HOMOEOSIS IN DROSOPHILA: A NEW ENHANCER OF POLYCOMB AND RELATED HOMOEOTIC MUTATIONS

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ABSTRACT

A new recessive lethal mutation in *Drosophila melanogaster*, Enhancer of Polycomb [*E(Pc)*], and chromosomal deficiencies lacking this locus act as dominant enhancers of the Polycomb mutant syndrome in adults. Thus, although *E(Pc)*/+ flies are phenotypically normal, this locus is haplo-abnormal with respect to its effect on the Polycomb phenotype. Recombinational and deficiency mapping localize the *E(Pc)* locus on chromosome *2* proximally and very closely linked (~0.1 map unit) to the engrailed gene. *E(Pc)* enhances the expression of all Polycomb point mutations examined including that of a deficiency, indicating that this interaction does not depend on the presence of an altered Polycomb gene product. In several respects the mutations extra sex comb, lethal(4)29, and Polycomblike resemble those at the Polycomb locus. In the presence of *E(Pc)*, recessive alleles of extra sex comb and lethal(4)29 are rendered slightly pseudodominant, and the homoeotic effects of Polycomblike heterozygotes are also enhanced. However, *E(Pc)* does not affect the expression of dominant mutations within the Bithorax gene complex (*Cbx*) or Antennapedia gene complex ($Antp^{Ns}$, $Antp^{73b}$, $Antp^{Scx}$, $Antp^{EJW15}$, Scr^{Msc}) which give homoeotic transformations resembling those of the Polycomb syndrome. Available evidence from the study of adult phenotypes suggests that mutations at *E(Pc)* do not result in homoeotic changes directly but instead modify the expression of a specific set of functionally related homoeotic variants.

HOMOEOTIC mutations cause the replacement of one body part by another. For *Drosophila melanogaster*, a large number of homoeotic mutations affecting the adult fly have been described (see OUWENEEL 1976), and it has often been suggested that at least some of these lesions affect loci that normally act to control embryonic determinative decisions. Recent studies, beginning with the seminal work of E. B. LEWIS (1978), have demonstrated that the identities of metameric segments of larvae are modified by some homoeotic mutations. LEWIS'S (1978, 1981, 1982) studies have focused on the Bithorax gene complex (BX-C), a huge gene cluster located on the right arm of the third chromosome (*3R*). LEWIS has argued that derepression of particular sets of BX-C genes establishes the identities of segments posterior to the mesothorax for both embryonic tissues and imaginal primordia. Mutations within the Antennapedia complex (ANT-C), a gene cluster located in proximal

3R, are associated with homoeosis or other developmental abnormalities of more anterior segments of embryos and adults (LEWIS *et al.* 1980a,b; WAKIMOTO and KAUFMAN 1981).

A number of other homoeotic mutations disrupt determinative decisions within the apparent developmental domains of the BX-C and ANT-C. One example, Polycomb (*Pc*), is a haplo-insufficient locus on the left arm of the third chromosome (PURO and NYGREN 1975; DENELL 1978). Adult flies heterozygous for dominant mutant alleles at this locus show variable partial homoeotic transformations of meso- and metathoracic legs to prothoracic leg and of antenna to leg (LEWIS 1947; HANNAH-ALAVA 1958), as well as wing to haltere (LEWIS 1978), ventral to dorsal wing (S. TIONG, personal communication) and anterior to posterior abdominal segments (LEWIS 1978; CAPDEVILA and GARCIA-BELLIDO 1981; DUNCAN and LEWIS 1982). Lethal embryos homozygous for *Pc* mutations exhibit homoeotic abnormalities of head segments and posterior transformations of thoracic and abdominal segments (LEWIS 1978; DUNCAN and LEWIS 1982; DENELL and FREDERICK 1983). Studies of mutant phenotypes and interactions with BX-C mutants and duplications indicate that Polycomb mutations alter BX-C expression; one possibility which has been suggested is that the Polycomb$^+$ gene product acts as a repressor of BX-C genes (LEWIS 1978; DUNCAN and LEWIS 1982; CAPDEVILA and GARCIA-BELLIDO 1981). As will be discussed, mutations at extra sex comb (*esc*), lethal(4)29 (*l(4)29*), and Polycomblike (*Pcl*) share many characteristics with Polycomb variants; these may also result in altered BX-C function.

In this report, we describe a second chromosome locus where mutations dramatically enhance the adult phenotype of Polycomb heterozygotes. In addition, such mutations also enhance *esc*, *l(4)29* and *Pcl* but do not affect the expression of any BX-C or ANT-C variants thus far examined.

MATERIALS AND METHODS

Many of the mutations utilized in these experiments are described in Table 1. Other mutations and chromosomes are described by LINDSLEY and GRELL (1968) or referenced in the text. Flies were raised on standard corn meal-yeast-molasses-agar medium at 25° and were usually anesthetized with ether and inspected under a dissecting stereomicroscope. With respect to the Polycomb syndrome, transformations of meso- and metathoracic legs to prothoracic leg and fourth to fifth tergite were scored in males, and transformations of antenna to leg and wing to haltere were scored in both sexes. To assess the degree of leg transformation, the number of sex comb bristles on meso- and metathoracic legs was counted; the extent of other transformations was scored with respect to arbitrary classes. For the wing to haltere transformation, wings with wild-type morphology were assigned a score of 0, those with a slightly serrated posterior margin were scored 1, those with a small amount of haltere tissue appearing at the posterior margin of the wing but not beyond the intersection of the fifth vein and posterior cross vein were scored 2 and those with large patches of haltere tissue extending beyond this intersection were scored 3. Classes of antenna to log transformation were scored according to the criteria of POSTLETHWAIT and SCHNEIDERMAN (1971), in which a wild-type phenotype is assigned a score of 1 and a maximally transformed appendage is given a 7. Both the wing and antennal transformations show a much higher penetrance and expression in females than in males, and, thus, the female data are presented in Table 2. Results for males showed apparent enhancement for the same genotypes as females but were associated with fewer significant differences, presumably due to the lower degree of expression. The appearance of pigmented patches on the anterior fourth tergite of males is diagnostic of a

Genetic variants utilized in this study

Locus	Location	Variant	Adult phenotype[a]	Chromosomal morphology[b]	Reference
Antennapedia	3-47.7	*Antp*^{73b}	Antenna to leg (het)		KAUFMAN, LEWIS and WAKIMOTO (1980)
		Antp^{Ns}	Antenna to leg (het and homo)		DENELL (1973)
		Antp^{EfW15}	2nd and 3rd to 1st leg (het)		LEWIS *et al.* (1980b)
		Antp^{Scx}	2nd and 3rd to 1st leg (het)		DENELL (1973)
Contrabithorax	3-58.8	*Cbx*^{1}	Wing to haltere (het and homo)		LEWIS (1978)
engrailed	2-62.0	*en*^{1}	Posterior to anterior compartment in wing and other structures (homo)		LINDSLEY and GRELL (1968)
		en^{LA7}	Lethal (homo)		KORNBERG (1981)
		Df(2R)en^{28}	Lethal (homo)	*Df(2R)47B3;47B9-14+ In(2R)47B9-14;48A1-2+ Df(2R)48A1-2;48B-C1*	EBERLEIN (1982)
		Df(2R)en^{30}	Lethal (homo)	*Df(2R)48A3-4;48C6-8*	EBERLEIN (1982)
		Df(2R)en^{A}	Lethal (homo)	*Df(2R)47D3;48A4-5*	BOWNES and ROBERTS (1981)
		Df(2R)en^{B}	Lethal (homo)	*Df(2R)47E3-6;48B2*	BOWNES and ROBERTS (1981)
		Df(2R)en^{SFX31}	Lethal (homo)	*Df(2R)48A;48B5*	KORNBERG (1981)
		T(2;3)en^{SFX37}	Lethal (homo)	*T(2;3)46C1;48A;84F*	KORNBERG (1981)
		T(2;3)Es	Lethal (homo)	*T(2;3)48A-B;84E*	THE SEATTLE-LA JOLLA DROSOPHILA LABS (1971)
extra sex comb	2-54.9	*esc*^{1}	2nd and 3rd to 1st leg (homo)		LINDSLEY and GRELL (1968)
lethal(4)29	4-(within *Df(4)G*)	*l(4)29*	2nd and 3rd to 1st leg and other effects (homo)		GEHRING (1970)
Polycomb	3-48	*Pc*^{1}, *Pc*^{3}, *Pc*^{R1}	2nd and 3rd to 1st leg and other effects (het)		LEWIS (1947); LEWIS (1980); S. TIONG (unpublished)
		Pc^{T3}	2nd and 3rd to 1st leg (homo)		S. TIONG (unpublished)
		Df(3L)Pc^{MK}	2nd and 3rd to 1st leg (het)	*Df(3L)78A3;79E1-2*	R. E. DENELL (unpublished)
Polycomblike	2-84	*Pcl*^{1}, *Pcl*^{W6}	2nd and 3rd to 1st leg and other effects (het)		I. M. DUNCAN (1982), J. WILLIAMS (unpublished)
		Df(2R)11B	2nd and 3rd to 1st leg and other effects (het)	*Df(2R)54F6-55A1,2;55C1-3*	I. M. DUNCAN (1982)
		Df(2R)Pcl^{W5}	2nd and 3rd to 1st leg and other effects (het)	*Df(2R)55A-B;55C*	J. WILLIAMS (unpublished)
		In(2R)Pcl^{W4}	2nd and 3rd to 1st leg and other effects (het)	*Df(2R)55A;57A*	J. WILLIAMS (unpublished)
Sex comb reduced	3-47.7	*In(3R)Src*^{Msc}	1st to 2nd leg, and 2nd and 3rd to 1st leg (het)	*In(3R)84B1-2;84F*	LEWIS *et al.* (1980b)

[a] For each phenotype, the abbreviation in parentheses indicates whether the variant is in homozygous (homo) or heterozygous (het) condition.
[b] Normal if not described.

TABLE 2

Effects of various second chromosome genotypes on adult Pc *expression*

Second chromosome	Polycomb syndrome				
	MS to PRO leg	MT to PRO leg	Wing to haltere	Antenna to leg	4th to 5th tergite
en^{28}	6.50 ± 2.11**	4.02 ± 2.03**	2.68**	1.84**	2.04**
Control	2.12 ± 1.60	0.91 ± 1.04	1.06	1.08	1.04
en^{SFX31}	7.41 ± 2.75**	4.96 ± 2.81**	2.06**	2.04**	1.04**
Control	1.99 ± 2.21	0.76 ± 1.29	0.56	1.02	0.36
$^{a}en^{A}$	5.40 ± 2.16**	2.48 ± 1.59**	2.32**	1.42**	1.38**
Control	3.73 ± 2.42	1.76 ± 1.89	1.12	1.00	0.70
$^{a}en^{B}$	8.82 ± 2.59**	6.79 ± 3.44**	2.66**	2.70**	1.32**
Control	4.86 ± 2.79	2.59 ± 2.39	1.12	1.04	0.78
en^{30}	6.63 ± 2.79**	4.22 ± 2.30**	1.58**	1.00	0.92
Control	3.70 ± 2.36	1.45 ± 1.61	0.94	1.00	1.04
en^{SFX37}	4.86 ± 2.20**	2.65 ± 1.76**	1.12	1.02	0.60
Control	2.90 ± 2.03	1.55 ± 1.50	1.02	1.00	0.62
en^{LA7}	1.73 ± 1.52**	0.56 ± 0.74*	1.62*	1.02	1.16
Control	1.04 ± 1.12	0.44 ± 0.57	1.30	1.02	0.98
en^{1}	4.16 ± 3.06*	2.05 ± 2.36**	0.62	1.00	0.34
Control	3.49 ± 2.97	1.54 ± 1.62	0.84	1.00	0.42
E(Pc)	10.19 ± 1.46**	7.52 ± 2.60**	2.70**	2.04**	1.74**
Control	6.85 ± 2.04	3.89 ± 1.95	1.02	1.00	0.88
Sp L Pu Pin	0.60 ± 0.98	0.31 ± 0.52	0.68*	1.00	0.70
Control	0.49 ± 0.88	0.28 ± 0.49	0.36	1.00	0.78

In each cross, Pc^{3}/*TM3, Sb* females were mated with males bearing various second chromosomes over *SM5, Cy*. Progeny heterozygous for the indicated maternal second chromosome and for Pc^{3} were scored with respect to the homoetic transformations presented (see MATERIALS AND METHODS) relative to *SM5*/+; Pc^{3}/+ siblings (controls). Each number represents the mean (± standard deviation for leg transformations) of 50 flies; for legs all 100 were scored, but for other structures only the 50 leftsides were second. MS = mesothoracic; PRO = prothoracic; MT = metathoracic.

[a] These chromosomes are certain recombinants between the *Sp L Pu Pin* chromosome and chromosomes bearing either $Df(2R)en^{A}$ or $Df(2R)en^{B}$.

* $0.05 > P > 0.01$ for tested second chromsome *vs.* control.

** $P < 0.01$.

fourth to fifth tergite transformation. Fourth tergites with a wild-type pattern of pigmented cuticle were assigned a score of 0, whereas homoeotically transformed tergites were given scores of 1, 2 or 3 when pigment covered less than one-half, more than one-half and the entire tergite, respectively. Statistical comparisons between mutant classes and appropriate controls varied depending on the parameter being considered. The numbers of meso- or metathoracic sex comb bristles were compared using a *t*-test, whereas the degrees of other transformations were compared using the nonparametric Kolmogorov-Smirnov test.

In mapping experiments, *Sp Bl* L^{rm} Pu^{2} Pin^{B} (CRAMYER 1980)/*E(Pc)*, $Df(2R)en^{30}$ or $Df(2R)en^{B}$ females were mated to *E(Pc)*/*In(2LR)SM5*, al^{2} *Cy* lt^{v} cn^{2} sp^{2} males. Male progeny receiving the *SM5* chromosome were scored for the presence of *Sp, L, Pu* and *Pin* (*Bl* could not be accurately scored) and were backcrossed to *E(Pc)*/*SM5* females to determine whether a lesion failing to complement *E(Pc)* for viability was present. Male progeny recognized as carrying a recombinant chromosome were tested by scoring the progeny from a further cross to Pc^{3}/*In(3LR)TM3, ri* p^{p} *sep Sb* bx^{34e} e^{s} to determine whether that chromosome enhanced the Polycomb syndrome.

RESULTS

In a preliminary experiment to screen a large number of heterozygous autosomal deficiencies for those modifying the expression of $Pc^3/+$, we found that a chromosome bearing *Df(2R)en*^{28} markedly enhanced all aspects of the Polycomb adult syndrome examined (Table 2). This deficiency also enhanced the expression of the less extreme alleles Pc^1 and Pc^{R1} (data not shown). As will be discussed, this enhancement showed specificity, as the deficiency did not modify the expression of ANT-C and BX-C mutations sharing phenotypic similarities with Polycomb.

Df(2R)en^{28} deletes the homoeotic engrailed (*en*) locus. To determine whether enhancement of the adult Polycomb phenotype is associated with this deficiency (as opposed to being localized elsewhere on the deficiency-bearing second chromosome) and to assess the involvement of the engrailed locus itself, we examined the effect of various other rearrangements and engrailed point mutations on Polycomb expression (see Table 2 and Figure 1). Previous studies

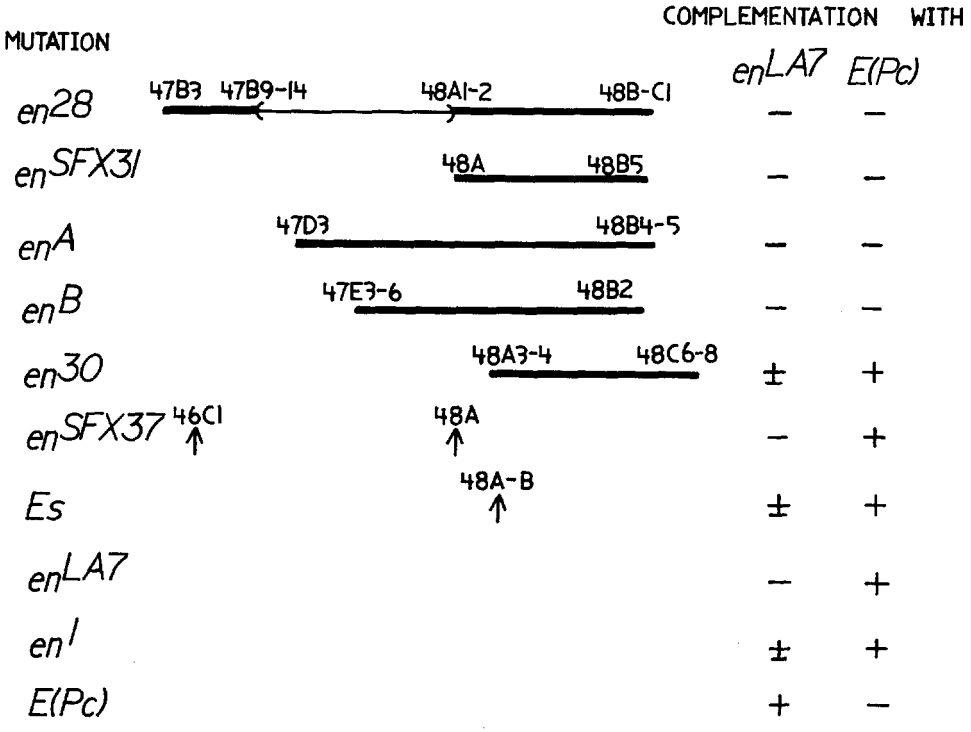

FIGURE 1.—The breakpoints within polytene chromosome region 47–48 associated with *en* and *E(Pc)* are indicated, as well as the results of complementation tests for these variants with en^{LA7} and *E(Pc)*. For the complementation tests, + indicates that heterozygous combinations were viable and morphologically normal, ± indicates they were viable but showed an interruption of the fourth wing vein and − indicates that they were lethal. Thick solid lines show the extent of chromosomal deficiencies, whereas the inversion is indicated by parentheses, and translocation breakpoints are indicated by arrows.

of Polycomb have shown that mutant expression appears to depend upon culture conditions and background genotype (HANNAH-ALAVA 1964; DENELL 1973). In Table 2 the level of expression among control classes varied from cross to cross, presumably due to uncontrolled background genotype; this interpretation is supported by the observation that reciprocal crosses gave similar results (data not shown). Thus, statistical comparisons were made only between experimental and control sibs. Deficiencies en^{SFX31}, en^{A} and en^{B} resembled en^{28} in enhancing all aspects of the Polycomb syndrome tested. In contrast, $Df(2R)en^{30}$ significantly enhanced only the extra sex comb phene and wing to haltere transformation, and $T(2;3)en^{SFX37}$ enhanced the extra sex comb phenotype exclusively. Engrailed mutations en^{1} and en^{LA7} displayed little or no enhancing effect. (The apparent effects of en^{LA7} and en^{1} on expression of the extra sex comb effect and that of $Df(2R)en^{30}$ on the wing transformation, although statistically significant, are of such a small magnitude in relation to the effect of various background genotypes that we do not believe them to be biologically meaningful.) Finally, *T(2;3)Es* [associated with a dominant neomorphic mutation at the engrailed locus (B. BAKER, personal communication)] showed low viability in combination with Pc^{3}, but those flies recovered displayed no Polycomb enhancement. The observation that no engrailed lesion affects Polycomb expression in a manner comparable with engrailed deficiencies indicates the existence of a separate, tightly linked, haplo-insufficient locus responsible for this behavior.

RUSSELL and EBERLEIN (1979) isolated three ethylmethanesulfonate-induced recessive lethal mutations that failed to complement $Df(2R)en^{28}$ for viability but did complement one another and en^{1}. When we tested these mutations in heterozygous condition, one of them, designated as Enhancer of Polycomb [*E(Pc)*], markedly enhanced Polycomb expression. In the absence of a Polycomb mutant allele, *E(Pc)*/+ flies were phenotypically normal. In doubly heterozygous flies, this mutation enhanced all aspects of the Polycomb syndrome in a manner comparable to the deficiencies described earlier (Table 2). The *E(Pc)*-bearing second chromsome is not associated with a detectable polytene chromosome rearrangement.

Flies that are Pc^{3}/Pc^{3}/+ (DUNCAN and LEWIS 1982) or pharate adults that are Pc^{2}/Pc^{T2} (S. TIONG, personal communication) may display a strong transformation in which both the anterior and posterior compartments of the mesothoracic leg and the anterior compartment of the metathoracic leg are prothoracic in nature. By comparison, the leg transformations of *E(Pc)*/+; Pc^{3}/+ are less extreme; the transformation appears to be restricted to more distal segments (tibia and tarsus) of the meso- and metathoracic legs in most (if not all) cases. Although the anterior compartment of these segments of the mesothoracic leg is strongly transformed, the lack of prominant markers for the corresponding posterior compartment makes it unclear whether homoeosis also occurs there. With respect to the wing transformation, haltere tissue usually appears only within the posterior compartment, although such homoeotic tissue develops in the anterior wing compartment in rare cases. Finally, although in some cases doubly heterozygous males show fourth tergites which are nearly

completely pigmented (and thus resemble fifth tergites), they do not display posterior transformations of other abdominal tergites to the extent described by DUNCAN and LEWIS (1982) for $Pc^3/Pc^3/+$ males. Thus, although heterozygosity for *E(Pc)* acts as a strong enhancer of Polycomb expression, the severity of the resulting phenotype does not reach that of flies bearing two copies of the antimorphic mutant allele Pc^3 (DUNCAN and LEWIS 1982).

DENELL (1978) showed that flies heterozygous for a deficiency including the Polycomb locus, constructed from elements of Y;autosome translocations, display the homoeotic leg transformation; the haplo-abnormal nature of this locus has been confirmed by DUNCAN and LEWIS (1982). In the former work, it was not clear whether other aspects of the syndrome are also expressed by deficiency heterozygotes. To determine whether the effect of *E(Pc)* requires an interaction with an altered product of the Polycomb locus, we examined the effect of this modifier on flies heterozygous for $Df(3L)Pc^{MK}$, a new deficiency of the locus; the results are presented in Table 3. With one exception, all aspects of the syndrome are clearly enhanced. These data provide no convincing evidence of an antenna to leg transformation in either the control or in $E(Pc)/+$; $Df(3L)Pc^{MK}/+$ flies, thus calling into question whether this particular aspect of the syndrome is expressed in a haplo-abnormal manner. Based on the enhancement of the haplo-abnormal phenotype observed in this experiment, we can conclude that the effect of *E(Pc)* is locus rather than allele specific.

Localization of E(Pc): Figure 1 summarizes the results of experiments to localize *E(Pc)* further by complementation and deficiency mapping. As expected, this mutation is lethal when heterozygous with the same deficiencies that enhance all aspects of the Polycomb syndrome. However, *E(Pc)* is viable in heterozygous combination with other rearrangements and point mutations affecting the engrailed locus. Although these data indicate that *E(Pc)* is a locus separate from engrailed, we must still consider why $Df(2R)en^{30}$ and $T(2;3)en^{SFX37}$ enhance the leg transformation phenotype of Polycomb mutations. For en^{30} a recombination experiment was performed to determine whether the behavior of this rearrangement is influenced by linked modifiers. Second chromosomes from $en^{30}/Sp\ L\ Pu\ Pin$ females were recovered balanced with *SM5* and tested to determine whether they carried the deficiency and whether they affect Polycomb expression (Table 4). [A preliminary experiment demonstrated that the *Sp L Pu Pin* chromosome does not carry Polycomb modifiers (Table 2).] Among 147 recombinants analyzed, enhancement of the extra sex comb effect was found to be strictly correlated with the presence of $Df(2R)en^{30}$. Likewise, the conclusion that this deficiency does not enhance the antennal and tergite effects of the Polycomb syndrome was confirmed for all recombinants tested. (The possible effect of this deficiency on wing transformation was too subtle to be assessed with the qualitative scoring used here.) We believe that $Df(2R)en^{30}$ [and probably $T(2;3)en^{SFX37}$ as well] modifies Polycomb expression because of a concomitant partial inactivation of the $E(Pc)^+$ locus. In heterozygous combination with other engrailed mutations, en^{30} acts as a leaky *en* allele (EBERLEIN 1982), and a similar effect on the nearby $E(Pc)^+$ locus is reasonable.

TABLE 3

Effect of E(Pc) *on the expression of* Df(3L)Pc^{MK}

Progeny genotype	Polycomb syndrome				
	MS to PRO leg	MT to PRO leg	Wing to haltere	Antenna to leg	4th to 5th tergite
E(Pc)/+; Df(3L)Pc^{MK}/+	6.30 ± 2.39**	3.73 ± 2.22**	1.50**	1.05	1.95**
SMF5/+; Df(3L)Pc^{MK}/+	1.63 ± 1.73	0.78 ± 1.33	0.05	1.00	0.50

The parental cross was *Df(3R)Pc^{MK}/TM3* females times *E(Pc)/SM5* males. Twenty male and 20 female progeny of each genotype indicated were scored for the severity of various homoeotic effects as described in the MATERIALS AND METHODS. MS = mesothoracic; PRO = prothoracic; MT = metathoracic.

** $P < 0.01$ for *E(Pc) vs. SM5* progeny.

TABLE 4

Results of experiments to map E(Pc) *and deficiencies of this locus by recombination*

Interval	Standard map distance[a]	Variant mapped		
		Df(2R)en^{30}	*Df(2R)en^{B}*	*E(Pc)*
Sp-E(Pc)	40.0[b]	16.5	14.6	16.6
E(Pc)-L	10.0[b]	5.5	3.8	7.8
L-Pu	25.0	20.6	14.2	21.9
Pu-Pin	10.3	17.2	16.0	11.6
Total scored		272	212	397

The map distances presented were derived by testing the offspring of females bearing a *Sp L Pu pin* chromosome heterozygous with the indicated *E(Pc)* variant.

[a] Taken from LINDSLEY and GRELL (1968).

[b] Standard distances for *en* rather than *E(Pc)* are presented.

Moreover, observations of weak Polycomb phenotypes indicate that the leg transformation is the aspect most sensitive to enhancement by the *E(Pc)* mutation.

To map the Enhancer of Polycomb locus and to confirm that the recessive lethality and effect on Polycomb expression are due to a single mutation, recombinant chromosomes from *E(Pc)/Sp L Pu Pin* females were recovered and individually tested for their ability to complement *E(Pc)* for viability and to modify Polycomb expression. Table 4 presents the mapping results with respect to the lethal effect of *E(Pc)*, *en^{30}* and *en^{B}*. As expected all three variants map between *Sp* and *L*. Although the *Sp* to *E(Pc)* distance appears to be considerably underestimated, the similarlity of the three sets of data suggests that this effect depends on the dominant markers utilized. The recessive lethality and enhancement of Polycomb effects of *E(Pc)* were completely correlated in all 205 recombinant chromosomes examined.

To map the relative positions of engrailed and Enhancer of Polycomb, we crossed *E(Pc)/en^{LA7} L^{rm}* females to *Df(2R)en^{28}/SM5* males and performed the reciprocal cross as a control. Among 10,271 progeny from the control cross, two second chromosomes (one *L* and one *L^{+}*), viable heterozygous with the

deficiency, were recovered; presumably, these exceptions arose by reversion of en^{LA7} and *E(Pc)* or by male crossing over. The chromosome bearing a putative reversion of *E(Pc)* did not enhance any aspect of the adult Polycomb syndrome, which is consistent with our interpretation based on other data that both lethality and enhancement are due to a single genetic lesion. Among 13,167 progeny recovered from the experimental cross, 15 were viable heterozygous with the deficiency. Twelve of these were L^+, indicating the *E(Pc)* lies to the left of engrailed. The others, which were *L*, presumably represent convertants or revertants of en^{LA7} or tandem duplications which resulted from unequal crossovers flanking the *E(Pc)-en* interval (GELBART and CHOVNICK 1979). Thus, Enhancer of Polycomb maps approximately 0.1 units proximal to engrailed (the estimated distance depends on whether one wishes to adjust the data for possible exceptions). This value is consistent with our interpretation that *E(Pc)* is a locus separate from but close to engrailed.

Interactions with other homoeotic mutations: We have also examined the effect of heterozygosity for $Df(2R)en^{28}$ or *E(Pc)* on the expression of other mutations giving adult homoeotic effects in common with Polycomb. The expression of two dominant ANT-C mutations associated with antenna to leg transformations ($Antp^{NS}$ and $Antp^{73b}$) and three causing leg transformations ($Antp^{Scx}$, $Antp^{EfW15}$ and Scr^{Msc}) was not enhanced by the *E(Pc)* mutation or deletion. Likewise, the expression of Contrabithorax (*Cbx*), a dominant BX-C mutation causing wing to haltere transformation, was not affected by either lesion. Three other BX-C genotypes (Ubx^1/+ heterozygotes and bx^{34e} or *abx* bx^3 *pbx* homozygotes (see LEWIS 1981)) were also unaffected.

In contrast, mutations at three other loci which share phenotypic similarities with Polycomb were enhanced. *esc* and *l(4)29*, both recessive mutations, showed a slight pseudodominant meso- and metathoracic leg to prothoracic leg transformation in the presence of *E(Pc)*/+; the proportion of males penetrant for this trait was 3.4% for *esc*/+ and 6.8% for *l(4)29*/+. In this respect, these homoeotic lesions resembled Pc^{T3}, a recessive hypomorphic allele (S. TIONG, personal communication), which showed a leg transformation in 7.2% of *E(Pc)*/+; Pc^8/+ males examined.

Dominant mutations or deficiencies of the haplo-abnormal Polycomblike gene also share adult phenotypic effects with Polycomb mutants (DUNCAN 1982). We examined the effect of *E(Pc)*/+ on the meso- and metathoracic leg to prothoracic leg transformation and fourth to fifth tergite transformation of males which were heterozygous for several Polycomblike mutations and deficiencies; these data are presented in Table 5. For every comparison, the extent of the Polycomblike homoeotic transformation is significantly enhanced relative to $E(Pc)^+$ control siblings. A control cross shows that Pcl^{W6}/*Sp L Pu Pin* and Pcl^{W6}/*SM5* males are quite similar in their extent of homoeotic transformation, indicating that the *SM5* chromosome is not itself a modifier of Polycomblike expression.

DISCUSSION

We have presented evidence that a recessive lethal mutation, *E(Pc)*, acts as a dominant enhancer of the adult homoeotic syndrome associated with Polycomb

and some other mutations. Recombinational and deficiency mapping localize this mutation on chromosome 2, proximal and very closely linked to the engrailed locus. We interpret the data presented to indicate that the Enhancer of Polycomb locus is haplo-abnormal and that *E(Pc)* is probably an amorph. For the antenna to leg, wing to haltere and tergite phenes, heterozygosity for *E(Pc)* or for deletions lacking this locus enhances Polycomb to a similar extent, whereas other included recessive lethal mutations or nondeficient engrailed alleles have no such effect. For the leg transformations the comparison between the deficiencies and *E(Pc)* is complicated by the observation that some chromosomes bearing engrailed lesions [$T(2;3)en^{SFX37}$ and $Df(2R)en^{30}$] are also associated with an enhancement. If, as we have argued earlier, inactivations of engrailed itself have little or no effect on the Polycomb leg transformation, then, we can again conclude that the effect of *E(Pc)* and of deletions of the locus are equivalent.

The effect of the *E(Pc)* mutation appears to be locus specific rather than allele specific. Heterozygosity for *E(Pc)* enhances the expression of all Polycomb alleles tested, including Pc^{T3} (a hypomorphic recessive allele), Pc^3 (an extreme antimorphic allele, DUNCAN and LEWIS 1982) and $Df(3L)Pc^{MK}$. In this respect, the only anomalous observation is the failure of $E(Pc)/+$; $Df(3L)Pc^{MK}/+$ flies to express an antennal transformation, even though $E(Pc)/+$ $Pc^1/+$ flies (which are quite similar for other aspects of the syndrome) clearly express that homoeotic phenotype (T. SATO, unpublished observation). At least for all other aspects of the Polycomb syndrome considered, we can conclude that enhancement does not depend on a direct interaction with a mutationally altered Polycomb gene product.

Developmental role of Enhancer of Polycomb: One current hypothesis as to the regulation of genes that control segment-specific cuticle markers is that "... the differentiation of most or all body segments is under the direct control of genes located in the BX-C and ANT-C" (DUNCAN and LEWIS 1982). However, mutations at loci elsewhere in the genome are also associated with homoeotic phenotypes similar to lesions within these complexes. In some cases it has been suggested that these other loci code for products regulating the BX-C (and possibly the ANT-C); alternatively, these gene products may play other roles necessary for normal determinative events or mutations may disrupt developmental events such that homoeoesis results indirectly. An examination of the effect of BX-C wild-type gene dosage on the Polycomb syndrome strongly suggests that this mutation does result in altered BX-C expression, and LEWIS (1978) argued that Polycomb$^+$ codes for a direct negative regulatory molecule acting on genes within this complex (see also DUNCAN and LEWIS 1982; CAPDEVILA and GARCIA-BELLIDO 1981). Alternatively, STRUHL (1981) and DENELL and FREDERICK (1983) have suggested that Polycomb mutations disrupt the normal transmission of determined states. LEWIS (1978; DUNCAN and LEWIS 1982) and CAPDEVILA and GARCIA-BELLIDO (1981) have further suggested that the normal gene product of Regulator of bithorax acts as a positive regulator of the BX-C.

TABLE 5

Comparison of male sibs bearing E(Pc) *or an* E(Pc)$^+$ *balancer for the degree of homoeotic expression of heterozygous mutations or deficiencies*

Genotype	Polycomblike syndrome		
	MS to PRO leg	MT to PRO leg	4th to 5th tergite
Df(2R)11B/E(Pc)	1.24 ± 1.11**	0.38 ± 0.63**	1.24**
Df(2R)11B/SM5	0.30 ± 0.70	0.04 ±0.20	0.40
Df(2R)Pcl^{W5}/E(Pc)	1.28 ± 1.02**	0.56 ± 0.75*	1.32**
Df(2R)Pcl^{W5}/SM5	0.52 ± 0.73	0.32 ± 0.58	0.32
In(2R)Pcl^{W4}/E(Pc)	2.48 ± 1.30**	1.38 ± 1.07**	1.64**
In(2R)Pcl^{W4}/SM5	1.50 ± 1.39	0.64 ± 0.93	0.60
Pcl^{W6}/E(Pc)	2.72 ± 1.60**	0.68 ± 0.73**	1.56**
Pcl^{W6}/SM5	0.92 ± 0.89	0.24 ± 0.43	1.00
Pcl^{1}/E(Pc)	0.46 ± 0.64**	0.20 ± 0.49*	1.00**
Pcl^{1}/SM5	0.18 ± 0.48	0.04 ± 0.20	0.56
Pcl^{W6}/Sp L Pu Pin	0.30 ± 0.57	0.00 ± 0.00	0.18
Pcl^{W6}/SM5	0.42 ± 0.70	0.14 ± 0.35	0.24

In each case, *E(Pc)/SM5* females were crossed to males bearing a Polycomblike lesion and a second chromosome balancer, and 25 males of each genotype were scored for leg and fourth tergite transformations as described previously. A control is also included, in which *SP L Pu Pin/SM5* females were crossed to *Pcl^{W6}/SM5* males. MS = mesothoracic; PRO = prothoracic; MT = metathoracic.

* $0.05 > P > 0.01$ for *E(Pc) vs. SM5* sibs.

** $P < 0.01$.

In terms of its phenotypic effect and interaction with the BX-C, the Polycomb locus is by no means unique. Mutations at the *esc* locus (STRUHL 1981) and *Pcl* locus (DUNCAN 1982) share some adult and larval phenotypic effects with Polycomb variants, and these authors suggest that these loci also disrupt BX-C expression. In addition, zygotes homozygous for *l(4)29* and originating from heterozygous mothers are cuticularly normal as larvae (J. WILLIAMS and R. E. DENELL, unpublished observations) and die as pharate adults displaying a homoeotic syndrome similar to the mutations cited before (GEHRING 1970; DUNCAN and LEWIS 1982; DUNCAN 1982). When ovaries are transplanted from homozygous female larvae into *l(4)29*$^+$ hosts, homozygous zygotes resulting from these implants die as late embryos that express a lethal syndrome similar in many features to those of *Pc* and *esc* (J. WILLIAMS and R. E. DENELL, unpublished observations). Thus, *l(4)29* has a maternal effect, as do Polycomb (DENELL 1982) and extra sex comb (STRUHL 1981); no evidence for a maternal effect of Polycomblike has been observed (DUNCAN 1982). Mutations at the Regulator of bithorax-trithorax locus are also associated with a maternal effect (CAPDEVILA and GARCIA-BELLIDO 1978, 1981; INGHAM and WHITTLE 1980).

In the context of these not yet well-understood developmental interactions, we must now consider the effects of mutations that modify the degree of

homoeotic expression. Some modifiers appear to be nonspecific in that they affect the expression of both homoeotic and nonhomoeotic variants. Examples of this sort include the RNA polymerase II mutation Ultrabithorax-like (MORTIN and LEFEVRE 1981) and the class of genetic variants known as Minutes, which modulate the expression of a number of homoeotic mutations (SINCLAIR, SUZUKI and GRIGLIATTI 1981; S. R. DALEY and R. E. DENELL, unpublished observations). In contrast, *E(Pc)* appears thus far to modify the expression only of homoeotic mutations and to do so with some specificity. In addition to *Pc* mutations, *E(Pc)* enhances the homoeotic transformations of the phenotypically similar variants *esc*, *l(4)29* and *Pcl* as well. This result further supports the conclusion that these loci are functionally related. However, no evidence exists that indicates *E(Pc)* directly modifies the expression of mutations in the BX-C or ANT-C associated with homoeotic transformations resembling Polycomb. This result suggests that *E(Pc)* does not modify mutations at Polycomb and related loci because it is itself a homoeotic variant. Such a suggestion is consistent with the normal cuticular phenotype of adult *E(Pc)* heterozygotes and of lethal homozygous late embryos and larvae (T. SATO, P. H. HAYES, and R. E. DENELL, unpublished observations). The effect of *E(Pc)* to enhance some adult phenotypes of mutations at the Polycomb, Polycomblike and lethal(4)29 loci is not dependent on the presence of an altered product of these genes, because *E(Pc)* enhances the phenotypes of $Df(3L)Pc^{MK}$, *Df(2R)11B*, $Df(2R)Pcl^{W5}$ and *l(4)29* (an apparently amorphic allele, DUNCAN 1982). Either the normal role of Polycomb and the other loci depends on the presence of the $E(Pc)^{+}$ gene product or else the developmental perturbation(s) resulting from the *E(Pc)* mutation somehow exacerbates the homoeotic effect of these variants.

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