Genet. Res., Camb. (2004), **84**, *pp.* 71–79. *With* 1 *figure.*
DOI: 10.1017/S0016672304007074 Printed in the United Kingdom

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

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(*Received 6 November 2003 and in revised form 22 April and 22 July 2004*)

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*^{mottled4} 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 & Périquet, 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*^{ah}] 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 *I* 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 embryo mortality (Picard *et al*., 1977; Lavige, 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 (Tschiersch *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*^{mottled4} (w^{m4}) 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, MRF$^{23\cdot5}$/Cy$^{23\cdot5}$, is an IQ strain kindly provided by Dr G. Yannopoulos (Yannopoulos *et al*., 1983, 1987). The MRF$^{23\cdot5}$ 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 MRF$^{23\cdot5}$/Cy$^{23\cdot5}$ 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 MRF$^{23\cdot5}$ 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 MRF$^{23\cdot5}$/Cy$^{23\cdot5}$ 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 permissivity.

(v) w^{m4} *variegation test*

To investigate the polymorphism of chromatin compaction on the *white*^{mottled4} (w^{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^{m4}/w^{m4} females and males of 13 RE strains. Two *white*^{mottled4} strains were used: the red-eyed *Su(var)2-5*^{05}/*InCy white*^{mottled4} strain, a haplo-suppressor of *Su(var)2-5* (Eissenberg *et al*., 1992), is used to estimate the enhancer effect polymorphism, and the white-eyed *T21A*/*CyO white*^{mottled4} strain, a triplo-enhancer of *Su(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 *white*^{mottled4} 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 permissivity and reactivity. In the case of permissivity, 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 permissivity (% 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 permissivity 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 permissivity or reactivity level. For the gruta-hs225 strains, strain 48·1, strain 53·1 and the two yctf strains, only the permissivity 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 permissivity respectively.

(ii) *Hereditary transmission of permissivity and reactivity*

The hereditary transmission of permissivity 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, permissivity and reactivity were re-estimated and sometimes differed slightly from the values in Table 1. To reveal any difference between the permissivity (% GD) and reactivity (% SF) of the reciprocal F1 progenies, we constructed two parameters: ΔParents, which is the difference between the mean % GD or % SF of the two parental strains (strain A – strain B), and ΔF1, which is the difference between the mean % GD or % SF of the F1 progeny of the two reciprocal crosses (female A × male B – female B × male A). To detect strong maternal inheritance of permissivity 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 permissivity and reactivity depended on the sense of the parental cross, whereas this is true for only permissivity 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 ΔParents difference. These differences between the reciprocal crosses could be due to maternal inheritance. However, in two crosses (HJ30/A4 and 72/yctf) the permissivities of the parental strains did not differ but those of the F1 did. In both cases, one F1 progeny had a level of permissivity lower than either of the parental strains. These results cannot, therefore, simply be attributed to maternal inheritance.

For crosses with a |ΔF1| of less than 20%, and a |ΔParents| value of more than 20% (Table 2), where a zygotic inheritance can be postulated, two situations are observed. In the first, the permissivity or reactivity of the F1 progenies is intermediate between those of

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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$^+$)	34·87	195	99·45	723
72	B^{SV} Yy$^+$/Binscy	26·27	276	69·82	328
99B	ry^{506}, P(ry$^+\Delta$2,3) 99B	80·18	217	35·00	200
137	WT (I-CAT)	42·37	321	97·18	638
148	WT (I-CAT)	42·70	274	91·76	437
178	WT (I-CAT)	86·67	165	22·98	496
412	WT	60·33	300	99·76	409
3032	y mwh	73·33	285	30·65	496
30800	w$^+$ Y/y w^a	72·28	285	29·12	340
36300	a px or	85·23	44	35·73	557
70900	ru h th st cu sr es ca/TM3	0·00	121	32·54	295
b375	y ac sc pn w^{e59}	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	27·78	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
p1m2	cn	99·31	72	54·87	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^a, 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 ct f	36·32	117	10·71	280
yctf-2	y ct 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).

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 ($r=0{\cdot}12$, 41 df, after arc sin $\sqrt{}$transformation). However, it was noted that strains

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Table 2. *Hereditary transmission of permissivity and reactivity: % GD and % SF of the F1 progeny from reciprocal crosses between RE strains*

Strain A					Strain B				ΔParents		F1 progenies from: females A × males B				females B × males A				ΔF1	
	% SF	nSF	% GD	nGD		% SF	nSF	% GD	nGD	SF	GD	% SF	nSF	% GD	nGD	% SF	nSF	% GD	nGD	SF	GD
s6	26·3	133	0·3	304	Wood	48·0	200	54·6	24	−21·7	−54·3	26·8	224	0·9	382	25·2	420	1·2	297	1·6	−0·3
s6	26·3	133	0·3	304	A4	50·7	150	92·0	158	−24·4	−91·7	22·4	237	23·9	201	36·7	308	78·7	167	−14·3	−54·8
s6	26·3	133	0·3	304	gruta	95·7	328	21·0	290	−69·4	−20·7	24·6	778	3·3	573	85·2	419	14·4	456	−60·6	−11·1
carnaval	18·9	302	13·5	196	A4	50·7	150	91·8	158	−31·8	−78·3	45·9	638	45·8	296	39·8	480	53·2	597	6·1	−7·4
carnaval	18·9	302	13·5	196	HJ30	86·3	313	98·6	150	−67·4	−85·1	36·9	884	54·6	544	46·4	403	69·4	309	−9·5	−14·8
cn	93·2	340	27·6	145	A4	50·7	150	91·8	158	42·5	−64·2	96·3	217	94·8	201	40·1	342	96·1	282	56·2	−1·3
72	73·6	276	23·5	935	yctf	9·1	230	26·0	1394	64·5	−2·5	40·1	292	21·9	436	30·5	295	49·8	420	9·6	−27·9
yctf	9·1	230	26·0	1394	Wood	48·0	200	54·6	24	−38·9	−28·6	57·7	208	23·9	67	53·9	180	38·8	210	3·8	−14·9
yctf	9·1	230	26·0	1394	gruta	95·7	328	21·0	290	−86·6	5·0	71·5	130	33·3	51	81·1	302	27·8	273	−9·6	5·5
Wood	48·0	200	54·6	24	HJ30	86·3	313	98·6	150	−38·3	−44·0	39·4	368	95·0	70	51·3	300	98·3	177	−11·9	−3·3
Wood	48·0	200	54·6	24	A4	50·7	150	91·8	158	−2·7	−37·2	47·1	153	46·3	450	53·9	78	66·6	341	−6·8	−20·3
HJ30	86·3	313	98·6	150	A4	50·7	150	91·8	158	35·6	6·8	76·0	309	96·8	202	27·1	170	60·6	414	48·9	36·2
A4	50·7	150	91·8	158	gruta	95·7	328	21·0	290	−45·0	70·8	64·0	300	78·8	226	79·5	190	79·1	222	−15·5	−0·3

nGD, number of dissected flies; nSF, number of tested eggs; nt, not tested; ΔParents, difference between the mean % GD or % SF of the two parental strains (strain A – strain B); ΔF1, difference between the mean % GD or % SF of the F1 progeny of the two reciprocal crosses (female A × male B – female B × male A).

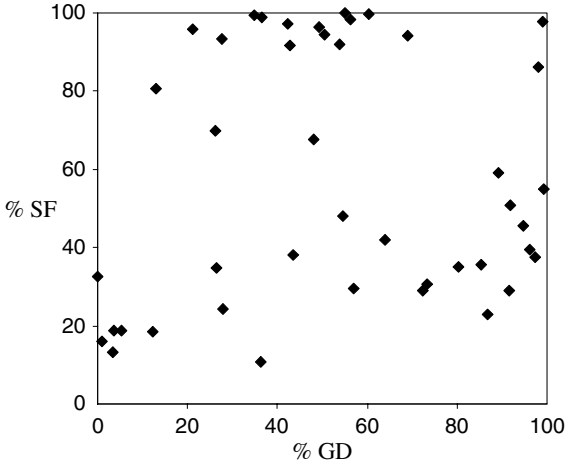

Fig. 1. Reactivity level (% SF) with regard to permissivity level (% GD) for 43 RE strains.

with high reactivity present an intermediate level of permissivity and, similarly, strains with high permissivity mostly have an intermediate level of reactivity, though this might reflect only their level of relatedness (Table 1).

(iv) Su(var)3-9 *test*

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).

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

To investigate the sensitivity of chromatin compaction in different RE contexts we used two *white*^{mottled4} strains: a *Su(var)2-5*^{05} variant which is a haplo-suppressor of position-effect variegation, *su(var)2-5*^{05}/*InCy white*^{mottled4} (Eissenberg *et al.*, 1992), and a *Su(var)3-7* variant which is triplo-enhancer of position-effect variegation, *T21A/CyO white*^{mottled4}. The *Su(var)3-9* variant could not be used because the strain does not possess the *white*^{mottled4} locus.

The red-eyed *Su(var)2-5*^{05}/*InCy white*^{mottled4} strain, a haplo-suppressor of *Su(var)2-5* (Eissenberg *et al.*, 1992), is used to estimate the enhancer effect polymorphism. The enhancer effect is detected by variegated or white-eyed [Cy^{+}] F1 males which are expected to be red-eyed due to their haplo-suppressor *Su(var)2-5* status. This phenotype is called 'no red-eyed', which does not take into account the number and size of spots in the eyes. The enhancer effect is quantified by the percentage of males with no

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Table 3. *Level of permissivity (% GD) of triplo-enhancer* Su(var)3-9 *transgenic lines*

	Control line		Triplo-enhancer *Su(var)3-9* lines					
	car24		5v		31v		61v	
Line	*n*	% GD	*n*	% GD	*n*	% GD	*n*	% GD
Replicates	92	3·8	79	70·3	45	28·9	27	59·3
	110	2·3	94	79·3	42	44·0	64	64·8
	49	7·1	136	42·3	34	44·1		
	29	8·6	26	69·2	80	41·9		
	25	0·0	30	86·7				
	25	6·0						
	44	4·5						
Total	374	4·1	365	63·4	201	39·8	91	63·2

n, number of dissected flies.

Table 4. *Enhancer and suppressor effects polymorphism in 13 RE strains using the* w^{m4} *variegation test*

	w^{m4}/w^{m4} *Su(var)2-5*^{05}*/InCy* F0 female no red-eyed F1 males				w^{m4}/w^{m4} *T21A/CyO* F0 female no white-eyed F1 males					
RE strains males	[Cy]		[Cy$^+$]		[Cy]		[Cy$^+$]		Permissivity GD (%)	Reactivity SF (%)
	%	*n*	%	*n*	%	*n*	%	*n*		
b375	14·9	87	0	116	100·0	93	87·5	96	<20	<20
s6	15·9	88	0	103	100·0	85	58·8	85	<20	<20
Wood	49·0	102	0	115	92·7	96	13·8	123	20–80	20–80
72	32·2	90	0	81	94·9	99	37·2	86	20–80	20–80
53·1	12·0	92	0	96	97·5	120	57·5	113	20–80	>80
est	90·8	65	0	73	84·9	73	0	62	20–80	>80
cn	74·6	71	0	77	94·2	86	15·3	85	20–80	>80
36300	93·5	93	0	81	81·5	92	3·1	98	>80	20–80
JA	39·6	91	0	107	99·0	101	63·6	77	>80	20–80
pf2	70·7	41	0	39	93·6	109	14·7	136	>80	20–80
paris2	41·2	102	0	93	96·2	133	26·5	102	>80	20–80
HJ325	48·9	88	0	79	100·0	90	35·2	71	>80	>80
48·1	89·6	77	9·0	89	76·4	123	15·1	119	>80	>80

Progeny of w^{m4}/w^{m4} *Su(var)2-5*^{05}*/InCy* F0 female: the enhancer effect is quantified by the percentage of variegated or no red-eyed [Cy$^+$] F1 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^{m4}/w^{m4} *T21A/CyO* F0 female: the suppressor effect is quantified by the percent of no white-eyed [Cy$^+$] 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).

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 $white^{mottled4}$ locus (Table 4). Only [Cy$^+$] 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

https://doi.org/10.1017/S0016672304007074 Published online by Cambridge University Press

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*^{mottled4} strain, a triplo-enhancer of *Su(var)3-7* (Reuter *et al*., 1990; Cléard *et al*., 1997), is used to estimate the suppressor effect polymorphism. The suppressor effect is detected by 'no white-eyed' [Cy$^+$] 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*^{mottled4} locus (Table 4). This polymorphism can be classified as having a high suppressor effect for b375 strain (% no-white-eyed males $>80\%$), a no-suppressor 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 ($r=-0{\cdot}88$, $p<0{\cdot}001$, after arc $\sin\sqrt{}$transformation) between the percentage of no red-eyed F1 [Cy] males from crosses with *Su(var)2-5*^{05}*/InCy white*^{mottled4} females and the percentage of no white-eyed F1 [Cy] males from crosses with *T21A/CyO white*^{mottled4} 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 *et al*., 1999). Similar characteristics have already been described for reactivity in the IR system. In other respects, Bucheton *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*^{mottled4} 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*^{05}*/InCy white*^{mottled4} 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*^{mottled4} strain the percentage of no white-eyed males in the [Cy$^+$] F1 males is less than 20%. Conversely the b375 strain shows a suppressor effect because in the cross with the *Su(var)2-5*^{05}*/InCy white*^{mottled4} 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*^{mottled4} strain the percentage of no white-eyed males in the [Cy$^+$] 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 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*^{mottled4} locus and permissivity or reactivity. This could be due to the fact that white^{mottled4} 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 *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 $w^{mottled4}$ locus variegation.

We would like to thank E. Châtelet and C. Gomez for their helpful technical assistance; M. Delattre for providing the *Su(var)2-5*^{05}/*InCy white*^{mottled4} and *T21A*/*CyO white*^{mottled4} strains; A. Bucheton and M. Balakireva for helpful discussions and for providing the *Su(var)3-9* RE transgenic lines; H. Pinon, D. Anxolabéhère, I. Busseau and D. Finnegan for providing different RE strains and lines; E. Bonnivard, I. Boussy and the anonymous referees for their pertinent criticisms of this manuscript; and M. Ghosh for language revision. This work was supported by 'Projet Genome CNRS – 1998: Elements transposables et génomes' and Paris 6 and Paris 7 Universities.

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