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Factors contributing to the hybrid dysgenesis syndrome in *Drosophila virilis*

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Summary

A hybrid dysgenesis syndrome in *Drosophila virilis* is associated with the mobilization of at least four unrelated transposable elements designated *Helena*, *Paris*, *Penelope* and *Ulysses*. We carried out 42 crosses between eight strains differing in transposable element copy number in order to assess their contributions to hybrid dysgenesis. Linear regression and stepwise regression analysis was performed to estimate the correlation between the difference in euchromatic transposable element number between the parental flies of different strains involved in the crosses and the percentage, in the progeny of these crosses, of males with atrophic gonads. Male gonadal atrophy is a typical manifestation of the *D. virilis* hybrid dysgenesis syndrome. About half the variability in the level of male gonadal atrophy can be attributed to *Penelope* and *Paris/Helena*. Other factors also seem to play a significant role in hybrid dysgenesis in *D. virilis*, including maternally transmitted host factors and/or uncontrolled environmental variation. In the course of this work a novel transposable element named *Telemac* was found. *Telemac* is also mobilized in hybrid dysgenesis but does not appear to play a major causative role.

1. Introduction

A hybrid dysgenesis syndrome takes place in the progeny of certain crosses between strains of *D. virilis* (Lozovskaya *et al.*, 1990). The dysgenic traits in the F_1 progeny include high frequencies of male and female sterility as well as gonadal atrophy. Male recombination, chromosomal non-disjunction, transmission ratio distortion, and the appearance of numerous visible mutations at different loci are also observed in the progeny of the F_2 and later generations. This hybrid dysgenesis syndrome is unusual in the fact that four unrelated transposable elements (*Helena*, *Paris*, *Penelope* and *Ulysses*) are all mobilized in the same dysgenic cross (Sheinker *et al.*, 1990; Petrov *et al.*, 1995; Evgen'ev *et al.*, 1997). In the course of this work, a novel *D. virilis* transposable element, named *Telemac*, was also cloned and key regions of this element were sequenced. *Telemac* belongs to the *BEL*-related class of transposable elements with terminal direct repeats, and is unrelated to *Helena*, *Paris*, *Penelope* or *Ulysses*. It is shown that *Telemac* is also mobilized in the same dysgenic cross where *Helena*, *Paris*, *Penelope* and *Ulysses* are simultaneously mobilized.

Two possible explanations, not mutually exclusive, have been proposed that may account for these observations: one hypothesizes the superposition of two or more different systems of hybrid dysgenesis, each representing a different transposable element; the other hypothesizes that the mobilization of a single element directly or indirectly (through chromosome breakage or possibly because they share a common pathway in the host) triggers the mobilization of others (Petrov *et al.*, 1995; Lozovskaya *et al.*, 1995; Evgen'ev *et al.*, 1997).

There is qualitative evidence that *Penelope* plays an important role in hybrid dysgenesis in *D. virilis* (Evgen'ev *et al.*, 1997), but there are no data about the role of other transposable elements, or any other factors, in the causation of this syndrome. Knowing

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which transposable elements or factors are involved in the causation of this syndrome is essential for distinguishing the above-mentioned hypotheses.

To identify possible candidates involved in the causation of the hybrid dysgenesis syndrome, the following was done: (a) the distribution and number of euchromatic copies of *Helena*, *Paris*, *Penelope*, *Telemac* and *Ulysses* was determined in several *D. virilis* strains and (b) a set of 42 crosses was performed, and in the progeny of these crosses the frequency of males with atrophied gonads (a typical characteristic of the *D. virilis* hybrid dysgenesis syndrome; Lozovskaya *et al.*, 1990) was estimated. Regression and stepwise regression analysis was then performed to ascertain whether any correlation could be detected between the difference in euchromatic transposable element copy number between the individuals (of different strains) used as parents in a particular cross, and the percentage of males without atrophic gonads in the progeny of that cross. Significant correlations were found, as expected, in relation to *Penelope*, but also in relation to *Paris*/*Helena*. *Penelope* alone can explain 51% of the variability in the results while *Paris*/*Helena* can explain 32% of this variability. Together these transposable elements explain as much as 55% of the variability in the results. The remaining variability may suggest the involvement of other, as yet unidentified transposable elements, other factors such as host cytoplasmic factors or uncontrollable environmental variation. The effect of host cytoplasm was tested by performing a set of pairs of reciprocal crosses. The analysis of these results suggest that host cytoplasmic factors are also involved in the *D. virilis* hybrid dysgenesis syndrome.

2. Materials and methods

(i) Drosophila virilis *strains*

Strains 104, 149, 9, 2 and 160 are described in Lozovskaya *et al.* (1990). Strain w^{11} is described in Petrov *et al.* (1995). The China (15010-1051.47), Chile (15010-1051.51) and Mexico (15010-1051.48) strains were obtained from the The National *Drosophila* Resource Species Center at Bowling Green, Ohio. Strain 104 was obtained from V. G. Mitrofanov. All the crosses were performed 'en masse' on standard cornmeal–molasses medium at 25 °C.

(ii) *DNA extraction*

DNA from P1 clones was extracted using the plasmid DNA miniprep protocol (Hartl & Lozovskaya, 1995). For plasmids smaller than 20 kb, the Quiagen QIAprep Spin Plasmid Miniprep Kit was used. For each strain, genomic DNA from 50 *D. virilis* flies was prepared as described in Sambrook *et al.* (1989).

(iii) *Cloning and sequencing of* Telemac

The transposable element *Telemac* was cloned from P1 clone Dv10-06, from a *D. virilis* genomic library (Lozovskaya *et al.*, 1993). For purposes described elsewhere, this particular P1 clone was included in a randomly chosen set of clones for which the sites of hybridization with *D. virilis* polytene chromosomes have been determined (Vieira *et al.*, 1997). After digesting the P1 clone with *Eco*RI and *Pst*I, the fragments were separated in an agarose gel and extracted from the gel. The DNA from each band was used as a probe for *in situ* hybridization with polytene chromosomes from larvae of strain 9. Several fragments gave the original multiple-site hybridization pattern, and the smaller fragments were subcloned. DNA sequencing was performed with an Applied Biosystems model 373A DNA sequencing system with the ABI PRISM Dye Termination cycle-sequencing Kit, using the transposon-facilitated DNA sequencing method of Strathmann *et al.* (1991).

To generate clones that would allow us to determine the approximate size of the particular *Telemac* copy analysed, we subcloned random fragments averaging 14 kb in size, from the same Dv10-06 P1 clone, using the sequence-scanning protocol described by Nurminsky *et al.* (1996).

(iv) *Determination of the transposable element content within strains*

(a) *PCR amplification*

Standard PCR amplification conditions were 25 cycles of denaturation at 94 °C for 30 s, primer annealing (adjusted for individual primer pairs) for 30 s, and primer extension at 72 °C for 3 min. For *Penelope*, 5′-TTGGTGTTAGTGCCCTGAAGA-3′ and 5′-TTGTAGTTTAGTCGTTTGTAG-3′ primers (49 °C annealing temperature) were used, which are expected to result in the amplification of a 2615 bp fragment. For *Paris* 5′-GCCATTAGCATCCATTACAGC-3′ and 5′-CAAAGGAAAATGTGCTAAGGA-3′ primers (53 °C annealing temperature) were used, which are expected to result in the amplification of a 973 bp fragment. For *Ulysses* 5′-GAAGTGCGACAGAAACCAATA-3′ and 5′-GTCCGTCCTGCCACTGCTAAG-3′ primers (57 °C annealing temperature) were used, which are expected to result in the amplification of a 4149 bp fragment. For *Telemac* 5′-ACCAGTCAGTCTTTCGTTTTG-3′ and 5′-CGATGTGACTGCTTGTGTTGC-3′ primers (40 °C annealing temperature) were used, which are expected to result in the amplification of a 1760 bp fragment. For *Helena* 5′-GATTTTAATGCGGGTGGTCTT-3′ and 5′-CAACAACTGCGGTGGCTCAAC-3′ primers (55 °C annealing temperature) were used, which are

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expected to result in the amplification of a 363 bp fragment.

None of these conditions results in the full transposable element amplification but rather in the amplification of internal fragments of variable size from the above-mentioned transposable elements.

(b) *Probes for Southern hybridization and* in situ *hybridization*

Probes were obtained from plasmids containing either a 1·9 kb *Paris* sequence with flanking genomic sequences, an 800 bp DNA fragment containing *Ulysses*, or a 2·6 kb RNA fragment containing *Helena* with flanking genomic sequences. These plasmids were kindly provided by D. Petrov. A *Penelope* PCR probe, 2615 bp long, was used, which was made using the PCR primers described above. A 3 kb fragment that contains the transposable element *Telemac*, with flanking genomic sequence, was used for *in situ* hybridization, and a subfragment of 2 kb was used for Southern hybridization.

(c) *Southern hybridization*

Genomic DNA was cleaved with the appropriate restriction enzymes, transferred onto Hybond-N nylon members (Amersham), and hybridized with the above-mentioned probes as described in Sambrook *et al.* (1989).

(d) In situ *hybridization*

In situ hybridizations were performed as described in Vieira *et al.* (1997).

3. Results

(i) *Characteristics of the* D. virilis *transposable element* Telemac

Telemac has been cloned and several key regions sequenced from P1 clone Dv10-06 (Vieira *et al.*, 1997). The particular copy of *Telemac* present in this P1 clone has a size of about 10 kb. It should be emphasized that we have no reason to believe that this copy of *Telemac* is complete. DNA sequences from both ends of *Telemac* have been obtained (accession numbers AF009439 and AF009440), which revealed that this transposable element has direct repeats 430 bp in size. Database searches at the amino acid level with sequences internal to the element revealed significant similarity only with an uncharacterized *D. melanogaster* cDNA fragment (accession number U11691) as well as with a putative protein encoded by a transposable element called *BEL* from *D. melanogaster*, which is 6·5–7·3 kb in size and contains 361 bp direct repeats (Lindsley & Zimm, 1992). On the basis of this similarity we assign *Telemac* to the *BEL* class of transposable elements with terminal direct repeats.

Table 1. In situ *localization of 10 new sites of* Telemac *in the* D. virilis w^{11} *dysgenic strain*

Chromosome[a]					
X	2	3	4	5	6
None	23D 23F/G	33F	40A 41D 45D 48C 48E	51F 53F	None

[a] The *in situ* localizations were determined according to the photomap of Gubenko & Evgen'ev (1984).

(ii) *Simultaneous mobilization of several unrelated transposable elements*

When females from strain 9 are crossed to males from strain 160, the hybrid dysgenesis syndrome is observed. One typical characteristic of this syndrome is the appearance of numerous visible mutations at different loci in generations reared from the F_1 progeny of this cross. Several dysgenic strains have been establish from individuals with visible mutations, including strain w^{11}. The comparison, by *in situ* hybridization, of the euchromatic localizations of a particular transposable element in the *D. virilis* w^{11} dysgenic strain and in its two parental strains, strain 9 and strain 160, can be used to infer transposable element mobilization. Petrov *et al.* (1995) used this approach to show that four unrelated transposable elements, namely *Paris*, *Helena*, *Ulysses* and *Penelope*, are all simultaneously mobilized in the same dysgenic cross. This same approach was used by us to show that *Telemac* is also mobilized in this particular dysgenic cross. In addition to *Telemac* sites present in strains 9 and 160 that are present in strain w^{11}, there are also 10 new sites (Table 1). Therefore, *Telemac* is the fifth transposable element, unrelated to any of the four previously described, shown to be mobilized in this particular dysgenic cross. This is in contrast with the *P-M* and *I-R* hybrid dysgenesis syndromes in *D. melanogaster*, in which only one specific transposable element is mobilized (Berg & Howe, 1989).

(iii) *Transposable element distribution*

Fig. 1 shows the distribution of *Telemac* (*A*), *Ulysses* (*B*), *Penelope* (*C*), *Paris* (*D*) and *Helena* (*E*) in eight

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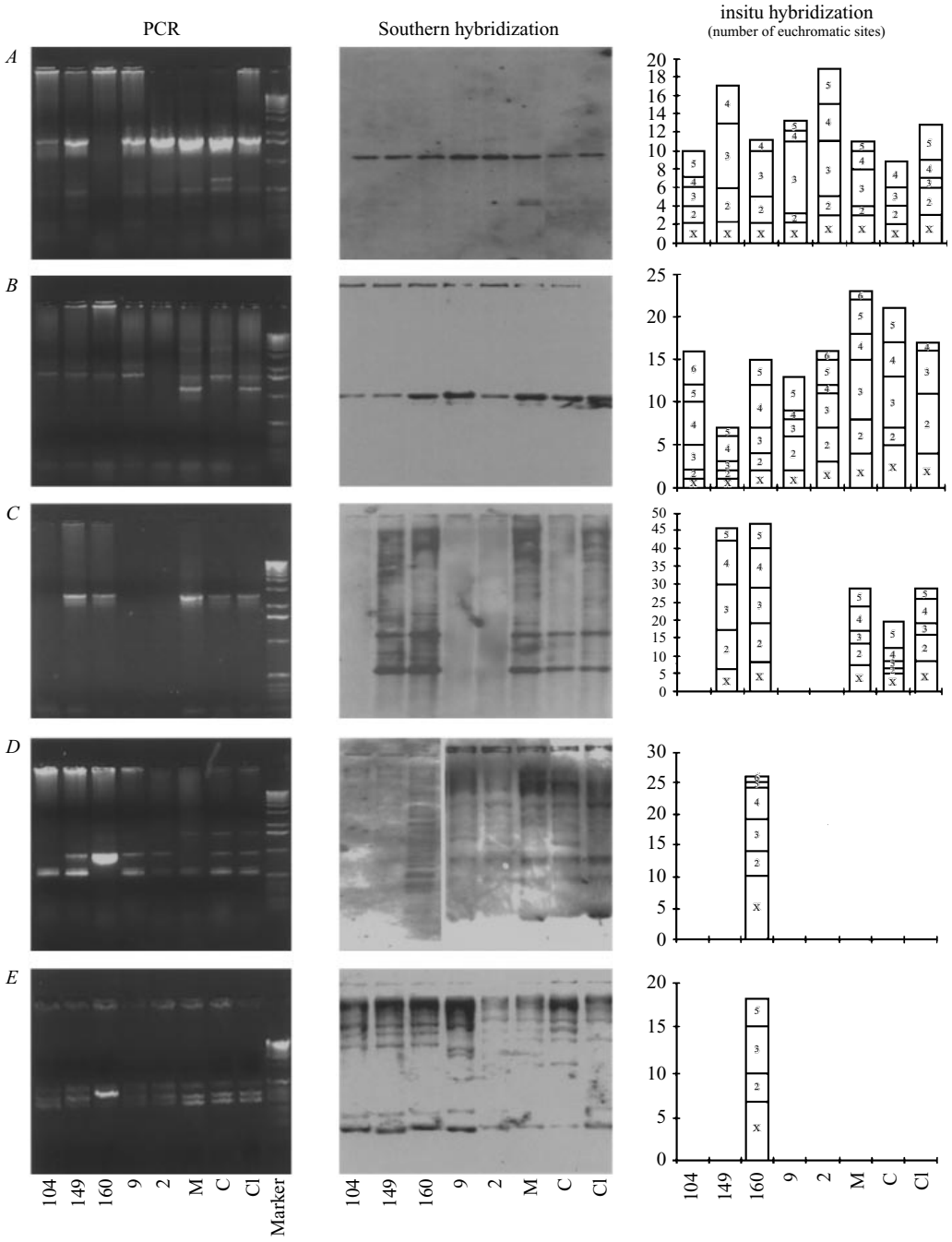

Fig. 1. Distribution of *Telemac* (*A*), *Ulysses* (*B*), *Penelope* (*C*), *Paris* (*D*) and *Helena* (*E*) in several different *D. virilis* strains as inferred from PCR experiments, Southern hybridization and *in situ* hybridization. The strains are: 104, 149, 160, 9, 2, Mexico (M), China (C) and Chile (Cl). In the PCR results, the marker lane is a lambda DNA/*Hind*III fragment marker. Note the PCR 'false negatives' for *Telemac* (*A*) in strain 160 and for *Ulysses* (*B*), in strain 2. These results are reproducible but conflicting with the hybridization and *in situ* data. It seems likely that in these strains the primers for PCR do not support amplification. In the case of Southern hybridization with *Ulysses* (*B*), *Penelope* (*C*) and *Helena* (*E*) genomic DNA was restricted with *Eco*RI. In the case of *Telemac* (*A*), genomic DNA was restricted both with *Eco*RI and *Hind*III. In the *Paris* (*D*) case, in the Southern hybridization on the left (three lanes) genomic DNA was restricted with *Pst*I and *Spe*I, while in that on the right (five lanes), genomic DNA was restricted with *Eco*RI.

strains studied (strains 160, 9, 2, China, Chile, Mexico, 104 and 149) as inferred from PCR experiments, Southern hybridization and *in situ* hybridization. These three methods were used because of consistency. A failure to amplify a DNA fragment by PCR may be due to a failure in primer anealing, and not to the absence of the template. Furthermore, it is very difficult to infer relative copy numbers from the relative intensity of PCR fragments. Copy numbers are also difficult to determine by Southern hybrid-

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Table 2. *Frequency of males with normal gonads in the progeny of 42 crosses involving several* D. virilis *strains*

Cross	Proportion	*n*	Cross	Proportion	*n*
160 × 160	0·99	112	9 × 160	0·13	226
160 × 9	0·96	125	9 × 9	0·97	117
160 × C	0·95	185	9 × C	1·00	281
160 × Cl	0·98	103	9 × Cl	0·98	302
160 × M	0·92	285	9 × M	0·99	149
160 × 2	0·97	201	9 × 2	1·00	128
C × 160	0·61	197	Cl × 160	0·98	107
C × 9	0·99	176	Cl × 9	0·99	119
C × C	0·97	109	Cl × C	0·99	110
C × Cl	0·99	161	Cl × Cl	0·99	112
C × M	0·99	131	Cl × M	0·99	209
C × 2	0·96	139	Cl × 2	0·98	150
M × 160	0·76	284	2 × 160	0	654
M × 9	0·99	107	2 × 9	0·99	102
M × C	1·00	117	2 × C	0·98	242
M × Cl	1·00	129	2 × Cl	0·52	133
M × M	0·85	127	2 × M	0·60	184
M × 2	0·99	279	2 × 2	0·98	121
104 × 149	0·99	216	149 × 104	0·98	336
2 × 149	0·28	312	2 × 104	0·95	109
104 × 160	0·54	70	149 × 160	0·98	52

Mexico (M), China (C) and Chile (Cl) were used in the crosses.

ization, from the relative intensity of the bands or from the number of bands, particularly if only large fragments hybridize with the DNA probe. Neither the PCR nor the Southern hybridization approach gives us information about whether the DNA fragements being detected are euchromatic or heterochromatic. *In situ* hybridization allow us to determine the number of euchromatic copies of a given transposable element but not of heterochromatic copies. The results obtained by the different techniques are complementary and therefore are discussed together. From the analysis of Fig. 1 it is evident that the five transposable elements analysed here are present in all the strains studied, except for *Penelope*, which is absent from strains 104, 9 and 2. *Telemac*, *Ulysses* and *Penelope* all have both euchromatic and heterochromatic sites, whereas *Paris* and *Helena* seem to have only euchromatic sites in strain 160. Strain 160 is a well-established laboratory strain and it was originally obtained by crossing strain 104 to strain 149 (Lozovskaya *et al*., 1990). Surprisingly, as assayed by PCR, Southern hybridization and *in situ* hybridization, strain 160 has many more copies of *Paris* and *Helena* than its parental strains (104 and 149) together. However, the male progeny of both reciprocal crosses 104 × 149 and 149 × 104 do not show any abnormal gonad formation (Table 2). It is unclear what conditions may have occurred that led to this increase in the *Paris* and *Helena* copy number. It is possible that other, as yet unidentified transposable elements may have been affected as well.

(iv) *Regression and stepwise regression analysis*

From *in situ* hybridization experiments, the number of euchromatic copies of each transposable element was estimated in eight different strains (104, 149, 160, 9, 2, China, Chile, Mexico), assuming that each hybridization signal corresponds to a single transposable element. In addition, the frequency of normal males in the progeny of a set of 42 crosses was estimated. These crosses consist of all possible pairwise crosses involving six of the strains studied (160, 9, 2, China, Chile, Mexico) plus six crosses involving strains 104 or 149 with some of the above.

In general, hybrid dysgenesis syndromes are observed when the males involved in the cross have more copies of a particular transposable element than the females (Berg & Howe, 1989). Therefore, the difference in transposable element euchromatic copy number between the parents (of different strains) used in the 42 crosses was calculated for each of the five transposable elements here analysed. When this difference is negative (meaning that there is a greater number of transposable element sites in the female than in the male used as parents), a value of zero is assigned to this variable. Regression and stepwise regression analysis was performed between this variable and the transformed frequency of males with normal gonads in the progeny of crosses between the strains. The regression analysis assumes that the data are normally distributed and this is not the case when percentages are used. Therefore, the frequency values

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Table 3. *Regression and multiple stepwise regression analysis between the differences in euchromatic transposable element content in the parental individuals of several crosses and the percentage of males without gonadal atrophy in the progeny of these crosses* (*involving several different* D. virilis *strains*)

	Penelope	*Paris* or *Helena*[a]	*Telemac*	*Ulysses*	All (stepwise regression)	
R	−0·717**	−0·570**	+0·237	+0·131	*Penelope*	−0·576**
					Paris or *Helena*[a]	−0·286*
R^2	0·514	0·324	0·056	0·017		0·554

The data consists of all possible pairwise crosses involving six of the strains studied (strains 160, 9, 2, China, Chile, Mexico) plus six crosses involving strains 104 or 149 with those above, where the frequency of male gonadal atrophy in the progeny of these crosses was determined (see Table 2).
* Significant at the 5% level; ** significant at the 1% level.
[a] *Paris* and *Helena* could not be analysed separately because these two variables are completely correlated.

were transformed by taking the sine of the square root of these values. Also, because in the multiple stepwise regression analysis some of our variables are partially correlated (which may influence what variables are going to be included in the final analysis), we used the adjustments α to remove = 0·05 and α to enter = 0·05, suggested by Wilkinson (1990) for such cases. The results of these regressions are shown in Table 3.

In both regression analyses the effects of *Paris* and *Helena* cannot be separated because the presence and copy number of the elements are completely correlated; in fact, both elements are present as euchromatic copies only in strain 160.

Penelope alone can explain about 51% of the variation in the results, while *Paris*/*Helena* alone can explain 32% of the variation in the results. Both values are significant at the 5% level. *Penelope* and *Paris*/*Helena* are also both significant (at the 5% level) in the stepwise regression analysis and together can explain 55% of the variation in the results. Therefore, *Penelope* and *Paris*/*Helena* seem to be main variables in the *D. virilis* hybrid dysgenesis syndrome.

(v) *Factors other than transposable element copy number involved in* D. virilis *hybrid dysgenesis*

To determine whether factors other than transposable element copy number are involved in the *D. virilis* hybrid dysgenesis syndrome, males and females of two different strains were first crossed (in reciprocal combinations separately), and the female progeny of both these crosses mated separately with males from a variety of strains. In this paper, a cross symbolized as $X \times Y$ means that females from strain X were mated with males from strain Y; reciprocal crosses are presented in the general form $(X \times Y) \times Z$ and $(Y \times X) \times Z$. The levels of normal gonadal development in the male progeny of both these crosses is then determined (in general between 100 and 200 males were analysed). If only the information on the chromosomes is relevant then it would be expected that the result of pairs of reciprocal crosses should be similar. However, as shown in Fig. 2, this is only true for the seven crosses to the right of the break in the *X*-axis. For the other seven crosses presented in this figure (to the left of the break), there is a significantly different result when the reciprocal crosses are compared ($P < 0{\cdot}05$). The difference between the reciprocal crosses ranges from as little as a few per cent to as much as 15-fold.

The levels of normal gonadal development in the male progeny of the reciprocal crosses may be compared with those observed in the male progeny of the crosses $X \times Z$ and $Y \times Z$, which are indicated by the black dots, one for each cross, in Fig. 2. These comparisons were done to determine whether it is possible to predict the result of the reciprocal crosses $(X \times Y) \times Z$ and $(Y \times X) \times Z$ from the results of the crosses $X \times Z$ and $Y \times Z$. In the cases in which the results of the reciprocal crosses are similar to each other, there is a strong tendency for the level of normal gonadal development in the $(X \times Y) \times Z$ and $(Y \times X) \times Z$ crosses to be similar to that in either the $X \times Z$ or $Y \times Z$ cross, whichever is larger. However, several of the reciprocal crosses clearly deviate from this tendency. The exceptions to the general tendency do not follow any particular rules. In some cases, the observed values are greater than expected from the general tendency; in other cases, the observed values are smaller than expected. In some cases the gonadal development of the male progeny is strongly affected by the strain of the grandmother, in other cases by the strain of the grandfather, and in some cases neither.

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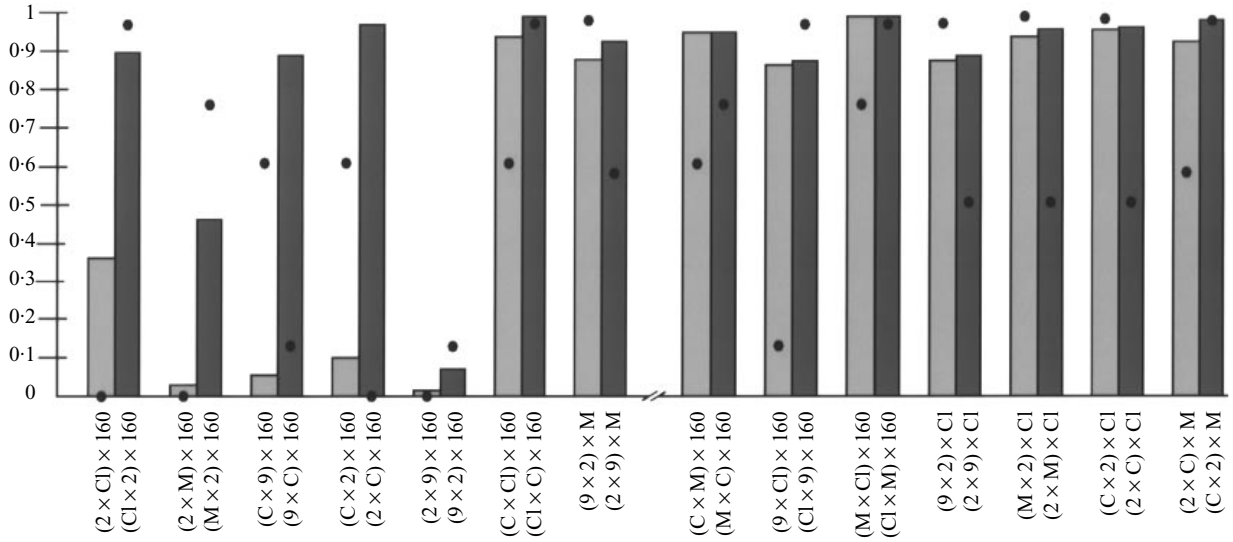

Fig. 2. Proportion of males without atrophic gonads in the progeny of several crosses. The designation $(X \times Y) \times Z$ means that females of strain X were mated with males of strain Y and then the female progeny were mated with males of strain Z. The black dots represent the value obtained when the grandmother of that specific reciprocal cross is mated with the male of that cross; for example, the dot over $(2 \times Cl) \times 160$ is the percentage of males without atrophic gonads in the cross 2×160; and the dot over $(Cl \times 2) \times 160$ is the corresponding value for males in the cross $Cl \times 160$.

4. Discussion

Two possible and not mutually exclusive explanations have been proposed for the unusual simultaneous mobilization of at least five (counting *Telemac*) unrelated transposable elements in the *D. virilis* hybrid dysgenesis syndrome: one hypothesizes the superposition of various different systems of hybrid dysgenesis, each representing a different transposable element, which break down following the hybrid cross; if this is the full explanation, then since at least five unrelated transposable elements are simultaneoulsy mobilized, in principle five superimposed hybrid dysgenesis systems should be present in the particular dysgenic cross studied. This model encounters difficulty with *Ulysses* and *Telemac*, which have nearly the same copy number among the strains studied.

A second hypothesis is that the mobilization of a single element triggers that of others, directly or perhaps indirectly through chromosome breakage or possibly because they share a common pathway in the host (Petrov *et al*., 1995; Lozovskaya *et al*., 1995; Evgen'ev *et al*., 1997). That, in *D. melanogaster*, host genes play an important role in the control of transposition has been shown for the case of transposable elements that are mobilized both by reverse transcription of an RNA intermediate (*gypsy*) or transpose through DNA (*P* element). Transposition of the retrotransposon *gypsy* is controlled by a host gene called *flamenco* (Pelisson *et al*., 1994; Prud'homme *et al*., 1995) while transposition of a *P* element has been shown to be controlled by a host gene coding for a 97 kDa protein (Siebel & Rio, 1990; Siebel *et al*., 1992, 1994).

Recently, Evgen'ev *et al*. (1997) presented qualitative evidence that *Penelope* plays an important role in hybrid dysgenesis in *D. virilis*, although the role of other transposable elements was not investigated. Therefore, it was of interest to extend this analysis to determine whether other transposable elements, or other unidentified factors, may also be involved in the causation. Only by determining which factors are involved in the causation, is it possible to understand why several unrelated transposable elements are simultaneously mobilized. The approach is to perform regression and stepwise regression analysis between the difference in euchromatic transposable element copy number between the parental flies (from different strains; Fig. 1) and the percentage of males with normal gonads in the progeny of the crosses (Table 2), as described in Section 2. As expected, in the linear regression analysis a significant correlation is found with respect to *Penelope* (Table 3). However, a significant correlation is also found with respect to *Paris*/*Helena* (the effects of *Paris* and *Helena* cannot be separated because the presence and copy number of the elements are completely correlated). *Penelope* alone can explain about 51% of the variation in the results, while *Paris*/*Helena* alone can explain 32% of the variation in the results (Table 3). In the stepwise regression analysis, both *Penelope* and *Paris*/*Helena* are significant and together can explain 55% of the variation in the results. Therefore, both analyses support the idea that *Penelope* and *Paris*/*Helena* are main variables in the *D. virilis* hybrid dysgenesis

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syndrome. However, because only in strain 160 can we find euchromatic copies of *Paris* and *Helena*, this result should be interpreted with caution. Also, we do not necessarily imply that *Telemac* and *Ulysses* do not play a role in hybrid dysgenesis in *D. virilis*. The failure to reveal any correlation between these transposable elements and the percentage of males with normal gonads in the progeny of the different crosses may reflect our inability to recognize which transposable elements interfere with a particular developmental pathway – in this case, with the formation of a normal male gonad.

The fact that 45% of the variation in our results cannot be explained does not necessarily imply that other factors are involved in the causation. It is still possible that *Penelope* and *Paris/Helena* are the only variables involved in the causation and that the fit of the regression is not better than observed simply due to our inability to determine what percentage of the total number of euchromatic copies of *Penelope* and *Paris/Helena* in each strain are 'autonomous' copies (that are able to induce hybrid dysgenesis in *D. virilis*). Under this hypothesis, consider the following crosses: 2 × Chile; 2 × Mexico; 9 × Chile and 9 × Mexico. None of the strains involved in these crosses has euchromatic copies of *Paris* and *Helena* (Fig. 1). Therefore males from Chile and Mexico must have at least some 'autonomous' *Penelope* copies because about 40% of the male progeny of the crosses involving males from either of these strains and females from strain 2 have atrophied gonads (Table 2). Furthermore, both strains 2 and 9 have no *Penelope* copies (either euchromatic or heterochromatic) as assayed by PCR, Southern hybridization and *in situ* hybridization (Fig. 1). Therefore, it was to be expected that the male progeny of the crosses involving males from Chile or Mexico strains and females from strain 9 should present at least some gonadal atrophy, which is not the case (Table 2). The contrast in the use of strain 2 or strain 9 females suggests that other factors are involved in the causation. These other factors may be (but are not necessarily) transposable elements. Host cytoplasmic factors may also be involved in the causation. In order to evaluate this hypothesis, another set of crosses was performed. Males and females of two different strains were first crossed (in reciprocal combinations separately) and their female progeny mated (separately) with males from a variety of strains (for the sake of simplicity the pairs of reciprocal crosses are presented in the general form $(X \times Y) \times Z$ and $(Y \times X) \times Z$). The chromosome content of $(X \times Y)$ and $(Y \times X)$ females is the same. Therefore, if host cytoplasmic factors are not involved in hybrid dysgenesis then the results in terms of normal gonadal development in the male progeny of the reciprocal crosses $(X \times Y) \times Z$ and $(Y \times X) \times Z$ should be similar. This is only true for half the crosses presented in Fig. 2 (the ones to the right of the break in the *X*-axis). For the other crosses presented in this figure (to the left of the break), there is a significantly different result when the the reciprocal crosses are compared ($P < 0{\cdot}05$). The difference between the reciprocal crosses ranges from as little as a few per cent to as much as 15-fold.

We were also interested in determining whether it was possible to predict the result (in terms of normal male gonadal development) in the progeny of the reciprocal crosses $(X \times Y) \times Z$ and $(Y \times X) \times Z$, from the percentage of males with normal gonads in the progeny of the crosses $X \times Z$ and $Y \times Z$ (these are indicated by black dots, one for each cross, in Fig. 2). When the results of the reciprocal crosses are similar to each other, there is a tendency for the level of normal gonadal development in the $(X \times Y) \times Z$ and $(Y \times X) \times Z$ crosses to be similar to that in either the $X \times Z$ or $Y \times Z$ cross, whichever is larger. There are clear exceptions to this general tendency. However, they do not follow any particular rules. Some of the observed values are greater and some smaller than expected from the general tendency. The gonadal development of the male progeny can be strongly affected by the strain of the grandmother, by the strain of the grandfather, or neither.

In conclusion, although regression on *Penelope* and on *Paris/Helena* accounts for about half the variation in male gonadal atrophy, and regression on other still unidentified transposable elements may account for additional variation in the results, the analysis of several aspects of the data suggests that maternally transmitted host factors are also involved in the hybrid dysgenesis syndrome in *D. virilis*.

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