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Genetic and molecular analyses of vg^{al}: a spontaneous and unstable mutation at the *vestigial* locus in *Drosophila melanogaster*

CLAUDE BAZIN[1,*], FRANÇOISE LEMEUNIER[2], GEORGES PERIQUET[3] AND JOËL SILBER[1]

[1] *Laboratoire de Génétique Quantitative et Moléculaire, Université Paris 7, CNRS URA 693, France*
[2] *Laboratoire de Biologie et Génétique Evolutives, CNRS UPR 2411–Gif sur Yvette, France*
[3] *IBEA, Université F Rabelais, CNRS URA 1298–372000 Tours, France*

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Summary

We describe herein, a new unstable mutant of the *vestigial* locus, isolated from a French natural population. From this mutant *vestigial*^{almost} (vg^{al}) wild-type flies (vg^{al+}) and extreme *vg* phenotypes (vg^{e}) arose spontaneously without genomic shock. The occurrence of vg^{al+} or vg^{e} alleles depends mostly on the breeding temperature; vg^{al+} revertants arose principally at low temperature (21 °C) and vg^{e} at 28 °C. These events occur mainly in the male germ line and the phenomenon appears to be premeiotic. Our results with *in situ* hybridization experiments and Southern blots show that the vg^{al} mutation is due to a 2 kb DNA insertion, which is a deleted *hobo* element. Genetic and molecular analyses show that two distinct events may underly the wild-type revertants. One is the excision of the resident *hobo* element, the other a further deletion (about 300 bp in the example characterized herein). The vg^{e} mutation is probably due to a deletion of *vestigial* sequences flanking the *hobo* insertion.

1. Introduction

The *vestigial* mutant, first described by Bridges & Morgan (1919), is characterized by wing atrophy. Other pleiotropic effects also occur: halteres are reduced, fertility is lowered and development is lengthened at 25 °C (Le Menn *et al.* 1987). The mutant phenotype is thermosensitive. Wing size increases with the breeding temperature. A large variety of *vestigial* mutants have been induced by gamma rays by Alexandrov & Alexandrova (1987). One of them, the vg^{83b27} mutant, defines a second complementation group: vg^{83b27}/vg heterozygotes display a wild-type wing phenotype. The *vestigial* locus has been cloned by Williams & Bell (1988). The nature of the molecular lesions for several recessive or lethal *vestigial* alleles was determined, and found to be due to either deletion of *vg* sequences or insertions. The classical *vg* mutant is due to the insertion of a *412* element. The vg^{83b27} homozygotes produce an extreme wing phenotype (Alexandrov & Alexandrova, 1987; Williams & Bell, 1988) and molecular analyses of vg^{83b27} revealed a 4 kb deletion of DNA from the central region of the locus. A *vg*-specific 3·8 kb transcript has been identified by Williams *et al.* (1990) and all the classical alleles have predicted effects on this transcription unit. The severity of the effect is approximately proportional to the reduction of the wing phenotype. The vg^{83b27} deletion maps entirely within a 4·5 kb *vestigial* intron, as defined by the 3·8 kb transcription unit. Many of the molecularly characterized classical *vestigial* viable alleles are due to DNA insertions, but so far none of them has been described as being particularly unstable.

Herein, we describe a novel *vestigial* allele isolated from a French natural population. The mutant *vestigial*^{almost} has wings with terminal notches and lateral excisions, but no pleiotropic effects on halteres, viability or fertility were observed. This mutant is very unstable and gives, with high frequency, wild-type revertants (vg^{al+}) and derivatives with an extreme wing phenotype (vg^{e}). The vg^{al+} revertants were observed both in the mutant stock as well as in the F_1 progeny of particular crosses. The production of revertants and derivatives is thermosensitive and is observed mainly but not exclusively in the male germ line.

* Corresponding author.

2. Materials and methods

(i) *Strains*

Flies were kept in bottles at 21, 25 or 28 °C. The temperatures 21 and 25 °C were used for the genetic analysis. Standard corn, yeast and sugar medium is used throughout. The wild-type strains used were: Oregon^R (Or^R); Harwich (H), a *P*-type reference strain; Canton-S (C), Oregon^S (OR^S) and Hikon (HK), *M*-type reference strains. Oregon^S (OR^S) is also *hobo*$^+$, with active *hobo* elements (kindly provided by Dr Gelbart), while Hikon (HK) is *hobo*$^-$. The mutant strains used were: *vg*^{B} and *vg* (Bowling Green Drosophila Center), their phenotypes are described in Lindsley & Grell (1968); *vg*^{83b27} (Alexandrov & Alexandrova, 1987) from Dr Bell and *vg*^{almost} (*vg*^{al}) isolated by Dr Fleuriet and Dr Periquet in a natural population from Languedoc-Roussillon (France, 1983). From the *vg*^{al} strain two kinds of new phenotypes arose spontaneously. The first type is wild-type flies, with the *vg*$^{al+}$/*vg*^{al} genotype, where the *vg*$^{al+}$ allele is dominant. Two independent revertant strains (*vg*$^{al+1}$ and *vg*$^{al+2}$) are analysed in this study. The second derived phenotype is comparable to the classical *vestigial* mutant phenotype. Some of these flies have the *vg*^{al}/*vg*^{e} genotype. The *vg*^{e} homozygotes display a very pronounced mutant phenotype (*vestigial*^{extreme}; *vg*^{e}) and females are sterile, so the *vg*^{e} mutation is maintained as a balanced stock *vg*^{e}/*Cy SM5*.

(ii) PM *and* hobo *system gonadal dysgenesis assays*

The standard diagnostic test crosses were performed for measuring gonadal (GD) sterility: at 28 °C for the *PM* system (Kidwell *et al.* 1977) and at 25 °C for the *hobo* system (Yannopoulos *et al.* 1987; Blackman *et al.* 1987). The standard crosses were: ♀ C × ♂ *vg*^{al} and ♀ *vg*^{al} × ♂ H for the *PM* system, and ♀ HK × ♂ *vg*^{al}, ♀ *vg*^{al} × ♂ OR^S and ♀ HK × ♂ OR^S for the *hobo* system.

(iii) vg^{al+} *genetic reversion*

The rate of reversion to *vg*$^{al+}$ was determined in bottles in homozygous *vg*^{al} stocks, and in crosses involving the *vestigial* mutant or *vg*/*vg*^{al} heterozygotes. All wild-type revertants and putative derivatives were genetically tested with the *vg*^{B} strain (the *vg*^{B} allele has a deletion of the entire *vestigial* locus). The reversion rate was estimated as the quotient:

(*vg*$^{al+}$ heterozygotes)/(total no. of flies analysed).

The statistical analysis was done with a χ^2 test. The reversion frequency was estimated by the quotient of independent events (these appeared in independent experiments in our analysis). The statistical analysis was done with the exact Fisher test.

(iv) *Analysis of clusters of revertants and derivatives*

Twenty-five pairs of *vg*^{al} flies were kept in vials at 21 °C, and 16 pairs at 25 °C. The number of genetic revertants (*vg*$^{al+}$) and derivatives (*vg*^{e}) was scored in the F_1. For the experiment at 25 °C, one F_1 pair was taken at random and the F_2 was analysed at 25 °C.

(v) *Molecular analysis*

In situ hybridizations were performed with *P*, *FB*, *mdg4*, *hobo* and *412* elements as probes on polytene chromosomes of the *vg*^{al} strain. Slides were treated with 0·07 N-NaOH for 2 min and hybridized at 37 °C with a solution of a biotinylated denaturated DNA probe. Hybridization conditions were 3 × SSC, 50% formamide and 8% dextran. Hybridization sites were visualized by a streptavidin horseradish peroxidase conjugate and finally with H_2O_2 dialinobenzidine. Slides were then stained by orcein aceto lactic.

Genomic DNA for Southern hybridizations and genomic libraries was prepared according to Ish-Horowitz *et al.* (1979) and repurified by spermine precipitation (Hoopes & McClure, 1981). All gels for Southern hybridization analyses were blotted onto Genescreen plus membranes using the capillary blot protocol recommended by the manufacturer (Dupont). For Southern gels, 5 μg of DNA/lane were used. After hybridization the filters were washed according to Genescreen Plus specifications. DNA probes were made from restriction fragments resolved on low melting agarose gels. We are grateful to J. Williams for the gift of all the *vestigial* probes. Culturing and storage of bacterial or lambda phage, preparation of DNA, restriction analyses and plasmid sub-cloning [pbluescribe (*pBS*)] were performed according to standard methodology (Maniatis *et al.* 1982). For the *vg*^{al} library, genomic DNA was entirely digested by *Eco*R I and fragments between 3 and 4 kb, purified in 0·5% agarose gel by electroelution onto dialysis membranes were cloned in λ*gt10* and subcloned in *pBS* (Williams & Bell, 1988).

3. Results

(i) *Phenotypic analysis*

As is shown in Fig. 1*a*, the *vg*^{al} wing has terminal notches and lateral excisions, although some phenotypic variability is observed within the stock. Halteres and scutellar bristles are wild type. Viability and fertility are normal. No gonadal atrophy was observed within the strain at any temperature tested. In contrast, wings and halteres of the *vg*^{e} derivative flies (Fig. 1*b*) are dramatically reduced and scutellar bristles are erect or sometimes absent. Various thoracic and leg abnormalities could be observed in both sexes. The *vg*^{e} females are sterile with partially developed ovaries. No sterility was observed in homozygous *vg*^{e} males.

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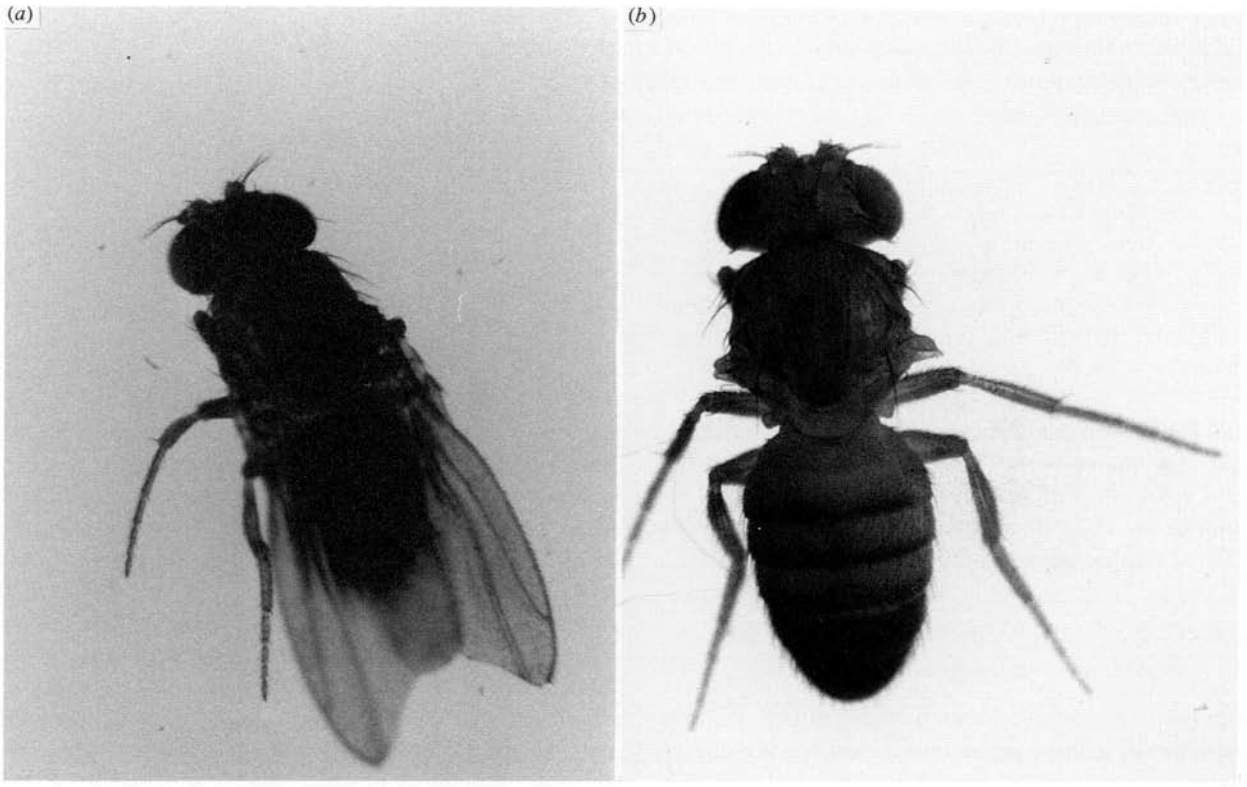

Fig. 1. (*a*) vg^{al} phenotype: the vg^{al} wings have terminal notches and lateral excisions, while the halteres are wild type. (*b*) vg^{e} phenotype: wings and halteres of the vg^{e} mutant are dramatically reduced, postscutellar bristles are erect, sometimes absent. The vg^{e} females are sterile with partially developed ovaries.

The vg^{al+} revertant phenotype is wild type and no pleiotropic effects were observed. Williams *et al.* (1990) have shown that the vg^{83b27} mutant, isolated by Alexandrov & Alexandrova (1987), defines a second complementation group at the *vestigial* locus. Crosses were done to determine in which complementation group the vg^{al} mutant, the vg^{e} derivative and vg^{al+} revertants could be classified. For this classification, the wing phenotype was divided into 6 classes (Fig. 2). The phenotype of F_1 progeny of various crosses (Table 1) shows that the vg^{al} mutation belongs to the same complementation group as *vg* (vg/vg^{83b27} and vg^{al}/vg^{83b27} are wild type) and the vg^{al} allele is recessive with respect to the vg^{+} allele, and partially recessive with *vg* and vg^{B}. The vg^{e} allele gives a mutant phenotype with *vg* as well as with vg^{83b27}; so it does not complement either of the two complementation groups. This allele is completely recessive with vg^{+}, and gives strong mutant phenotypes (classes IV, V and VI) when heterozygous with the vg^{al}, *vg*, vg^{83b27} and vg^{B} alleles. Morover various thoracic anomalies and female sterility could be observed in vg^{e}/vg^{B} heterozygotes. On the basis of our results, two kinds of vg^{al+} alleles have been differentiated, one is completely dominant with respect to vg^{B} (vg^{al+1}) and the other one is only partially dominant (vg^{al+2}).

Like all other *vg* alleles, the vg^{al} mutant is thermosensitive, but, unlike the others, the increase of the wing phenotype is positively correlated with a decrease of the breeding temperature. The vg^{al} stock

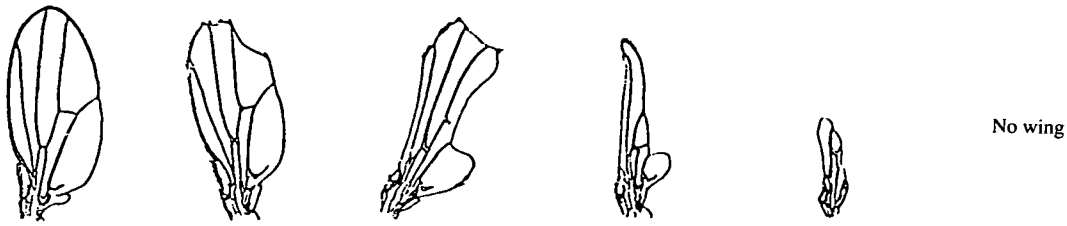

Fig. 2. The wing phenotype is arbitrarily divided into 6 classes, and ranges from class 1 for wild type to class 6 for no wing.

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Table 1. *Phenotype classification for the different genotypic combinations of* vg *alleles. All numeral designation represent the phenotypes for the respective heterozygotes. The numerals correspond to those in Fig. 3*

Alleles	*vg*	vg^{e}	vg^{al}	vg^{al+1}	vg^{al+2}
vg^{+}	I	I	I	I	I
vg^{83b27}	I, II	VI	I, II	I	I
vg^{al}	IV	V	III	I	—
vg^{B}	V	VI*	V	I	II
vg	V	V	IV	I	I

* Females are sterile.

reared for several generations at 21 °C tends to exhibit a wild-type phenotype. Thus, our analyses tested the genetic instability of vg^{al} when it was reared for 1 or 2 generations at 21 °C and then again when there was a shift of temperature (21–25 °C) (see Tables 3, 5).

(ii) *Relation of the* vg^{al} *mutant with the* PM *and* hobo *hybrid dysgenesis systems*

Since the vg^{al} mutant was isolated in a natural population, it seemed important to test the possibility that our results would or would not be correlated with known hybrid dysgenesis systems. At the molecular level, the vg^{al} strain harbours both *P* and *hobo* sequences (data not shown). In Table 2, we analysed the activity potential and the susceptibility of vg^{al} in the *PM* and the *hobo* hybrid dysgenesis systems by scoring for gonadal dysgenesis in F_1 females in standard crosses. Crosses with the reference wild-type strains for the *PM* system [H (*P*) and C (*M*)] lead us to classify vg^{al} as a weak M^{s} type. In the *hobo* system, vg^{al} is a weak $hobo^{+}$ strain, since we observe 20% gonadal atrophy in the cross ♀ HK × ♂ vg^{al}, and 47% in the control (♀ HK × ♂OR^{s}). The *vg* stock used in our experiments is an *M* and $hobo^{-}$ strain (result not shown). Since we observed similar results with the vg^{al} mutant and a $vg^{al(4)}$ strain, where the *vg* background was introduced by four backcrosses (results not shown), we assume that the *vg* genotype does not play any role in the instability observed in our outcrosses.

(iii) *Genetic analysis*

In Table 3, we present the intrastrain phenotypic reversions observed. Class V (*vg*) and class I (+) phenotypes were scored and potential genetic revertants were tested. The switch of temperature was performed, due to the number of wild-type phenocopies observed in the vg^{al} stock reared at 21 °C. For experiments 1 and 3 the flies were reared respectively at 28 and 21 °C for two generations and then shifted to 25 °C for the experiment. We observed significantly more class V wing phenotypes when vg^{al} flies were reared at 28 °C than when they were reared at 21 °C. No crosses were done with these flies to ascertain the existence of a genetic change, but as these flies give extreme phenotypes (class VI) in their progeny, we assumed that these flies are genetic derivatives. Class I phenotype was observed more often when flies were either reared and tested at 21 °C (experiment 4) or reared at 21 °C for 2 generations and tested at 25 °C (experiment 3). We tested genetically (with the vg^{B} allele), all wild-type individuals (class I) found in Table 3. As tabulated, some of them (1/7 at 28 °C, 2/3 at 25 °C and 14/80 at 21 °C) were genetic revertants and had a vg^{al+}/vg^{al} genotype. Statistical tests revealed that not only are vg^{al+} genetic revertants more frequent when the flies are reared at 21 °C (1·16%) for the mean of experiments 3 and 4, than for those reared at 25 °C (9×10^{-4}) or 28 °C (2×10^{-4}), but also that they are more frequent when there is a shift of temperature (experiment 3) than when flies are tested at 21 °C (experiment 4).

The existence of a large number of genetic revertants or derivatives suggests that they are the result of premeiotic events. The occurrence of clusters of genetic revertants and derivatives has been tested by the genetic analysis of the progeny of single couples. For both revertants and derivatives, the existence of clusters of various sizes is observed (Table 4). At 25 °C, the presence of genetic revertants seems to be independent in F_1 and F_2. Revertants and derivatives

Table 2. *Induction of GD sterility (measured as % of gonadal dysgenesis in* F_1 *females) among the progeny of different* PM *crosses at 28 °C and* $hobo^{+}$, $hobo^{-}$ *crosses at 25 °C*

	PM crosses (28 °C)		*hobo* crosses (25 °C)		
	♀ vg^{al} × ♂ H	♀ C × ♂ vg^{al}	♀ vg^{al} × ♂ OR^{s}	♀ HK × ♂ vg^{al}	♀ HK × ♂ OR^{s}
Atrophic gonads	42/112	1/328	3/296	70/364	106/226
%	37·5	0·3	1·0	19·2	47·0

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Table 3. *Phenotypic reversion of* vg^{al} *to* vg (*class V*) *and* vg^{+} (*class I*), *and genetic reversion to* vg^{al+} *at different temperatures* (*21, 25 and 28 °C*)

Expt no.	Temperature (°C) of vg^{al} breeding G1, G2	Temperature (°C) of Experiment G3	Number of secondary events: Phenotypic classes V	Phenotypic classes I	Heterozygotes* vg^{al+}/vg^{B} (10^{-4})	Total of flies analysed
1	28	25	90	7	1 (2)	4817
2	25	25	0	3	2 (9)	2282
3	21	25	5	35	26 (226)	1150
4	21	21	3	80	14 (61)	2291

* Each wild-type phenotypic revertant was crossed with the vg^{B} mutant. In this column we give the number of genetic vg^{al+} revertants, and in parentheses their frequency expressed as the quotient, i.e. (no. of heterozygotes)/(total no. of flies analysed).

Table 4. *Appearance of heterozygotes* vg^{al}/vg^{e} *and* vg^{al}/vg^{al+} *in the progeny of single pairs of* vg^{al} *flies bred at 21 or 25 °C*

Pair no.	Progeny F_1 at 25 °C: Total	vg^{al}/vg^{e}	vg^{al}/vg^{al+}	Progeny F_2 at 25 °C: Total	vg^{al}/vg^{e}	vg^{al}/vg^{al+}	Pair no.	Progeny F_1 at 21 °C: Total	vg^{al}/vg^{e}	vg^{al}/vg^{al+}
1	290	1	—	186	—	—	1	149	—	1
2	169	—	—	74	4	—	2	125	—	1
3	155	8	—	123	—	—	3	78	—	4
4	106	1	—	—	—	—	4	164	—	1
5	73	—	—	97	3	—	5	50	3	—
6	109	—	1	74	—	—	6–25	1580	—	—
7–16	1203	—	—	1014	—	—	—	—	—	—
	2605	10	1	1668	7	0	—	2146	3	7

Vials have been renumbered to group them according to type of exception produced, with those giving no exceptions combined at the end.

were never observed in the same progeny vial. We aimed to estimate if the genetic reversion to vg^{al+} was sex-dependent. We analysed, in Table 5, the progeny in the female germ line cells (cross ♀ $vg^{al} \times$ ♂ vg), and in the male germ line cells (cross ♀ $vg \times$ ♂ vg^{al}). As the intrastrain genetic reversion was thermosensitive, these experiments were done with vg^{al} parents reared for 2 generations at 21 or 25 °C. We observed that the reversion rate is higher in the male germ line (mean of the ♀ $vg \times$ ♂ vg^{al} crosses is $1{\cdot}9 \times 10^{-3}$) than in the female one (mean of the ♀ $vg^{al} \times$ ♂ vg cross is $2{\cdot}7 \times 10^{-4}$). It is also higher when vg^{al} males and females are reared at 21 °C, and is highest in the progeny of males reared at 21 °C and tested at 25 °C. The statistical analysis shows that there is no difference in the progeny of the ♀ $vg \times$ ♂ vg^{al} cross and the reciprocal cross when they are reared and tested at 25 °C ($P = 0{\cdot}2$). The mean genetic reversion frequency

Table 5. *Frequency of* vg^{al+} *heterozygotes observed in the* F_1 *progeny of* vg *crossed with* vg^{al}

Temperature (°C) of vg^{al} breeding G1, G2	Temperature (°C) of Experiment G3	Crosses ♀	Crosses ♂	F_1 progeny total	Heterozygotes vg^{al+}: Total	(Reversion rate 10^{-4})	Independent	(Reversion frequency 10^{-4})
25	25	vg^{al}	vg	8050	0		0	
25	25	vg	vg^{al}	16826	7	(4·1)	4	(2·4)
21	25	vg^{al}	vg	1161	2	(17)	2	(17)
21	25	vg	vg^{al}	1780	28	(157)	1*	(5·6)
21	21	vg^{al}	vg	1741	1	(5·7)	1	(5·7)
21	21	vg	vg^{al}	3900	10	(25)	2*	(5·1)

* On account of the large number of vg^{al+}, we are not sure that there is only one or two events in those experiments.

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Table 6. *Rate of wild-type* vg^{al+} *heterozygotes obtained in the progeny of backcrosses of* F_1 (♀ vg × ♂ vg^{al}) × vg *or* vg^{al}

Crosses			No. of flies analysed	No. of heterozygotes			
Female		Male		Total	(Reversion rate 10^{-4})	Independent	(Reversion frequency 10^{-4})
vg	×	F_1	9643	0		0	
vg^{al}	×	F_1	4064	8	(20)	4	(10)
F_1	×	*vg*	12828	8	(6)	5	(4)
F_1	×	vg^{al}	4952	7	(14)	3	(6)
vg	×	vg^{al}	16826	7	(4)	4	(2·5)
vg^{al}	×	*vg*	8050	0		0	

for both male and female germ cells can be estimated at $1{\cdot}6 \times 10^{-4}$ (4/24876) at 25 °C. There are also, no statistical differences in the genetic reversion frequency in females reared at 21 °C and tested at 25 °C ($1{\cdot}7 \times 10^{-3}$) versus those reared at 21 °C and tested at 21 °C ($5{\cdot}7 \times 10^{-4}$). The mean frequency is 10^{-3} at 21 °C and is significatively different from that estimated at 25 °C ($1{\cdot}6 \times 10^{-4}$, $P = 0{\cdot}03$). Large numbers of vg^{al+} revertants were observed in the ♀ *vg* × ♂ vg^{al} progeny reared at 21 °C, but we cannot be sure that they are all from single clusters. If they are, the reversion frequency in male and female germ lines is similar and can be estimated at 7×10^{-4} in the germ line of flies reared at 21 °C. This frequency is significantly higher than in flies reared at 25 °C ($P = 0{\cdot}022$). These results imply that the reversion in vg^{al} (reversion rate and reversion frequency) is highly thermosensitive and the range is maximum when there is a shift of temperature.

In order to study the instability of the vg^{al} allele in heterozygous flies, individuals originating from male germ line F_1 progeny (from ♀ *vg* × ♂ vg^{al}) were crossed with *vg* or vg^{al} at 25 °C (Table 6). F_1 males gave significantly more genetical revertants in their progeny when they were crossed with vg^{al} females than with *vg* females ($P = 0{\cdot}007$). Moreover, the ♀ vg^{al} × ♂ F_1 cross gave more revertant progeny (4/4064) than the control ♀ vg^{al} × ♂ *vg* cross (0/8050) tested at 25 °C ($P = 0{\cdot}012$). This could indicate that the high percentage observed in the first cross is not due only to female vg^{al} or male F_1 instability. On the contrary, there were no statistical differences in the progeny of ♀ F_1 × ♂ *vg* and ♀ F_1 × ♂ vg^{al}. The result does not seem to reflect only the frequency of the intragenic recombination, because the frequency ($4{\cdot}4 \times 10^{-4}$) is too high and clusters occurred. The reversion frequency in these crosses is similar to that of the controls where the reversion was tested in the germ lines (female and male) at 25 °C ($1{\cdot}6 \times 10^{-4}$, $P = 0{\cdot}053$).

(iv) *Molecular analysis*

The vg^{al} mutant displays properties typical of insertion mutations. To test which element is involved in the vg^{al} mutation, we performed *in situ* hybridizations on polytene chromosomes using the *P. gypsy*, *FB*, *412* and *hobo* elements as probes. Only the *hobo* element

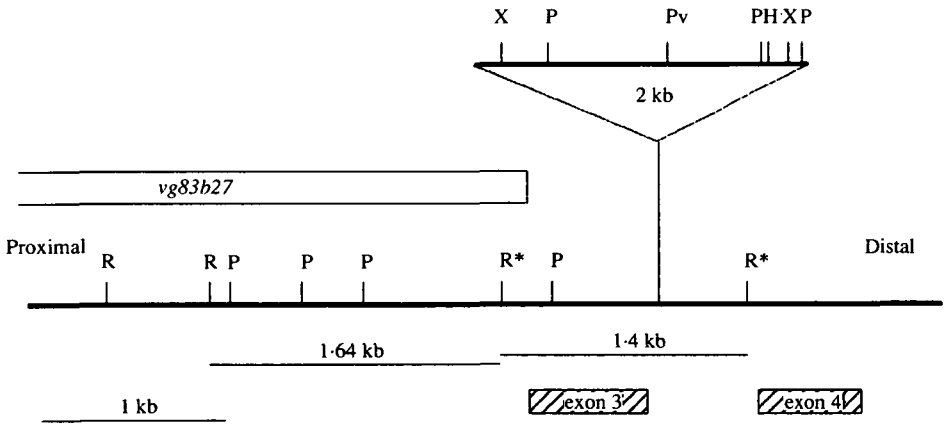

Fig. 3. Partial physical map of the *vestigial* locus. The open bars (□) above the restriction map designate the vg^{83b27} deletion, the bars (▨) below the restriction map denote the exons 3 and 4. The *412* insertion in the classical vestigial mutation is located within the 1·4 kb fragment but distal to exon 3 sequences (Williams *et al.* 1990). The 3·4 kb cloned DNA is designated R*, the triangle designates the 2 kb insertion involved in the vg^{al} mutation. The probes used for the Southerns analyses are *Eco*R I fragments of 1·4 and 1·64 kb. The restriction sites are: R, *Eco*R I, P, *Pst* I, H, *Hinc* II, X, *Xho* I and Pv, *Pvu* II.

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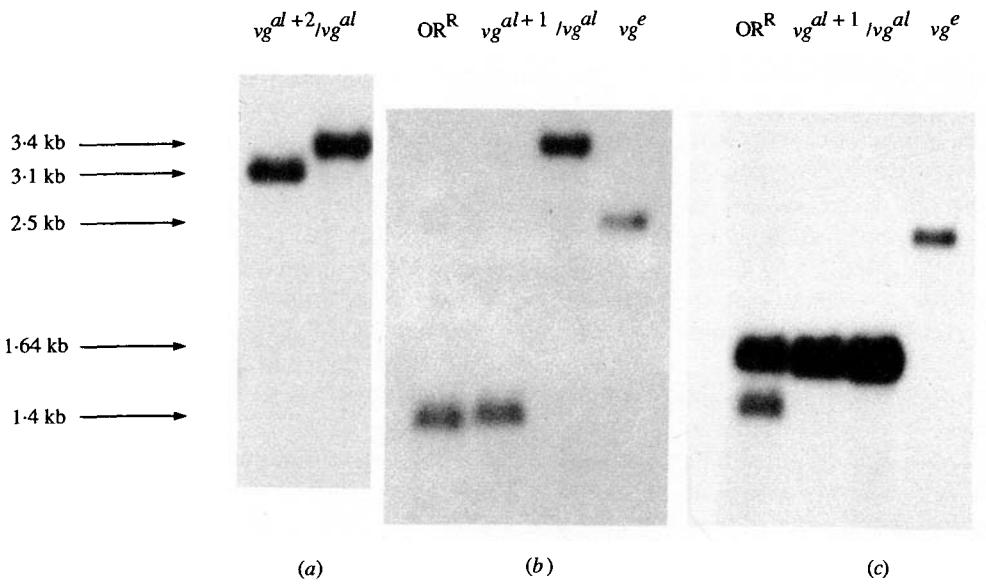

Fig. 4. Southern blot analysis of vg^{al}, vg^{al+1}, vg^{al+2}, vg^e and OR^R strains. The DNA was digested with *Eco*R I and probed with the 1·4 kb *Eco*R I fragment (*a* and *b*) and then (*c*) probed with the 1·64 kb *Eco*R I *vestigial* fragment from the vg^+ locus (Fig. 3). In the OR^R wild-type strain there is a polymorphism for the 1·64 kb *Eco*R I fragment as indicated by the two hybridization fragments.

hybridizes in the 49 D region, where the *vg* gene is located (Lindsley & Grell, 1968). In addition to a specific hybridization of the *hobo* element at the *vestigial* locus, the presence of a *gypsy* element was observed in the nearby region 50/51 (results not shown).

The *vestigial* locus has been cloned and characterised by Williams & Bell (1988). We give in Figure 3 a partial physical map of the locus. We have done a molecular analysis of vg^{al}, vg^{al+1}, vg^{al+1} vg^e compared to the wild-type OR^R locus. We used several probes spanning the entire *vestigial* locus and the only difference found was in the 1·4 kb *Eco*R I fragment (results not shown). The size of this fragment is about of 3·4 kb in DNA from vg^{al} flies, 3·1 kb in DNA from vg^{al+2} flies and 1·4 kb in DNA from vg^{al+1} flies (Fig. 4*a*, *b*). To analyse the vg^e mutation, genomic DNA was digested with *Eco*R I and the same Southern blot was first hybridized with the 1·4 kb probe and next with the 1·64 kb probe (see Fig. 3). For these sequential hybridizations, the same 2·5 kb *Eco*R I fragment hybridizes in the vg^e lane (Fig. 4*b*, *c*). An explanation of this could be that the *Eco*R I restriction site marking the boundary of the genomic 1·4 and 1·64 kb *Eco*R I fragments is missing and that there is a deletion of *vestigial* sequences in the vg^e strain that include this site. The 3·4 kb *Eco*R I fragment of vg^{al} was cloned into *λgt10* and sub-cloned into *pBS*. The restriction map is given in Fig. 3. A 2 kb DNA insertion is located in the 1·4 kb *Eco*R I fragment. There are no restriction sites of *Eco*R I, *Bam*H I, *Sal* I, *Hind* III, *Sac* I, *Bgl* II or *Xba* I in this insertion, but we find *Xho* I, *Hinc* II, *Pvu* II and *Pst* I. This sequence hybridizes with the *hobo108* element (Streck *et al.* 1986). The distance between the two *Xho* I restriction sites is about 2·6 kb in the *hobo108* element and here is about 1·6 kb, so this insertion is likely an internally deleted *hobo* element. Surprisingly, we found a *Pvu* II restriction site in this sequence, which is not present in the *hobo108* sequenced element. This site could be a polymorphism site or a site created at the deletion junction.

4. Discussion

The vg^{al} mutant displays the property of instability often observed for insertion mutants. It is the first time that an unstable mutant has been observed at the *vestigial* locus. Molecular analysis allowed us to show a 2 kb DNA insertion located in the same 1·4 kb *Eco*R I fragment where an insertion of a *412* element produced the classical *vestigial* mutant (Williams & Bell, 1988). This location explains why vg^{al} belongs to the first complementation group. *In situ* hybridization and the cloning of the vg^{al} mutation show that the mutation is due to the insertion of a deleted *hobo* element.

The genetic analysis of the vg^e derivative allows us to think that a major molecular event alters the structure of the *vestigial* locus. Indeed, vg^e does not complement mutants of the first complementation group, such as *vg* and vg^{np} (results not shown), or the mutant of the second complementation group comprised of vg^{83b27}. Moreover, this strain displays complete female sterility. This sterility disappears in the heterozygous condition except for vg^B/vg^e heterozygotes. This observation may be related to the sterility observed in vg^B/vg^{nw} and vg^B/vg^{NO2} heterozygotes (Silber & Goux, 1978), where vg^{nw} and vg^{NO2} are deletions of *vg* sequences (Williams & Bell, 1988). Our

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genetical results suggest that the vg^e mutation may be due to a deletion, and molecular analyses (Fig. 4) show that this alteration is a deletion of *vestigial* sequences close to the vg^{83b27} deletion. That could explain why vg^e does not complement either with mutations of the first or of the second complementation group. We can postulate from vg^e an explanation similar to that for the dpp^{Blk} allele, where Blackman *et al.* (1987) observed a deletion of flanking genomic DNA with a deleted *hobo* element at the deletion site. This has to be confirmed by the cloning of the vg^e mutation.

The genetic analysis of two revertant vg^{al+} strains allows us to think that at least two kinds of different molecular events could lead to a wild-type phenotype. The vg^{al+1}/vg^B heterozygote is completely wild type, similar to vg^+/vg^B heterozygotes. That is not the case with vg^{al+2}/vg^B heterozygotes which have a mutant phenotype. On the basis of our molecular analysis, we assume that the vg^{al+1} reversion results in a complete excision of the deleted *hobo* element, and that vg^{al+2} results in partial DNA excision (300 bp) in the region involved in the mutation. This deletion could be in the *hobo* element since vg^{al+2} has a wild-type phenotype.

It would not be the first time that phenotypic reversion is accomplished by different mechanisms. The vg^{ni} revertant strain is due to a further insertion of a *roo* element in the 5′ part of the *412* element of the *vg* mutant (Williams & Bell, 1988). Another mutation, cut^{MR2} can either excise the *gypsy* element or insert the *jockey* element into the *gypsy* insertion. The two events lead to a wild-type phenotype (Mizrokhi *et al.* 1985; Leigh-Brown *et al.* 1989).

In our genetic analyses, we show that clusters of various size are observed for both revertants and derivatives, and suggest that the reversion is premeiotic. In material and methods, we defined the reversion frequency which represents independent events of reversion and the reversion rate which take into account the cluster size. The genetical reversion frequency range to vg^e is around $1{\cdot}3 \times 10^{-4}$ at 21 and 25 °C. Moreover, class V (*vestigial*) phenotypes are most frequently observed at 28 °C (result not shown) and at 25 °C, if the flies' parents were reared at 28 °C. The genotypes of these flies were not tested but we assume that most, if not all, were genetical derivatives. For vg^{al+}, the reversion rate is sex-dependent. For any temperature we tested in our experiments, it is higher in the male germ line than in the female germ line. It is also temperature dependent, and is higher when the vg^{al} parents were reared at 21 °C. We observe the same result with the reversion frequency. It is higher when flies reared at 21 °C were tested at 21 or 25 °C than when they were reared and tested at 25 °C. These results indicate that what matters for increasing the vg^{al+} genetic reversion is the temperature at which the parents' germ line (especially males) is formed. Similar results have been described by Ronsseray (1986) for the cytotype in the *PM* system and by Bucheton (1979) for reactivity in the *IR* system.

We wanted to test the possibility of an abnormal segregation in vg/vg^{al} heterozygotes issued from a dysgenic *hobo* cross ♀ $vg \times$ ♂ vg^{al}. No wild-type revertants are observed in the progeny of ♀ $vg \times$ ♂ F_1. In contrast, 8 revertants appeared in the progeny of ♀ $vg^{al} \times$ ♂ F_1 (4 of which are independent, appearing in separate bottles). The simplest conclusion would be that these revertants resulted from reversion events occurring in the female vg^{al} germ cell line. However, the frequency of this reversion ($4/4064 = 9{\cdot}8 \times 10^{-4}$) is not only statistically higher than that observed in the female vg^{al} germ line, but also higher than that observed in the vg^{al} germ lines at 25 °C ($4/24876 = 1{\cdot}6 \times 10^{-4}$, $P = 0{\cdot}017$). Another hypothesis would be that the vg^{al} allele is particularly unstable in the heterozygous state, but this is inconsistent with there being no revertant in the ♀ $vg \times$ ♂ F_1 cross (0/9643). The explanation of these results is probably rather complex. In parallel to our results, we can examine those of Leigh Brown *et al.* (1989). In their analysis of the cut^{MR2} strain the intrastrain reversion frequency (cut^+) is $1{\cdot}1 \times 10^{-5}$, but when crossed with *MRh12/Cy*, they observed F_1 revertants at a frequency of 5×10^{-4}. That would mean that the frequency of reversion in F_1 depends in this case, as in ours, on the genotype of the parents. It would also mean that the two genotypes could interact with each other by a molecular alteration occurring after the zygote formation. Further work is needed to decide between these hypotheses, especially those using 'marked' strains.

The instability of vg^{al} follows more or less the same pattern as other mutations due to an insertion of a *hobo* element. *Hobo* elements may produce hybrid dysgenesis but the instability of the system is rather complex. *Hobo* is not only unstable in dysgenic crosses but also in established stocks (Yannopoulos *et al.* 1987; Blackman *et al.* 1987; Hatzopoulos *et al.* 1987; Lim, 1988; Blackman & Gelbart, 1989). The vg^{al} strain fits in the $hobo^+$ group and possesses several *hobo* elements. The instability studied here, appears to be sex and thermodependent.

From the results presented herein, two points emerge about *hobo* elements, which have not yet been described so far as we know. The first point concerns the size of clusters, which seems to be dependent in our results on the breeding temperature (larger at 21 than at 25 °C) and on the parent germ line (larger in male than in female). The second point is the thermosensitivity of the type of genetic and probably molecular events. Indeed, we postulate that a temperature of 28 °C could favour the vg^e appearance by causing deletions in the host gene. In contrast, at 21 and 25 °C we observed principally wild-type revertants which can result either from an excision (vg^{al+1}), more or less precise, or of a partial deletion (vg^{al+2}). The molecular analyses of vg^e and vg^{al+2} will show us, if the *hobo* element is still there and how it is implicated in

those deletions. It would be interesting to investigate whether or not the genetic reversions are always due to the same kind of molecular events and if they are correlated with the *hobo* element insertion. The screening of several independent wild-type revertants and derivatives will allow us to answer to these questions.

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