Is hobo permissivity related to I reactivity and sensitive to chromatin compaction in Drosophila melanogaster?

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Summary

In Drosophila melanogaster, the hobo transposable element is responsible for a hybrid dysgenesis syndrome. It appears in the germline of progenies from crosses between females devoid of hobo elements (E) and males bearing active hobo elements (H). In the HE system, permissivity is the ability of females to permit hobo activity in their progeny when they have been crossed with H males. Permissivity displays both intra- and inter-strain variability and decreases with the age of the females. Such characteristics are reminiscent of those for the reactivity in the IR system. The reactivity is the ability of R females (devoid of I factors) to permit activity of the I LINE retrotransposon in the F1 females resulting from crosses with I males (bearing I factors). Here we investigated permissivity properties in the HE system related to reactivity in the IR system. Previously it had been shown that reactivity increases with the number of Su(var)3-9 genes, which increases chromatin compaction near heterochromatin. Using the same lines, we show that permissivity increases with the number of Su(var)3-9 genes. To investigate the impact of chromatin compaction on permissivity we have tested the polymorphism of position-effect variegation (PEV) on the white+ mottled locus in RE strains. Our results suggest a model of regulation in which permissivity could depend on the chromatin state and on the hobo vestigial sequences.

1. Introduction

In Drosophila melanogaster, the hobo transposable element (like the P transposon and the I LINE element) is responsible for a hybrid dysgenesis syndrome (Blackman et al., 1987; Yannopoulos et al., 1987). This syndrome appears in the germline of progenies from crosses between (E) females devoid of euchromatic hobo sequences and (H) males bearing active hobo elements. In the hobo system (HE system), the syndrome includes thermosensitive sterility (greatest at 25 °C) involving gonadal atrophy (GD, gonadal dysgenesis), chromosomal breaks and rearrangements, mutations and male recombination.

The HE dysgenic system can be tested in different and complementary ways. Strains can be classified according to the following: (1) The presence/absence of full-size hobo elements leading to H/E strains respectively (Streck et al., 1986). Moreover, some strains can bear only deleted hobo elements; they are classified as DH strains. (2) The gonadal atrophy generated in the F1 females of dysgenic crosses (Yannopoulos et al., 1987; Stamatis et al., 1989). (3) The capacity to mobilize hobo reporter elements (Blackman et al., 1989; Ho et al., 1993; Smith et al., 1993; Bazin & Higuet, 1996). Points 2 and 3 refer to hobo activity, but a fourth point is sometimes investigated and this is the potential for repressing hobo activity. This repression could result either from self-regulation by hobo or from host factors that interfere with hobo activity (Pascual & Pérıquet, 1991; Ho et al., 1993; Yannopoulos et al., 1994). When different strains are tested for the first three properties, no correlation can be found between GD sterility, the rates of hobo[white+] and hobo[vg+] reporter gene mobilization and the number of full-size and deleted

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hobo elements (Bazin & Higuet, 1996). The inability to detect a significant correlation between the parameters of hobo activity results from the low rate of hobo reporter gene mobilization. Moreover hobo reporter gene mobilization occurs not only in the classical dysgenic cross (E females × H males), but also in crosses between H females × H males and H females × E males (Blackman et al., 1987; Lim, 1988; Bazin & Higuet, 1996). Thus to detect and quantify hobo activity in different strains the best parameter is GD sterility. We define permissivity as the ability of females to permit hobo activity in their progeny when they have been crossed with males harbouring active hobo elements and it is measured by the percentage of GD sterility. Permissivity presents a maternal effect as reactivity and susceptibility in the IR and PM systems, respectively. We had previously shown that permissivity displays both intra- and inter-strain variability and also that it decreases with the age of the females but is independent of the age of the males (Bazin et al., 1999).

Interestingly, such characteristics are reminiscent of those described for the reactivity level in the IR system. In this system there are two kinds of strain: R strains devoid of the factor (active I LINE element) and I strains bearing I factors. The hybrid dysgenesis syndrome is expressed in particular as thermosensitive sterility (at 22 °C) of the F1 females (SF) from crosses involving R females and I males (Bucheton et al., 1984). This sterility is due to embryonic mortality (Picard et al., 1977; Lavigne, 1986), which decreases as the SF and R females age (for a review see Brégliano et al., 1980). Reactivity is the ability of R females to permit activity of I factor, measured by the embryo mortality in the progeny of the F1 females (SF) resulting from crosses between R females and I males. Reactivity is defined as a maternally inherited but chromosomally determined cellular state that has been shown to undergo heritable, cumulative and reversible changes in response to aging and some environmental conditions (Bucheton & Brégliano, 1982). Moreover, ancestral sequences for I and hobo are present in all Drosophila melanogaster strains, and could be vestiges of ancient invasions (Bucheton et al., 1986; Stacey et al., 1986; Daniels et al., 1990).

One of our goals was to find out whether permissivity has genetic characteristics described in the IR system such as high inter-strain variability, and sensitivity to chromatin compaction. Indeed, Bucheton et al. (2001) had shown that reactivity increases with the number of Su(var)3-9 genes, which increases chromatin compaction near heterochromatin. Here we investigated hereditary transmission of reactivity and permissivity and their inter-strain variability. Using the Su(var)3-9 lines, we found an increase in permissivity in the presence of an additional copy of Su(var)3-9 (introduced as transgene). As the Su(var)3-9 gene manifests a triplo-enhancer effect on position-effect variegation (Tschiertsch et al., 1994), this suggested that chromatin compaction may affect the level of permissivity. To analyse this effect in more detail we have investigated the polymorphism of position-effect variegation on the white

\[ w^{mutloaded} \]

locus in 13 RE strains. We propose a model of regulation in which permissivity could be the result of the hobo vestigial sequences, which could have regulatory effects according to the chromatin state at their locus.

2. Materials and methods

(i) Strains

The Drosophila melanogaster strains analysed (Table 1) were kept at 23 °C under standard laboratory conditions by mass culture on a cornmeal-sugar-yeast-agar medium. All strains were maintained by breeding only from young flies. Their status with regard to the IR, PM and HE systems are R, M and E respectively. In Table 1 they are grouped according to their relatedness.

The reference H strain, MRF235/Cy235, is an IQ strain kindly provided by Dr G. Yannopoulos (Yannopoulos et al., 1983, 1987). The MRF235 chromosome is a lethal wild second chromosome carrying the 23-5 MRF elements described as inducing GD sterility. The Cy chromosome is a balancer second chromosome bearing the Curly (Curly wing, II.61.1) dominant mutation. This H reference strain is used as a controlled source of the transposase that induces hobo GD sterility.

(ii) GD and SF sterility assays

The hobo permissivity and I reactivity of the females of different RE strains were measured at 23 °C. The standard cross was 5–10 RE females × 5–10 MRF235/Cy235 males. The females laid their eggs over a period of 3 or 4 days. GD sterility was estimated in the [Cy+] F1 progeny bearing the MRF235 chromosome from the percentage of dystrophic ovaries, and SF sterility was measured from the percentage of embryo mortality amongst the offspring of 5-day-old [Cy] F1 females.

(iii) Hereditary transmission

To identify hereditary transmission of permissivity and reactivity, F1 female progeny from both reciprocal crosses between two RE strains were analysed. These F1 females were crossed with MRF235/Cy235 males; their levels of permissivity and reactivity were estimated from the percentage GD of the F2 [Cy+] females and the percentage of embryo mortality...
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(\%SF) from [Cy] F2 females respectively. Thirteen reciprocal crosses were performed for the F1 hereditary transmission tests. Two replicates were performed for each cross.

(iv) Su(var)3-9 test
A. Bucheton and M. Balakireva have introduced an additional copy of the Su(var)3-9 gene by transgenesis into the car24 RE strain genome (unpublished data). Three independent transgenic lines (5v, 31v and 61v) and a car24 line, kindly provided by A. Bucheton, were used to detect a putative effect of this gene on permisivity.

(v) \(w^{\text{m4}}\) variegation test
To investigate the polymorphism of chromatin compaction on the \(w^{\text{m4}}\) locus (for a review see Weiler & Wakimoto, 1995; and Wallrath, 1998), the position-effect variegation on this locus has been measured in F1 male progeny obtained by crosses between homozygous \(w^{\text{m4}}/w^{\text{m4}}\) females and males of 13 RE strains. Two \(w^{\text{m4}}\) strains were used: the red-eyed \(Su(\text{var})2-5\text{98}/InCy \text{ white}^{\text{m4}}\) strain, a haplo-suppressor of \(Su(\text{var})2-5\) (Eissenberg et al., 1992), is used to estimate the enhancer effect polymorphism, and the white-eyed \(T21A/CyO \text{ white}^{\text{m4}}\) strain, a triplo-enhancer of \(Su(\text{var})3-7\) (Reuter et al., 1990; Cléard et al., 1997), is used to estimate the suppressor effect polymorphism. The F1 male progeny are screened for the maintenance or not of the \(w^{\text{m4}}\) parental phenotype.

3. Results
(i) Inter-strain variability
The IR and HE status of 43 laboratory strains was determined (Table 1). The distributions showed a high level of variability for both permisivity and reactivity. In the case of permisivity, we observed totally permissive strains (more than 80 \% GD), non-permissive strains (less than 10 \% GD) and intermediate levels between these extremes. A similar phenomenon was detected for reactivity; the absence of non-reactive strains is due to the basal level of embryo mortality present in all strains (data not shown).

Estimations of permisivity (\% GD) and reactivity (\% SF) of these 43 strains revealed high inter-strain variability. However, some of this variability could reflect ancient intra-strain variability due to relatedness between laboratory strains. In Table 1, the strains are grouped according to their relatedness. In some cases, the related strains display high variability for both permisivity and reactivity. An example is the HJ30 and HJ325 strains derived from hikon by isofemale lines (J. C. Bregliano, personal communication): the hikon strain has 57 \% GD and 30 \% SF, whereas HJ30 and HJ325 have 92 \% GD, 30 \% SF and 98 \% GD, 86 \% SF respectively. Similar variability was seen in strains 137, 148 and 178 that are I-CAT transgenic lines.

In other cases, variability was found only for either the permisivity or reactivity level. For the gruta-hs225 strains, strain 481, strain 53-1 and the two yctf strains, only the permisivity displays differences (Table 1). Conversely, in the related e-ew-est strains only the reactivity is affected. Such intra-strain variability has been described by Bucheton et al. (1976) and Bazin et al. (1999) for reactivity and permisivity respectively.

(ii) Hereditary transmission of permisivity and reactivity
The hereditary transmission of permisivity and reactivity in different strains was tested in the F1 female progeny of the 13 reciprocal crosses between two strains. The results are presented in Table 2. For some parental strains, permisivity and reactivity were re-estimated and sometimes differed slightly from the values in Table 1. To reveal any difference between the permisivity (\% GD) and reactivity (\% SF) of the reciprocal F1 progenies, we constructed two parameters: \(\Delta\text{Parents}\), which is the difference between the mean \% GD or \% SF of the two parental strains (strain A – strain B), and \(\Delta\text{F1}\), which is the difference between the mean \% GD or \% SF of the F1 progeny of the two reciprocal crosses (female A \(\times\) male B – female B \(\times\) male A). To detect strong maternal inheritance of permisivity and reactivity, we required a difference of 20 \% between the means of the two F1. In one case, HJ30/A4, the level of both F1 permisivity and reactivity depended on the sense of the parental cross, whereas this is true for only permisivity in the three F1 progenies s6/A4, Wood/A4 and 72/yctf and for only reactivity in the two F1 progenies s6/gruta and cn/A4. In all cases, the effect of the sense of the cross seems to be independent of the \(\Delta\text{Parents}\) difference. These differences between the reciprocal crosses could be due to maternal inheritance. However, in two crosses (HJ30/A4 and 72/yctf) the permisivities of the parental strains did not differ but those of the F1 did. In both cases, one F1 progeny had a level of permisivity lower than either of the parental strains. These results cannot, therefore, simply be attributed to maternal inheritance.

For crosses with a \(|\Delta\text{F1}|\) of less than 20 \%, and a \(|\Delta\text{Parents}|\) value of more than 20 \% (Table 2), where a zygotic inheritance can be postulated, two situations are observed. In the first, the permisivity or reactivity of the F1 progenies is intermediate between those of
the two parental phenotypes (gruta/s6, yctf/Wood for permissivity; s6/A4 for reactivity; and carnaval/HJ30, yctf/72, A4/carnaval, A4/gruta for both). In the second, in some crosses, one parental phenotype displays dominance/recessivity (cn/A4, Wood/HJ30 and Wood/A4 for permissivity; yctf/Wood, yctf/gruta for reactivity; and s6/Wood, Wood/HJ30 for both).

(iii) Correlation between permissivity and reactivity

Due to the similarities and differences between permissivity and reactivity, we investigated the correlation between these two properties. Fig. 1 shows reactivity level (% SF) as a function of permissivity level (% GD). Three situations can be defined in terms of their permissivity-reactivity status: strains with high reactivity (>80% SF) and variable levels of permissivity; strains with high permissivity (>80% GD) and variable levels of reactivity; and strains in which permissivity and reactivity are both less than 80%. The first two situations show that the two parameters are independent; moreover no significant correlation was detected ($r = 0.12, 41$ df, after arc sin $\sqrt{\text{transformation}}$). However, it was noted that strains

Table 1. Level of permissivity (% GD) and reactivity (%SF) of 43 RE strains

| Strain    | Genotype       | % GD | nGD  | % SF | nSF  |
|-----------|----------------|------|------|------|------|
| 48-1      | e P(neo +)     | 99.05| 421  | 97.88| 472  |
| 53-1      | P(neo +)       | 54.87| 195  | 99.45| 723  |
| 72        | B Y+/y Binscy  | 26.27| 276  | 69.82| 328  |
| 99B       | ry +, P ry + A2,3 99B | 80.18| 217  | 35.00| 200  |
| 137       | wt (I-CAT)     | 42.37| 321  | 97.18| 638  |
| 148       | wt (I-CAT)     | 42.70| 274  | 91.76| 437  |
| 178       | wt (I-CAT)     | 86.67| 165  | 22.98| 496  |
| 412       | wt             | 60.33| 300  | 99.76| 409  |
| 3032      | y mwh          | 73.33| 285  | 30.65| 496  |
| 30800     | w + Y/y w a   | 72.28| 285  | 29.12| 340  |
| 36300     | a px or       | 85.23| 44   | 35.73| 557  |
| 70900     | ru h th cu sr es ca/TM3 | 0.00| 121  | 32.54| 295  |
| b375      | y ac sc pn w 699| 3.40| 162  | 13.37| 389  |
| bzz       | al dp b pr cn  | 26.47| 153  | 34.94| 953  |
| Ch-n      | wt             | 56.25| 64   | 98.33| 120  |
| cn        | e cn           | 27.59| 145  | 93.24| 340  |
| DCxF-U2   | In(3LR)DcxF/Sb | 63.89| 198  | 42.00| 200  |
| e         | e              | 43.41| 364  | 38.06| 310  |
| ew2       | e              | 27.35| 117  | 24.32| 333  |
| est       | e st           | 53.85| 156  | 92.06| 126  |
| carnaval  | m f car        | 5.28 | 142  | 18.87| 302  |
| gruta     | wt             | 21.03| 290  | 95.73| 328  |
| hs225-0   | gruta P.transgenic line | 49.17| 121 | 96.48| 199  |
| hs225-1   | gruta P.transgenic line | 55.09| 226 | 100.00| 662 |
| hs225-2   | gruta P.transgenic line | 36.52| 204 | 98.77| 570  |
| hikon     | wt             | 56.93| 137  | 29.68| 603  |
| HJ30      | hikon isogenic line | 91.67| 102 | 29.00| 200  |
| HJ325     | hikon isogenic line | 97.96| 98  | 86.26| 313  |
| JA        | y w            | 97.41| 135  | 37.50| 200  |
| jazz      | b pr cn        | 12.31| 65   | 18.45| 542  |
| plm2      | cn             | 99.31| 72   | 54.97| 390  |
| A4        | cn             | 91.80| 158  | 50.70| 150  |
| paris2    | cn             | 94.79| 48   | 45.68| 324  |
| pf2       | cn             | 96.05| 76   | 39.48| 347  |
| s6        | y, w, sn       | 0.97 | 414  | 16.04| 480  |
| sef8      | se             | 50.59| 85   | 94.40| 232  |
| vest-1    | v e st         | 48.18| 165  | 67.72| 316  |
| vest-2    | v e st         | 13.02| 192  | 80.74| 379  |
| WE        | w              | 68.93| 272  | 94.17| 412  |
| Wood      | wt             | 54.59| 185  | 48.00| 200  |
| yctf-1    | y et f         | 36.32| 117  | 10.71| 280  |
| yctf-2    | y et f         | 3.67 | 245  | 18.81| 335  |
| zola      | y w            | 89.27| 396  | 59.02| 327  |

nGD, number of dissected flies; nSF, number of tested eggs; wt, wild-type; genotypes are described in Lindsley & Zimm (1992).
Table 2. Hereditary transmission of permissivity and reactivity: % GD and % SF of the F1 progeny from reciprocal crosses between RE strains

| Strain B | Parents females A | nSF | % SF | % GD | nSF | % SF | % GD |
|----------|-------------------|-----|------|------|-----|------|------|
|          | males B           |     |      |      |     |      |      |
|          | females B         |     |      |      |     |      |      |
|          | males A           |     |      |      |     |      |      |
|          | females A         |     |      |      |     |      |      |

Table of values for each strain combination:

- Strain A: % SF, % GD, nSF, nGD
- Strain B: % SF, % GD, nSF, nGD

Fig. 1. Reactivity level (% SF) with regard to permissivity level (% GD) for 43 RE strains.

To investigate the sensitivity of chromatin compaction on the level of permissivity, the original car24 strain and its three derived transgenic Su(var)3-9 lines were analysed for their permissivity. In all transgenic lines bearing an additional copy of the Su(var)3-9 gene the permissivity was at least 10-fold higher than in the control car24 line. This is true for each transgenic line and for all replicates (Table 3).

(v) Enhancer or suppressor effects polymorphism in 13 RE strains

To test the impact of chromatin compaction on the level of permissivity, the original car24 strain and its three derived transgenic Su(var)3-9 lines were analysed for their permissivity. In all transgenic lines bearing an additional copy of the Su(var)3-9 gene the permissivity was at least 10-fold higher than in the control car24 line. This is true for each transgenic line and for all replicates (Table 3).

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(iv) Su(var)3-9 test

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red-eyed phenotypes. A same quantification in [Cy] brothers which do not have a haplo-suppressor status, reveals a polymorphism of genes implicated in the chromatin compaction at the white<sup>mutted</sup> locus (Table 4). Only [Cy<sup>+</sup>] males from the 48.1 cross give the no red-eyed phenotype (9%). The no red-eyed phenotype in [Cy] males reveals a polymorphism within RE strains (Table 4). A first group is constituted of the 36300, est and 48.1 strains, in which more than 80% of males have the no red-eyed phenotype; this can be due to an enhancer effect. Two other groups were comprised by b375, s6 and 53.1 strains, with a no red-eyed percentage less than 20%; and all other strains, which had an intermediate

| RE strains | [Cy] | [Cy<sup>+</sup>] | [Cy] | [Cy<sup>+</sup>] |
|------------|------|-----------------|------|-----------------|
| b375       | 14-9 | 87              | 0    | 116             |
| s6         | 15-9 | 88              | 0    | 103             |
| Wood       | 49-0 | 102             | 0    | 115             |
| 72         | 32-2 | 90              | 0    | 81              |
| 53-1       | 12-0 | 92              | 0    | 96              |
| est        | 90-8 | 65              | 0    | 73              |
| cm         | 74-6 | 71              | 0    | 77              |
| 36300      | 93-5 | 93              | 0    | 81              |
| JA         | 39-6 | 91              | 0    | 107             |
| pf2        | 70-7 | 41              | 0    | 39              |
| paris2     | 41-2 | 102             | 0    | 93              |
| HJ325      | 48-9 | 88              | 0    | 79              |
| 48-1       | 89-6 | 77              | 9-0  | 89              |

Progeny of w<sup>m4</sup>/w<sup>m4</sup> Su(var)2-5<sup>8S</sup>/InCy F0 female: the enhancer effect is quantified by the percentage of variegated or no red-eyed [Cy<sup>+</sup>] males which are expected to be red-eyed due to their haplo-suppressor Su(var)2-5 status; this effect is also quantified within diplo Su(var)2-5 [Cy] brothers.

Progeny of w<sup>m4</sup>/w<sup>m4</sup> T21A/CyO F0 female: the suppressor effect is quantified by the percent of no white-eyed [Cy<sup>+</sup>] F1 males which are expected to be white-eyed due to their triplo-enhancer of Su(var)3-7 status; this effect is also quantified within diplo Su(var)3-7 [Cy] brothers.

n, number of F1 males analysed.

For permissivity and reactivity three levels are used: low, <20%; intermediate, >20% and <80%; high, >80% (see Table 1).
percentage of no red-eyed. These two groups could result from no or weak enhancer effects or from suppressor effects.

The white-eyed T21A/CyO white\textsuperscript{mottled}\textsubscript{A} strain, a triplo-enhancer of Su(var)3-7 (Reuter \textit{et al.}, 1990; Cléard \textit{et al.}, 1997), is used to estimate the suppressor effect polymorphism. The suppressor effect is detected by ‘no white-eyed’ [Cy\textsuperscript{+}] F1 males which are expected to be white-eyed due to their triplo-enhancer of Su(var)3-7 status. It is quantified by the percentage of males with the no white-eyed phenotype; this phenotype describes flies with either variegated or coloured-eyed phenotypes whatever the intensity of the colour. In the [Cy] brothers which do not have triplo-enhancer status, quantification of the different phenotypes reveals, as above, a polymorphism of genes implicated in chromatin compaction at the white\textsuperscript{mottled}\textsubscript{A} locus (Table 4). This polymorphism can be classified as having a high suppressor effect for b375 strain (% no-white-eyed males > 80%), a no-suppresser or enhancer effect in the Wood, est, cn, 36300, pf2 and 48-1 strains (% no white-eyed males less than 20%), and a weak suppressor effect for the other strains. In the [Cy] males, the 48-1 strain differs from the others because the percentage of no-white-eyed males is less than 80%; this is according to the enhancer effect detected previously.

More generally, there is a negative correlation ($r = -0.88$, $p < 0.001$, after arc sin\textsuperscript{3} transformation) between the percentage of no red-eyed F1 [Cy] males from crosses with Su(var)2-5\textsubscript{GS}/\textit{InCy white}\textsuperscript{mottled}\textsubscript{A} females and the percentage of no white-eyed F1 [Cy] males from crosses with T21A/CyO white\textsuperscript{mottled}\textsubscript{A} females. Moreover, no significant correlation has been detected between each of these and the permissivity or the reactivity levels of the strains.

4. Discussion

We had previously defined permissivity in the HE system as the ability of females to permit hobo activity. The characteristics of permissivity included maternal effect, variability both within and between E strains and a decrease with age of E tested females (Bazin \textit{et al.}, 1999). Similar characteristics have already been described for reactivity in the IR system. In other respects, Bucheton \textit{et al.} (2001) had shown that reactivity increases with the number of Su(var)3-9 genes, which increase chromatin compaction near heterochromatin.

Here we have analysed the permissivity and reactivity of 43 RE strains. We have shown that the amplitude of the variability of permissivity between strains is similar to that observed for reactivity. On the other hand it had been shown that reactivity level in the IR system was maternally transmitted (Bucheton & Brégliano, 1982). Here we have found different hereditary transmissions for permissivity and reactivity, suggesting that they could be regulated by different mechanisms. In addition hereditary transmission of permissivity and reactivity appears similar in eight experiments of the 13 analysed, and independent in five experiments. Moreover, reactivity has been shown to be sensitive to the number of Su(var)3-9 genes, suggesting sensitivity to chromatin compaction; we reveal herein that permissivity is as well.

To investigate this sensitivity, the polymorphism of the chromatin compaction at the w\textsuperscript{mottled}\textsubscript{A} locus in RE strains was estimated. Our results reveal the existence of polymorphism in our strains. Some of them, such as strains 36300, 48-1 and est, show enhancer effects, because in the cross with the Su(var)2-5\textsubscript{GS}/\textit{InCy white}\textsuperscript{mottled}\textsubscript{A} strain the percentage of no red-eyed males is more than 80% in the [Cy] F1 males and in the cross with the T21A/CyO white\textsuperscript{mottled}\textsubscript{A} strain the percentage of no white-eyed males is in the [Cy\textsuperscript{+}] F1 males is less than 20%. Conversely the b375 strain shows a suppressor effect because in the cross with the Su(var)2-5\textsubscript{GS}/\textit{InCy white}\textsuperscript{mottled}\textsubscript{A} strain the percentage of no red-eyed males in the [Cy] F1 males is less than 20% and in the cross with the T21A/CyO white\textsuperscript{mottled}\textsubscript{A} strain the percentage of no white-eyed males in the [Cy\textsuperscript{+}] F1 males is more than 80%. All other strains have intermediate status and are difficult to classify with regard to enhancer or suppressor effects. We have searched for a putative correlation between the on the one hand levels of variegation and permissivity and on the other hand levels of variegation and reactivity. In both cases no significant correlation has been found.

Whereas there are several similarities between permissivity and reactivity, we did not detect any correlation between the two parameters % GD and % SF. This lack of correlation could be due to the fact that these two parameters are not directly correlated but rather each is correlated with a third. This third parameter could be the chromatin compaction as detected by the Su(var)3-9 experiments. However, we did not find a correlation between the level of variegation at the white\textsuperscript{mottled}\textsubscript{A} locus and permissivity or reactivity. This could be due to the fact that white\textsuperscript{mottled}\textsubscript{A} does not reveal the status of the chromatin all along the chromosome but just at the X pericentromeric region. Other reporter sites need to be tested to investigate the chromatin status of the other chromosomes. Alternatively, this could be the consequence of the omission of polymorphic factors which are specific to each system, such as the ancestral sequences described in IR and HE systems. The differences in sensitivity to chromatin compaction could result from different defective ancestral hobo and I sequences locations. Indeed ancestral I elements are pericentric but hobo homologous sequences are not restricted to this region (Galindo \textit{et al.}, 2001).
Our results could suggest a model in which the regulation of permissivity by hobo ancestral sequences depends on the level of chromatin compaction around these sequences. In the light of our data, one way to look for hobo vestigial sequences that could act on regulation would be to compare the location and/or the sequences of hobo defective elements in strains with levels of variegation of less than 20%, and with different levels of permissivity. The search for these sequences could be facilitated by using the sequenced Drosophila genome and by knowing the hybridization sites on the chromosomes of the protein complex implicated in $w^{wmottled}$ locus variegation.

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