A Hybrid Dysgenesis Syndrome in *Drosophila virilis*

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ABSTRACT

A new example of "hybrid dysgenesis" has been demonstrated in the F₁ progeny of crosses between two different strains of *Drosophila virilis*. The dysgenic traits were observed only in hybrids obtained when wild-type females (of the Batumi strain 9 from Georgia, USSR) were crossed to males from a marker strain (the long-established laboratory strain, strain 160, carrying recessive markers on all its autosomes). The phenomena observed include high frequencies of male and female sterility, male recombination, chromosomal nondisjunction, transmission ratio distortion and the appearance of numerous visible mutations at different loci in the progeny of dysgenic crosses. The sterility demonstrated in the present study is similar to that of P-M dysgenesis in *Drosophila melanogaster* and apparently results from underdevelopment of the gonads in both sexes, this phenomenon being sensitive to developmental temperature. However, in contrast to the situation in *D. melanogaster*, hybrid dysgenesis was observed in *D. virilis* only when the male parent was a long-established laboratory strain.

MATERIALS AND METHODS

*Drosophila* strains: The following wild-type strains were used: strain 2 (collected in Kutaisi, in 1970, Georgia USSR); strain 9 (Batumi, collected in 1970, Georgia USSR); strain S9 (Seychelle Islands, collected in 1984); and strain 101 (obtained from Japan in 1968). The following marker strains, obtained in 1967 from W. STONE (University of Texas) were used: strain 104, gl (chromosome VI); strain 109, w Bx y (I); strain 110, th, gp-L₂ (III), st (V); and strain 142, st es (V). In addition, we used: strain 149, b (II), tb, gp-L₂ (III), ed (IV), pe (V) (obtained from Japan in 1968) and strain 160, b (II), tb gp-L₂ (III), ed (IV), pe (V), gl (VI) (constructed by us in 1975 by combining the strains 149 and 104 mentioned above). All strains were obtained from the *Drosophila* collection of the Institute of Developmental Biology of the USSR Academy of Sciences. The genetic markers mentioned were described earlier (GUBENKO and EVGEN'EV 1984).

Crosses performed to study hybrid dysgenesis: A cross involving strain 9 females and strain 160 males is designated "cross A" while the reciprocal cross is designated "cross B." Parental strains were mated en masse.

To evaluate male recombination we studied the frequency of crossing over in chromosome V, between the markers st and es which had been transferred by repeated back crosses from strain 142 into the Batumi strain 9. The resulting strain (Batumi, st es) has been crossed to strain 160, and...
TABLE 1

Influence of temperature on the level of sterility of F1 hybrids obtained in A and B crosses between Batumi strain 9 and strain 160 of D. virilis

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Sterile females/299</th>
<th>Sterile males/255</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18°</td>
<td>53/76 69</td>
<td>43/73 59</td>
</tr>
<tr>
<td>25°*</td>
<td>461/480 96</td>
<td>113/120 94</td>
</tr>
<tr>
<td>29°</td>
<td>26/60 44</td>
<td>51/70 74</td>
</tr>
<tr>
<td>Cross B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18°</td>
<td>0/16 0</td>
<td>5/17 30</td>
</tr>
<tr>
<td>25°*</td>
<td>2/28 7</td>
<td>1/10 5</td>
</tr>
<tr>
<td>29°</td>
<td>8/35 23</td>
<td>1/36 3</td>
</tr>
</tbody>
</table>

Cross A involves strain 9 females and strain 160 males, while the reciprocal cross is designated cross B. The flies of parental strains and hybrid individuals from egg to imago were grown at corresponding temperature. All data except those marked with an asterisk were obtained in four replicate mass crosses with the range of variabilities of about 2–4%. The data on sterility were treated by G-test (SOKAL and ROHLF 1969), the differences were considered to be significant if the probability of difference due to chance was <1%, s, significant; NS, not significant.

male recombination was estimated in the progeny of two males crossed to st es females from the parental strain.

A search for visible mutations was carried out among the progeny of F1 individuals from the A and B crosses. To investigate the stability of dysgenesis-induced mutations, sn and w mutations found in the offspring of the dysgenic cross, were transferred by nine repeated back crosses into Batumi strain 9. sn/+ or w/wo females from the resulting strains were mated to strain 160 males, and subsequently F1 sn (or w) males were crossed with the original sn (or w) strain females. F2 flies were examined for revertants.

The frequency of X chromosome nondisjunction was studied when F1 females obtained in A or B crosses were mated to males carrying the "white eye"-marker (strain 109). The F2 progeny was examined for sterile w males.

The influence of temperature on the level of sterility, was studied when the parental flies were grown at 18°, 25° or 29° before the crosses were performed and the development of hybrid flies from egg to imago also took place at the corresponding temperature. To examine the reproductive system, the gonads were dissected in a drop of phosphate buffer (ASHBURNER 1970). We used strains with "synthetic" karyotypes to study the genetic bases of sterility. These strains carry individual chromosomes of Drosophila lummai in heterozygous condition in otherwise an intact genome of D. virilis (strain 160) (EVGEN'EV and SIDOROVA 1976).

RESULTS AND DISCUSSION

General description of sterility: Hybrid females and males from cross A (strain 9 female × strain 160 male) and cross B (strain 160 female × strain 9 male) which were grown at 18°, 25° or 29° were tested for their ability to produce offspring when mated individually to highly fertile flies from the wild-type Batumi strain 9.

As Table 1 shows, the highest level of sterility in both males and females was exhibited in the progeny of cross A at 25°. The females from cross A may be divided into two distinct categories. The majority are totally eggless, while others exhibit various abilities to lay eggs. Therefore, in subsequent investigations, we classified females as "sterile" if no eggs were produced under experimental conditions and "fertile" otherwise.

In order to analyze the phenomenon further, we performed crosses, in both directions, using various strains of D. virilis (Table 2). It is evident that all of the strains studied can be categorized as follows: P-like strains (strains 160, 149); M-like strains (strains 2, 9), and Q-like or neutral strains (strain 110). Additional crosses involving strains 160, 9 (Batumi) and several other strains of D. virilis (see MATERIAL AND METHODS) enable us to conclude that strains 104, 109 and S9 are M-like, while strain 101 represents a typical Q-like strain (data not shown). P-like strains (strains 160 and 149), when used as male parents in crosses with M-like (but not Q-like) females give rise to F1 progeny exhibiting high levels of sterility. Significant sterility (up to 20%) observed in the strain 149 and strain 160 intraspecific crosses (Table 2) is probably due to the presence of multiple recessive markers, five and six respectively, in the autosomes of these strains.

Examination of the reproductive system: Hybrid females and males from crosses A and B were grown at 25°, aged 5 days in mating vials and dissected to study the gonads. The investigation showed that a high proportion of hybrid males and females from cross A are characterized by rudimentary testes and ovaries, while no abnormalities were seen in any of the cross B hybrids. It is interesting that in both sterile males and females of cross A, all somatic tissues appear normal. Figure 1 illustrates typical cases of gonadal underdevelopment in both males and females in com-
Hybrid Dysgenesis in *D. virilis*

FIGURE 1.—Effects of hybrid dysgenesis on the reproductive system: (A) Reproductive system of a normal *D. virilis* male (strain 9). (B) Reproductive system of a normal *D. virilis* female (strain 9). (C) Reproductive system of a dysgenic male obtained in cross “A.” (D) Reproductive system of a dysgenic female obtained in cross “A.”

Comparison with normal gonads of *D. virilis*. Although panels C and D of Figure 1 show instances of bilateral atrophy, unilateral atrophy was also sometimes observed. These unilaterally affected individuals may be either sterile or fertile. It is evident from Table 1 that the maximum level of sterility was manifested at intermediate temperature (25°C), while both high (29°C) and low (18°C) temperature has a curative effect. It is worth mentioning that in *D. melanogaster* GD-sterility due to P-M dysgenesis was observed only when dysgenic hybrids were reared above 25°C.

**Male recombination:** Although recombination is not normally observed in the *D. melanogaster* or *D. virilis* males, a low frequency of male crossing over has been independently observed several times (Hirai et al. 1973; Green 1980). Male recombination represents one of the most consistent attributes of the P-M system. To investigate male recombination in *D. virilis* we have studied crossing over between the st and es markers of chromosome V; in females, the recombination frequency between the markers is 15.4% (Evgen'ev 1971). The markers were transferred by nine back crosses into the Batumi strain 9.

**TABLE 3**

<table>
<thead>
<tr>
<th>Type of cross</th>
<th>Number of crossovers/2 flies</th>
<th>Percent recombination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross A</td>
<td>5/8,814</td>
<td>0.057</td>
</tr>
<tr>
<td>Cross B</td>
<td>0/10,962</td>
<td>0</td>
</tr>
</tbody>
</table>

Both reciprocal crosses, strain 9, st es females mated to strain 160 males (cross A) and vice versa (cross B) were performed and recombination was estimated in the F1 males. Table 3 summarizes the results of this study.

It is noteworthy that the level of sterility in the strain 9, st es × strain 160 crosses was very similar to that observed in the original A and B crosses. Independent investigation carried out to estimate male recombination between the tb and gp markers (chromosome III) also showed significant recombination (0.08%) between these genes in the progeny of cross A. Thus recombination in the hybrid males from crosses A may occur in both the third and fifth chro-
mosomes. However, we cannot at present specify whether the male recombination associated with 
hybrid dysgenesis in *D. virilis* is the result of premeiotic 
or meiotic events.

**X chromosome nondisjunction and transmission ratio distortion:** To study the frequency of *X* 
chromosome nondisjunction hybrid females from crosses A and B were crossed with *white* males and the 
progeny of such crosses were screened for *w* individuals. 
The results of these experiments are shown in Table 4. To estimate the frequency of nondisjunction indicated in Table 4, we took into account only three sterile *w* males. The fertile *w* male and *w* female independently produced in cross A are evidently the result of mutational events. Thus the approximate frequency of mutations at the *white* locus in a dysgenic cross is $10^{-3}$.

Since distortion of the transmission ratio is a typical feature of P-M dysgenesis (Hiraizumi 1971; Kidwell and Kidwell 1976), we decided to determine the transmission frequencies for Batumi strain 9 and strain 160 chromosomes in the progeny of crosses A and B. We crossed the F1 male progeny of A and B crosses with females from the marker strain 160 and estimated a transmission frequency *K* value (where *K* = number of progeny homozygous for a certain strain 
160 chromosome/total progeny) for strain 160 chromosomes. Table 5 illustrates transmission frequencies for chromosome *III* in crosses A and B.

It is evident from Table 5 that transmission is significantly reduced for a strain 160 chromosome *III* carrying the *tb* and *gp* markers. Further analysis (data not shown) suggests that the transmission ratio for other chromosomes is also reduced significantly. Hence the situation described *D. virilis* dysgenesis appears to be very similar to that found in P-M dysgenic hybrids where a tendency exists for *P* strain-derived chromosomes to be transmitted less frequently than *M* strain-derived chromosomes (Kidwell, Kidwell and Sved 1977). Probably, chromosomes derived from strain 160 are “eliminated” among the progeny of a dysgenic cross, as has been demonstrated for the T-OOT chromosome of *D. melanogaster* (Hiraizumi 1977).

**Enhanced mutability associated with hybrid dysgenesis:** In the progeny of cross A, we identified many 
visible mutations. Interestingly, mutations were found in the progeny (F2, F3, F4, etc.) of both dysgenic females and males. The most active site was the *sn* (*singed*) locus, where we isolated seven independent mutations. In addition to *sn*, other loci with moderately high frequencies of mutation were *white* (3), *Delta* (4), *dusky* (2), *Stretched* (3) and *abnormal abdomen* (6). Besides these well-known loci where repeated mutational events occurred and several other known loci where single mutations were found, we have isolated several mutations which so far have not been found in *D. virilis* e.g., “apterous” (*apt*), “lozenge” (*lz*) “ocelliless” (*oc*), “arch” (*arc*), etc.

Instability is a characteristic feature of some dysgenesis-induced mutations. We transferred the *sn* and *w* mutations into Batumi strain 9 by repeated back crosses and F8 *sn*+/+ and F8 *w*/*w* females were crossed to strain 160 males to study the stability of these single alleles obtained in the progeny of dysgenic crosses. It is noteworthy that *sn*/*+* females were used in the present study, because in the Batumi genotype, *sn*/*sn* females are completely sterile. Hybrid males, *sn* and *w*, respectively, were backcrossed to the females from the original mutant strains (*sn* and *w*) and the frequency of wild type females was estimated. While the experiments failed to demonstrate the reversion of the *white* mutation to the wild-type state (data not shown), the *sn* mutation was found to be moderately unstable and reverted to wild-type state with the frequency of $3.8 \times 10^{-2}$.

**Genetic control of sterility:** To determine the roles of individual chromosomes of strain 160 in provoking sterility we utilized stains with “synthetic” karyotypes obtained by us. These strains represent strain 160 carrying different, single *D. lummei* chromosomes in heterozygous condition. Preliminary experiments have demonstrated that Fl hybrids obtained in reciprocal crosses between *D. virilis* (strain 160) and *D. lummei* exhibit high levels of fertility (up to 90%) when crossed with strain 9. Thus, the presumptive genetic elements of strain 160 chromosomes which control sterility in interstrain crosses behave as recessives when combined with *D. lummei* chromosomes. Table 6 summarizes the results of crosses where strains het-

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### Table 4

**X chromosome nondisjunction in the progeny of hybrid females**

<table>
<thead>
<tr>
<th>Origin of F1 hybrid females</th>
<th>Total number of F2 flies</th>
<th>Number of w-flies in F2</th>
<th>Percent of nondisjunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross A</td>
<td>1922</td>
<td>4</td>
<td>0.16</td>
</tr>
<tr>
<td>Cross B</td>
<td>2220</td>
<td>(1 fertile)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5

**Transmission frequency of chromosome III from strain 160 carrying *tb* and *gp* markers in the progeny of hybrid males**

<table>
<thead>
<tr>
<th>Origin of F1 hybrid males</th>
<th>Total number of F2 flies</th>
<th>Number of <em>tb gp</em>/<em>tb gp</em> flies in F2</th>
<th><em>K</em> value ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross A</td>
<td>1288</td>
<td>415</td>
<td>0.32 ± 0.013</td>
</tr>
<tr>
<td>Cross B</td>
<td>1830</td>
<td>864</td>
<td>0.47 ± 0.011</td>
</tr>
</tbody>
</table>

The difference between the data of two crosses is highly significant, the probability of difference due to chance according to *G*-test is $<1\%$. 

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**TABLE 6** Percent of *F₁* hybrid sterility in crosses between Batumi strain 9 females and "synthetic" strain males

<table>
<thead>
<tr>
<th>Type of cross</th>
<th>Percent of <em>F₁</em> male sterility</th>
<th>Percent of <em>F₁</em> female sterility</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 × 9 (control)</td>
<td>94 (120)</td>
<td>96 (488)</td>
<td></td>
</tr>
</tbody>
</table>

Numbers of flies are indicated in parentheses. The data on sterility were treated by *G*-test, the deviations from control were considered to be significant if the probability of difference due to chance was <5%. s, significant.

erozygous for different *D. lummei* chromosomes were used to obtain *F₁* hybrids (A cross) with strain 9. The data indicate clearly that the high level of sterility manifested in dysgenic crosses is under polygenic control. All large autosomes of strain 160 probably carry factors for sterility. Independent experiments, where different individual chromosomes of strain 160 were transferred into strain 9 (data not shown) confirmed this conclusion and showed that the microchromosome (VI) from strain 160 does not seem to carry any sterility factors. Several years ago one of us isolated dispersed repeats from the genome of *D. virilis* and demonstrated that these repeats (pDV-elements) may be mobilized by interspecific crosses (ZELENTSOVA et al. 1986; VASHAKIDZE et al. 1989). Moreover, the transpositions were demonstrated when males of the marker strain 160 were crossed to *D. lummei* or *D. littoralis* females. Naturally, when we discovered the new type of dysgenesis in *D. virilis* described above, we supposed that pDV-elements were probably responsible for all manifestations of dysgenesis including the occurrence of mutations. However, Southern and in situ hybridization experiments using mutant strains showed this not to be so. Thus we failed to detect a pDv element in either white allele or in a singed allele obtained in the progeny of dysgenic crosses.

Recently we have isolated from a white eye mutation obtained in dysgenic crosses another repeated element which we have named "Ulysses." Molecular analysis of this element and interspecific transformations may uncover a role for it in the hybrid dysgenesis syndrome.

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**LITERATURE CITED**


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