Analysis of vestigial\(^w\) (vg\(^w\)):
a mutation causing homoeosis of haltere to wing
and posterior wing duplications in Drosophila melanogaster

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SUMMARY

vg\(^w\) is a homozygous lethal mutation killing embryos prior to formation of the syncitial blastoderm. In heterozygous condition it causes duplications of the posterior wing, ranging from very small duplications of the axillary cord and alar lobe to large duplications including much of the wing blade and the posterior row of bristles. No anterior margin structures are ever observed. The thorax is sometimes slightly abnormal, but rarely shows large duplications. The size of the wing is related to the number of pattern elements deleted or duplicated.

Heterozygous vg\(^w\) flies also show homoeosis of the haltere to wing. This occurs in the capitellum, where wing blade is observed, but no wing margin structures are found. As with the bithorax (bx) mutation which transforms anterior haltere to anterior wing this aspect of the phenotype is repressed by the Contrabithorax (Cbx) mutation. The transformed haltere discs show more growth than wild-type haltere discs.

Flies heterozygous for vg\(^w\) also show a high frequency of pupal lethality, those forming pharate adults generally show the most extreme vg\(^w\) phenotype.

No cell death has been observed in the imaginal discs of third instar larvae, suggesting that if the wing defects result from cell death this must occur early in development. The homoeosis in the haltere discs and duplications of the wing disc are reflected by the altered morphology and growth of these discs.

There are some minor differences in the expressivity of the phenotype when flies are reared at different temperatures. Chromosome substitutions suggested that all aspects of the phenotype related to the vg\(^w\) mutation and that other mutations had not occurred in the stock. Cytological analysis indicated that vg\(^w\) is a deletion or inversion on the right arm of chromosome 2 from 47F/48A to 49C.

Complementation studies with various mutants thought to be located within the deletion, or inversion and which affect wing morphology have been undertaken.

Cbx causes transformations of wing to haltere; this occurs in the posterior compartment far more frequently than in the anterior compartment. Cbx; vg\(^w\) flies have wings where one of the duplicates is no longer present, presumably transformed to haltere, though this is difficult to identify. One copy of the axillary cord, alar lobe etc, the structures commonly duplicated in vg\(^w\), are present, but they are the anterior duplicate rather than the original posterior copy of these structures. Thus Cbx acts upon genuine posterior structures but not those posterior structures in vg\(^w\) which form in anterior wing locations, suggesting that although these structures differentiate into posterior wing, to the Cbx gene product the cells are still 'anterior'.

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INTRODUCTION

There are several approaches to the analysis of growth and the development of pattern. In many systems the most helpful answers can be obtained by direct experimental manipulation of the tissues and organs being studied. This has been valuable in *Drosophila* especially in the determination of how growth and pattern are regulated during duplication and regeneration of the imaginal discs. It is, however, far more difficult to analyse how growth and pattern formation are regulated in normal *Drosophila* development. One way of at least finding some of the features involved is to analyse mutations which interfere with either growth, such as mutations causing cell death, or with pattern, such as the homoeotic mutants which have replacement of one body structure with another, or mutations causing morphological defects such as deletions and duplications of pattern elements in the larva or adult. It is hoped that detailed studies of these mutants will enable us to understand how the wild-type genotype regulates and controls growth and the establishment of pattern in *Drosophila*.

The *vestigial (vg)* mutations in *Drosophila* cause deletions of parts of the wing. The size and nature of the deletions depend upon the particular vg allele present in the stock (Lindsley & Grell, 1968; Waddington, 1940). The size of the mutant wing is related to the number of pattern elements which differentiate; smaller wings have fewer pattern elements. The vg wing phenotype seems to be the result of position-specific cell death in some of the vg mutants (Fristrom, 1968, 1969; Bownes & Roberts, 1981). The size of the presumptive wing blade is reduced in vg wing discs indicating altered growth characteristics in this region during larval development. The capitellum of the haltere is also reduced in size in the vg mutants.

Several overlapping deletions are known which include the vg locus, and many of these are homozygous lethals affecting embryonic development (Lindsley & Grell, 1968). A new dominant vg allele was recently isolated by Shukla, and named vestigial wingless. The preliminary report in *Drosophila* Information Service (Shukla, 1980) indicated that it not only caused a dominant wing reduction, but also caused homoeosis of haltere to wing in some offspring. As only one other vg mutant causes dominant wing reduction, vg\(^W\) and is a chromosomal inversion, and none of the other vg alleles caused homoeotic transformations, we have investigated this new mutant in more detail.

One of our first observations was that in fact the vg\(^W\) mutation causes duplication of posterior wing structures and a deletion of anterior wing structures. This duplication was in marked contrast to our studies on vg which caused no duplications of wing structures (Bownes & Roberts, 1981).

The paper describes the phenotype of vg\(^W\) wings, halteres and wing and haltere discs in detail and presents cytological evidence to show that it is a deletion or inversion on the second chromosome including the vg locus. Complementation studies were undertaken with other vg mutations. The chromosomal
Studies of the vg\textsuperscript{W} mutation

defect extends close to, or includes the engrailed (en) gene, which causes anterior wing duplications, so preliminary complementation studies with engrailed mutants are described (Garcia-Bellido, Ripoll & Morata, 1973; Lawrence & Morata, 1976; Morata & Lawrence, 1975, 1978). The genetics suggests that vg\textsuperscript{W} is an inversion.

Homoeotic mutations alter the state of determination in specific presumptive adult cells. This leads to a substitution of pattern elements in the adult and altered growth characteristics in the affected imaginal disc. The mutant bithorax (bx) causes homoeosis of anterior haltere to anterior wing and postbithorax (pbx) transform the posterior haltere to posterior wing. (Morata & Garcia-Bellido, 1976; Lewis, 1963, 1964, 1967, 1968, 1978). A further mutant from the bithorax complex Contrabithorax (Cbx) represses the homoeosis caused by bx, and itself causes transformations of wing to haltere (Lewis, 1963, 1964; Morata, 1975). Thus we finally studied the interactions between Cbx and vg\textsuperscript{W} in the hope that it would provide some insight into the mechanism of action of the vg\textsuperscript{W} mutation.

MATERIALS AND METHODS

Maintenance of flies

All flies were maintained on freshly-yeasted, sugar, cornmeal, yeast and agar medium at 25 °C, unless otherwise stated in the Results Section.

Mutations used

The mutations used for characterizing vg\textsuperscript{W} are listed in Table 1. For description of the symbols in marked stocks see Lindsley & Grell (1968). This table also supplies information on the phenotype of the mutants, who supplied the stock, and its chromosomal location, if it is a previously studied deficiency. Where more than one stock is listed for maintaining the mutation, the first was supplied to us, and the others were constructed in our lab to facilitate identification of chromosomes in genetic crosses.

Our wild-type stock was Oregon R, OrR.

Other stocks used were: Binsey; rucuca.

Binsey; TM3

Examination of adults

Adults resulting from various crosses were first examined using a dissecting microscope, then the thorax was mounted between two coverslips using Gurr's water mounting media. Detailed analysis of structures present was made using Zeiss Nomarski Interference optics.

Examination of embryos

Embryos were collected for 2–3 h, washed and dechorionated in 3 % sodium hypochlorite. They were lined up on double-sticky tape, covered in Voltalef oil and observed immediately and subsequently at frequent intervals until hatching, using Zeiss Nomarski interference optics.
<table>
<thead>
<tr>
<th>Mutant</th>
<th>Phenotype</th>
<th>genetic location</th>
<th>Supplied by</th>
<th>reference (not all refs to each mutant are listed)</th>
<th>Stocks used</th>
</tr>
</thead>
<tbody>
<tr>
<td>$vg^W$</td>
<td>heterozygote; vestigial wing, homoeosis of haltere wing homozygote; lethal.</td>
<td>Df(2R) 47F–49C</td>
<td>Shukla</td>
<td>Shukla, 1980 and this manuscript.</td>
<td>$vg^W / In(2L)t In(2R)Cy Roi cn^2 bw or sp^2; al dp b cn vg^W bw$</td>
</tr>
<tr>
<td>$vg^B$</td>
<td>homozygote; lethal. heterozygote; occasional wing nicks.</td>
<td>Df(2R) 49D3/4–50 A2/3</td>
<td>Bull</td>
<td>Bull 1966</td>
<td>$vg^B / In(2L)t In(2R)Cy Roi cn^2 bw or sp^2.$</td>
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<tr>
<td>$vg^D$</td>
<td>homozygote; lethal. heterozygote; sparse hairs and bristles on thorax.</td>
<td>Df(2R) 49C1/2–49 E2/6</td>
<td>Cal. Tech. Stock Centre</td>
<td>Bull 1966</td>
<td>$vg^D / In(2L)t In(2R)Cy Roi cn^2 bw or sp^2.$</td>
</tr>
<tr>
<td>$vg^S$</td>
<td>homozygote; lethal. heterozygote; wing nicks in some flies.</td>
<td>Df(2R) 49B12/C1–49F1/50A1</td>
<td>Cal. Tech. Stock Centre</td>
<td>Bridges, Morgan &amp; Schultz 1938</td>
<td>$vg^S / Cy L4 sp^2$</td>
</tr>
<tr>
<td>$vg^U$</td>
<td>homozygote; lethal. heterozygote; vestigial wings</td>
<td>In(2R) 49C1/2–50C/2</td>
<td>Cal. Tech. Stock Centre</td>
<td></td>
<td>$vg^U / In(2L)t In(2R)Cy Roi cn^2 bw or sp^2.$</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Genetic Location</td>
<td>Source</td>
<td>Notes</td>
<td></td>
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<tr>
<td>vg</td>
<td>homozygote; vestigial wings.</td>
<td>placed between 49C1/2-50C/2</td>
<td>Cal. Tech. Stock Centre</td>
<td>Bridges &amp; Morgan 1919, Fristrom 1968</td>
<td></td>
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<tr>
<td>Cbx</td>
<td>homozygote; heterozygote; homoeosis of wing → haltere.</td>
<td>89E1/2</td>
<td>Lewis, 1978, Morata 1975</td>
<td></td>
<td></td>
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<tr>
<td>bx&lt;sup&gt;34e&lt;/sup&gt;</td>
<td>homozygote; homoeosis of anterior haltere → anterior wing.</td>
<td>89D</td>
<td>Lewis, 1978, Morata &amp; Garcia-Bellido 1976</td>
<td></td>
<td></td>
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<tr>
<td>en&lt;sup&gt;L,1004&lt;/sup&gt;</td>
<td>homozygote; lethal abnormal segments in embryo.</td>
<td>48A</td>
<td>Lawrence, Nusslein-Volhard &amp; Wieschaus 1980</td>
<td></td>
<td></td>
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<tr>
<td>en&lt;sup&gt;I&lt;/sup&gt;</td>
<td>homozygote; anterior wing duplications.</td>
<td>48A</td>
<td>Lawrence &amp; Morata 1976</td>
<td></td>
<td></td>
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<tr>
<td>en&lt;sup&gt;A&lt;/sup&gt;</td>
<td>homozygote; lethal.</td>
<td>Df(2R)47D3-48B4/5</td>
<td>Gubb per. comm.</td>
<td></td>
<td></td>
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<tr>
<td>en&lt;sup&gt;B&lt;/sup&gt;</td>
<td>homozygote; lethal.</td>
<td>Df(2R)47E3-48B2</td>
<td>Gubb per. comm.</td>
<td></td>
<td></td>
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<tr>
<td>en&lt;sup&gt;30&lt;/sup&gt;</td>
<td>homozygote; lethal.</td>
<td>Df(2R)48A-48C6/8</td>
<td>Russell per. comm.</td>
<td></td>
<td></td>
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Fig. 1. (a) Drawings of the dorsal and ventral wing structures. (b) Drawing of the haltere structures. (Figure taken from Bownes & Seiler, 1977. Copyright Alan R. Liss Inc.)
Studies of the \( v_{g}^{W} \) mutation

Examination of imaginal discs

Imaginal discs were dissected from \( v_{g}^{W} \) heterozygotes at the beginning, middle and end of the third larval instar. They were stained with a Ringer's solution (Chan & Gehring 1971) containing neutral red and trypan blue which stains dead cells blue. They were then observed using Zeiss Nomarski Interference optics.

Cytological analysis of chromosomes

Salivary glands were dissected into 3:1 ethanol:propionic acid to fix them, the nuclei were stained with aceto-orcein, and they were squashed in 45% acetic acid to prepare the polytene chromosomes. The squash preparations were observed with Zeiss phase optics.

RESULTS AND DISCUSSION

(A) The phenotype of \( v_{g}^{W} \) wings and halteres

Identifying the phenotype of abnormal wings and halteres depends upon our detailed knowledge of the structure of the wild-type wing and haltere. These are shown as line drawings, Fig. 1 (taken from Bownes & Seiler, 1977) and as photographs in Figs 2a and 3a, and it will be essential to refer to particular structures shown in these figures throughout the text.

Homozygotes

When \( v_{g}^{W} \) heterozygotes are mated 27% of the embryos die prior to migration of nuclei to form the syncitial blastoderm, and no further lethality is observed prior to larval hatching. This is consistent with \( v_{g}^{W} \) homozygotes being lethal very early in development. We have not investigated whether any nuclear divisions occur in these embryos. Since a \( v_{g}^{W} \) egg fertilized by a wild-type sperm

<table>
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<th>ABBREVIATIONS USED THROUGHOUT FIGURES</th>
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<td>Wing abbreviations: PS, pleural sclerite; DC, dorsocentral bristles; PA, postalar bristle; Scu, scutellar bristles; SA, supraalar bristles; NP, notopleural bristles; ANWP, anterior notal wing process; Teg, tegula; AS 1–4, axillary sclerites 1–4; Sc 4, group of 4 sensilla campanoformia; AC, axillary cord; SC25, group 25 sensilla campanoformia; AL, alar lobe; PR, posterior row; DR, double row; TR, triple row; D Co., distal costa; M Co., medial costa; P Co., proximal costa; SC12, group 12 sensilla campanoformia; HP, humeral plate; UP, unnamed plate; PNWP, posterior notal wing process; PAA, prealar apophysis; YC, yellow club; PVR, proximal ventral radius; Sc4, group 4 sensilla campanoformia; Sc3, group 3 sensilla campanoformia; Sc5, group 5 sensilla campanoformia; AP, axillary pouch; PS, pleural sclerite; PWP, pleural wing process. WB, wing blade. VWS ventral wing surface; DWS dorsal wing surface.</td>
</tr>
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Haltere abbreviations: MS, metathoracic spiracle; S, scabellum; d, dorsal; v, ventral; C, capitellum; CS, capitelar sensilla; P, pedicel; PS, pedicellar sensilla; SS, scabellar sensilla; MP, metathoracic papillae; MB metathoracic bristle group.
Studies of the \( \text{vg}^\text{w} \) mutation

Table 2. The phenotype of \( \text{vg}^\text{w} \) at 25 °C

<table>
<thead>
<tr>
<th>Flies showing phenotype</th>
<th>(%)</th>
</tr>
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<tbody>
<tr>
<td>Haltere -&gt; wing homoeosis</td>
<td>83</td>
</tr>
<tr>
<td>Haltere absent</td>
<td>14</td>
</tr>
<tr>
<td>Haltere normal</td>
<td>1</td>
</tr>
<tr>
<td>Haltere abnormal</td>
<td>3</td>
</tr>
<tr>
<td>Wing-large posterior duplications (Classes 5 &amp; 6 Fig. 5)</td>
<td>51</td>
</tr>
<tr>
<td>Wing-large posterior duplications and small haltere-like patches of tissue</td>
<td>7</td>
</tr>
<tr>
<td>Wing-small posterior duplication (Classes 1–4 Fig. 5)</td>
<td>46</td>
</tr>
<tr>
<td>Duplications or bristle misarrangement within thorax</td>
<td>10</td>
</tr>
<tr>
<td>Total number of half flies scored</td>
<td>140</td>
</tr>
</tbody>
</table>

will develop normally, yet a similar egg fertilized by a \( \text{vg}^\text{IV} \) sperm dies early in embryogenesis, one might conclude that a gene product which is defective in \( \text{vg}^\text{IV} \) zygotes is required very early in development for normal embryogenesis to proceed. This would be an unusual example of an early zygotic effect on development, as in general, there is no evidence for new gene activity prior to cellularization of the blastoderm (Wright, 1970; Zalokar, 1976; Anderson & Lengyel, 1979; McKnight & Miller, 1976).

**Heterozygotes**

Flies heterozygous for \( \text{vg}^\text{w} \) show a number of morphological abnormalities. They often have missing halteres or the halteres are transformed into wing. The thoraces sometimes show bristle misarrangements and small local duplications. The penetrance of these phenotypic variations is given in Table 2, and examples of the phenotype are shown in Figs. 2–4. Some flies have abnormal legs, but this aspect of the \( \text{vg}^\text{w} \) phenotype has not yet been analysed in detail.

Rearing the flies at 18 °C or 29 °C does not significantly alter the types of defect observed at 25 °C. At 18 °C the wings tend to be smaller than at 25 °C, which is consistent with other \( \text{vg} \) mutants where the wings are larger when flies are reared at higher temperatures.

It is difficult to establish which regions of the haltere are transformed to wing, since no wing margin is observed (Fig. 4b). Very occasionally \( \text{vg}^\text{IV} \) flies show some metathorax transformation to mesothorax (Fig. 2b) suggesting that

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Fig. 2. \( \text{vg}^\text{w} \)/+ (a) Shows a fly with a relatively small wing duplication and homoeotic transformation of haltere to wing. (b) An example where there is some mesothorax in the metathoracic segment (ms). (c) Shows a fly with abnormal bristle orientations on the mesothorax.
Fig. 3. Types of wing duplications found in vg\(^{wa}\)/+ flies. (a) Wild-type wing. (b) Large wing duplication including posterior row and much of the wing blade. (c) Smaller wing duplication also including posterior row. (d) Small wing duplication extending to the alar lobe. (d) No wing blade, but the axillary cord is duplicated.
Fig. 4. Halteres. (a) Wild type. (b) vg\textsuperscript{W}/+ showing typical homoeotic transformation to wing. (c) vg\textsuperscript{W}/+; Cbx/+ showing that the homoeosis is repressed but that the structures on the anterior hinge marked X in (a) are no longer present. (d) vg\textsuperscript{W}/en\textsuperscript{L,304}. homoeotic transformation now includes posterior margin bristles.
anterior transformations similar to bithorax are occurring. The wings of \(vg^w\) flies never differentiate anterior margin structures, which may explain why they are not observed in the transformed halteres, since in other \(vg\) mutants both the wing and haltere are defective in similar positions. However, \(vg^w\) wings can differentiate posterior margin, yet these structures are never observed in the transformed halteres. It is likely then, that the homoeosis is genuinely similar to that caused by the bithorax mutation and restricted to the anterior compartment of the wing. However, because of the nature of the \(vg^w\) mutation, we cannot be sure of this, since it cannot be directly observed. The duplications observed in the posterior region of the wing were very variable in size, ranging from very large duplications, including the posterior row and most of the posterior wing blade (Fig. 3b–d) to extremely small duplications, showing only duplicated axillary cord and no wing blade (Fig. 3e). The structures of the ventral hinge are usually present in one copy and structures of the dorsal hinge are sometimes present, again only in one copy. The costa and anterior wing margin are never present. A classification scheme for the wing duplications was devised according to which areas of the wing are deleted, present once or duplicated. The structures actually present in 52 \(vg^w\) wings were scored. A fate map of the wild-type wing disc showing the cells which give rise to these structures is shown in Fig. 5a. The fate map and nomenclature is that of Bryant (1975). We then divided the wing up into regions and these are shown on the wing disc in Fig. 5b. The region marked X is a region which does not differentiate any bristles or identifiable cuticular markers and thus we cannot determine its behaviour in \(vg^w\). The regions selected were (1) ventral hinge, (2) dorsal hinge, (3) the groups of dorsal sensilla on the proximal dorsal radius, (4) the axillary cord, (5) the alar lobe and its associated wing blade, (6) the posterior row of bristles with associated wing blade and (7) the costa, anterior margin with triple and double row of bristles with associated wing blade. Thoracic defects were not included in this classification scheme. The wings were then divided into classes according to whether these regions were deleted, partially or completely, present once, or duplicated. Class 1 has one copy of region 1 and a duplicated region 4; all the other regions are deleted (Fig. 5c), Class 2 is similar to Class 1, except that region 5 is now also present and duplicated (Fig. 5d). Class 3 shows again one copy of region 1 and duplicated regions 4 and 5, but regions 2 and 3 are now also present in one copy (Fig. 5e). Class 4 is similar to 3 except that region 3 becomes duplicated (Fig. 5f). Class 5 is similar to class 3 except that region 6 now appears in duplicated form (Fig. 5g). Class 6 shows one copy of regions 1 and 2, and regions 3, 4, 5 and 6 are duplicated (Fig. 5h).

The general pattern, then, is that region 7, the anterior margin and costa, is always deleted. That region 1, in the ventral hinge, is always present, but never duplicated, and that the axillary cord (region 4) is always present and always duplicated. As the duplicated wings increase in size, the alar lobe and posterior row, regions 5 and 6, appear but are always duplicated, and the dorsal hinge,
region 1, appears but is never duplicated. Only the sensillae of the dorsal radius appear sometimes in one copy and sometimes duplicated.

It is possible that such a pattern could arise by cell death followed by duplication though it is difficult to explain why the ventral hinge would always fail to duplicate. The failure of vg to regenerate the anterior margin structures of region 7 as a result of the proposed cell death, would be expected, since vg has been shown to have a position-specific inability to produce anterior margin structures (Bownes & Roberts, 1981).

The distribution of wings falling into the various classes is shown in Fig. 5. It should be noted that duplications do not follow the compartment boundary in any of the classes observed.

A large amount of pupal lethality was observed in the vg stock. As the homozygous embryos appeared to die very early, this lethality was presumably due to the heterozygous mutation. The fate of 190 pupae was followed and 48% of them failed to emerge. Of these 40% died early and the other 60% formed pharate adults. Twenty of the pharate adults were mounted and the morphology of the 40 wings and halteres was examined in detail. Twenty-nine of the halteres showed transformations to wing and the remainder had no haltere. The transformations were similar to those observed in adults which emerged. The wing duplications fell into the same classes as those described for adults, but their distribution was different. Only 8% fell into classes 4 and 5 with large wing duplications, whereas 50% of the adults had been in these two classes. Thus those pupae which fail to emerge show a higher penetrance of the more extreme wing phenotypes.

The dominant effects of the vg mutation are very varied, causing pupal lethality, wing duplications and deletions and homoeosis of the haltere to wing. The mutation therefore has pleiotropic effects and disturbs many aspects of normal development.

\[(B)\] Development of vg wing and haltere discs

Studies of third instar vg wing discs were carried out to establish if any morphological defects could be observed and to see if cell death could be detected in the presumptive wing blade.

Haltere discs of vg were relatively normal in morphology, but were usually much larger than the wild-type haltere discs, presumably as a result of the increased cell number in the region transformed to wing.

The mature third instar vg wing discs were extremely interesting. Some were small discs with reduced presumptive wing blade areas (Fig. 6b) and presumably these give rise to the flies with very little or no wing blade. Other discs had very obvious duplications of the folds normally seen in a wild-type disc and presumably these develop into wings with large duplications. (Fig. 6c, d).

There was no indication of regions of cell death after trypan blue staining of discs although this technique will not detect individual dying cells.
Fig. 5. Classification of wing duplications (details of scheme used are in the text). □ = deleted region. ■ = some structures in this region are present in 1 copy. ✎ = some structures in the region are duplicated.
Mid-third- and early-third-instar larval wing discs were found showing the duplicated fold structure appearing (Fig. 6c, f) and again they showed no regions of cell death. All the discs shown in Fig. 6 had been stained for cell death.

These results are in marked contrast to findings with vg wing discs. These showed large areas of cell death in the presumptive wing blade region (Fristrom, 1968; Bownes & Roberts, 1981). Thus if the vg\textsuperscript{w} duplicated wing phenotype does result from regulation within the wing disc after cell death, this cell death must occur early in development.

(C) Are there mutations elsewhere in the vg\textsuperscript{w} stock contributing to the abnormal development of the flies?

As the vg\textsuperscript{w} stock showed so many defects in development and organization of the adult, it seemed possible that other mutations had been induced in the stock at the same time as the vg mutation, and that these were contributing to the phenotype of the stock.

We therefore systematically substituted various chromosomes from the stock, with known chromosomes and checked the phenotype.

The original stock supplied was vg\textsuperscript{w}/+. We produced balanced stocks, vg\textsuperscript{w}/SM1, and vg\textsuperscript{w}/In(2L)t In(2R)Cy Roi cn\textsuperscript{2} bw or sp\textsuperscript{2} to do these experiments.

The 1st chromosomes were substituted with Binscy/Binscy, and the Binscy; vg\textsuperscript{w}/SM1 stock still showed the same phenotype indicating that no mutations on the 1st chromosomes were involved.

The 3rd chromosomes were substituted with rucuca and TM3, to produce vg\textsuperscript{w}/+; rucuca/TM3 flies which again showed the typical vg\textsuperscript{w} phenotype indicating that the third chromosome was also not involved.

Finally, vg\textsuperscript{w} was recombined into a marked 2nd chromosome to substitute the second chromosome either side of the vg\textsuperscript{w} phenotype.

Thus all aspects of the vg\textsuperscript{w} are attributable to defective genes in the vg region of the second chromosome. We cannot, of course, rule out the possibility that there is more than one mutation in this region.

(D) Cytological analysis

As all aspects of the phenotypes were likely to be the result of one mutation, and vg point mutations cause only a reduction in size of the wing and haltere, or sometimes only wing nicks when heterozygous with other vg alleles, it seemed possible that vg\textsuperscript{w} may in fact be a deletion or chromosomal rearrangement.

Polytene chromosome squash preparations were made from the salivary glands of third instar vg\textsuperscript{w}/+ larvae. As can be seen in Fig. 7 there is a deletion or inversion from 47F/48A to 49C. Thus the affected region includes vg, bic, scaborous (sea) and some material which is also absent in some engrailed (en) deletions and thus the mutation may also include the en gene. Two en deletions from 47D3–48B4/5, Df (en)-A; and from 47E3–48B2, Df (en)-B were supplied by D. Gubb (Cytological Analysis unpublished data of D. Gubb). A further en
deletion from 48A–48C6/8 was supplied by M. Russell (cytological analysis unpublished data of M. Russell). Whether \( vg^w \) is a deletion or inversion will be established prior to the J.E.E.M. discussion (see page 74).

(E) Complementation studies with other \( vg \) mutations

The \( vg^w \) mutant was originally assigned to the \( vg \) locus because \( vg^w/vg \) heterozygotes showed no wings at all and showed an increased expressivity of the vestigial phenotype compared to either mutant alone (Shukla, 1980). There are several other deletions which include the \( vg \) gene and complementation studies with these deletions were undertaken.

The extent of these deficiencies is described in Table 1, and Fig. 8 shows which regions of the right arm of chromosome 2 are deleted by each mutation.

We found that \( vg^w/vg^c \) flies showed no wings and no halteres, but the thorax was still present. The \( vg^w/vg^D \) and \( vg^w/vg^B \) flies however, showed an even more extreme phenotype where the dorsal meso-thorax was often also deleted. These two genotypes were semi-lethal and many of the progeny died before eclosion. The \( vg^w/vg^S \) flies showed an intermediate phenotype between \( vg^w/vg \) and \( vg^w/vg^B \). In no case was any homoeosis observed since the haltere was completely deleted and for similar reasons it was not possible to detect any of the typical \( vg^w \) wing duplications.

Only the \( vg^w/vg^U \) flies were lethal and failed to emerge as adults. This is of some interest since \( vg^U \) is the only \( vg \) mutation with a dominant vestigial wing phenotype. Analysis of \( vg^U/+ \) flies showed that the wings lacked margin structures and were very similar in phenotype to \( vg \) homozygotes. No duplications of any structures were seen in the \( vg^U \) wings and no homoeosis was seen in the halteres. Thus, although it has a dominant vestigial phenotype, it does not resemble \( vg^w \) in other respects. However, we did analyse \( vg^U/+ \) late-third-instar wing discs and discovered that they showed no cell death in the wing blade region, which is an aspect of its development which is similar to \( vg^w \), rather than \( vg \).

As \( vg^w \) was not lethal in combination with either \( vg^B \), \( vg^C \), \( vg^D \) or \( vg^S \), yet \( vg^w \) and \( vg^C \) overlap by several bands, it seems likely that \( vg^w \) is an inversion rather than a deletion since the homozygous deletion which would be produced by \( vg^w/vg^C \) heterozygotes would be unlikely to be viable.

In combination with \( vg^U \) lethality of the \( vg^w/vg^U \) progeny was observed (\( vg^U \) is an inversion). When \( vg^U \) is combined with other \( vg \) deletions varying results are seen. The \( vg^U/vg^S \) flies survive and are fertile, the \( vg^U/vg^B \) and \( vg^D/vg^U \) flies survive, but are sterile and the \( vg^U/vg^C \) progeny are lethal before eclosion (Seiler & Bownes, unpublished). The \( vg^C \) deletion extends further to the left
Fig. 7. Polytene chromosomes of vg\textsuperscript{w}/+. (a) Whole of second right arm. (b) Detail of deleted or inverted region.
Fig. 8. Drawing of the region of chromosome 2 we have studied. Banding pattern was taken from *Journal of Heredity* 30 (1930, Bridges) and is typical of the salivary gland chromosomes of late third-instar larvae. (Copyright American Genetic Association). The figure also shows the extent of all the deletions studied (see also Table 1). Solid horizontal lines represent known deleted regions, broken horizontal lines may be deleted. $vg^U$ is an inversion. $vg^W$ may be an inversion.
along the chromosome than $v_g^B$, $v_g^D$ and $v_g^S$ and thus it seems that lethality in combination with $v_g^U$ occurs when the chromosome deletions clearly include the $v_g^U$ breakpoint of the inversion.

Another interesting point from these studies is that the cytological analysis suggests that $v_g^W$ and $v_g^B$ do not in fact overlap, yet they are both $v_g$ mutants. It seems likely that careful analysis of the position of the right-hand breakpoint of $v_g^W$ and the left-hand breakpoint of $v_g^B$ could give the precise location of the $v_g$ gene itself. It is possible that the cytological analysis will show that $v_g^W$ and $v_g^B$ do just overlap, but if they do not, they may actually split the $v_g$ gene.

(F) Does $v_g^W$ delete the engrailed gene

The left-hand breakpoint of the $v_g^W$ deletion overlaps with some deletions which include the *engrailed* (*en*) gene. *Engrailed* causes anterior structures to replace posterior structures in the wing a very different phenotype to $v_g^W$, where no anterior structures are observed and the posterior wing which is present is often duplicated. We are therefore trying to establish if $v_g^W$ also deletes the *en* gene. For this purpose we have obtained two *engrailed* deletions from Dr Gubb (unpublished, one *en* deletion, from Dr Russell (unpublished) *en* and a lethal *en* point mutation (*en* $^{L1304}$) produced by Nüsslein-Volhard & Wieschaus (1980). Unfortunately only *en* $^{L1304}$ had the appropriate markers for the final complementation crosses to be set up immediately (see Table 1). For the other four complementation crosses the other stocks in Table 1 are being constructed. The $v_g^W/en$ $^{L1304}$ flies survive to be adults initially suggesting that $v_g^W$ does not include *en*. However, the $v_g^W/en$ $^{L1304}$ flies show a slightly different phenotype to $v_g^W/+$ . The wings, although they show duplications, are more disrupted, some forming wings with holes in, with the dorsal and ventral wing blade fused around the hole, making a doughnut shape. Wing margin was often observed on the transformed halteres (Fig. 4d). Several flies with defective legs were also observed. It seems then, that $v_g^W$ and *en* $^{L1304}$ do interact. It should be noted that complementation tests with a dominant mutation never provide such clear cut results as one usually finds with recessive mutations. The best interpretation of this data would be that $v_g^W$ is an inversion with one breakpoint close to *engrailed*, but that the breakpoint does not split the *engrailed* gene.

Preliminary tests, using *Dfen* $^A$, *Dfen* $^B$ and *en* $^1$, without markers which enable us to distinguish $v_g^W/balancer$ chromosome, from $v_g^W/en$ suggest that $v_g^W/en$ $^1$, $v_g^W/Dfen$ $^A$ and $v_g^W/Dfen$ $^B$ do survive. This is indicated by the number of progeny with a $v_g^W$ type phenotype compared to other genotypes of progeny expected from each cross. There is no indication that there is lethality of a class of flies carrying $v_g^W$. In all three crosses there was a high proportion of flies with abnormal legs. There were more flies with duplications in the thorax than with $v_g^W/+$, but whether these were all $v_g^W/en$ flies or due to interactions with the genetic background is not yet known. Again these results suggest that $v_g^W$ is an inversion since it is not lethal when heterozygous with quite large *en* deletions.
Studies of the \textit{vg}^w \ mutation

The complementation crosses with \textit{en}^1 and the three \textit{en} deletions in marked stocks should help to decide if \textit{vg}^w does delete \textit{en} and to localise the \textit{en} gene by comparisons of the break points of the \textit{en} deletions and the \textit{vg}^w deletion. This data should be available at the J.E.E.M. meeting in three months' time where this manuscript will be presented (see page 74).

\textbf{(G) Interactions of \textit{vg}^w with \textit{Cbx}}

The mutation \textit{Contrabithorax} (\textit{Cbx}) causes dominant transformations of wing to halteres (Lewis, 1963) Fig. 9.a. It is 100\% effective in posterior parts of the wing, but has lower penetrance in more anterior wing regions (Morata, 1975). In combination with \textit{bx}, the \textit{Cbx} mutation prevents the homoeosis of halteres to wing, thus \textit{Cbx} clearly is acting on anterior wing structures in this case too, as \textit{bx} affects only the anterior halteres. We tested if \textit{Cbx} would be able to inhibit the homoeosis shown by \textit{vg}^w which would suggest that the homoeosis was occurring by similar genetic and metabolic pathways to \textit{bx}. The interactions of \textit{Cbx} with \textit{vg}^w was also investigated in the wing.

\textit{Cbx} completely inhibited the homoeosis in \textit{vg}^w. As seen in Fig. 4.c there is no wing blade in the capitellum. It is interesting that the haltere now formed is incomplete. The rows of sensillae found in the hinge are present but the hairy regions in the anterior margin of the Scabellum and pedicel are deleted. (Compare Fig. 4.a and Fig. 4.c.) This suggests that \textit{vg}^w does cause a failure of the anterior haltere to develop, as in \textit{vg}, and it is this wing which prevents the appearance of anterior wing margin in the transformed halteres.

The wing phenotype of \textit{vg}^w/+/\textit{Cbx}+/+ was extremely unusual and may provide insight into the mechanism of action of \textit{Cbx} and \textit{vg}^w. The \textit{vg}^w phenotype usually shows a duplication of the posterior wing, the 2nd posterior being in mirror-image symmetry to the original posterior and replacing anterior structures. When \textit{Cbx} is also present the original posterior is no longer seen. Presumably it is transformed to haltere but so little tissue is present that we cannot be sure about this. The duplicated posterior, lying in an anterior position is still present. This phenotype is shown in Figs. 9.b–e and was true of all \textit{Cbx}/+; \textit{vg}^w/+/wings. Thus \textit{Cbx} is clearly acting in the original posterior wing. Since we now have none of the original posterior wing tissue to compare with the duplicate, we cannot tell if it is acting to a small extent in the duplicated posterior, but clearly it does not simply act more effectively in 'posterior'-determined wing cells. It may be that \textit{Cbx} acts in a position-specific way, but other alternatives are also presented in the general discussion.

\textbf{GENERAL DISCUSSION}

The \textit{vg}^w deletion causes several different morphological defects in the wing and haltere of \textit{Drosophila melanogaster}. We cannot, at present, separate the various pleiotropic effects from one another. It would be interesting to know if
Fig. 9. Wings showing interactions with Cbx. (a) Cbx/+ wing and haltere. The posterior wing is transformed to haltere. (b)-(d) Are all Cbx/+; vg"+. (b) The duplicated posterior remaining is shown in relation to the thorax for orientation. (c) Detail of the posterior present in reversed polarity and in an anterior location. (d) Detail of the remaining structures in relation to the haltere. (e) Normal organization and orientation of the axillary cord and alar lobe. Particularly note the location of the AC and wing hinge (WH) in these figures.
smaller deletions or point mutations in that region could be found which cause just haltere-to-wing transformations, or just the wing duplications, or if many genes must be rearranged to produce this complex pattern of developmental abnormalities.

There are three aspects of the phenotype to be discussed. (1) The wing duplications of vg\(^w\) in relation to other vg mutations. (2) The relation of en mutations to vg\(^w\). (3) The interactions in the wing and haltere when both vg\(^w\) and Cbx are present.

(1) Wing duplications

It is not clear why vg\(^w\) wings show such specific patterns of duplication. The duplicate wing could arise by either incorrect specification during development of the disc by a trans-determination-like event, or by cell death and regulation phenomena. As the vg mutation shows cell death in the wing discs, the latter might be the most favoured explanation. However, third instar vg\(^w\) wing discs show no signs of cell death. It is possible that the dead cells are pushed into the lumen of the disc and therefore not detected by our staining technique though it is unlikely that we would never catch some cells before they are extruded. We shall test this by sectioning discs. The other dominant vg allele, vg\(^U\) also shows no cell death in late development of the wing discs, yet it does not duplicate.

It is possible that in both vg\(^U\) and vg\(^w\) the cell death occurs very early and in one case the nature of the wound healing is such that no duplication occurs. The phenotypes seen in vg\(^W\) wings are difficult to explain by early cell death and regeneration since ventral hinge structures never duplicate and they certainly should have time to do so. Early X-irradiation-induced cell death does lead to duplication of wing hinge (Postlethwait, 1975). Some further experiments may help to solve this problem. Regeneration studies on the vg\(^w\) disc will determine if it can duplicate hinge structure or ever regenerate anterior margin structures. Longer periods of culture of the wing discs in an adult abdomen prior to metamorphosis should enable us to determine if the regulation process is incomplete during differentiation \textit{in vivo} and extra growth allows duplication of the wing hinge.

We shall also induce clones of marked cells early in development. Comparison of the shape and size of these clones with those found in wild-type wings should help to establish if there is cell death early in the development of the vg\(^w\) wing discs. Similar studies were undertaken with the wingless (wg) mutation (Morata & Lawrence, 1977\textit{a}, 1978) where distal structures of the wing and haltere are replaced by a duplication of the thorax. In some ways vg\(^W\) is a slightly less extreme, but related phenotype to wg. In this case the clonal analysis provided no evidence for early cell death and regeneration so there is a precedent for mutations with large duplications arising by mechanisms other than cell death and regulation.
(2) The engrailed mutation

The *engrailed*\(^1\) (*en*\(^1\)) mutation causes anterior wing structures to appear in the posterior compartment of the wing. (Garcia-Bellido & Santamaria, 1972; Morata & Lawrence, 1975; Lawrence & Morata, 1976). The relationship of homeotic genes and compartments has been reviewed by Morata & Lawrence (1977b). It has been proposed that the insect is not just divided into segments, but is further divided into compartments or polyclones (Garcia-Bellido *et al.* 1973, 1976; Crick & Lawrence, 1975). These are groups of clonally related cells which remain segregated during differentiation. As development proceeds the polyclones are further subdivided into smaller compartments. In the wing the first clonal separation is into anterior and posterior, and subsequently there is a separation to dorsal and ventral within those compartments. Garcia-Bellido (1975) and Morata & Lawrence (1975) proposed that the process was controlled by selector genes and that when a group of cells divides into two, a given selector gene becomes activated in one polyclone and inactivated in the other. The evidence obtained with *en*\(^1\) suggested that it was the selector gene for anterior and posterior compartments and that the defective *en*\(^1\) gene product causes posterior cells to become partially anterior in phenotype. The 'leaky' nature of *en*\(^1\) was therefore held responsible for the failure of complete transformation of posterior to anterior wing. Thus the *en* gene has been built into some models for segmentation in *Drosophila* (Hayes, Girton & Russell, 1979; Deak, 1980). Several other *en* point mutations have since been found and some are embryonic lethals (Nüsslein-Volhard & Wieschaus, 1980) which disturb segmentation in the embryo. Various deletions have also been isolated in order to establish if *en* is a selector gene (Gubb, pers. comm.; Russell, pers. comm.) and the *vg*\(^W\) mutation described in this paper perhaps also includes *en*. If *en*\(^1\) were 'leaky', then one might expect a hemizygous *en*\(^1\) fly to show a more extreme phenotype. However, they show a weaker phenotype (Gubb, pers. comm.; Russell, pers. comm.). Given this information and the dominant effects of *vg*\(^W\), it is difficult to predict how *vg*\(^W\) and *en* would interact. The *vg*\(^W\)/*en*\(^{L1304}\) combination showed an interaction which led to some leg defects and slight modifications of the dominant *vg*\(^W\) phenotype but these flies did survive even though *en*\(^{L1304}\) is a homozygous lethal and thus it is unlikely that *vg*\(^W\) deletes or has a breakpoint in *en*. These facts taken together suggest to us and to D. Gubb that the *en* mutations produce novel gene products which disturb development either in the embryo or in leg and wing discs depending upon the allele present.

There are other mutations which cause wing duplications and the replacement of anterior structures by posterior structures, such as *apterous-blot* (Whittle, 1979) and *apt\(^r\)* (Roberts & Bownes, 1981), which have phenotypic features in common with *engrailed* but do not appear to behave as selector genes (Whittle, 1979). Perhaps *engrailed* is actually similar in action to *apt\(^{blt}\)*. A comparison of *apterous* wings with *engrailed*, *vg*\(^W\) and *wingless* wings, along with investigations
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of their interactions may help us to understand how the various mutations all interfere with the organisation of the wing in different, but specific ways.

The rudimentary (ru) mutants (which also have reduced wing) have been shown to be defective in pyrimidine biosynthesis (Norby, 1973; Falk, 1976). The vestigial mutants also have disturbed nucleotide synthesis (Silber, 1980). Analysis of the metabolic deficiencies of several wing mutants, including those described above, may show common mechanisms of action at the metabolic level.

(3) Interactions between vestigial\textsuperscript{w} and Contrabithorax

\textit{Cbx} is one of the dominant genes of the \textit{bithorax} series of homoecotic mutants which cause homoecotic transformations between the mesothorax, metathorax and the abdominal segments (Lewis, 1963, 1964, 1978). When heterozygous or homozygous, \textit{Cbx} causes transformations of the wing to haltere. This happens 100\% of the time in the posterior wing and less frequently, from 11\% - 91\% in various regions of the anterior wing and thorax when homozygous. It is thought that \textit{Cbx} is a mutation which damages a regulator site next to \textit{Ubx} (Lewis, 1978) and that as a result of this the \textit{bx}\textsuperscript{+} and \textit{postbithorax}\textsuperscript{+} (\textit{pbx}) gene products are derepressed and consequently over-produced in a constitutive fashion (Morata, 1975; Garcia-Bellido, 1977). The \textit{bx}\textsuperscript{+} gene product is required to produce anterior haltere and the \textit{pbx}\textsuperscript{+} gene product to produce posterior haltere. Thus \textit{bx} mutant genes cause a transformation of anterior haltere to anterior wing and \textit{pbx} mutant genes cause a transformation of posterior haltere to posterior wing (Lewis, 1978; Hayes \textit{et al.} 1979). \textit{vg}\textsuperscript{w} causes homoeosis of haltere to wing and determination of whether this is \textit{bx}-like or \textit{pbx}-like or both is confused by the fact that the \textit{vg}\textsuperscript{w} also prevents differentiation of margin structures, especially the anterior margin. \textit{Cbx} is able to repress the homoeosis found in the haltere as a result of the \textit{bx} and \textit{pbx} mutations. Since we have found that \textit{Cbx} can also repress the transformation of haltere to wing in \textit{vg}\textsuperscript{w} one could propose that \textit{vg}\textsuperscript{w}, by a related metabolic pathway to \textit{bithorax}, produces homoeosis of haltere to wing, but \textit{Cbx} over-produces \textit{bx}\textsuperscript{+} and \textit{pbx}\textsuperscript{+} in the haltere and thus the effect of \textit{vg}\textsuperscript{w} is overcome by a competitive type of interaction. \textit{Cbx} would also be expected to covert the \textit{vg}\textsuperscript{w} wing to haltere. We found that this was true in the posterior region, but that the duplicated posterior in an anterior location was not affected. The fact that \textit{Cbx} acted to transform only the original posterior, could be explained in several ways. It is possible that \textit{Cbx} acts in a position-specific, rather than a determination-specific fashion and thus, as in flies carrying the \textit{Cbx} mutation alone, cells in posterior locations are affected more than cells in anterior locations. This suggests that \textit{Cbx} could be acting after any cell death and duplication had occurred, or that no regulative processes are involved in the \textit{vg}\textsuperscript{w} wing duplications. An alternative is that the posterior region is transformed to haltere, but that during duplication it gives rise to wing. This kind of process has been observed in the discs of other homoecotic mutants (Adler, 1978), although since \textit{Cbx} is expected to affect the whole disc to some extent, it would
seem more likely that after cell death the Cbx posterior would regulate and produce cells which would differentiate into haltere. Cbx does sometimes act in the anterior of the wing and convert it to haltere, this may well still be happening when it is in combination with vg\textsuperscript{IV}, but since one no longer has the original posterior for size comparisons, we cannot detect this.

Studies of interactions of vg\textsuperscript{IV} with other homeoetics in the bithorax series may not only help us to understand the mechanism of action of vg\textsuperscript{IV}, but also the mode of action of some of the bithorax mutations.

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A summary of additional information on vg\textsuperscript{W} obtained between submission of the manuscript and presentation of the paper at the meeting

Cytological analysis (reference Results page 63)

We have now established that vg\textsuperscript{W} is an inversion with breakpoints at 47F 15-16(48A1-2 and at 49E4/49E5 (I should like to thank David Gubb, Mike Ashburner and Chris Redfern for independently confirming these breakpoints). The accurate position of the right-hand breakpoint shows that vg\textsuperscript{W} and vg\textsuperscript{B} do overlap and hence clarifies the situation discussed on page 68.

Crosses with engrailed mutants (reference Results page 69)

The vg\textsuperscript{W} flies were crossed to the engrailed strains, en\textsuperscript{1} Dfen\textsuperscript{a0} Dfen\textsuperscript{B}, Dfen\textsuperscript{4}, in such a way that the en/\textit{vg}\textsuperscript{W} progeny could be distinguished from the \textit{vg}\textsuperscript{W}/Balancer chromosome progeny by virtue of their eye colour. In all cases the \textit{vg}\textsuperscript{W}/en progeny survived. There was some variability in the phenotypes between crosses. For example, \textit{vg}\textsuperscript{W} × en\textsuperscript{1} often showed extra regions of thorax close to the wing hinge and the wings were often small; \textit{vg}\textsuperscript{W} × en\textsuperscript{30} often produced flies with haemolymph between the wing surfaces but with large wing blade areas; \textit{vg}\textsuperscript{W} × en\textsuperscript{B} often had very small wings with duplications and bristle rearrangements within the thorax, and finally \textit{vg}\textsuperscript{W} × en\textsuperscript{A} gave progeny with very abnormally shaped wings. However, in all cases these phenotypes were found amongst the \textit{vg}\textsuperscript{W}/Balancer and the \textit{vg}\textsuperscript{W}/en progeny and thus seem to result from interactions with the genetic background rather than being due to interactions of \textit{vg}\textsuperscript{W} and en.

These results indicate that \textit{vg}\textsuperscript{W} does not actually split the en gene at the lefthand breakpoint of the inversion. The breakpoints do suggest, however, that en is included in the inverted region and is thus in a new chromosomal location.
Implications of the extra information

The inversion present in \(vg^w\) is not lethal when heterozygous with large deletions covering its breakpoints, which suggests that the homozygous lethality of \(vg^w\) is not due to the genes split at the breakpoints of the inversion. Further mutations may lie just outside or within the inversion. It may be possible, however, to separate the lethality from the interesting dominant aspects of the \(vg^w\) phenotype. It is possible that some aspects of the \(vg^w\) phenotype are the result of new or untimely gene products which result from the activation of normally inactive genes or perhaps the constitutive activation of normally tissue-specific genes due to the new location of the genes in the genome. Inversions may bring genes into close proximity to different promoter sequences or place them in differently structured regions with respect to chromatin organisation, and thus alter their normal regulation.

REFERENCES


