The *ash-1*, *ash-2* and *trithorax* Genes of *Drosophila melanogaster* Are Functionally Related

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**ABSTRACT**

Mutations in the *ash-1* and *ash-2* genes of *Drosophila melanogaster* cause a wide variety of homeotic transformations that are similar to the transformations caused by mutations in the *trithorax* gene. Based on this similar variety of transformations, it was hypothesized that these genes are members of a functionally related set. Three genetic tests were employed here to evaluate that hypothesis. The first test was to examine interactions of *ash-1*, *ash-2* and *trithorax* mutations with each other. Double and triple heterozygotes of recessive lethal alleles express characteristic homeotic transformations. For example, double heterozygotes of a null allele of *ash-1* and a deletion of *trithorax* have partial transformations of their first and third legs to second legs and of their halteres to wings. The penetrance of these transformations is reduced by a duplication of the *bithorax* complex. The second test was to examine interactions with a mutation in the *female sterile (1)* homeotic gene. The penetrance of the homeotic phenotype in progeny from mutant mothers is increased by heterozygosity for alleles of *ash-1* or *ash-2* as well as for *trithorax* alleles. The third test was to examine the interaction with a mutation of the *Polycomb* gene. The extra sex combs phenotype caused by heterozygosis for a deletion of *Polycomb* is suppressed by heterozygosity for *ash-1*, *ash-2* or *trithorax* alleles. The fact that mutations in each of the three genes gave rise to similar results in all three tests represents substantial evidence that *ash-1*, *ash-2* and *trithorax* are members of a functionally related set of genes.

**GENES** of *Drosophila* which give rise to homeotic mutations can be classified according to whether they normally "function selectively in particular segments" or are "required in all segments" for the correct expression of those genes which do function selectively in particular segments (STRUHL 1985). Examples of the former class include genes of the *bithorax* complex (LEWIS 1978; BENDER et al. 1985; AKAM 1983; SANCHEZ-HERRERO et al. 1985) and the *Antennapedia* complex (DENELL et al. 1981; WAKIMOTO, TURNER AND KAUFMAN 1984; CARROLL et al. 1986; MAHAFFEY and KAUFMAN 1987; GLICKSMAN and BROWER 1988). Loss of function mutations in these genes only cause transformations in specific segments and the products of these genes are normally only expressed in those specific segments.

By contrast, mutations in the latter class of genes can cause transformations in all or nearly all of the segments. Mutations in *ash-1*, *ash-2* or *trithorax* can cause homeotic transformations affecting all of the imaginal discs (Table 1). The nature of most transformations caused by *ash-1*, *ash-2* or *trithorax* mutations is similar to transformations caused by mutations in the former class of genes which "function selectively in particular segments." So, an individual homozygous for a loss of function mutation in any one of the *ash-1*, *ash-2* or *trithorax* genes could be described as expressing a *proboscipedia* transformation (labial to leg/antenna), and an *arista-pedia* transformation (arista to tarsus), and a *Sex combs reduced* transformation (prothorax to mesothorax), and an *Ultrabithorax* transformation (metathorax to mesothorax), etc.

The similarity in the spectrum of homeotic transformations caused by mutations in *ash-1*, *ash-2* and *trithorax* and evidence that double mutations in *ash-1* and *ash-2* caused an enhanced phenotype led to the hypothesis that these genes represent a functionally related set (SHEARN, HERSPERGER and HERSPERGER 1987). Three independent lines of genetic evidence are presented here to support this hypothesis. The rationale of the genetic tests used is that if the products of these three genes are involved in the same cellular function then loss of function mutations in *ash-1* and/or *ash-2* should show similar interactions with mutations in other genes as have already been reported for loss of function mutations in *trithorax*.

**MATERIALS AND METHODS**

**Mutant stocks:** The *ash-1* (3-49; 76B-D) and *ash-2* (3-76; 96A) genes were originally identified in a screen for third chromosome, late larval/early pupal lethals which cause imaginal disc defects (SHEARN et al. 1971). The symbol *ash* is an acronym for the kinds of imaginal disc defects caused by different alleles of these two genes: discs absent, small, or homeotic. All of the *ash-1* and *ash-2* alleles used in this study have been previously described (SHEARN, HERSPERGER and HERSPERGER 1987). The *trithorax* (trx, 3-54; 88B) gene
is also on the third chromosome. It was originally identified by a mutation called Regulator of bithorax which was isolated by E. B. Lewis. The deletion of trithorax used in this study Df(3R)redPg3, was also isolated by E. B. Lewis. It deletes 88A10-88C2-3. The EMS induced allele, trx<sup>-</sup>, was isolated by Kennison and Tamkun (1988) as a dominant suppressor of the extra sex combs phenotype of a Polycomb mutation. Df(3R)P9 is a deletion of the entire bithorax complex (BX-C); Dp(3;1)P115 is the duplication segregant of the transposition Tp(3;1)P115 which includes the entire BX-C (Lewis 1978). The maternal-effect lethal mutation, fi(1)k<sup>h</sup>, was isolated by Gans et al. (1975) as a temperature-sensitive, female-sterile mutation. Its homeotic phenotype was described by Forquignon (1981). The fi(1)k<sup>h</sup> gene (1-21) is uncovered by Df(1)<sup>sm<sup>128</sup></sup> (Leevre and Johnson 1973) which deletes 7D1-7D5-6. The deletion of Polycomb used, Df(3L)Asc, was isolated by G. Jurgen and described by Haynie (1983) and Capdevila, Botas and Garcia-Bellido (1986). For a description of the mutations and balancer chromosomes used see Lindley and Grell (1968) and Lindley and Zimm (1985, 1986, 1987).

**Interaction crosses:** All cultures were maintained at 20° in 10-dram shell vials on a medium of cornmeal, autolized yeast, molasses, and agar with Tegosept added as a mold inhibitor. Each vial was seeded with a suspension of live yeast. All crosses were done at 20° except those with Df(3L)Asc, which were done at 27° in order to maximize the penetrance of the extra sex combs phenotype. As pointed out by Kennison and Tamkun (1988) and as observed in these studies the penetrance and expressivity of the transformations caused by the mutations studied is sensitive to growth conditions. To minimize this source of variability, a standard procedure was adopted for all crosses described here. Five females and five males were placed in a vial and transferred every 24 h for 4-10 days.

**Interactions of ash-1, ash-2 and trithorax mutations with each other:** All of the ash-1 and ash-2 mutations are on chromosomes with the recessive marker mutation red Malpighian tubules (red, 3-53.6). The deletion of trithorax, Df(3R)redPg3, is also a deficiency of the red gene. In crosses between flies which are heterozygous for these mutations and balancer chromosomes, the relevant double and triple heterozygous progeny can be recognized by the eye color caused by homozygosis for red. Comparisons of the numbers of such progeny with the numbers of their sibs heterozygous for balancer chromosomes is based that none of these mutations had a dramatic effect on viability even as double or triple heterozygotes (data not shown).

**Effect of BX-C gene dosage on penetrance of homeotic transformations:** Flies heterozygous for both ash-1<sup>1H<sup>60</sup></sup> and Df(3R)redPg3 have transformations of the metathorax to mesothorax which resemble those observed in BX-C mutants. To examine whether flies heterozygous for both a deletion of BX-C and either ash-1<sup>1H<sup>60</sup></sup> or Df(3R)redPg3 also expressed such transformations, ash-1<sup>1H<sup>60</sup></sup> and Df(3R)redPg3 heterozygotes were mated to flies of the genotype Df(3R)<sup>P9</sup>/Df(3R)<sup>P9</sup>. To examine the effect of a duplication of BX-C on the penetrance of homeotic transformations caused by heterozygosis for both ash-1<sup>1H<sup>60</sup></sup> and Df(3R)redPg3, females heterozygous for Df(3R)redPg3 were mated to males of the genotype Dp(3;1)P115; ash-1<sup>1H<sup>60</sup></sup>/red/ TM1 (derived from crossing Dp(3;1)P115; Df(3R)P115/TM1 females to ash-1<sup>1H<sup>60</sup></sup>red/TM3 males). The female progeny of the cross which are marked with red are heterozygous for both ash-1<sup>1H<sup>60</sup></sup> and Df(3R)redPg3 and have three doses of BX-C; the male progeny which are marked with red are also heterozygous for both ash-1<sup>1H<sup>60</sup></sup> and Df(3R)redPg3 but have two doses of BX-C.

**Interactions with a mutation in the fi(1)k<sup>h</sup> gene:** The effect of ash-1 and ash-2 mutations on the penetrance of homeotic transformations caused by maternal fi(1)k<sup>h</sup> homozygosis was compared to that previously described by Digan et al. (1986) for Df(3R)redPg3. Females hemizygous for fi(1)k<sup>h</sup> were generated by crossing females of the genotype Df(1)<sup>n128</sup>/Base<sup>1</sup> to males of the genotype fi(1)k<sup>h</sup>/Y. The hemizygous females [fi(1)k<sup>h</sup>/Df(1)<sup>n128</sup>] were mated to males with the genotype GI<sup>mutant</sup> where mutant stands for an allele of ash-1, ash-2, or trithorax or a deletion of BX-C. These males were generated by crossing GI/TM1 females to males heterozygous for an allele of ash-1, ash-2, or trithorax or a deletion of BX-C. For each cross the GI<sup>+</sup> progeny served as the control. This was necessary because, as can be seen in the control column of Table 4, even at 20° the penetrance of homeotic transformations in the progeny of mothers hemizygous for fi(1)k<sup>h</sup> varies and can be as high as 9%. For each mutation tested, the significance of the difference in penetrance between the sibling experimental and control flies was evaluated using the G-test (Sokal and Rohlf 1969).

**Interaction with a mutation in the Polycomb gene:** Female heterozygous for a deficiency which includes the Polycomb gene, Df(3L)Asc, were mated to males heterozygous for alleles of ash-1, ash-2, or trithorax. Male progeny were examined for the presence of sex comb teeth on their mid and hind legs using a stereomicroscope at 30X magnification. This method of analysis provides a conservative estimate of the degree of suppression, since a leg with a single sex comb tooth bristle is scored the same as one with a complete sex comb. The t-test was used to evaluate the significance of the difference between the mean number of legs with sex comb teeth per male heterozygous for the deficiency of Polycomb alone compared to those heterozygous for that deficiency and an allele of ash-1, ash-2, or trithorax.

**RESULTS**

**Phenotype of mutant alleles:** A wide variety of homeotic transformations are caused by mutations in the ash-1, ash-2, or trithorax genes (Table 1). Transformations affecting all of the imaginal discs have been recognized (Shearn et al. 1971; Ingham and Whittle 1980; Shearn 1980; Ingham 1981, 1985; Shearn, Hersperger and Hersperger 1987). There are only two differences in the variety of transformations caused by mutations in these genes. One difference is that none of the ash-2 mutations so far examined express the posterior wing to anterior wing transformation. The highest penetrance observed for this transformation among ash-1 mutations is 50% for ash-1<sup>1H<sup>10</sup></sup> homozygotes (Shearn, Hersperger and Hersperger 1987). Most other alleles of ash-1 do not express this transformation at all. This incomplete penetrance probably indicates that many of the ash-1 alleles that have been examined are leaky alleles. The fact that none of the alleles of ash-2 so far studied (Shearn, Hersperger and Hersperger, 1987; A. Shearn, unpublished observation) express this transformation of posterior wing to anterior wing may indicate that the alleles of ash-2 so far studied are also leaky alleles. The only other difference in the variety of transformations listed in Table 1 involves the trans-
formation of posterior abdominal segments to anterior abdominal segments. That transformation was only observed for the spontaneous, nonlethal allele, *trx*<sup>1</sup> (Ingham and Whittle 1980). Homozygous clones of lethal alleles of *trithorax* do not express such transformations in the abdomen (Ingham 1985) nor do homozygous clones of *ash-1* or *ash-2* lethal alleles (A. Shearn, unpublished observation).

**Interactions of *ash-1*, *ash-2* and *trithorax* mutations with each other:** If the similarity in the variety of homeotic transformations caused by mutations of *ash-1*, *ash-2* and *trithorax* reflects that the products of these genes are involved in the same cell function, then mutations in any one of these genes should enhance the phenotype caused by mutations in either of the other two genes. Previous studies indicated that such interactions did occur. Double heterozygotes of a weak allele of *ash-1*, (ash-<sup>1<sup>III-10</sup></sup>) and a deletion of *trithorax* showed a slightly enhanced phenotype (Capdevila and Garcia-Bellido 1981). Double homozygotes of *ash-1<sup>1<sup>III-10</sup></sup>* and either *ash-2*<sup>703</sup> or *ash-2*<sup>1803</sup> also expressed an enhanced phenotype (Shearn, Hersperger and Hersperger 1987). The results presented in Table 2 provide additional evidence of such interactions. Flies heterozygous for either *ash-1*<sup>RF605</sup>, a putative null allele, or *Df(3R)red*<sup>RF605</sup>, a deletion of *trithorax*, express no detectable transformations of halteres to wings or third legs to second legs (data not shown). Capdevila and Garcia-Bellido (1981) also found a low penetrance of such transformations (0–1%) among flies heterozygous for deletions of *trithorax*. However, among flies heterozygous for both *ash-1*<sup>RF605</sup> and *Df(3R)red*<sup>RF605</sup>, 52.4% or 21.8% (depending on the maternal genotype) express partial transformations of halteres to wings and/or partial transformations of third legs to second legs (Table 2). Examples of such transformations are presented in Figure 1. The haltere transformations are recognized by the presence of scutellar bristles on the metanotum and/or wing margin bristles on the capitellum. The third leg transformations are recognized by the presence of apical bristles on the distal tibia. Some double heterozygotes, 3.5% or 40.7% (depending on the maternal genotype), also express a partial transformation of first legs to second legs (prothoracic to mesothoracic). This transformation is most easily recognized by the presence, on first legs, of sternopleura and/or apical bristles which are characteristic of second legs. In males, this prothoracic to mesothoracic transformation also causes a reduced number of sex comb teeth on the basitarsus of first legs. Clearly, the degree of penetrance of the prothoracic to mesothoracic transformation compared to the metathoracic to mesothoracic transformation does not depend only on the zygotic genotype but also depends on the maternal genotype. For example, the penetrance of prothoracic to mesothoracic transformations is greater than the penetrance of metathoracic to mesothoracic transformations if *Df(3R)red*<sup>RF605</sup> is maternally derived (Table 2). This is evidence that there is a maternal as well as a zygotic component to the interaction between these genes.

Double and triple heterozygotes of other alleles of

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**TABLE 1**  
Homeotic transformations caused by mutations in the *ash-1*,  
*ash-2* or *trx* genes

<table>
<thead>
<tr>
<th>Transformation</th>
<th>Gene&lt;sup&gt;+&lt;/sup&gt;</th>
<th><em>ash-1</em></th>
<th><em>ash-2</em></th>
<th><em>trx</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Proboscis → leg and/or antenna</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antenna → leg</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Humerus → wing</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leg 1 → leg 2</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Posterior wing → anterior wing</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haltere → wing</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leg 3 → leg 2</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>abd 2-7 → abd 1</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Genitalia → leg and/or antenna</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>+</sup> <sup>+</sup> means that homozygous mutant alleles of this gene have been reported to cause the indicated transformation. Superscript indicates reference to the original data in footnotes.

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**TABLE 2**  
Penetrance of homeotic transformations in double and triple heterozygotes of *ash-1*, *ash-2* and *trx* mutations

<table>
<thead>
<tr>
<th>Genotype&lt;sup&gt;+&lt;/sup&gt;</th>
<th>No. flies</th>
<th>Percent transformation&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ash-1</em></td>
<td><em>trx</em></td>
<td><em>ash-2</em></td>
</tr>
<tr>
<td><strong>RF605</strong></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td><em>Df(3R)red</em>&lt;sup&gt;RF605&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td><strong>RF605</strong></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td><em>Df(3R)red</em>&lt;sup&gt;RF605&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td><em>703</em></td>
</tr>
<tr>
<td>+</td>
<td><em>Df(3R)red</em>&lt;sup&gt;RF605&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td><em>1803</em></td>
</tr>
</tbody>
</table>

<sup>+</sup> <sup>+</sup> Maternally derived genes are indicated in boldface.

<sup>+</sup> pro → meso = partial transformation of prothorax to mesothorax, i.e., leg 1 to leg 2; meta → meso = partial transformation of metathorax to mesothorax, i.e., leg 3 to leg 2 and/or haltere to wing.
**Figure 1.**—Partial metathoracic transformations in ash-

+/+ Df(3R)redP93 double heterozygotes. A, Arrow indicates trans-
formation of metathoracic notum to mesothoracic notum; sc indi-
cates scutellum of normal mesothoracic notum; cap indicates capi-
tellum of haltere. B, Arrow indicates transformation of capitellum to
wing blade (wb); dashed line indicates border between trans-
formed area and normal haltere. C, Third leg with apical bristle (ab) characteristic of second leg.

**TABLE 3**

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>pro meso meta meso</th>
<th>No. flies</th>
</tr>
</thead>
<tbody>
<tr>
<td>ash-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF605</td>
<td>+ + + Df(3R)P9</td>
<td>176</td>
</tr>
<tr>
<td>+ + + Df(3R)P9</td>
<td></td>
<td>140</td>
</tr>
<tr>
<td>+ + + Df(3R)redP9</td>
<td></td>
<td>223</td>
</tr>
<tr>
<td>+ + + Df(3R)redP9</td>
<td></td>
<td>172</td>
</tr>
<tr>
<td>RF605 + + + Df(3R)redP9</td>
<td></td>
<td>143</td>
</tr>
</tbody>
</table>

*Maternally derived genes are indicated in boldface.
† pro → meso = partial transformation of prothorax to meso-
thorax, i.e., leg 1 to leg 2; meta → meso = partial transformation of metathorax to mesothorax, i.e., leg 3 to leg 2 and/or haltere to
wing.
‡ Males derived from the cross of +/+; Df(3R)redP9/Bal females
to Dp(3:1)P115/Y; ash-1RF605 red/Bal males.
§ Females derived from the cross of +/+; Df(3R)redP93/Bal females
to Dp(3:1)P115/Y; ash-1RF605 red/Bal males.

gotes for the double mutant, ash-1III-10ash-2703 and
Df(3R)redP93. This is much higher than the sum of
the penetrance with ash-1RF605 alone (9.8) and
ash-2703 alone (15.6).

**Effect of BX-C gene dosage on the penetrance of
homeotic transformations:** Since trithorax mutations
cause homeotic transformations of the haltere and
third leg which are similar to those caused by muta-
tions in the Ultrabithorax gene of the BX-C (Lewis
1968; Ingham and Whittle 1980), several investi-
gators have looked for and found interactions be-
tween trithorax and Ubx mutations (Ingham 1980;
Capdevila and García-Bellido 1981; Capdevila,
Botas and García-Bellido 1986; Sató and Denell
1987). The effect of BX-C gene dosage on the
penetrance of homeotic transformations caused by
heterozygosis for ash-1RF605 and/or Df(3R)redP93 is
presented in Table 3. Among flies heterozygous for
ash-1RF605 and Df(3R)P9, which deletes the entire BX-
C, 14.8% express transformations from metathorax
to mesothorax, but only if the ash-1RF605 mutation
is derived from the mother. If the ash-1RF605 mutation
is derived from the father the penetrance is zero,
which indicates that this interaction depends upon an
ash-1 maternal-effect. Among flies heterozygous for
Df(3R)redP93 and Df(3R)P9 only 2.2% express trans-
fomrations from metathorax to mesothorax if the
Df(3R)redP93 mutation is derived from the mother. A
similar low penetrance was observed by Capdevila
and García-Bellido (1981) for flies deficient for
both trithorax and BX-C when the trithorax deficiency was derived from the mother. The penetrance of metamorphic to mesothoracic transformations in male flies with two doses of the BX-C but heterozygous for maternally derived $D_f(3R)red^{93}$ and paternally derived $ash-1^{RF605}$ is 26.7%, a value that is much higher than the penetrance in flies heterozygous for either maternally derived $D_f(3R)red^{93}$ (2.2%) or paternally derived $ash-1^{RF605}$ (0%) and hemizygous for BX-C. Moreover, 48.3% of these males heterozygous for maternally derived $D_f(3R)red^{93}$ and paternally derived $ash-1^{RF605}$ also express a partial transformation of first leg to second leg (pro $\rightarrow$ meso). The penetrance of both transformations is significantly reduced ($P < 0.005$ according to G-test) among sibling females heterozygous for maternally derived $D_f(3R)red^{93}$ and paternally derived $ash-1^{RF605}$ but which also have a duplication, i.e., three doses, of the entire BX-C. The difference in sex between these two classes of progeny is not primarily responsible for the difference in penetrance. This conclusion is based on analyzing the penetrance of these transformations among sibling males and females which were heterozygous for maternally derived $D_f(3R)red^{93}$ and paternally derived $ash-1^{RF605}$ and which had two doses of the BX-C (Table 2). Among 216 progeny, the penetrance of prothoracic to mesothoracic transformations was 40.7% and the penetrance of metamorphic to mesothoracic transformations was 21.8%. Neither of these values are significantly different ($P > 0.05$ according to G-test) than those for the males of that identical genotype reported in Table 3 (48.3% and 26.7%, respectively).

**Interactions with a mutation in the $fs(1)h^+$ gene:**
The $fs(1)h^+$ gene was originally identified by a recessive, temperature-sensitive, X chromosome mutation (originally called 1456 and now called $fs(1)h^+$) which at a restrictive temperature is both a maternal-effect lethal and a pupal lethal (GANS, AUDIT and MASSON 1975). It was discovered subsequently that progeny, derived from homozygous $fs(1)h^+$ mothers in which oogenesis occurred at an intermediate temperature (23°), exhibited a substantial frequency of missing halteres and/or third legs and a low frequency of homeotic transformations of the haltere to wing and third leg to second leg (FORQUIGNON 1981). If the progeny of homozygous $fs(1)h^+$ mothers were also heterozygous for a deletion of trithorax the frequency of metamorphic to mesothoracic homeotic transformations was markedly increased (FORQUIGNON 1981). The consequence of the interaction of the $fs(1)h^+$ maternal-effect and the trithorax zygotic effect can be interpreted either as an enhancement of the $fs(1)h^+$ maternal-effect resulting in an increased frequency of progeny expressing homeotic transformations or as an enhancement of the recessive trithorax mutation causing trithorax to act as a semidominant mutation.

As a criterion for showing that $ash-1$ and $ash-2$ mutations behave like trithorax mutations, alleles of $ash-1$ and $ash-2$ and double mutants of $ash-1$ and $ash-2$ have been tested for interactions with the $fs(1)h^+$ mutation. For comparison, a deletion of BX-C and an EMS-induced allele of trithorax were also tested. The penetrance of metamorphic homeotic transformations in $D_f(3R)red^{93}$ heterozygotes derived from $fs(1)h^+$ hemizygous mothers is just as great as in $D_f(3R)P9$ heterozygotes derived from $fs(1)h^+$ hemizygous mothers (Table 4). DIGAN et al. (1986) also observed a higher penetrance (43%) among $D_f(3R)red^{93}$ heterozygotes derived from $fs(1)h^+$ hemizygous mothers. Heterozygosis for an EMS-induced allele (tra$^{57}$) increases the penetrance but to a lesser extent (Table 4). The penetrance of these transformations in progeny derived from $fs(1)h^+$ hemizygous mothers is much higher than in progeny derived from $fs(1)h^+$ homozygous mothers.

Heterozygosis for $ash-1^{RF605}$ increases the penetrance of homeotic transformations in progeny derived from $fs(1)h^+$ hemizygous mothers to nearly the same extent as does heterozygosis for a deletion of BX-C or trithorax (Table 4). Heterozygosis for other $ash-1$ alleles ($ash-1^{TN402}$ and $ash-1^{III-10}$) also increases the penetrance of homeotic transformations in progeny derived from $fs(1)h^+$ hemizygous mothers, although to significantly lower levels ($P < 0.005$ according to the G-test) than does $ash-1^{RF605}$ (Table 4). Based on the phenotype of homozygous larvae, $ash-1^{TN402}$ is considered a less extreme loss of function mutation than $ash-1^{RF605}$ and $ash-1^{III-10}$ is considered a less extreme loss of function mutation than $ash-1^{TN402}$ (SHEARN, HERSPERGER and HERSPERGER 1987). So, the increase in penetrance appears proportional to the loss of $ash-1$ function.

Of the two $ash-2$ mutations that were tested as heterozygotes, only one, $ash-2^{1803}$, causes a small but significant increase in the penetrance of homeotic transformations in progeny derived from $fs(1)h^+$ hemizygous mothers (Table 4). Heterozygosis for the double mutant $ash-1^{III-10}.ash-2^{1803}$ causes a net increase in penetrance (experimental-control) of 38.8%. The sum of the net penetrance caused by $ash-1^{III-10}$ (24.0%) and the net penetrance caused by $ash-2^{1803}$ (6.3%) is 30.3%. So, the sum of the net penetrance caused by each mutation alone is less than the net penetrance caused by the double mutant. The net penetrance caused by heterozygosis for $ash-1^{III-10.ash-2^{1803}}$ (38.8%) is less than that caused by heterozygosis for $ash-1^{RF605}$ (52.2%). Heterozygosis for $ash-1^{III-10.ash-2^{1803}}$ causes a larval lethal phenotype indistinguishable from that
TABLE 4
Penetration of metathoracic to mesothoracic transformations in mutant heterozygotes derived from mothers hemizygous for fs(1)h^1

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Experimental [+/mutation]^*</th>
<th>Control [+/+G1]</th>
<th>Significance^e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>Allele</td>
<td>No. flies</td>
<td>Percent transformed</td>
</tr>
<tr>
<td>BX-C</td>
<td>Df(3R)P9</td>
<td>92</td>
<td>58.7</td>
</tr>
<tr>
<td>trx</td>
<td>E5</td>
<td>399</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td>Df(3R)red^{99}</td>
<td>511</td>
<td>58.5</td>
</tr>
<tr>
<td>ash-1</td>
<td>III-10</td>
<td>281</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>703</td>
<td>853</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td>1803</td>
<td>327</td>
<td>58.4</td>
</tr>
<tr>
<td>ash-2</td>
<td>703</td>
<td>226</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>1803</td>
<td>414</td>
<td>11.1</td>
</tr>
<tr>
<td>ash-1 and ash-2</td>
<td>III-10</td>
<td>240</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td>703</td>
<td>205</td>
<td>45.9</td>
</tr>
</tbody>
</table>

^* +/mutation indicates heterozygous for the mutant allele (in the mutation column.
^*** Indicates a probability of <0.005 (according to the G-test) that the difference between experimental and control is due to chance.

caused by homozygosis for ash-1^{RF605} (SHEARN, HERSPERGER and HERSPERGER 1987) which was interpreted as the null phenotype.

Interaction with a mutation in the Polycomb gene:
The dominant extra sex combs phenotype observed in adult males heterozygous for Polycomb mutations is sensitive to the gene dosage of trithorax. The extra sex combs phenotype of Pc^+/+ is suppressed by homozygosis for a deletion of trithorax and enhanced by homozygosis for a duplication of trithorax (CAPDEVILLA and GARCIA-BELLIDO 1981). As shown in Table 5, ash-1 and ash-2 mutations also suppress this phenotype. Control males, heterozygous for a deletion of the Polycomb locus, Df(3L)Asc, express an extreme extra sex combs phenotype when raised at 27°C. The mean number of legs with sex comb teeth/male fly was 5.8. Most of the males examined had sex comb teeth on all six legs and none had sex comb teeth on less than five legs. Normal males only have sex comb teeth on two legs, the prothoracic (or first) pair of legs. In males which are heterozygous for Df(3L)Asc and also heterozygous for ash-1^{RF605} this phenotype is almost completely suppressed. The average number of legs with sex comb teeth is reduced to 2.4, i.e., close to normal (Table 5). For comparison, in males heterozygous for Df(3L)Asc and also heterozygous for Df(3R)red^{99}, the mean number of legs with sex comb teeth is 2.1 (Table 5). An allele of ash-2, also significantly suppresses the extra sex combs phenotype but to a lesser extent than does either ash-1^{RF605} or Df(3R)red^{99}. Males heterozygous for both Df(3L)Asc and ash-2^{RF605} have an average of 4.7 legs with sex comb teeth.

TABLE 5
Suppression by mutant heterozygotes of the extra sex combs phenotype caused by a deletion of the Polycomb gene

<table>
<thead>
<tr>
<th>Genotype^a</th>
<th>No. of legs with sex comb teeth</th>
<th>Significance^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pc ash-1</td>
<td>+</td>
<td>115 5.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>111 2.4 ± 0.6</td>
</tr>
<tr>
<td>Df(3L)Asc</td>
<td></td>
<td>88 2.1 ± 0.4</td>
</tr>
<tr>
<td>Df(3L)Asc</td>
<td>+</td>
<td>110 4.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1803</td>
</tr>
</tbody>
</table>

^a Maternally derived chromosomes are in boldface.
^b **** indicates a probability less than 0.001 that the difference between the mean of the experimental and the mean of the unsuppressed control (5.8) is due to chance.

DISCUSSION

The ash-1, ash-2 and trithorax genes are functionally related: Mutations in ash-1 and trithorax cause similar homeotic transformations. Three lines of genetic evidence have been presented here which imply that the products of these genes are functionally related. One, recessive null alleles of these genes as double heterozygotes show a substantial penetrance of homeotic transformations whereas as single heterozygotes they show no transformations. Two, heter-
ozygosis for null mutations in ash-1 or trithorax increases the penetrance of the maternal-effect homeotic phenotype caused by fs(1)h'. Three, heterozygosis for null mutations in ash-1 or trithorax suppresses the extra sex combs phenotype caused by heterozygosis for a deletion of the Polycomb locus. The ash-1 and trithorax genes appear to be part of a functionally related set that has been called the trithorax set (Shearn, Hersperger and Hersperger 1987). The results presented here define the properties expected for mutations in other genes which belong to this set.

Mutations of ash-2 express a similar variety of homeotic transformations as leaky alleles of ash-1 or trithorax (Table 1). The evidence that ash-2 is a gene which belongs to the trithorax set is as follows. One, double homozygotes of ash-2^703 or ash-2^1803 and leaky alleles of ash-1 express a strongly enhanced phenotype (Shearn, Hersperger and Hersperger 1987). Heterozygotes of one of those alleles, ash-2^703, or of the double mutant chromosome, ash-1^11-10-ash-2^703, and a deficiency of trithorax show an increased penetrance of homeotic transformations (Table 2). Two, ash-2^1803 increases the penetrance of the maternal-effect homeotic phenotype caused by fs(1)h' (Table 4). Three, ash-2^1803 partially suppresses the dominant extra sex combs phenotype caused by a heterozygosis for a deletion of the Polycomb locus (Table 5). Thus mutations of ash-2 exhibit all three of the properties expected for mutations in a gene of the trithorax set. However, they do so to a lesser degree than does a null allele of ash-1 or a deletion of trithorax. This may indicate that none of the ash-2 alleles tested, including ten alleles for which no data has been presented here, are null alleles. Analysis of the phenotype of ash-2 homozygotes and trans-heterozygotes also led to the conclusion that none of the twelve ash-2 alleles examined are null alleles (Shearn, Hersperger and Hersperger 1987; N. Tripoulas, E. Hersperger and A. Shearn, unpublished observations).

Other genes of the trithorax set: Capdevila and Garcia-Bellido (1981) showed that a deficiency of trithorax suppresses the extra sex combs phenotype caused by a Polycomb mutation (Pc^3/+) and that a duplication of the wild-type allele of trithorax enhances the phenotype of Pc^3/+. Based on these observations, Kenison and Russell (1987) screened the autosomes for other loci with a dosage dependent effect on Polycomb mutations. They identified several regions of the genome, including the trithorax region, in which an extra wild-type copy enhances the extra sex combs phenotype of Pc^R/+. To identify the relevant genes in such regions, Kenison and Tamkun (1988) screened for mutations which act as dominant suppressors of Polycomb. They identified 13 previously unknown genes in addition to new alleles of trithorax and Sex combs reduced. It seems quite likely that some, if not all, of these genes belong to the trithorax set. Mutations in some of these 13 genes have already been found to increase the penetrance of the maternal-effect homeotic phenotype caused by fs(1)h' (J. A. Kenison, personal communication). Despite the large number of mutations recovered in the screens of Kenison and Tamkun, it is unlikely that all of the genes of the set have yet been identified. They did not, for example, recover any mutations in ash-1 or ash-2 either of which can suppress the extra sex combs phenotype, as shown by the data in Table 5. Interestingly, they did recover a mutation, called kohtalo, which complements the lethality of ash-1 mutations but which is in the same cytogenetic region as ash-1, 76B-D (Shearn, Hersperger and Hersperger 1987; J. A. Kenison, personal communication). A mutation isolated by Kenison which fails to complement the lethality of both ash-1 and kohtalo is a deletion from 76B1,2 to 76D5 (A. Martinez-Arias and M. Ashburner, personal communication); heterozygosis for this deletion increases the penetrance of the maternal-effect of fs(1)h' to near 100% (A. Shearn, unpublished observation).

How could the products of the trithorax set of genes be functionally related: There are, at least, two different ways in which the products of the trithorax set of genes could be functionally related. They could function catalytically in a linear pathway like the sex-determination pathway (McKeeown et al. 1987) or they could function stoichiometrically as subunits of a multimeric protein. According to either model, mutations in any one of the genes would give rise to similar phenotypes because, ultimately, a single product is affected. It is not yet possible to exclude either model. However, if the former model were correct, one ought to be able to predict the rank order of phenotypes caused by double mutations based on the severity of the phenotypes caused by the component single mutations. This expectation is based on the idea that each mutation would reduce the level of the ultimate product to a given extent. This was not possible for ash-1 and ash-2 mutants (Shearn, Hersperger and Hersperger 1987). Data presented here emphasizes this point. The single mutant ash-2^703 and the double mutant, ash-1^11-10-ash-2^703 cause much higher penetrance of first leg to second leg transformations when heterozygous with a deletion of trithorax [15.5% and 82.1% respectively (Table 2)] than does the single mutant ash-2^1803 or the double mutant ash-1^11-10-ash-2^1803 [2.9% and 5.6% respectively (Table 2)]. However, ash-2^1803 and ash-1^11-10-ash-2^1803 increase the penetrance of the maternal-effect of fs(1)h' more than do ash-2^703 or ash-1^11-10-ash-2^703 (Table 4). Thus in one test ash-2^703 behaves as the stronger allele, while in another test ash-2^1803 behaves as the stronger allele. This pattern slightly favors the latter model,
that the products of these genes are subunits of a multimeric protein and that the different activities of this protein are differentially sensitive to changes in each subunit.

The regulation of segment specific homeotic genes by the trithorax and polycomb sets of genes: The homeotic transformations caused by mutations in any one of the trithorax set of genes is similar to those caused by loss of function mutations in genes of both the bithorax and Antennapedia complexes. This similarity implies that the trithorax set of genes regulates those segment-specific homeotic genes. Indeed, the fact that the enhanced penetrance caused by heterozygosis for both ash-1 and Df(3R)redP93 is much stronger than the interaction of either mutation with a deletion of the BX-C, Df(3RP9), implies that the products of these genes don’t regulate BX-C function independently. Moreover, it is unlikely that each gene of the trithorax set regulates those segment-specific homeotic genes independently, because there appears to be so many genes in the set. Rather, this regulation may occur via the multimeric protein which is hypothesized to be the ultimate product of the trithorax set of genes.

There is another set of functionally related genes, the polycomb set, which regulates genes of the bithorax and Antennapedia complex (STRUHL and AKAM 1985; WEDDE, HARDING and Levine 1986; Glicksman and Brower 1988). The polycomb set includes at least ten genes: extra sex combs (STRUHL 1981); pleiohomeotic (Gehring 1970; Denell, Hummels and Girton 1989); Polycomb (Denell and Frederick 1983); polycombotic (Shearn, Hersperger and Hersperger 1978; M. Phillips and A. Shearn, manuscript in preparation); Polycomblike (Duncan 1982); polyhomeotic (Dura, Brock and Santamaria 1985); Posterio r sex combs, Additional sex combs, and Sex combs on midleg (Jurgens 1985); and super sex combs (Ingham 1984). Jurgens (1985) has estimated that this set may include 40 genes. Of particular interest is the fact that mutations in the trithorax set can suppress the phenotype of mutations in the polycomb set. Ingham (1983) showed that homeozigosis for a null allele of trithorax suppresses the embryonic phenotype caused by homeozigosis for a null allele of extra sex combs. Capdevila and Garcia-Bellido (1981) showed that a deletion of trithorax suppresses the extra sex combs phenotype of a Polycomb mutation. Data presented here show that mutations in ash-1 and ash-2 also suppress the extra sex combs phenotype of a Polycomb mutation (Table 5). Capdevila, Botas and Garcia-Bellido (1986) hypothesized that normal segment identity requires a balance between trithorax and the polycomb set of genes. Now that it appears the trithorax gene is only one member of a set of functionally related genes, their hypothesis could be revised to state that normal segment identity requires a balance between the products of the trithorax set of genes and the polycomb set of genes. Molecular studies of genes of the trithorax and polycomb sets should lead to an understanding of the mechanism by which the products of these two sets of genes regulate segment specific homeotic genes.

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