CMLS, Cell. Mol. Life Sci. 54 (1998) 1283–1290
1420-682X/98/111283-08 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Research Article

Characterization of a hypermutable strain of *Drosophila simulans*

É. L. S. Loreto[a], A. Zaha[b], C. Nichols[c,*], J. A. Pollock[c] and V. L. S. Valente[d,]**

[a]Departamento de Biologia, Universidade Federal de Santa Maria, Santa Maria, RS (Brazil)
[b]Departamento de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS (Brazil)
[c]Center for Light Microscopic Imaging and Biotechnology and the Department of Biological Sciences, Carnegie Mellon University, Pittsburgh (Pennsylvania 15213, USA)
[d]Departamento de Genética, Caixa Postal 15053, Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre, RS (Brazil), Fax + 55 513192011, e-mail: valente@if1.if.ufrgs.br

Received 6 July 1998; received after revision 31 August 1998; accepted 1 September 1998

Abstract. A hypermutable strain of *Drosophila simulans* that originated from a single spontaneous mutant male was characterized. Seven different mutations were isolated from roughly 100 generations of offspring. The genetic analysis of the viable mutants showed two mutations on the X chromosome, one in the *lozenge* locus and the other in the *ruby* gene. The autosomic mutations characterized were a *dpp-heldout*-like, a *blistered*-like and a homoeotic dominant mutant with an antenna-to-leg transformation and ectopic eyes that we called *Zoinho-na-pata*.

Key words. Hypermutability; *lozenge*; *ruby*; *blistered*; *Drosophila simulans*.

Spontaneous mutations are rare events. The majority of these may be due to the insertion of moderately repetitive DNA or mobile elements [1]. In some particular crosses between certain strains, hypermutability occurs together with chromosomal rearrangements, male recombination, reversion of mutations, chromosome nondisjunction, and partial or complete sterility. These correlated genetic traits have been defined as hybrid dysgenesis [2]. Hybrid dysgenesis has been described in *Drosophila melanogaster* for the *P* element [3, 4]; the *I* element [5] and *hobo* [6–9]. The transposable element *Ulysses* is also responsible for hybrid dysgenesis in *D. virilis* [10].

Some *Drosophila* strains show only hypermutability and not the other traits associated with hybrid dysgenesis. The *gypsy* element, for example, produces germinal mutations in the *D. melanogaster* MS strain [11], and *mariner* transposition results in somatic and germinal mutations in some strains of *D. mauritiana* and *D. simulans* [12].

In *D. simulans*, the content of moderately repetitive DNA is one-seventh of that in the sibling species *D. melanogaster* [13]. Therefore, if mobile DNA elements are the primary agents of mutagenesis, a lower frequency of spontaneous mutations could be expected in this species. However, spontaneous mutations at the white locus and genetic instability associated with transposable elements have also been demonstrated in *D. simulans* [14], indicating that there are active transposable elements in this species.

* Present address: Department of Pharmacology, Vanderbilt University School of Medicine, MRBII, Nashville (Tennessee 37232-6000, USA).
** Corresponding author.

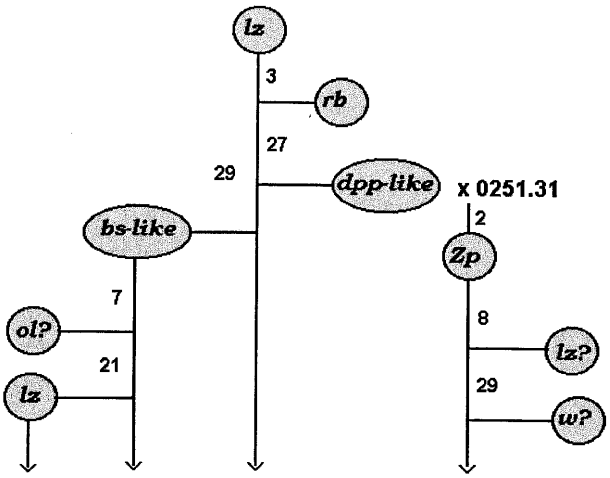

Figure 1. Schematic representation of the appearing order of mutants in *D. simulans* hypermutable strain (Dshs). The mutants were the following: *lz*, *lozenge*; *rb*, *ruby eyes*; *dpp*-like, *decapentaplegic* heldout like; *Zp*, *Zoinho-na-pata*; *w*?, *white*; *bs*, *blistered*-like; *ol*?, *occeli-less*; 0251.31 (marker strain – BG). The arrows represent strains maintained in our laboratory. The numbers represent the total generations since each mutant appeared.

The present study corresponds to the characterization of one hypermutable strain of *D. simulans* in an attempt to contribute to the knowledge of genetic instability in strains of *D. simulans*.

Materials and methods

Origin of the hypermutable strain. The *D. simulans* 'hypermutable' strain (Dshs) originated from a single spontaneous mutant male, encountered in a freshly collected wild sample in Estação Experimental Agronômica de Guaíba-UFRGS, Southern Brazil (30° 50′ S; 51° 39′ W). This mutant has eyes of reduced size, glistening surface and colour alteration (dark red). These phenotypes resemble that of the *lozenge* mutant of *D. melanogaster*. Homozygous females are sterile, so, for maintenance of this mutant, F_2 males are crossed with a wild-type *D. simulans* strain (Eld A) coming from the same location. In the course of the strain maintenance other spontaneous mutants arose (described below).

Genetic analysis of the mutants. For the chromosome localization of the first mutant and genetic mapping of the other sex-linked genes, a *yellow* (*y*) strain (1-0.0) was used as genetic marker. A *D. simulans ruby* (*rb*) strain from the Bloomington Stock Center #2320 rb[1] was used to perform an allelism test with our *rb* mutant.

For the chromosome localization of the autosomic mutant genes, mass-mating crosses were performed between the homozygous mutant line and the BG 14021-1251.42 strain (Bowling Green Stock Center – BG). This strain is homozygous for the markers *nt* (2-0.0), *pm* (2-104.5), *st* (3-40.0) and *e* (3-60). F_1 and F_2 were analysed for mutant classes.

For genetic mapping, a strain was constructed with the markers *e* (3-60), from strain 0251.33 (BG), and *ry* (3-), from strain 2211 (Bloomington Stock Center). Homozygous mutant females were crossed to males homozygous for all markers. F_1 females were backcrossed to males of the marker strain. The offspring were analysed in order to score phenotype classes.

For analysis of the temperature effect in the expression of *blistered*-like mutant phenotype, F_1 and F_2 of crosses between the *bl*-like strain and two different strains (wild-type Eld A and *yellow*) were reared at 22 °C and at 29 °C and scored for the appearance of the blistered phenotype.

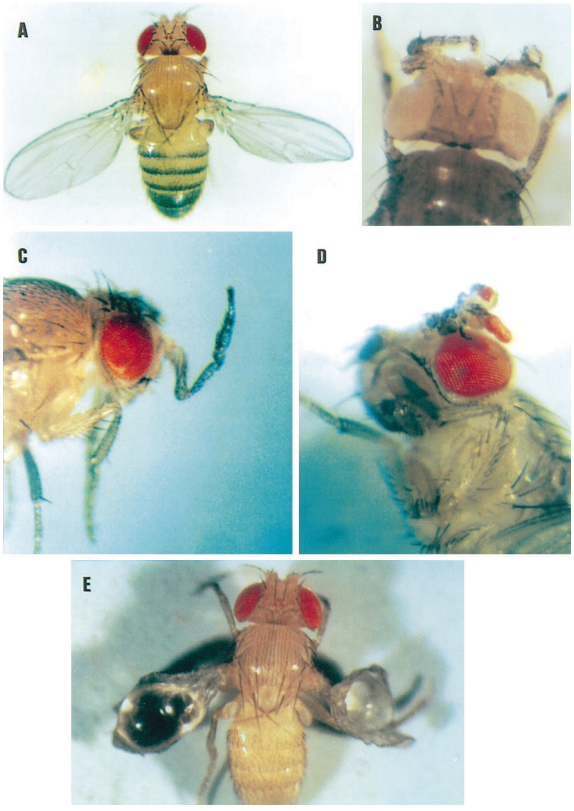

Figure 2. (*A*) *D. simulans dpp*-like strain showing heldout wings phenotype. (*B*) *D. simulans* showing white eyes, resembling the *white* alleles of *D. melanogaster*. (*C*) *D. simulans Zoinho-na-pata* strain showing antenna-to-leg transformation and an ectopic eye in the homoeotic leg. (*D* and *E*) *D. simulans blistered*-like (*bs*-like) strain in that flies show wings with blisters.

Southern blot analysis. Genomic DNA of each strain was prepared from roughly 200 adult flies according to the method described by Jowett [15]. DNA samples (approximately 5 µg from each strain) were digested with restriction endonucleases, following the recommendations of the manufacturers. The DNA fragments were then submitted to electrophoresis on 0.8% agarose gels, and transferred to nylon membranes. Fragments of genomic DNA from around the *P*-element insertion of lz^{1ArB1} were used as a probe for the *lozenge* gene [16, 17]. The plasmids *pUC1813* and *pUC109H* (kindly provided by Dr. R. Blackman, University of Illinois, USA) were used as a probe for the *decapentaplegic* gene.

Results and discussion

The hypermutable strain

The mutations that arose from the *lozenge* mutant, and the number of generations that passed until the appearance of each additional mutation are represented in figure 1. After three generations of main-tenance of the *lozenge* mutant in laboratory, a second X-linked mutation appeared. The double homozygous with *lozenge* present light yellow eyes. This new mutant had brown eyes. Genetic analysis of the mutant indicated that it is an allele of the *ruby* gene (see below). After 27 generations, one fly with 'heldout' wings was identified. The phenotype of this mutant resembles the *decapentaplegic* 'heldout' allele of *D. melanogaster*. Thus, we called this mutant '*dpp*-like' (fig. 2A). In an attempt to map the '*dpp*-like' gene, we crossed this mutant with a strain bearing markers on the second chromosome (BG-0251.31). Another new mutant, homoeotic and dominant, then appeared in the F_2 of this cross. The phenotype expression of this new mutant is antennae-to-leg transformation, similar to *Antennapedia* mutants, and an ectopic eye formation similar to those described for constructs of the *eyeless* gene [18] and *dachshund* [19]. Due to the uniqueness of such a phenotype as a spontaneous mutant we think that it deserves special attention. This mutant was further analysed elsewhere (É. L. S. Loreto et al., unpublished results). We suggest that this mutant may be either an *Antp* allele with a remarkable phenotype or another gene that can activate both *Antp* and/or *eyeless* ectopically. We call this mutant '*Zoinho-na-pata*' (*Zp*), which in the Portuguese vernacular means 'a little eye in the leg' (fig. 2C and D). After 8 generations of maintenance of the *Zp* strain a new male with a *lozenge* phenotype appeared, but it was sterile. In the 29th generation, a male with *white* phenotype arose in this strain, however it, too, was sterile (fig. 2B).

In the propagation of the original *lozenge* strain, the 29th generation produced a wing mutant. In this mutant, the defects range from wings with intervein blisters to completely ballooned wings. The phenotype of this mutant was similar to that of the *blistered* (*bs*) of *D. melanogaster*, so we called this mutant *blistered*-like (*bs*-like, fig. 2E). Seven generations later in this strain, one fly with a phenotype resembling *ocelli-less* occurred. Once again, this fly was sterile. In the 21st generation a new *lozenge* mutant appeared (fig. 1). Allelic complementation tests showed that this mutation targeted the same locus affected in the first mutant observed (*lozenge*).

Some phenotypic alterations appeared in the Dshs strain that suggested the occurrence of somatic mutations. For example, some female flies that did not develop one side of the dorsal thorax can be seen in figure 3B and C. These flies produced 67 wild-type

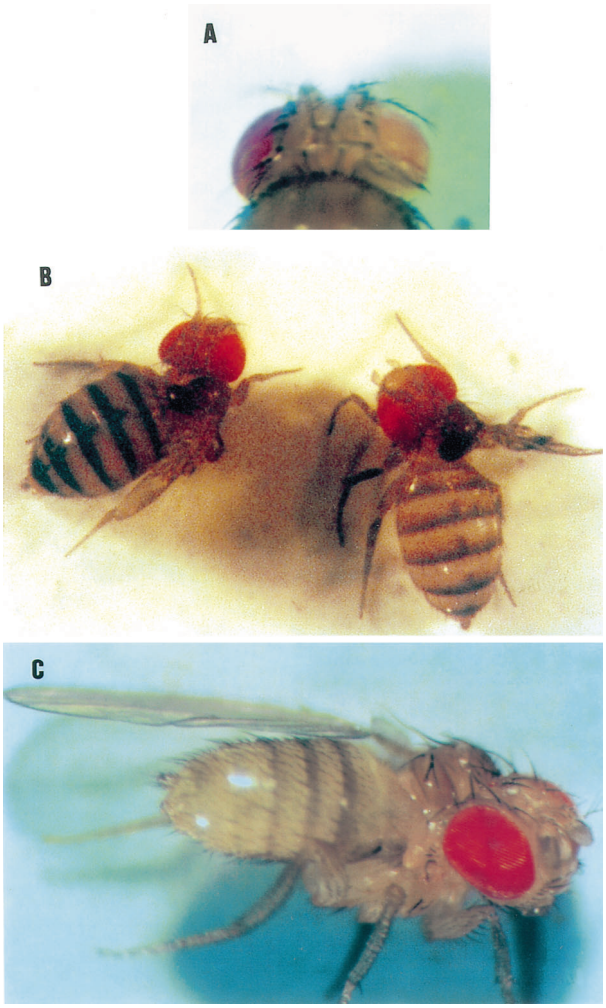

Figure 3. (*A*) *D. simulans* showing one wild-type eye and another with *lozenge*/*ruby* phenotype. (*B* and *C*) Flies showing half of the thorax missing and wings with malformations.

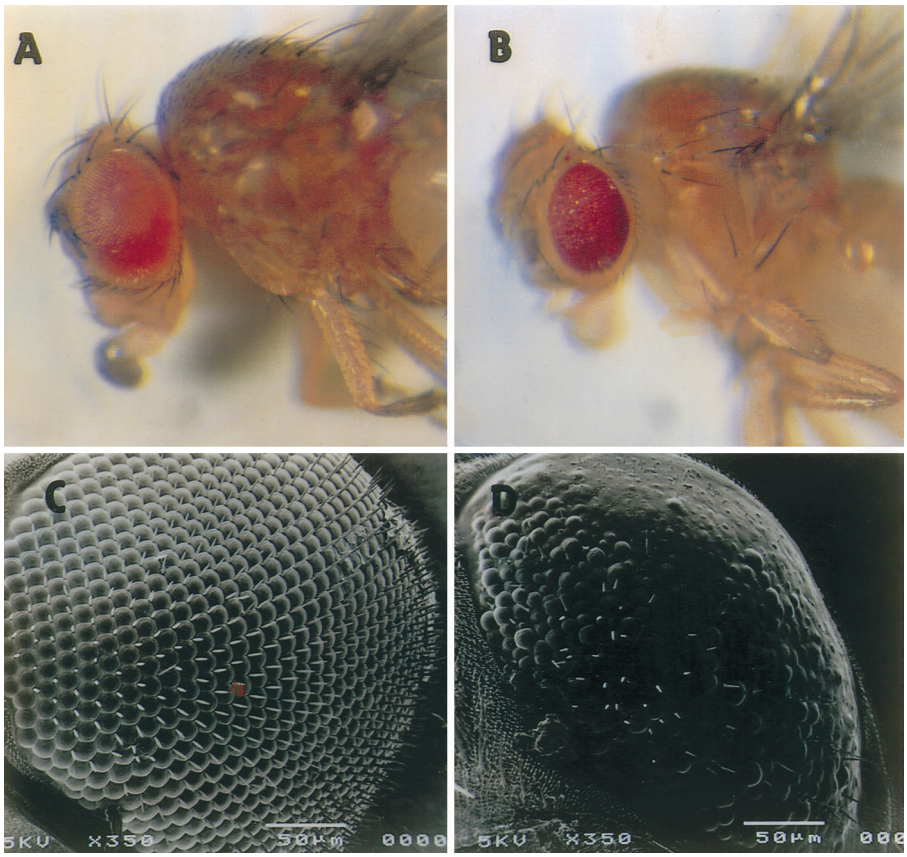

Figure 4. (*A*) *D. simulans* wild-type to eye phenotype. (*B*) *lozenge* mutant showing eyes characterized by reduced size, and a glistening surface appearance and changes in pigmentation. (*C*) Scanning microscopy of a wild-type eye. (*D*) Scanning microscopy of a *lozenge* mutant eye.

offspring in the F_1 and 215 wild-type flies in the F_2, suggesting that this phenotype may be due to a somatic alteration.

One female that had one wild-type eye and one eye with a *lozenge-ruby* phenotype (fig. 3A) was found in the crosses performed in the maintenance of the *lozenge* strain (Eld A females × *lozenge*/*ruby* males). The occurrence of somatic nondisjunction of the X chromosome is a possible explanation for this remarkable phenotype.

Genetic analysis of the mutants

The *lozenge* mutant. This mutant shows a phenotype similar to *D. melanogaster*'s *lozenge* mutants: the eyes are reduced in size, and the surface has a glistening appearance and altered pigmentation (fig. 4). The tarsal claws are reduced. Homozygous females are sterile; spermathecae and parovaria are absent. As can be seen in table 1, this gene is clearly X-linked because *lz* males crossed with wild-type females produced wild-type F_1. The F_2 produced both *lz* and wild-type males, but only wild-type females. In table 2, the results of genetic mapping for the *lozenge* locus shows the map distance from the *yellow* marker to be 24.2 cM. This is slightly different from the distance that is found for *D. melanogaster* (27.7). However, differences in map distances are frequently found between *D. melanogaster* and *D. simulans* [20, 21].

The molecular analysis of the *lozenge* region of this mutant by Southern hybridization experiments indicates that there may be an insertion or rearrangement in the *lz* gene. Figure 5 presents rough restriction maps of *D. simulans lozenge* region and the *D. simulans lozenge* mutant. The membrane shown in figure 6 was hybridized with the *Sal*/*Sal* 7 kb probe. The *Hin*dIII digested DNA of wild-type flies, and *rb* flies showed 5.5-kb and 2-kb hybridizing bands, whereas the *lozenge* mutant showed a 5.2-kb hybridizing band. That result suggested that the defect could represent an insertion in

the adjacent fragment to this genomic region. In the *Sal*I digested DNA, no differences were observed; however, a larger band appeared in the *lz* mutant in the *Eco*RI digested DNA. When the membrane was hybridized with the *Sal*/*Sal* 6-kb probe (fig. 6B), smaller bands appeared in all digestions of the *lz* mutant, compared with the wild-type and *rb* controls, suggesting a rearrangement in the *lz* region for this mutant. No significant differences were observed in hybridizations with the BH5 probe (fig. 6C) and the *Sal*/*Sal* 3-kb probe (data not shown). Together, the data suggest the occurrence of an insertion within the *Sal*/*Sal* 6-kb genomic region. However, further studies will be necessary to identify and completely describe this alteration.

The *ruby* mutant. Crosses between *rb* mutant males and wild-type females (Eld A) showed that this mutation is recessive and sex-linked (table 3). In the F_1 generation, all offspring were wild-type. However, in the F_2, males were *rb* and wild-type, while all of the females were of the wild-type. Genetic mapping for this gene is presented in tables 4 and 5. The map distance as determined by crosses between *yellow* males and the *rb* females in this strain of *D. simulans* was around 7.5 cM. In the opposite crosses (*yellow* females × *rb* males), the map distance was 6.9 cM. Therefore, the average distance between *yellow* and *ruby* is 7.2 cM, practically the same as the position for *ruby* in *D. melanogaster* (7.4 cM). The results of the allelism test to the *rb* mutant described here and the rb[1] allele of *D. simulans* # 2320 strain are presented in table 6. As can be seen, both F_1 and F_2 show *rb* phenotypes, indicating that the mutant described here is an allele of the *ruby* gene.

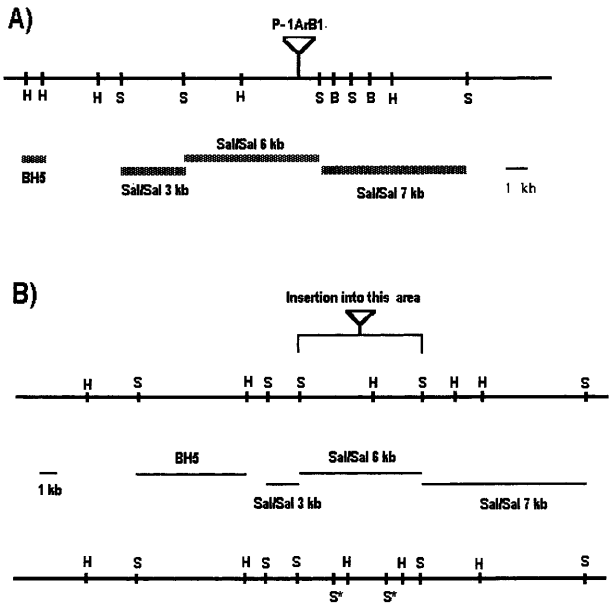

Figure 5. (*A*) The genomic region surrounding the *P*-1ArB insertion element within the *D. melanogaster* lz^{1ArB1} strain. The fragments representing the probes used in the Southern analysis are indicated by the bars below the map [16, 17]. (*B*) The upper figure shows a rough restriction map of the Eld A *D. simulans* region that corresponds to the *D. melanogaster* lz^{1ArB1} genomic region. The lower figure shows a rough restriction map of the *D. simulans* *lz*-like mutant for the same region. The polymorphisms detected by Southern analysis correspond primarily to the *Sal*/*Sal* 6-kb genomic region. The lines in the middle show which fragments hybridize to the *D. melanogaster* probes from the lz^{1ArB1} genomic region. S*, only one of these *Sal*I sites is present, but the exact site it undetermined.

Table 1. Number of *lozenge* and wild-type flies in the F_1 and F_2 of crosses between the *lozenge* mutant males and wild-type (Eld A) females.

	F_1	F_2
Males	467 wild-type	621 wild-type 608 *lz*
Females	438 wild-type	1115 wild-type

Table 2. Genetic mapping of the *D. simulans lozenge* gene. *D. simulans lz* males were crossed with *yellow* females. The F_1 and F_2 offspring were screened for phenotypic classes. The recombinants between *y* and *lz* correspond to the sum of the frequencies of the wild-type and the frequencies of the double mutant *lz*/*y* (24.2%).

	F_1	F_2
Females	798 wild-type	1037 wild-type 1104 yellow
Males	806 *y*	865 (38.2%) *lz* 852 (37.6%) *y* 283 (12.5%) *lz*/*y* 265 (11.7%) wild-type

The *decapentaplegic*-like gene. This mutant is an autosomic recessive one. Homozygous mutant flies show heldout wings, and heterozygous flies are wild-type. Mapping of the mutant locus was attempted by crossing the mutant line to markers from all chromosomes. The frequencies of parental and recombinant phenotypes in the F_2 indicated that the gene is on the left arm of the second chromosome, since we did not find recombinants between *nt* and this mutant (table 7). The *D. melanogaster decapentaplegic* gene is localized on left arm of the second chromosome (2-4.0). The *dpp* locus is a 55-kb genetic unit required for proper pattern formation during the embryonic and imaginal development of the organism. Its expression is essential for the growth and differentiation of the 19 imaginal discs. Some mutations in a specific 3′ regulatory region of this gene, called the *disk-ho* region, produce flies with a heldout wings posture [20].

The *D. simulans dpp*-like mutant described here exhibits heldout wings. However, based on results of two differ-

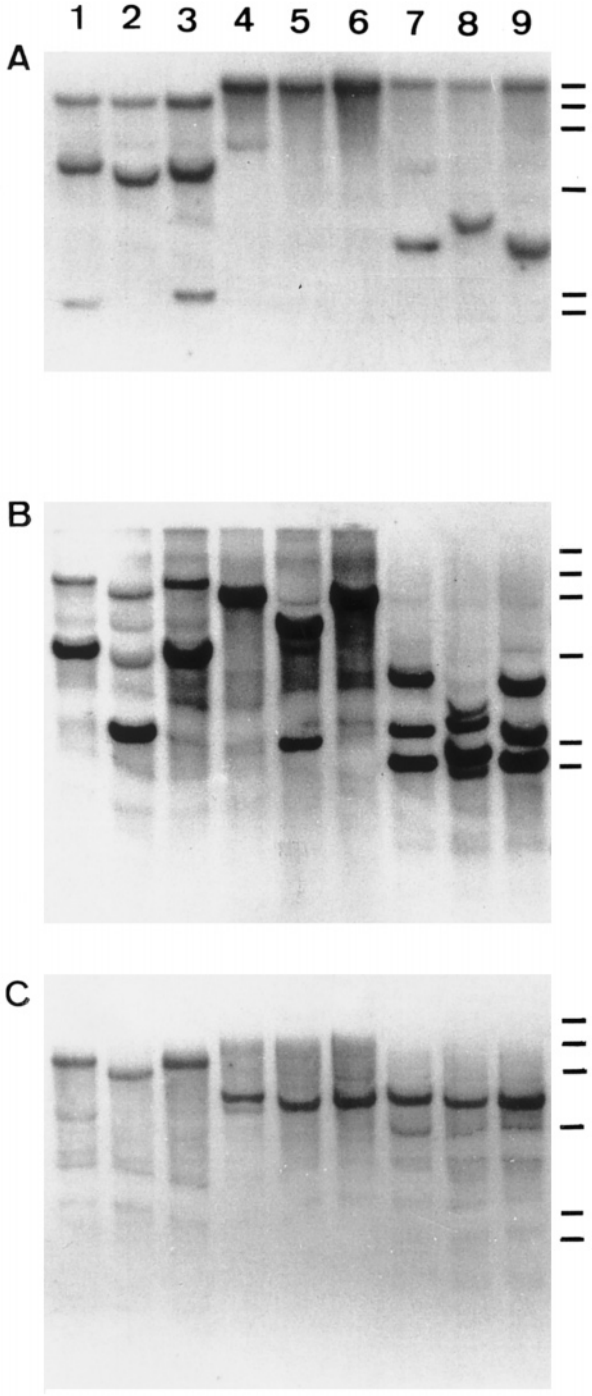

Figure 6. Southern blot of genomic DNA of a *D. simulans lozenge* mutant; one wild-type strain (Eld A) and a *ruby* mutant (but wild-type to *lozenge* phenotype). The membrane was hybridized with three different probes, corresponding to 25 kb of *lozenge* gene (see fig. 5). (*A*) *Sal*/*Sal* 7 kb; (*B*) *Sal*/*Sal* 6 kb; (*C*) BH5. Approximately 5 μg of genomic DNA of each strain was digested with restriction enzymes in the following order: (1) Eld A digested with *Hin*dIII; (2) *lozenge (lz)* digested with *Hin*dIII; (3) *ruby* (*rb*) digested with *Hin*dIII; (4) Eld A digested with *Sal*I; (5) *lozenge* (*lz*) digested with *Sal*I; (6) *ruby* (*rb*) digested with *Sal*I; (7) Eld A digested with *Eco*RI; (8) *lozenge* (*lz*) digested with *Eco*RI; (9) *ruby* (*rb*) digested with *Eco*RI. Bars on the right represent the λ *Hin*dIII fragments (24 kb; 9.5 kb; 6.8 kb; 4.3 kb; 2.3 kb and 2 kb).

Table 3. Number of *ruby* and wild-type flies in the F_1 and F_2 of crosses between the *ruby* mutant males and wild-type (Eld A) females.

	F_1	F_2
Males	217 wild-type	216 wild-type 223 *rb*
Females	237 wild-type	415 wild-type

Table 4. Genetic mapping of the *D. simulans ruby* gene. *D. simulans yellow* males were crossed with *ruby* females. The F_1 and F_2 offspring were screened for phenotypic classes. The recombinants between *y* and *rb* correspond to the sum of the frequencies of the wild-type and the frequencies of the double mutant *rb*/*y* (7.5%).

	F_1	F_2
Females	470 wild-type	860 (48.8%) wild-type 903 (51.2%) *rb*
Males	313 wild-type	601 (48.2%) *rb* 552 (44.3%) *y* 54 (4.3%) *rb*/*y* 40 (3.2%) wild-type

ent methodological approaches, we believe that this mutation is not an allele of the *dpp* gene. The F_2 frequencies of crosses between double mutant male (*dpp*-like/*nt*) and wild-type (Eld A) females is 10.1% for *nt* recombinant and 9.8% for *dpp*-like recombinant (in 2209 F_2 screened). Although the F_2 is not the best choice for genetic mapping, this recombination frequency indicates that the distance of this mutant gene to *nt* is greater than 4.0 cM, which is the distance from *nt* to *dpp* in *D. melanogaster*.

Southern blot analysis of the *dpp* gene region provided a second line of evidence that the mutated locus is unlikely to be *dpp*. Using a *D. melanogaster dpp* genomic DNA fragment corresponding to the disk-ho region as probe, no differences were detected among the *dpp*-like mutant and four *D. simulans* strain used as wild-type controls (data not shown).

The *blistered*-like gene. The wings of *Drosophila* are derived from two ephithelial layers of the wing disc. During wing development, intervein cells are responsible for connecting and holding the two surfaces of the wing together. Intervein cells differentiate a highly specialized system of cytoskeletal supports anchored in integrin-mediated basal lamina [21, 22]. Mutations in integrin genes such as *inflated* (*if*), *myospheroid* (*mys*) and *blistered* (*bs*) result in defective dorsoventral adhesion of intervein cells, leading to the formation of wing blisters [22, 23]. The *D. simulans blistered*-like mutant shows blisters in the wings with variable expression and incomplete penetrance. As can be seen in figure 10, crosses between *bs*-like with two different strains – one wild-

Table 5. Genetic mapping of the *D. simulans ruby* gene. *D. simulans rb* males were crossed with *yellow* females. The F_1 and F_2 offspring were screened for phenotypic classes. The recombinants between *y* and *rb* correspond to the sum of the frequencies of the wild-type and the frequencies of the double mutant *rb*/*y* (6.9%).

	F_1	F_2
Females	578 wild-type	
Males	603 *y*	902 (47.2%) *rb*
		877 (45.9%) *y*
		63 (3.3%) *rb*/*y*
		70 (3.6%) wild-type

Table 6. Number of flies in F_1 and F_2 in the allelism test between the *ruby* mutant males and *D. simulans ruby (rb)* strain from Bloomington Stock Center # 2320 rb[1].

	F_1	F_2
Males	365 *rb*	457 *rb*
Females	338 *rb*	476 *rb*

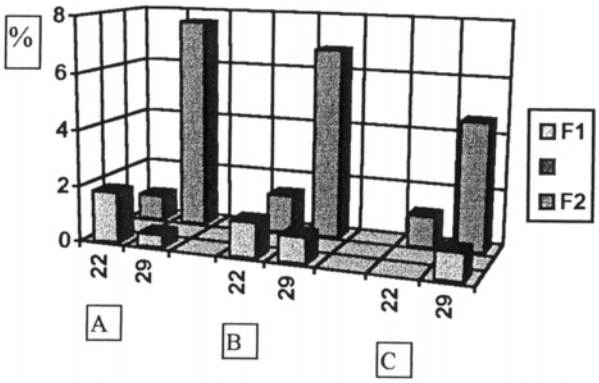

Figure 7. Frequencies of flies with blisters in the wing when reared at 22 °C and at 29 °C. (*A*) Column 1, F_1 of crosses between *bs*-like male and *yellow* female; column 2, F_2 of the same cross. (*B*) Crosses between *yellow* male and *bs*-like female. (*C*) Crosses between the *bs*-like male and Eld A female (wild-type).

type and the other yellow – produce F_1 and F_2 with blisters in the wings, although in low frequency. These crosses suggest that this mutation is dominant, with incomplete penetrance. Frequently, the affected flies show one wing with blisters and another free of any malformation. The expression of the phenotype of this mutation is also affected by temperature; the flies reared at 29 °C produce more offspring with wing blisters than flies reared at 22 °C (fig. 7). Interestingly, the mutations *blistered*-like and *Zoinho-na-pata* have the same pattern of expression: (i) incomplete penetrance; (ii) often just one body side is affected; and (iii) the phenotype expression is influenced by temperature.

Table 7. Frequencies of phenotype classes in the F_2 of crosses between the mutant *dpp*-like males and the females with the markers *nt*; *pm* (chromosome 2) and *st*; *e* (chromosome 3).

Class	*n*	%
Wild	637	35.55
dpp-like	168	9.38
dpp-like; *st*; *e*	49	2.73
dpp-like; *st*	7	0.39
dpp-like; *e*; *pm*	7	0.39
*dpp*like; *pm*	28	1.56
pm; *nt*	91	5.08
nt	140	7.81
e; *st*	245	13.67
e	70	3.90
e; *pm*	119	6.64
e; *pm*; *nt*	49	2.73
st	14	0.78
pm	140	7.81
nt; *st*	15	0.84
nt; *e*; *st*	13	0.73
	1792	100%

As can be seen in table 8, there is no linkage of the *bs*-like mutation with any of the markers of the third chromosome, indicating that this gene is probably on the second chromosome. Because the *D. melanogaster inflated* and *myospheroid* genes are on the X chromosome, the putative genes for which the *bs*-like mutant can be an allele are *blistered* (2:107.3) or *bloated* (2:58.5) [24].

Hypermutability. The genetic instability in Dshs is restricted to hypermutability, since gonadal dysgenesis and embryonic sterility were not observed (data not shown). The mutations described here are very stable, and no reversions of mutations were observed (at least in the *rb* and *dpp*-like strains that showed complete penetrance). In the other mutations that showed incomplete penetrance, revertants may be confused with the flies that did not express the phenotype. It has not yet

Table 8. Frequencies of phenotype classes in the F_2 of crosses between the mutant *bs*-like males and the females with the markers *st*; *e*; *ry* (chromosome 3).

Class	*n*	%
Wild-type	330	61.9
e; *sr*; *ry*	30	4.5
e	2	0.4
ry	58	10.9
st	20	3.8
e; *st*	16	3.0
e; *ry*	9	1.7
st; *ry*	6	1.1
bs-like	24	4.5
e; *st*; *ry*; *bs*-like	17	3.2
st	21	3.9
st; *bs*-like	1	0.2
e; *ry*; *bs*-like	5	0.9

conclusively been proven if a transposable element is the causal agent of the hypermutability within this strain. In situ hybridization with *gypsy*, *hobo*, *I*, *mariner* and 412 transposons with polytenic chromosomes from larvae of each mutant line did not show hybridization of the expected bands in the *lz* and *rb* mutants on the X chromosome (data not shown). Thus it is unlikely that these transposable elements are related to these mutations. Nevertheless, another transposable element may be the causal agent of this genetic instability. That remains to be demonstrated.

Acknowledgements. This research was supported by grants and fellowships of CNPq, CAPES, FINEP and FAPERGS (grant number 931017.6). The authors also thank Ms Nena B. Morales for technical assistance.

1 Harada K., Yukuhiro K. and Mukai T. (1990) Transposition rates of movable genetic elements in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **87:** 3248–3252
2 Bregliano J. and Kidwell M. G. (1983) Hybrid dysgenesis determinants. In: Mobile Genetic Elements, pp. 363–410, Shapiro J. (ed.), Academic Press, New York
3 Kidwell M. G., Kidwell J. F. and Sved J. A. (1977) Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. Genetics **86:** 813–833
4 Bingham P. M., Kidwell M. G. and Rubin G. M. (1982) The molecular basis of P-M hybrid dysgenesis: the role of the *P* element, a *P* strain-specific transposon family. Cell **29:** 995–1004
5 Picard G. (1976) Non-Mendelian female sterility in *Drosophila melanogaster*: hereditary transmission of I-factor. Genetics **83:** 107–123
6 Hatzopoulos P., Monastirioti M., Yannopoulos G. and Louis C. (1987) The instability of the TE-like mutation Dp(2:2)GYL of *Drosophila melanogaster* is intimately associated with the *hobo* element. EMBO J. **6:** 3091–3096
7 Lim J. K. (1988) Intrachromosomal rearrangements by *hobo* transposons in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **85:** 9153–9157
8 Yannopoulos G., Stamatis N., Monastirioti M., Hatzopoulos P. and Louis C. (1987) *hobo* is responsible for the induction of hybrid dysgenesis in strains of *Drosophila melanogaster* bearing the male recombination factor 23.5MRF. Cell **49:** 487–495
9 Sheen F., Lim J. K. and Simmons M. J. (1993) Genetic instability in *Drosophila melanogaster* mediated by *hobo* transposable elements. Genetics **133:** 315–334
10 Lozovskaya E. R., Scheinker V. S. and Evgen'ev M. B. (1990) A hybrid dysgenesis in *Drosophila virilis*. Genetics **126:** 619–623
11 Kim A. I., Belyaeva E. S. and Aslanian M. M. (1990) Autonomous transposition of *gypsy* mobile element and genetic instability in *Drosophila melanogaster*. Mol. Gen. Genet. **224:** 121–122
12 Jacobson J. W., Medhora M. M. and Hartl D. L. (1986) Molecular structure of a somatically unstable transposable element in *Drosophila*. Proc. Natl. Acad. Sci. USA **83:** 8684–8688
13 Dowsett A. P. and Young M. W. (1982) Differing levels of dispersed repetitive DNA among closely related species of *Drosophila*. Proc. Natl. Acad. Sci. USA **79:** 4570–4574
14 Inoue Y. H., Taira T. and Yamamoto M. (1988) Genetics of an unstable white mutant in *Drosophila simulans*: reversion suppression and somatic instability. Genetics **119:** 903–912
15 Jowett T. (1986) Preparation of nucleic acids. In: *Drosophila*: A Practical Approach, pp. 275–286, Roberts D. B. (ed.), IRL Press, Washington, DC
16 Crew J., Batterham P. and Pollock J. A. (1997) Developing compound eye in *lozenge* mutants of *Drosophila*: *lozenge* expression in the R7 equivalence group. Dev. Genes Evol. **206:** 481–493
17 Nichols C. D. (1997) Molecular Characterization of the *lozenge* Locus of *Drosophila melanogaster*, Carnegie Mellon University, PhD thesis
18 Halder G., Callaerts P. and Gehring W. (1995) Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. Science **267:** 1788–1792
19 Shen W. and Mardon G. (1997) Ectopic eye development in *Drosophila* induced by direct *dachshund* expression. Development **124:** 45–52
20 Johnston R. D. St., Hoffmann F. M., Blackman R. K., Segal D., Grimaila R., Padgett R. W. et al. (1990) Molecular organization of the *decapentaplegic* gene in *Drosophila melanogaster*. Genes Dev. **4:** 1114–1127
21 Fristrom D., Wilcox M. and Fristrom J. W. (1993) The distribution of PS integrins, Laminin A and F-actin during key stages in *Drosophila* wing development. Development **117:** 509–523
22 Fristrom D., Gotwals P., Eaton S., Kornberg T. B., Sturtevant M., Bier E. et al. (1994) *blistered*: a gene required for vein/intervein formation in wings of *Drosophila*. Development **120:** 2661–2671
23 Wilcox M., DiAntonio A. and Leptin M. (1989) The function of PS integrins in *Drosophila* wing morphogenesis. Development **107:** 891–897
24 Lindsley D. L. and Zimm G. G. (1992) The genome of *Drosophila melanogaster*, Academic Press, New York