
7. No significant difference in genetical behaviour exists between the New Orleans and normal strains. Nevertheless the markedly low viability and fertility of the stock with the recombination sixth chromosome is possibly due to some incompatibility between the two kinds of chromosomes.

Literature


