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Lack of correlation between dysgenic traits in the *hobo* system of hybrid dysgenesis in *Drosophila melanogaster*

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Summary

Currently in the *hobo* system of hybrid dysgenesis, strain classification is based on the presence/absence of the 2·6 kb *Xho* I restriction fragment. Using this criterion, strains are classified as: (1) H strains when full-size elements are detected by presence of a 2·6 kb *Xho* I restriction fragment; they can also contain internally deleted elements; (2) DH strains when only deleted elements are detected (*Xho* I restriction fragment less than 2·6 kb); (3) E strains, devoid of any restriction fragment equal to or less than 2·6 kb in length. In addition, the strains can be classified on their ability to generate gonadal atrophy (GD sterility) when males of a studied strain are crossed with females from an E strain (dysgenic cross). Here we try to define the nature of the dysgenic cross, which leads us to analyse the different components of the dysgenic syndrome and to look for eventual correlations between them. Molecular analysis, GD sterility tests, *hobo* mobilization with the haw strain and the vg^{al} strain, and hereditary transmission of the instability at the *vg* locus have been assayed in different strains. We show that the occurrence of GD sterility depends on the tested H strains as expected, but also on the E strains used. On the other hand we do not find any correlation between the different dysgenic parameters. Our data reveal that molecular and GD sterility tests are not sufficient to classify strains in the *hobo* system, and that all the components of the dysgenic syndrome must be taken into account. Our results are discussed with regard to active and full-size elements in relation to the structure of the S region where an amino acid sequence (TPE) presents a repetition polymorphism.

1. Introduction

Currently, most studies of the *hobo* dysgenic system in *Drosophila melanogaster* make use of the PM hybrid dysgenic system as a reference (for reviews see Louis & Yannopoulos, 1988; for reviews see Blackman & Gelbart, 1989). Strain classification in the *hobo* dysgenic system is usually realized at the molecular level using either the presence/absence of the 2·6 kb *Xho* I *hobo* fragment (Streck *et al.*, 1986; Blackman *et al.*, 1989) or the properties of *hobo* elements to generate gonadal atrophy in the F_1 females of dysgenic crosses (Yannopoulos *et al.*, 1987; Stamatis *et al.*, 1989). Apart from classification purposes, the ability of *hobo* elements to mobilize *hobo* marked elements as such is studied (Blackman *et al.*, 1987, 1989; Yannopoulos *et al.*, 1987; Lim, 1988; Sheen *et al.*, 1993; Smith *et al.*, 1993; Bazin *et al.*, 1993; Ho *et al.*, 1993; Calvi & Gelbart, 1994).

Several data suggest that the *hobo* system has similarities to but also important differences from the PM dysgenic system. The persistence of *hobo* instability in inbred stocks and the fact that high levels of activity are not restricted to the progeny of crosses between E females and H males both argue in favour of a different model of hybrid dysgenesis (Blackman *et al.*, 1987; Lim, 1988; Bazin *et al.*, 1991; Shenn *et al.*, 1993; Ho *et al.*, 1993).

In this study, we focus on the dysgenic syndrome using the classical tests: molecular analysis, GD sterility and *hobo* mobilization (with two reporter genes), using different E and H strains. Our purpose was (1) to determine and quantify the dysgenic syndrome in the *hobo* system, and (2) to find a robust method of classifying the strains in this system.

We show that E strains differ in their ability to reveal GD activity. In addition, no correlation has

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been found between GD sterility and rates of *hobo* mobilization, neither between these parameters and the presence of the potential full-size *hobo* element (2·6 kb *Xho* I fragment). So the *hobo* classification of strains can not simply be inferred from the presence of the 2·6 kb *Xho* I restriction fragment and of GD sterility. Our results are discussed with regard to the nature of full-size and active elements and their interactions.

2. Experimental procedure

(i) *Strains*

The *Drosophila melanogaster* strains used were reared and crossed at 23 °C on standard axenic medium. The mutant strains (Bowling Green Drosophila Center, Ohio, USA) *cinnabar* (*cn*) (II.-57.5), *brown* (*bw*) (II.-104.5), *vestigial* (*vg*) (II.67.0) and *yellow white forked* attached X strains are described in Lindsley & Grell (1968). The strains used were:

AL (Bazin *et al.*, 1991): This strain comes from a French population collected in 1982. The **AL-vg^{al}** mutant strain was isolated from the AL strain. The *vg^{al}* scalloped-wing phenotype corresponds to the *vg^{np}* phenotype (see Lindsley & Grell, 1968). The *vg^{al}* mutation results from the insertion of a deleted *hobo* element into a *vg* intron (Bazin *et al.*, 1993). The *vg^{al}* chromosome was recombined and isogenized with that of a *cn bw* E strain to obtain a multimarked *cn vg^{al} bw* strain named **vg^{al}***.

23.5 strain: This strain, 23.5*/Cy MRF, comes from Yannopoulos' laboratory. This is a classical H strain established from 23.5Δ/CyL4 MRF through the replacement of all its chromosomes, except the 23.5 MRF second chromosome, by those of the Cy/Sp E laboratory strain (Yannopoulos *et al.*, 1987).

CyHBL1 and **haw** strains: these transformed strains were kindly provided by Calvi and Gelbart (Calvi *et al.*, 1991; Calvi & Gelbart, 1994). CyHBL1 contains a stable *hobo* transposase source, P(*ry*$^+$, HBLI), located on the balancer Cy° second chromosome. HBL1 is a truncated HFL1 element. *y w* H(*w*$^+$, haw1), denoted **haw**, is a *white* E strain which carries two *h*(*w*$^+$) composite elements on the X chromosome. *h*(*w*$^+$) was constructed by replacing the 0·8 central *Eco*R I fragment of *hobo* with a 4·2 kb fragment of the mini-*white* gene. The haw eye-colour phenotype is orange but when one element is excised the colour more or less lightens (Calvi & Gelbart, 1994).

HK is a classical E strain without any *hobo* element, and **OR^s** is a classical H strain.

(ii) *Molecular analysis*

Southern blot. Genomic DNA (5 μg/line) was prepared for Southern hybridization according to Ish-Horowicz *et al.* (1979). Gels were blotted onto 'Genescreen plus' membranes using the capillary blot protocol recommended by the manufacturer (Dupont) and probed with a ^{32}P-labelled HFL1 element (Blackman *et al.*, 1989).

Concerning molecular *hobo* classification, genomic DNA digested with *Xho* I and hybridized with the *hobo* element reveals three categories of strains (McGinnis *et al.*, 1983; Streck *et al.*, 1986; Yannopoulos *et al.*, 1987; Blackman *et al.*, 1987):

H (*hobo*) strains, when full size elements are detected by the presence of a 2·6 kb *Xho* I restriction fragment. They can also contain internally deleted elements.
E (empty) strains when no *hobo* hybridization corresponding to an *Xho* I fragment of 2·6 kb or less is found.
DH strain when only deleted elements are present (*Xho* I fragment less than 2·6 kb) (Ho *et al.*, 1993; Calvi & Gelbart, 1994).

PCR amplification and sequencing. PCR amplification was performed using two primers, *h11* and *h6*, whose sequences correspond to bases 1756–1774 (5′-ATCC-ACCCGCAGCACATC-3′) and 2168–2152 (5′-GCT-GCTGCGCTACTGGCTGG-3′) in HFL1, respectively. Less than 10 ng of DNA were used in 50 μl of total reaction volume containing 0·5 units of *Taq* polymerase (Promega). Amplification was performed on the Trio-Thermoblock of Biometra for 30 cycles. Cycling conditions were 94 °C for 45 s, 60 °C for 2 min and 72 °C for 2 min. PCR products were cloned with the pGEM-T vector system (Promega) and sequenced with the T7™ polymerase according to the manufacturer (Pharmacia).

GD sterility assays. The 23·5, AL and AL-*vg^{al}* strains contain P elements and are classified as Q strains in the PM dysgenic system. To determine the *hobo* GD sterility, crosses were performed at 24 °C, because at such a temperature Q strains do not express P GD sterility. Therefore, assuming that there is no interaction between *hobo* and PM system, the GD sterility found can be attributed to *hobo* elements.

The standard cross is between HK females and 23·5 males (Yannopoulos *et al.*, 1987). GD sterility is estimated by the percentage of dystrophic ovaries: (number dystrophic/number dissected × 100).

(iii) Hobo *transposase activity* – hobo *mobilization*

Hobo mobilization occurs at a low rate and shows great heterogeneity between the F_1 germlines. So it is necessary to estimate this rate in individual F_1 germlines. The best estimate is the percentage of F_1 hybrid males with mobilization events in their progenies.

hobo *transposase tests involving the mobilization of the* hvg^{al} *element at the* vestigial *locus*. The *hvg^{al}* insertion

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Table 1. *Hereditary transmission of instability at the* vg *locus. The cross scheme follows an initial cross between CyHBL1 females and* vg^{al}* *males, and was denoted experiment A*

F^0	**A** cross: ♀CyHBL1 × vg^{al}*	
F_1	Crosses for F_2 progeny	
	A1 cross	**A2** cross
	32{1 F_1♀CyHBL1/*cn* vg^{al} *bw* × 2♂ vg^{al}*}	29{1 F_1♂CyHBL1/*cn* vg^{al} *bw* × 2♀ vg^{al}*}
F_2	Analysis of the F_2 progeny for the vg^+ and vg^e occurrences: estimation of %Mvg^+_1 and %Mvg^e_1	
	Crosses for F_3 progeny	
	45{1 F_2♀[Cy] × 2 F_2♂[*white*, vg^{al}]}	25{1 F_2♀[Cy] × 2 F_2♂[*white*, vg^{al}]}
	34{1 F_2♂[Cy] × 2 F_2♀[*white*, vg^{al}]}	24{1 F_2♂[Cy] × 2 F_2♀[*white*, vg^{al}]}
F_3	Analysis of the F_3 progeny for the vg^+ and vg^e occurrences: estimation of %Mvg^+_2 and %Mvg^e_2	
	Crosses for F_4 progeny	
	26{1 F_3♀ or ♂[Cy] × 2 F_3♂ or ♀[*white*, vg^{al}]}	43{1 F_3♀ or ♂[Cy] × 2 F_3♂ or ♀[*white*, vg^{al}]}
F_4	Analysis of the F_4 progeny for the vg^+ occurrence: estimation of %Mvg^+_3	

is stable in the vg^{al}* strain but it can be mobilized *in trans* by active *hobo* elements.

We have shown that vg^{al} reverses to the vg^+ phenotype and that three different and independent events can affect the hvg^{al} element: (1) more or less precise excision of the hvg^{al} element, (2) deletion or (3) insertions of variable sizes in the hvg^{al} element. The different $vg^{(+)}$ alleles generated are dominant on the vg^{al} allele and can be phenotypically detected in $vg^{al}/vg^{(+)}$ heterozygous flies.

In addition to the $vg^{(+)}$ reversions, vg^e mutations can occur. We have shown that they result from deletions, variable in size, of *vg* adjacent sequences and that the *hobo* element is still present. We can screen dominant vg^e alleles in vg^{al}/vg^e flies since their phenotype is *vestigial* (Bazin *et al.*, 1991, 1993).

To perform the mobilization test, the hybrid males from the vg^{al}* × H cross were singly backcrossed to vg^{al}* females and the progeny scored for exceptional phenotypes: *cn* vg^+ *bw* or *cn* vg^e *bw*. To eliminate similar phenotypes due to male recombination, only tests with no recombination between *cn* and *bw* (*cn* bw^+ and cn^+*bw*) were taken into account. All the exceptional progenies were crossed to *cn* vg^{al} *bw* mutant flies to ensure that the vg^+ or vg^e phenotypes were due to genetic events and not to *vg* penetrance or to somatic events.

The germline mobilization frequency (%M) was calculated as the number of germlines that had at least one mobilization event. The %M here can be subdivided into %Mvg^+ and %Mvg^e with respect to the different molecular rearrangements.

Statistical analysis were carried out with the exact Fisher test.

hobo *transposase tests involving the mobilization of* h(w^+) *from the X chromosome.* The haw females were crossed with different H males, then F_1 males were singly crossed to two or three *yellow white forked* attached X females. The progeny were scored for the appearance of exceptional individuals: coloured-eyes *yellow forked* females that result from an autosomal $h(w^+)$ transposition and less-coloured-eyes males that can result from $h(w^+)$ excision. The %M is calculated as above and we distinguish the %Mt (transposition) and the %Me (excision).

(iv) *Hereditary transmission*

The experimental protocol used to study the hereditary transmission of the hvg^{al} instability is described in Table 1. The vg^{al}* strain was crossed as male (experiment A) or female (experiment B) with the CyHBL1 strain. Hybrid Cy males or females were individually crossed with vg^{al}* flies to study the mobilization in the F_1 germline revealed in the F_2 progeny by *white* vg^+ (*cn* vg^+ *bw*) or *white* vg^e (*cn* vg^e *bw*) phenotypes. In preliminary experiments we did not find any difference between experiments A and B. Therefore, our analysis was carried on using only experiment A. At each generation, Cy males or females were crossed at random individually with *cn* vg^{al} *bw* homozygous siblings from the same cross. At the phenotypic level, we could not know which of the vg^{al} alleles is borne by Cy flies, and whether the HBL1 source of transposase was always linked with the Cy mutation; therefore both were checked in their progeny. Experiment A was followed for five generations. Since the F_2 and F_3 analysis did not reveal any statistical differences between males and females for hvg^{al} mobilization, the maternal or paternal CyHBL1 origin in the subsequent generations were not distinguished. vg^e mutations were screened only in the F_2 and F_3 progenies.

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3. Results

(i) *Molecular analysis*

On a molecular basis, the haw strain that contains only two $h(w^+)$ elements can be classified as E strain, and the CyHBL1 strain that has one active element, as H strain.

Fig. 1 shows the restriction patterns of genomic DNAs (OR^s, AL; AL-vg^{al}; vg^{al}*; HK and 23·5 strains) digested by *Xho* I and probed with HFL1 *hobo* element. The only strain devoid of *hobo* element is HK; all the others are H since they contain both full-size (2·6 kb *Xho* I fragment) and deleted elements.

The HFL1 element differs from *hobo* 108 by a 63 bp deletion located in the S region. The S region consists of tandem repeats of a 9 bp sequence ('actccagaa', TPE motif in the protein); there are ten perfect repeats in *hobo* 108, while there are only three perfect repeats in HFL1 (Blackman *et al.*, 1987; Calvi *et al.*, 1991). It was interested to test whether this region could be implicated in *hobo* activity and/or regulation. The S region was investigated using two primers (h11, h6) that surround it. There was only one PCR product in the CyHBL1 control strain, in 23·5 and in vg^{al}* strains, but there were three PCR products in the AL strain (Fig. 2). The CyHBL1, vg^{al}* and the two shortest AL PCR products were cloned and sequenced. Five repeats were found in vg^{al}*, three and five repeats in AL. In vg^{al}* deleted *hobo* elements were found (Fig. 1). One of them is the hvg^{al} sequenced element whose deletion includes the S sequence, the other being Th elements whose S region is also deleted; then the five repeats correspond to the full-size *hobo* element.

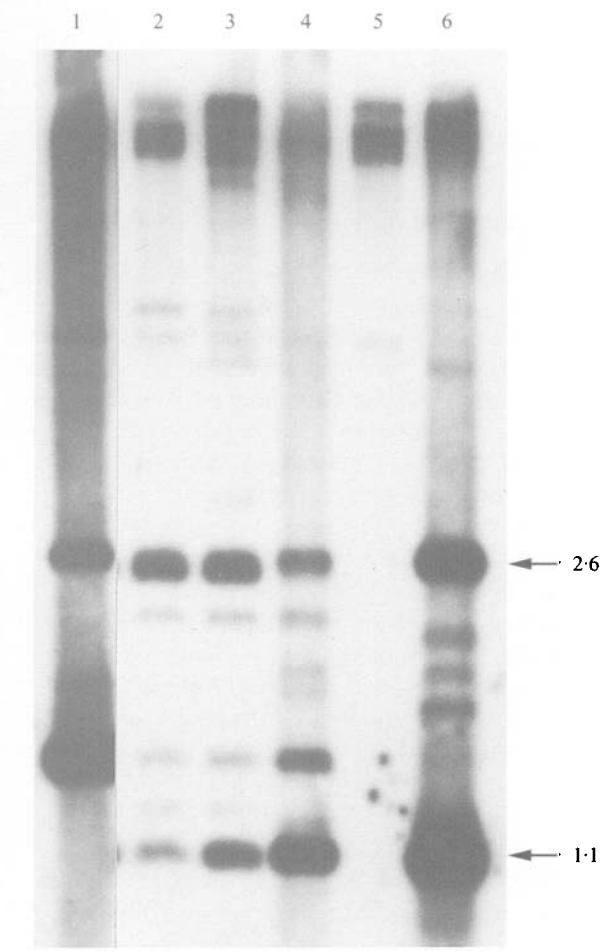

Fig. 1. Restriction pattern of genomic DNAs: lane 1, OrS; lane 2, AL; lane 3, AL-vg^{al}; lane 4, vg^{al}*; lane 5, HK; lane 6, 23·5, digested with *Xho* I and probed with HFL1 *hobo* element. Full-size element generates a 2·6 kb *Xho* I restriction fragment, the Th deleted elements a 1·1 kb fragment.

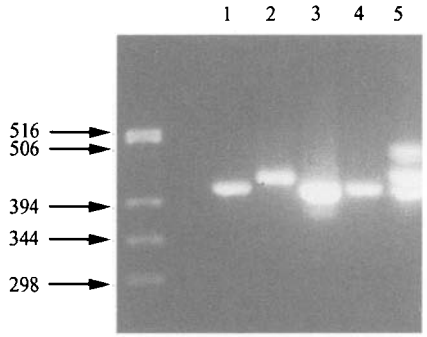

Fig. 2. PCR amplification product using h11 and h6 primers (see text). Lanes 1 and 4, CyHBL1 (412 bp°); lane 2, vg^{al}* (439 bp°); lane 3, 23.5 (412 bp), lane 5, AL (412°, 439° and 475 bp). °Sequenced products.

According to our investigation, at the molecular level the CyHBL1, 23·5 and AL strains contain the same kind of element (three perfect repeats), whereas vg^{al}* is devoid of such an element.

(ii) *Gonadal dysgenic sterility analysis*

Table 2 summarizes the gonadal dysgenic (GD) sterility detected in the F_1 females of various crosses at 24 °C. In the 23·5 and CyHBL1 strains, the females were classified according to their Cy phenotype because in the 23·5 strain the *hobo* active elements are located on the homologous MRF second chromosome whereas in the CyHBL1 strain the HBL1 element is on the Cy balancer chromosome.

The only *hobo* GD sterility detected was in crosses involving males 23·5. Thus, with respect to GD sterility, the only *hobo*+ strain is 23·5 and its activity is clearly correlated with presence of the MRF chromosome.

The 23·5-induced GD sterility varies with the strain tested: a high level is found with HK females (92%), a lower one with haw females (31%). Those strains are E strains and their only difference with regard to the *hobo* system is the presence of two $h(w^+)$ elements on the X haw chromosome. Such results have already been reported by Yannopoulos *et al.* (1994): two independent E strains exhibited different *hobo* GD sterilities when their females were crossed with 23·5 males. Those results suggest that E strains have different abilities to repress *hobo* GD sterility. Since

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Table 2. Hobo *activity and* hobo *repression measured by the percentage of GD sterility using different H strains and two different E strains*

H males	Phenotype of progeny	*hobo* activity E females		*hobo* repression H females		
		HK	haw	23.5	CyHBL1	Al-vg^{al}
AL	+	0·0 (40)	0·0 (68)	n.t.	n.t.	0·0 (20)
23.5	Cy	9·4 (101)	3·5 (56)	0·0 (30)	13·0 (26)	2·0 (95)
	MRF[a]	92·0 (169)	31·0 (30)		22·5 (40)	9·7 (128)
CyHBL1	Cy[a]	0·0 (40)	0·0 (55)	n.t.	0·0 (40)	0·0 (40)
	+	0·0 (40)	0·0 (40)			0·0 (40)
AL-vg^{al}	Cy			0·8 (124)	0·0 (20)	
	+	0·0 (91)	0·0 (40)	5·2 (210)		0·0 (45)

Number of dissected females in brackets, n.t., not tested.
[a] Chromosomes bearing *hobo* elements.

Table 3. *Frequencies of* cn vg^+ bw *occurrences in germlines of hybrid males from crosses involving various H strains and the multi-marked* cn, vg^{al} *and* bw *strain* (vg^{al} * *strain*)

	F_0 crosses				
Females:	vg^{al}*	AL	vg^{al}*	23·5	vg^{al}*
Males:	AL	vg^{al}*	23·5	vg^{al}*	CyHBL1
No. of F_1 males tested	15	42	9	17	143
%Mvg^+	0	28·5	11	0	11
Cluster size		1–8	39		1–6
Pr	0·124	0·004	0·384	0·093	0·085

$Pr = C_n^k p^k (1-p)^{n-k}$ is the probability of observing each result assuming that the %Mvg^+ (p) is the same in all crosses ($p = 29/226 = 0{\cdot}13$).

they are devoid of *hobo* sequences, such a phenomenon could result from interactions of E strain genomic factors with 23·5 factors implicated in *hobo* GD sterility.

In crosses involving AL-vg^{al} or CyHBL1 females and 23·5 males, GD sterility is 10% in the first cross and 22% in the second cross (Table 2). These sterilities imply that one full-size *hobo* element is not sufficient to repress GD sterility totally. In those cases one possibility is that *hobo* interactions can modulate 23·5 GD sterility. When 23·5 females are crossed with AL-vg^{al} males, 5% of GD sterility is observed. If this GD sterility reflects a *hobo* activity, we can conclude that in 23·5 there are not enough factors to repress *hobo* activity.

(iii) Hobo *transposase activity* – hobo *mobilisation*

Action of different H strains on hvg^{al} *mobilisation.* The protocol used to determine the *hobo* activities of different H strains on hvg^{al} focused only on the $vg^{(+)}$ allele occurrences. The vg^{al}* strain was crossed with different H strains and the hybrid males were back-crossed individually with vg^{al}* females. The progeny of each cross was screened for vg^+ *white*-eyed flies. No male recombination between *cn* and *bw* was detected. All vg^+ *white*-eyed flies were tested to confirm a wild-type *vg* allele (in some cases we confirmed hvg^{al} mobilization by molecular analysis; data not shown). In Table 3, except for the cross of vg^{al}* females with CyHBL1 males, only a few males were tested. To compare the %Mvg^+ found in the different crosses, the exact probability of each set of data was calculated using the binomial law, the null hypothesis being the lack of differences between crosses. Under this hypothesis, the probability of obtaining 28·5% (12/42) of vg^+ reversion in the AL female × vg^{al}* male cross is the only one to be significant ($Pr = 0{\cdot}004$). This result indicates that in this cross the %Mvg^+ is greater than in the other crosses where the %Mvg^+ do not differ significantly (mean value 9·2%).

Excision and transposition of two h(w^+) *reporter hobo elements.* The correlation between GD sterility induction and $h(w^+)$ mobilization was investigated in F_1 germlines from crosses between haw females and different H males. For this purpose the F_1 females were dissected to estimate GD sterility (Table 2) and the males (which had received the X haw chromosome from their mother) were singly crossed to *yellow white*

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Table 4. h(w+) *mobilization test: hybrid males from haw females and various H males were singly mated to three* y w f *attached X females, and their progenies analysed for transposition; no excision events were detected*

	F_0			
	Males			
Females haw	AL	23.5	CyHBL1	vg^{al}*
No. of F_1 males tested	95	51	52	189
Transposition				
%Mt	0	2	23	1
Cluster size		1	1–10	1

forked attached X females. The results are shown in Table 4. The %M was calculated as the percentage of hybrid F_1 males which gave at least one transposition event. If we consider that all the transposants result from the same transposition event, the mean cluster size can be estimated as $m = 3{\cdot}5$ for the CyHBL1 cross. This value leads to the postulate that *hobo* transposition could occur premeiotically; such a possibility has already been proposed by Blackman *et al.* (1987), Ho *et al.* 1993 and Bazin *et al.* (1991, 1993).

The CyHBL1 strain is the most active strain (%Mt = 23%) whereas the 23·5 strain, which gives the highest GD activity, does not mobilize $h(w^+)$ so much (Table 4). The transposition rate is statistically the same for the AL, 23·5 and vg^{al}* strains and can be estimated at 0·9%, which is lower than for CyHBL1. No excision was found in our experiments.

Those results reveal the independence of the ability of the strains to induce GD sterility and $h(w^+)$ mobilization, and also the differences between hvg^{al} and $h(w^+)$ mobilizations.

(iv) *Hereditary transmission of the instability at the* vg^{al} *locus*

Previous experiments have shown that a CyHBL1/*cn vg^{al} bw* strain is unstable for several generations while a CyHBL1/Sp haw strain is stable. Lim (1988), Ho *et al.* (1993) and Sheen *et al.* (1993) have described the possibility of selecting unstable and stable lines in the Uc strain. In our case, the question was to test whether an unstable line can become stable and, conversely, whether a stable line can reverse to an unstable line after a few generations. Hvg^{al} mobilization in hybrid males and females from initial crosses between vg^{al}* and CyHBL1 strains was followed during five generations (see Experimental Procedure). The data in Table 5 show that the $\%M_1$ ($+\%Mvg^+{}_1 + \%Mvg^e{}_1$) are statistically the same for A2, B1 and B2 experiments (mean value 9%), but significantly higher in the A1 experiment (31%). It is of interest to note that vg^+ and vg^e events contribute equally to the $\%M_1$.

During the subsequent generation the instability was statistically the same for males and females in A1,

Table 5. *Hereditary transmission of the instability of the* vg^{al} *mutation: evolution of the %M*vg$^+$ *and the %M*vg^e *in heterozygous CyHBL*1/cn vg^{al} bw *during three generations*

Initial cross	A ♀CyHBL1 × ♂ vg^{al}*		B ♀vg^{al}* × ♂CyHBL1	
	F_1 × vg^{al}*: F_2 analysis			
Experiment	A1	A2	B1	B2
	F_1 ♀	F_1 ♂	F_1 ♀	F_1 ♂
No. of flies tested	32	29	10	30
$\%Mvg^+{}_1$	15·6	3·4	0·0	10·0
Mean cluster size	1·2	1·0		2·0
$\%Mvg^e{}_1$	15·6	3·4	0·0	3·3
Mean cluster size	1·0	1·0		1·0
	$F_2 \times F_2$: [Cy] × [*white*, vg^{al}] (sib-mating): F_3 analysis			
	F_2	F_2		F_2
No. of flies tested	79	47		49
$\%Mvg^+{}_2$	32·9	17·0		18·4
Mean cluster size	3·5	3·7		3·4
$\%Mvg^e{}_2$	19·0	14·9		nd
Mean cluster size	1·7	1·8		
	$F_3 \times F_3$: [Cy] × [*white*, vg^{al}] (sib-mating: F_4 analysis			
	F_3	F_3		
No. of flies tested	26	43		
$\%Mvg^+{}_3$	19·2	7·0		
Mean cluster size	5·2	2·3		

nd, no data.

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A2 and B2 experiments. The $\%Mvg^{+}{}_{2}$ are homogeneous between A2 and B2 experiments (mean value 17·7%), but in the A1 experiment this percentage is significantly higher (32·9%, Fisher test $P < 5\%$). The $\%Mvg^{e}{}_{2}$ are similar in A1 and A2 experiments (mean value 17·5%).

In the F3 analysis, hvg^{al} is more unstable than in the F_2 analysis, but in the subsequent F_4 and F_5 analyses the $\%Mvg^{+}$ falls back to the F_2 analysis level (i.e. $\%Mvg^{+}{}_{1}$) (result not shown). However, the total number of events is still important and greater than in the F_1 germline. One hypothesis is that, for an unknown reason, hvg^{al} mobilization happens earlier in the F_2 germline than in the F_1 germline (in the F_3 and F_4 analyses, mean cluster sizes appear higher than in the F_2 analysis). To obtain the $\%Mvg^{+}{}_{2}$, sib-mating crosses have been performed, so the result of the F_3 analysis could be due to both maternal and paternal instabilities.

4. Discussion

In this study we wanted to define the components of the *hobo* dysgenic system in *Drosophila melanogaster*. For this purpose different strains were tested with regard to several characteristics of this system: at the molecular level in order to determine whether they were H (*hobo*) or E (empty) strains and with regard to the components of the classical hybrid dysgenic syndrome, GD sterility and mobilization of two reporter genes ($h(w^{+})$ and hvg^{al}). Our results are summarized in Table 6. They reveal that strains classified as H strains on molecular criteria alone are different with respect to their abilities to mobilize *hobo* reporter genes. In addition, no correlation was detected between the GD sterility induced and the mobilization of *hobo* reporter genes.

Our results reveal that E strains have different capacities to repress the *hobo* GD sterility induced by the 23·5 strain, as previously described by Yannopoulos *et al.* (1987) and Stamatis *et al.* (1989). As suggested by these authors this is probably due to a regulation induced by host factors. Moreover, at least in some cases, strains containing full-size elements are unable to induce *hobo* GD sterility. Our attention is therefore focused on trying to determine which kind and which quantity of full-size elements are required for GD sterility to occur.

In our experimental set-up used to estimate the rate of $h(w^{+})$ transposition, the most active strain was CyHBL1 (%Mt = 23%); all the others had statistically the same %Mt (0·9%). Given that in CyHBL1 there is only one active element whereas several full-size elements are present in other strains, it is important to determine whether all these full-size elements are active. If so, they probably differ greatly in their capacities to mobilize $h(w^{+})$ and it would be interesting to understand which factors are implicated. In AL, $vg^{al}*$ and 23·5, in addition to the full-size element, deleted elements are found which could be *Th1*, *Th2* and *Oh*, described as potential regulatory elements (Periquet *et al.*, 1990, 1994). Since these elements are absent in CyHBL1 but present in all the other strains analysed, it cannot be excluded that they are responsible for our observations. However, their presence in the 23·5 strain indicates that they probably do not regulate *hobo* GD sterility.

The presence of a 2·6 kb *Xho* I restriction fragment by itself does not allow prediction of GD sterility or *hobo* mobilization. Moreover our analysis shows that this molecular characteristic is not a reliable assay for predicting whether a *hobo* element is complete or active. Indeed, the PCR amplification result reveals a polymorphism of 18 bp which is not detectable by Southern analysis. At the protein level, this polymorphism affects a TPE motif, with three repeats in the putative transposase of CyHBL1, five in $vg^{al}*$, and probably three in 23·5. AL presents several transposase molecules with three, five and probably 10 TPE repeats. Although there is no biochemical argument to suggest that this motif could be important for transposase activity, an addition of several prolines could modify the secondary protein structure. In addition, the transposase activity in the $vg^{al}*$ strain

Table 6. *Detection of* hobo *activities in five H strains. The* h(w⁺) *mobilization experiment was performed with the haw strain that bears two* h(w⁺) *reporter genes on the X chromosome*

Strains	Molecular classification *Xho* I fragment		No. of TPE repeats	GD sterility tested with HK strain	*hobo* reporter gene mobilization	
	2·6 kb	1·1 kb			$h(w^{+})$ %Mt	hvg^{al} %Mvg⁺
23·5	+	+	3	92%	0·9	9
AL	+	+	3, 5, 10	0	0·9	28–9
AL-vg^{al}	+	+	nd	0		
$vg^{al}*$	+	+	5		0·9	0
CyHBL1	1 element	–	3	0	23·0	9

nd, no data.

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($h(w^+)$ experiment) is not related to a 3-TPE transposase but to a 5-TPE transposase. Such a result could imply that differences in *hobo* element activity (level and quality) depend on the number of TPE repeats in the S region.

Finally, no vg^+ reversion in the vg^{a1}* strain has been detected for several years. A reason for this stability could be that the full-size element detected in this strain is inactive or alternatively that a strict *hobo* regulation exists. Our results show that a *hobo* element with five TPE repeats (in the vg^{a1}* strain) is active on the $h(w^+)$ reporter gene; thus the vg^{a1}* stability could be due to strain-specific behaviour of this element as for its activity or its regulatory potential. Such strict regulation could result from genomic factors and/or *hobo* elements themselves. It is known that host factors can influence the appearance of *hobo* activity within strain (Ho *et al.*, 1993; Sheen *et al.*, 1993); in our experiments they are not controlled. In the hereditary transmission experiment, the most unstable germlines are the F_3 ones. Our hypothesis does not exclude the possibility that the vg^{a1}* regulatory system is disturbed in hybrids; thus the hvg^{a1} mobilization observed could result from the action not only of the HBL1 element but also of vg^{a1}* full-size element(s).

Hobo belongs to the *hobo*, *Ac*, *Tam* family (hAT) (Calvi *et al.*, 1991); it shares sequence homologies with *Ac*, *Tam*, *Tagl*, *Bg*, *Ac-pg* and *hermes* (Warren *et al.*, 1994) but the S region is not included in the highly conserved C-terminal sequences of these elements. For this reason, we believe that the S polymorphism observed here could be specific to the *hobo* element in relation to its behaviour. Moreover it would be interesting to test whether such a specificity exists outside *D. melanogaster*.

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