THE INTERACTION OF TWO COMPLEX LOCI, ZESTE AND BITHORAX IN *DROSOPHILA MELANOGASTER*[1]

T. C. KAUFMAN, S. E. TASAKA AND D. T. SUZUKI

Department of Zoology, The University of British Columbia, Vancouver, B.C., Canada

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ABSTRACT

It has been found that certain alleles of the zeste locus (z^a 1–1.0) have no phenotype of their own, but interact with certain alleles at the bithorax locus (*bx* 3–58.8). This interaction takes the form of an enhancement of the homeotic *bx* phenotype to a more extreme form—i.e., the metathorax is transformed into mesothorax in varying degrees depending on the *bx* allele used. This enhancement is somewhat reminiscent of the transvection effect described by LEWIS (1954). The characterization of the interaction thus far has shown that the enhancement only effects *bx* alleles which arise spontaneously, whereas the origin of the z^a allele is unimportant. The gene claret nondisjunctional was used for the production of gynandromorphs which showed that the enhancing ability of z^a, like the eye pigment change caused by *z*, is autonomous. The enhancement of one specific allele (bx^{34e}), which is temperature-sensitive, has allowed a delineation of the temperature-sensitive period of the bithorax locus to a period extending from the middle of the second larval instar to the middle of the third larval instar. These results, as well as those of other enhancer and suppressor systems in Drosophila, have revealed the possibility of the involvement of heterocyclic compounds in the control of cell determination and fate in *Drosophila melanogaster*.

LEWIS (1954) described a phenomenon in *Drosophila melanogaster* which he called the "transvection effect". It was discovered by an enhancement of the *bx* phenotype when a break occurred in the right arm of the third chromosome between the centromere and the bithorax locus (*bx* 58.8 salivary chromosome bands 89E 1–4). He found this enhancement to be a rather good method for detecting breakpoints in this region of the genome and most flies expressing the enhanced phenotype could be shown to have the predicted breakpoint, with one notable exception. This exception was an inversion in the *X* chromosome (*In(1)e(bx)*) with breakpoints at 3A and 4F. Later a point mutant of the enhancer of bithorax ($e(bx)^2$) locus was recovered (LEWIS 1959) and by recombination studies it was localized to 1.0 on the *X* chromosome.

At approximately the same time, GANS (1953) was carrying out experiments on a newly discovered locus, *zeste* (*z* *X*-1.0). Her studies showed that this new locus affects eye pigmentation only in females but apparently has no effect in males. This dimorphism is not due to sex *per se* but to the number of functional

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(i.e., non-mutant) white loci present in the genome. That is, the zeste locus requires two functional white loci for its own mutant phene to be expressed. The properties of the zeste locus have been studied further in development (CHEN 1948; BECKER 1960); in recombination (JUDD 1961; GREEN 1959b,c, 1963); and in variegating systems (GREEN 1967). GANS (cf LINDSLEY and GRELL 1968) also discovered another allele at the *z* locus which she called z^a and assumed it to be a subliminal allele at the *z* locus. In their study of band to gene relationships, JUDD, SHEN and KAUFMAN (1972) attempted to localize all of the existing genes in the *zeste white* interval and found that LEWIS's *e (bx)* locus was exposed by certain deficiencies which also exposed the known loci, giant (*gt*), technical knock out (*tko*), zeste (*z*) and lethal (*l*) zeste to white-1 (*l(1)zw1*). When complementation was tested between these four loci and *In(1)e(bx)*, it was found that all of the combinations were wild type with regard to enhancement of *bx* but that *In(1)e(bx)/z* yielded yellow-eyed (zeste) females. This finding led to the following study, in which it was discovered that *e(bx)* is actually an allele of zeste—more specifically an allele of the z^a type.

In order to study this interaction more intensely, we have carried out an extensive test of complementation between several alleles of the *z* and *bx* loci including several new alleles at each locus. We have also characterized the interaction of *z*, z^a and other *z* alleles with various alleles of the white and white ocelli (*wo*) loci. *wo* is a third chromosome mutant which has been shown to interact with *z* (RAYLE 1969). Further, various combinations of *z*, z^a, etc., and *bx*, *Ubx*, etc., have been made to study the interaction itself. Finally, the combined system has been studied developmentally for cellular autonomy by the use of gynandromorphs and temporally by the use of a temperature-sensitive allele of *bx*, bx^{s4e}.

MATERIALS AND METHODS

Flies were raised on standard cornmeal dextrose food. Complementation tests and most routine crosses were carried out at 22° ±1°. Larvae destined for use in salivary gland chromosome preparations were raised at 17° ±1°. The flies used in "shift" studies to delineate the temperature-sensitive period (tsp) for mutant expression were raised at 17° ±1° and 28° ±1° for various periods of time as described below. Most mutants used, and their descriptions, can be found in LINDSLEY and GRELL (1968). Those not listed or of special interest are cited in the text.

Complementation at the zeste locus: Eleven alleles at the zeste locus were used in this study and are listed in Table 1. All crosses of these alleles were performed *inter se* and the phenotype of the resultant heterozygous females determined. All of the zeste stocks were also made homozygous for the mutant white ocelli (*wo* 3–76.2), the phenotypes of these females were recorded and then the *inter se* crosses were repeated with *wo* in the genome and the male and female eye color phenotypes noted. Both *z* and z^a were crossed to an array of white alleles which are listed with their various properties in Table 2. The effect of these white alleles on the *z* and z^a genes was then noted in males, and in cis and trans position in females, i.e., suppression and nonsuppression of zeste eye color. An attempt was made to obtain intralocus recombination at the zeste locus. Various combinations of zeste alleles were made and crossed to $y\,z\,w^{rdp}+$ males; thus, a recombinant at zeste could show up as a wild-type eye color in the female offspring.

The mutant $w^{rdp}+$ is an intralocus duplication of the white gene (JUDD 1961b). One of the distinctive properties of this mutation is that the *z* locus is expressed in males bearing the duplication and a mutant zeste locus (they no longer are wild type as they possess two functional white loci). A second property and the one which was used in the above experiment is that

TABLE 1

Alleles and description of mutants at the zeste and bithorax loci

Mutant	Location	Description	Origin	Discoverer
z	X- 1.0	Yellow eye in female	Spont.	GANS
z^{58g}	X- 1.0	Yellow eye in female	Spont.	GLOOR
z^{p69a}	X- 1.0	Brown mottled eye in female	EMS	GELBART
z^{11G3}	X- 1.0	Eye + in female	X-ray	GANS
z^{a}	X- 1.0	Eye + in female, yellow in z/z^{a} female	Spont.	GANS
$z^{a\ e(bx)}$	X- 1.0		G-ray	LEWIS
z^{a68k}	X- 1.0		EMS	GELBART
z^{a69-1}	X- 1.0		EMS	GELBART
z^{a69-2}	X- 1.0		EMS	GELBART
z^{a69-3}	X- 1.0		EMS	GELBART
z^{a69-4}	X- 1.0		EMS	GELBART
bx	3–58.8	Transformation of anterior portion of halter to wing-like structure	Spont.	BRIDGES
bx^{3}	3–58.8		Spont.	STERN
bx^{34e}	3–58.8		Spont.	SCHULTZ
bx^{H1}	3–58.8		EMS	HOLDEN
bx^{H2}	3–58.8		Spont.	HOLDEN
bx^{H3}	3–58.8		Spont.	HOLDEN
bx^{H4}	3–58.8		Spont.	HOLDEN
bx^{T}	3–58.8		EMS	TASAKA
Ubx	3–58.8	Halteres enlarged in heterozygote homozygous lethal	Spont.	HOLLANDER
Ubx^{130}	3–58.8	Allele of Ubx in multiply inverted chromosome $TM2$	X-ray	LEWIS

females heterozygous for a w allele or a deficiency which is a dominant suppressor of zeste and $w^{rdp}+$ (i.e., $w^{-}/w^{rdp}+$) and homozygous for z express the zeste phenotype. The females, like the males, possess the two functional w loci which are necessary for zeste expression. The combinations of markers used and results are listed in Table 3.

Complementation at the bithorax locus: Eight alleles at the bx locus were studied, three of which have been extant for several years. The remaining five were discovered in our laboratory and have not been reported elsewhere. These mutants are listed with the z alleles in Table 1. All *inter se* crosses were performed and the phenotypes recorded, with the following exceptions. bx^{H2} and bx^{H4} could not be made homozygous or tested for complementation with each other as they both arose spontaneously in an *Sb*-bearing chromosome and, therefore, any combination of these two alleles would be lethal due to the homozygous lethality of *Sb*. The bx^{T} chromosome was only tested in combination with bx^{1}, bx^{34e} and bx^{H1}, as it was not discovered until late in the study. All of the bx alleles were also crossed to Ubx and Ubx^{130} and their phenotypes compared and recorded. No intralocus recombination was attempted at this locus.

Interaction of z and bx: The crosses used to test for the interaction of alleles at the z and bx loci are outlined in Figure 1. A stock in which the female flies carried *sbd Sb Ubx/T(2;3)Xa* on the third chromosome and the *FMA3,y²* attached-*X* chromosome was constructed. At the same time, stocks were constructed which carried various z and w allele combinations on the *X* chromosome and the bx alleles to be tested, either homozygous if viable and fertile in that condition or balanced to *T(2;3)Xa* if they were lethal or sterile. These were kept as male stocks by crossing to females with *FMA3,y²* as the *X* chromosome with the same third chromosome com-

TABLE 2

Mutants at the white locus

Allele	Locus subsite	Suppressor of zeste	Suppressor of zeste[a]	Affects male eye color	
				zeste[a]	zeste
w^a	3	—	—	*	*
w^{a2}	3	—	—	*	*
w^{bf}	2	—	—	*	*
w^{bf2}	2	—	—	*	*
w^{bl}	1–2	—	—	–	–
w^{Bwx}	1	—	—	–	–
w^{ch}	4	+	+	–	–
w^{cf}	1–2	—	—	–	–
w^{cf2}	1	—	—	–	–
w^{col}	1–2	—	—	–	–
w^{crr}	2–3	—	—	*	*
w^{crr2}	?	—	—	–	–
w^{e}	4	+	+	–	–
w^{h}	4	+	+	–	–
w^{i}	?	—	—	–	–
w^{sat}	?	—	—	–	–
w^{sp}	5	+	+	–	–
w^{sp3}	5	+	+	–	–
w^{11E4}	?	+	+	†	†
w^{65a25}	2–3	—	—	†	†
$w^{86.1}$	2&3	—	—	†	†
$w^{86.3}$	2&3	—	—	†	†
$w^{86.5}$	2&3	—	—	†	†
$w^{86.8}$	deficiency	+	+	†	†
$w^{rdp}+$	duplication	0	0	–	‡

Scoring of suppression:
— = not a dominant suppressor of zeste.
+ = dominant suppressor of zeste.
0 = dominant enhancer of z and z^a.
Scoring of male eye color affect:
* dilutes eye color in males hemizygous for z and w.
† male eye color bone white, therefore no dilution of pigment can be seen.
‡ male eye color zeste.

TABLE 3

Heterozygous ♀ parent			♂ parent	Progeny		
y^+ z $w^{rdp}+$ / y z^{11G3} w^+	×	y z $w^{rdp}+$/Y		♀♀ 18,627	all z
				♂♂ 18,833	
y^2 z^a w^a / y z^{11G3} w^+	×	y z $w^{rdp}+$/Y		♀♀ 16,042	all z
				♂♂ 14,165	
y z^{p69a} w^+ / y^+ z $w^{rdp}+$	×	y z $w^{rdp}+$/Y		♀♀ 38,193	all z
				♂♂ 22,048	one white eyed
y z^{p69a} w^+ / y^2 z^a w^a	×	y z $w^{rdp}+$/Y		♀♀ 21,114	all z
				♂♂ 13,861	

CROSS I

$z^{(*)}\ w^{(*)}\ ;\ bx^{(*)}/T(2;3)Xa\ \sigma\sigma\ \times\ FMA3, y^2\ ;\ sbd\ Sb\ Ubx/T(2;3)Xa\ \varphi\varphi$

$\downarrow$

$z^{(*)}\ w^{(*)}\ ;\ bx^{(*)}/sbd\ Sb\ Ubx\ \sigma\sigma\ \&\ FMA3, y^2\ ;\ bx^{(*)}/sbd\ Sb\ Ubx\ \varphi\varphi$

CROSS II

$z^{(*)}\ w^{(*)}\ ;\ bx^{(*)}/T(2;3)Xa\ \sigma\sigma\ \times\ z^{(*)}\ w^{(*)}/z^{(*)}\ w^{(*)}\ ;\ sbd\ Sb\ Ubx/T(2;3)Xa\ \varphi\varphi$

$\downarrow$

$z^{(*)}\ w^{(*)}\ ;\ bx^{(*)}/sbd\ Sb\ Ubx\ \sigma\sigma\ \&\ z^{(*)}\ w^{(*)}/z^{(*)}\ w^{(*)}\ ;\ bx^{(*)}/sbd\ Sb\ Ubx\ \varphi\varphi$

CROSS III

$z^{(*)}\ w^{(*)}\ ;\ bx^{(*)}/T(2;3)Xa\ \sigma\sigma\ \times\ z^{(*)}\ w^{(*)}/z^{(*)}\ w^{(*)}\ ;\ bx^{(*)}/T(2;3)Xa\ \varphi\varphi$

$\downarrow$

$z^{(*)}\ w^{(*)}\ ;\ bx^{(*)}/bx^{(*)}\ \sigma\sigma\ \&\ z^{(*)}\ w^{(*)}/z^{(*)}\ w^{(*)}\ ;\ bx^{(*)}/bx^{(*)}\ \varphi\varphi$

FIGURE 1.—Mating schemes to determine *z*, *w* and *bx* interactions. Only those progeny of interest are indicated; siblings containing balancer chromosomes are omitted. The females utlized in Cross 3 were obtained as balancer sibs of the progeny scored in Cross 2. The *'s above the symbols of *z*, *w* and *bx* indicate that any one of the alleles listed in Tables 1 and 2, including the wild-type, may be substituted.

plement. A third series of stocks was constructed in which the *X* chromosomes were hemi- or homozygous for various *z* and *w* allele combinations and carried *sbd Sb Ubx/T(2;3)Xa* on the third chromosome. As has been pointed out (LEWIS 1959), the enhancement phenomenon is most easily detected in flies heterozygous for a *bx* allele and *Ubx* (i.e., *bx/Ubx*). Therefore, when males carrying a *z* allele were to be tested for enhancement, they were selected from stock 2 and crossed to females from the first stock (Cross 1, Figure 1). This allowed for a comparison of the phenotypes of males carrying the *z* allele in question with its female sibs which were *z*+. When complementation of different *z* alleles was to be accomplished, males of the same type as above were mated to females of the third stock. This procedure also allowed for the comparison of the enhancement of various *z* alleles in both the male and female (Cross 2, Figure 1). Thirdly, when a comparison of the *bx* alleles in heterozygous condition with different combinations of *z* alleles was desired, females resulting from Cross 2, Figure 1, which were homozygous for different *z* alleles and heterozygous for the desired *bx* allele and *T(2;3)Xa* were collected as virgins. These females could then be crossed to males from stock 2 and the resultant phenotype assessed (Cross 3, Figure 1). As can be seen readily, the number of combinations of alleles at these three loci (*z*, *w* and *bx*) is extremely large; therefore we decided against any attempt at making all stocks isogenic. Therefore, very few cross comparisons from allele to allele, except in the broadest sense, will be made, and indeed a great deal of phenotypic variability was noted.

Autonomy of enhancement: Several attempts were made to cross an unstable ring *X* chromosome into a stock containing *sbd Sb Ubx/T(2;3)Xa* on the third chromosome. However, each attempt failed due to sterility of the first cross. Therefore, the gene, claret-nondisjunctional

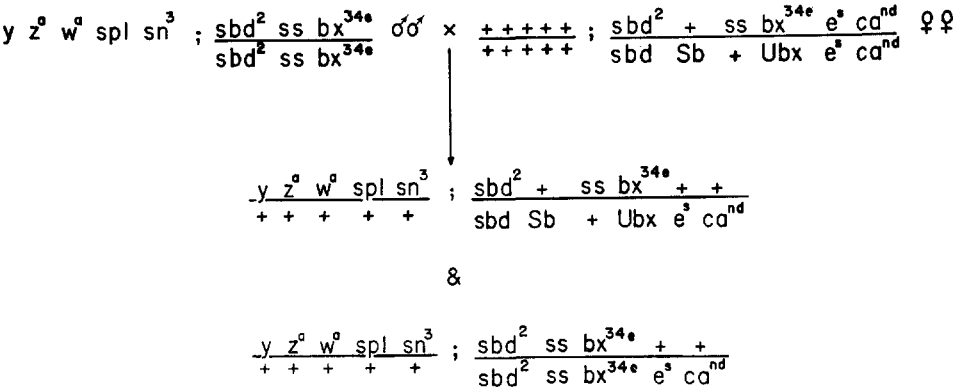

FIGURE 2.—Mating used to obtain gynandromorphs to study the autonomy of the enhancement of bithorax. Only the two female classes scored for loss of maternal (wild-type) *X* chromosome are indicated as progeny.

(*ca*nd 3-100.7), which causes a low percentage of gynandromorphic offspring from homozygous *ca*nd females (LEWIS and GENCARELLA 1952), was crossed onto a chromosome containing *bx*^{34e} and another chromosome containing *Ubx*. These two chromosomes are maintained by balancing to the multiply-inverted third chromosome *TM3, ri p*^{p} *sep bx*^{34e} *e*^{s}. When females were needed for the production of gynandromorphs, the two stocks were crossed (i.e., *sbd*^{2} *ss bx*^{34e} *e*^{s} *ca*nd/*TM3* ♂ ♂ × *sbd Sb Ubx e*^{s} *ca*nd/*TM3* ♀ ♀) and the appropriate females collected. These females were then crossed to males carrying *y z*^{a} *w*^{a} *spl* and *sn*^{3} on the *X* chromosome and were homozygous for *sbd*^{2} *ss bx*^{34e} on the third chromosome (Figure 2). The female offspring from this cross carrying the proper third chromosome complement (see Figure 2) were scored for the loss of the maternal *X* chromosome by the use of the *y*, *w*^{a}, *spl* and *sn*^{3} alleles. If this loss included the mesonotum of the fly, that tissue was scored as enhanced or not enhanced. The autonomy of the effect was most easily determined in bilateral mosaics which included both the thorax and abdomen.

Temperature sensitivity of bx^{34e}: Experiments to determine the time and extent of the "tsp" of *bx*^{34e} were carried out at 17° and 28°. The mutant phenotype is expressed at both temperature regimes. However, the extent of the mutant effect is strikingly different (Figure 4). At 17°, there is virtually no dorsal mesothoracic tissue present between the scutellum and the first abdominal segment. This temperature, then, is defined as the permissive, as the phenotype is closer to wild type. Conversely at 28°, there is a wide band of mesothoracic tissue bearing bristles and hairs between the scutellum and first abdominal segment. Therefore, we have defined this temperature as non-permissive (restrictive), as the phenotype is more mutant.

The cross used was as follows: *y z*^{a} *w*$^{sp-s}$; *sbd*^{2} *ss bx*^{34e} ♂ ♂ were mated in mass to virgin females from the *FMA3*, *y*^{2}; *sbd Sb Ubx/T(2;3)Xa* stock. Several crosses were set up and the adult flies were allowed to acclimate to the two separate temperature regimes. After four days at the two temperatures, eggs were collected every two hours and shifted to the opposite temperature regime at varying times during development at 12-hour intervals. This gave a rough estimation of the tsp and a further experiment was carried out to define more precisely its limits. It was found that the tsp did not involve the embryonic period; therefore, females in the second experiment were maintained at 22° ± 1° and their eggs were collected. At approximately 24 hours after deposition, the egg laying plates were inspected and newly hatched first instar larvae were selected and placed on new food dishes with 50 larvae per dish. These dishes were then placed at either 17° or 28° and a sample of four dishes was shifted to the opposite temperature every 6 hours, at which time the stage of development was also noted. Once the tsp had been delineated in this manner, first instar larvae were collected in the above manner and shifted up and down

through the tsp. That is, cultures were left at the permissive temperature through all of the life cycle except the tsp or a portion of it as well as the converse.

Scanning electron microscopy: Adult flies were etherized and their wings removed with a pair of fine scissors. These flies were then mounted on a scanning EM chuck by means of a drop of conductive silver paint. The chuck was placed in the SEM vacuum chamber and the flies viewed and photographed immediately, as too long a period of time in the vacuum resulted in distortion of the abdomen of the fly. We found this to be an easy (no preparation of the specimen was necessary) and very effective way to preserve and present phenotypic variations of our adult material.

Salivary gland chromosome analysis: Larvae were washed and sexed in Ringer's solution and dissected in 45% acetic acid. The salivary glands were then removed and transferred to a drop of lacto-aceto-orcein on a microscope slide and the gland was squashed beneath a cover slip. The cover slip was then sealed at the edges with clear fingernail polish. The slides were then examined within 24 hours.

RESULTS

The results of the complementation analysis are summarized in Table 4. The two alleles z and z^{58g} are similar in that they give, with two exceptions, yellow-eyed females in combination with the other alleles, i.e., all z^a type mutants. The z^a type alleles again are all similar in that they are all wild type with regard to eye color when complemented among themselves but give a yellow eye when heterozygous with z or z^{58g}. However, as in the case of z and z^{58g}, exceptional results are obtained with the two remaining alleles, z^{p69a} and z^{11G3}.

The first of these, z^{p69a} (zeste peppered), was discovered by GELBART (1971). The hemizygous male is wild type and the homozygous female has a brown mottled phenotype superimposed on a yellowish background. Heterozygotes of z^{p69a} and either z type alleles or z^a type alleles yielded a female whose eye color is between that of z and z^{p69a} homozygotes. The brown mottle in these heterozygotes

TABLE 4

Eye color phenotypes of females resulting from complementation analysis at the zeste locus

Mutant	z	z^{58g}	z^{p69a}	z^{11G3}	z^a	$z^{ae(bx)}$	z^{a68k}	z^{a69-1}	z^{a69-2}	z^{a69-3}	z^{a69-4}	z^-
z	z	z	z^m	+	z	z	z	z	z	z	z	z
z^{58g}	..	z	z^m	+	z	z	z	z	z	z	z	z
z^{p69a}	..	..	z^p	+	z^m	z^m	z^m	z^m	z^m	z^m	z^m	z^m
z^{11G3}	..	..	..	+	+	+	+	+	+	+	+	+
z^a	..	..	..	..	+	+	+	+	+	+	+	+
$z^{ae(bx)}$	..	..	..	..	..	+	+	+	+	+	+	+
z^{a68k}	..	..	..	..	..	..	+	+	+	+	+	+
z^{a69-1}	..	..	..	..	..	..	..	+	+	+	+	+
z^{a69-2}	..	..	..	..	..	..	..	..	+	+	+	+
z^{a69-3}	..	..	..	..	..	..	..	..	..	+	+	+
z^{a69-4}	..	..	..	..	..	..	..	..	..	..	+	+

z = yellow-eyed females.
z^m = zeste mottled, females have light brown spots on a yellow-brown background.
z^p = zeste peppered similar to z^m but somewhat darker in color.
+ = wild-type eye color.

is diluted and the eye becomes lighter in color, i.e., more yellowish. This dilution varies in degree from allele to allele, especially among the z^a types, but is detectable in every case. The second exceptional allele, z^{11G3}, deserves some special mention. It was originally recovered as an X-ray-induced "revertant" of z and was attributed to a change at the white locus (GANS 1953). Later data showed it to be a change at the zeste locus itself (LEFEVER 1966). This allele has the singular property of not only giving wild-type eye color when homozygous, as is the case with the z^a type alleles, but also gives wild-type eye color with all other alleles at the zeste locus. Therefore, it acts as if it was an "allelic suppressor of *zeste*" (LEFEVER 1966) by its recombinational position and by these complementation data. However, further analyses cited below will show that it is not a true reversion to the wild-type condition.

The results of the same complementation matrix, but now including the third chromosome gene white ocelli (*wo*), gave the first indication of the non-wild-type nature of z^{11G3}. This mutant (*wo*) has a generalized diluting effect on the alleles at the zeste locus. All of those combinations in Table 4 which give yellow eye color will give "white" eyes in combination with *wo*. The temperature sensitivity of zeste (BECKER 1960) still remains, however, and to obtain "white" eyed flies, they must be reared at 25° or higher. As the temperature is lowered, the eye becomes increasingly more pigmented until at 17° it reaches a pale buff color, but never attains a full zeste pigmentation. All those combinations which yield wild-type eye color (i.e., the z^a type alleles) are not affected by the addition of *wo* to the genome; they remain wild type. Here again, z^{11G3} proved to be an exception. The three combinations, z^{11G3}/z, z^{11G3}/z^{58g} and z^{11G3}/z^{p69a} all gave females with dark brown mottled eyes, a color usually classified as a "suppressed-zeste" phenotype. All other combinations of z^{11G3} remain wild type in the presence of *wo*. The other exceptional allele, z^{p69a}, also shows this dichotomy of dilutive effect. The combinations z^{p69a}/z and z^{p69a}/z^{58g} have the peppering removed from the eye and give a yellow eye color. All of the other combinations, with the exception of the aforementioned z^{11G3}/z^{p69a} do not appear to be diluted in eye color.

From these results we can draw an array of the zeste alleles which we have tested and rank them in their order of effect on eye color, the most mutant first, as follows: $z = z^{58g} > z^{p69a} > z^a = z^{ae(bx)} = z^{a68k} = z^{a69-1} = z^{a69-2} = z^{a69-3} = z^{a69-4} > z^{11G3} > z^+$.

The results obtained with a deficiency of z seem to place it in the same category as the z^a type alleles. However, since the existing deficiencies for z of which we are aware also include other loci around the zeste locus, a hemi- or homozygous fly cannot be obtained due to lethality. Therefore, we have not attempted to rank z^- in this array.

Interactions with the white locus: The interaction of z type alleles and the white locus has been well documented (GANS 1953; GREEN 1959a). Since we had found a secondary function for the z^a type of allele, we wanted to find if the previous relationship with the white locus and z was true of z^a. To this end, chromosomes with z, z^a and all of the white loci listed in Table 2 were constructed. It

was found that in those combinations at the zeste locus capable of giving yellow eye color, i.e., z/z and z/z^a, the dominant suppressing ability of mutants at the 4 and 5 subsites as well as a deficiency of the white locus held true; whereas, the inability of white alleles at sites 1, 2 and 3 to suppress zeste was also true. On the other hand, those combinations of z alleles which gave wild-type eyes, i.e., z^a/z^a, were not affected by any change at the white locus. The one exception to this was z^{11G3}. When z^{11G3} was made heterozygous for z or z^{58g} and w^{rdp+} (i.e., z^{11G3} w^+/z w^{rdp+}), the resultant females had a brown mottled eye color reminiscent of z^{p69a}. It therefore appears that z^{11G3} will express mutant phenotype (i.e., eye color change) if at least three doses of the white locus are also present in the genome.

Another point of interest resulted from this study. It has been known for some time (GREEN 1959a) that a male carrying both z and w^a has a considerably lighter eye color than a male with just w^a. We have found that other pigmented white alleles at sites 2 and 3 (i.e., w^{a2}, w^{bf}, w^{bf2} and w^{crr}) are also diluted in males carrying z in the same chromosome. When the same site 2 and 3 alleles are placed on a chromosome bearing z^a, the same dilution effect is observed. Therefore, the zeste locus is apparently having some effect on the metabolism of the male as well as the female and does not need two doses of the white locus to be expressed. This fact will become more evident below from the results of the interaction of z and *bx*.

Intralocus recombination: The results of the attempts at intralocus recombination are given in Table 3. Combinations of representatives from the four types (with regard to eye pigmentation) of zeste alleles were made. Recombinants should be recovered as wild-type females; however, as can be seen, no such progeny were found. The only exceptional progeny was a single white-eyed male, which proved to involve a change at the white locus rather than at zeste. These results parallel those of GELBART (1971) and JUDD (personal communication) in which autosomal inversions were used to increase crossing over and no recombinants were found. The chromosomes used in this study were examined to determine if there were any structural anomaly to account for the results, and none were found.

Complementation of bx alleles: The results of the complementation analysis at the *bx* locus are summarized in Table 5. There is no obvious pattern of complementation within the locus. That is to say, an allele which gives an extreme mutant phenotype when homozygous (i.e., bx^3) or heterozygous with *Ubx* or Ubx^{130}, only gives a comparatively moderate transformation when heterozygous for another apparently strong allele, bx^{H4}. On the other hand, two extremely weak alleles, as judged by their homozygous phenotypes (bx^1 and bx^{H1}), give the same moderate transformation as bx^3/bx^{H4} when they are heterozygous. This apparent inconsistency could be due to variations in genetic background, as was mentioned earlier; however, by comparing total severity of mutant effect in all of the various combinations, the following general range of mutant effect can be derived in order of least to most severe: $bx^T \sim bx^1 \sim bx^{H1} < bx^{H2} \sim bx^{H3} \sim bx^{34e} < bx^{H4} \sim bx^3$.

The locus can be broken down roughly into three types of alleles. The members of the first category (bx^T, bx^1 and bx^{H1}) are the weakest; they generally result in

TABLE 5

Phenotypes of the inter se *combinations of mutants at the bithorax locus*

Mutant	bx^1	bx^3	bx^{34e}	bx^{H1}	bx^{H2}	bx^{H3}	bx^{H4}	bx^T	Ubx^1	Ubx^{130}
bx^1	±	+	+	++	+	—	+	+	±	+
bx^3	..	+++	+++	+	+	++	++	0	+++	++++
bx^{34e}	..	..	++	++	+	±	++	+	++	+++
bx^{H1}	..	..	..	—	+	++	+	—	+	+
bx^{H2}	..	..	..	..	0	++	0	0	++	+++
bx^{H3}	..	..	..	..	..	+	++	0	++	+++
bx^{H4}	..	..	..	..	..	..	0	0	+++	++++
bx^T	..	..	..	..	..	..	..	—	+	+

— = no detectable change from wild-type.
± = variable transformation usually only seen in the third halter segment.
\+ = consistent transformation of third halter segment.
++ = consistent transformation of halter plus some mesothoracic tissue formed.
+++ = transformation of halter more mesothoracic tissue with definite bristle pattern, also metathoracic leg transformed.
++++ = extreme transformation of metathorax including halter→ wing; leg and wide band of mesothoracic tissue.
0 = not tested.

a slight enlargement of the third segment of the halter with the presence of a few terminal hairs and a very thick band of mesothoracic tissue between the scutellum and first abdominal segment. There is usually no involvement of the metathoracic leg. The second category (bx^{H2}, bx^{H3} and bx^{34e}) causes a moderate transformation. The halter is usually wing-like and bears a row of marginal bristles on the anterior edge; in more extreme cases, there is some indication of venation. There is usually a band of mesothoracic tissue between the scutellum and first abdominal segment, which generally extends the width of the animal. This tissue possesses thoracic bristles but they are generally hard to identify due to the restricted width of the mesothoracic tissue. The transformation of the metathoracic leg seems to be variable. The third and final group is the most extreme (bx^{H4} and bx^3). In these two mutants, the anterior portion of the halter is extremely wing-like, bearing marginal bristles and at least the marginal and second longitudinal veins. There is a wide band of mesothoracic tissue extending the width of the animal which bears identifiable thoracic bristles. There is also generally a second scutellum present and the metathoracic leg is usually transformed.

A somewhat unexpected result was found when the *bx* alleles were tested for enhancement of the phenotype by the Ubx^{130} chromosome. This is indicated in the last two columns in Table 5. If a given allele shows an enhancement of mutant phenotype, it will be more mutant (i.e., more transformed) over Ubx^{130} than *Ubx*. This was found to be the case with six out of the eight alleles tested. However, bx^{H1} and bx^T did not increase their mutant expression but remained the same. This finding could be due to the fact that in some cases, if the breakpoints of a "transvecting" chromosome (i.e., *TM2*, Ubx^{130}) are close to those of a chromosome being tested (in this case, bx^{H1} and bx^T) so that proper pairing can take place, the "transvection" effect is no longer extant (LEWIS 1954). We use the

term "transvection" here with caution as the original effect was described using chromosomes with a very different type of breakpoint and genetic constitution. The Ubx^{130} chromosome has one of its breakpoints very close to the bithorax locus and acts more like a complete inactivation of the entire bithorax gene complex (LEWIS personal communication). The *bx* alleles used in this study are presently being examined with other chromosomes which have true transvecting rearrangements to determine if the dichotomy of enhancement holds with these chromosomes as well. The chromosomes of bx^{H1} and bx^{T} were examined to determine if there were any breakpoints which could possibly account for this behavior, but none were found. This odd result will be shown to have a direct parallel in the results, given below, of the studies on the interaction of z^a and *bx*.

Interaction of z and bx: All eleven alleles of *z* were tested for their ability to enhance the phenotype of bx^{34e} and bx^{3}. This was accomplished as outlined in Cross 1 of Figure 1. The results were extremely clear-cut; *z*, z^{58g} and z^{p69a} had no effect on the phenotype of either bx^{34e}/Ubx or bx^{3}/Ubx. On the other hand, all of the z^a alleles (see Table 1) as well as z^{11Gs} had a definite effect on the *bx*, *Ubx* combinations. This effect is very similar to that produced by the Ubx^{130} chromosome (Figure 3). There is an increase in the extent of the mesothoracic tissue between the scutellum and first abdominal segment, there is some slight but variable transformation of the anterior portion of the halter to wing, and the metathoracic leg and pleurae become more mesothoracic. To determine if the z^a (enhancer) type allele was dominant to the *z* (non-enhancer) type allele, heterozygous females were produced of all combinations of *z* alleles on the *X* chromosome and either bx^{34e}/Ubx or bx^{3}/Ubx on the third chromosome. The results again were unequivocal; the z^a (enhancer) type allele is recessive. That is to say, any *z* allele when heterozygous for *z*, z^{58g} or z^{p69a} does not show enhancement of the *bx* phenotype. All combinations of z^a (enhancer) type alleles among themselves, however, lead to enhancement. This is also true of z^a/z^-; therefore, *z* is not acting simply as a null allele and thereby reducing the effectiveness of z^a. By referring back to Table 4 and the text with regard to the effect of *wo* on the *z* phenotype, it can be seen that any allele or combination of alleles at the zeste locus which has the potential to alter the eye color phenotype will not act as an enhancer of *bx*.

As has already been mentioned, certain alleles at the white locus act as dominant suppressors of *z* and z^a. It was, therefore, decided to determine if this third locus (*w*) would interact in any way with the enhancing effect of z^a. To this end, the various *z*, z^a and *w* combinations used earlier in this study were introduced into stocks which could be used in Crosses 1 and 2 of Figure 1. It was found that any change at the white locus had no effect on the ability of z^a (or the inability of *z*) to enhance either in males or females the phenotypes of bx^{34e}/Ubx or bx^{3}/Ubx. This result was, however, not true of all combinations of *bx* and *Ubx*, as mentioned below.

Since we now knew the effect of the various alleles of *z* and white on two alleles at the *bx* locus, we decided to determine the effects of the two *X*-linked loci on all alleles at *bx*. The effect of selected *z*, *w* combinations of the various *bx*, *Ubx*

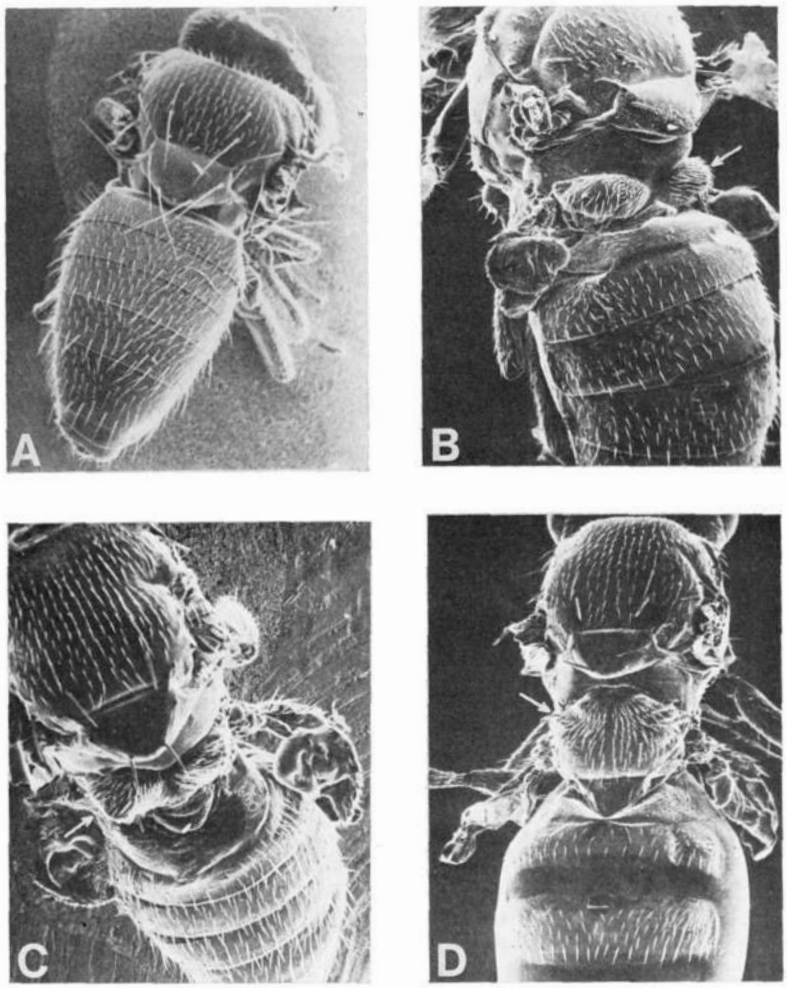

FIGURE 3.—Scanning electron micrographs of selected enhanced and non-enhanced flies. A. Oregon-R. Mag. 70X. B. $z^{+}w^{+}$;bx^{3}/*Sb Ubx*. Mag. 70X. C. $z^{+}w^{+}$;bx^{3}/*TH2*, Ubx^{130}. Mag. 70X. D. $z^{11G3}w^{+}$;bx^{3}/*Sb Ubx*. Mag. 70X. Arrows in B., C. and D. indicate dorsal mesothoracic tissue formed in the metathorax.

combinations is given in Table 6. Not all combinations of *z* and *w* tested are given, as they all gave the same results with no exceptions. As can be readily seen, bx^{H2}, bx^{H3} and bx^{H4} fall into the same category as bx^{34e} and bx^{3}. They are enhanced by all z^{a} type alleles but not by *z* types. The first exception to this is the bx^{1}/*Ubx* combination. It is not enhanced by *z* or z^{p69a} as would be expected; however, it also is not affected by *y* z^{a} w^{+}, z^{11G3} and z^{a69-1}. But when a white allele, either a suppressor or non-suppressor of zeste is placed in the genome, the phenotype is enhanced. This is true in females only if the white allele is homozygous. There is no dominant effect as in suppression of zeste; therefore, this interaction of z^{a} and white is probably of a different type than that of suppression of zeste.

TABLE 6

Effect of selected zeste and white combinations on mutants at the bithorax locus

	bx/Ubx	*bx³/Ubx*	*bx³⁴ᵉ/Ubx*	*bx^H1/Ubx*	*bx^H2/Ubx*	*bx^H3/Ubx*	*bx^H4/Ubx*	*bx^T/Ubx*
y z^a w+	—	+	+	—	+	+	+	..
y z^a w^a2	+	+	+	—	+	+	+	..
y z^a w^bf	+	+	+	—	+	+	+	..
y z^a w^sp-3	+	+	+	—	+	+	+	..
y z^a w^h	+	+	+	—	+	+	+	..
z^11G3	—	+	+	—	+	+	+	—
z^a69-1	—	+	+	—	+	+	+	..
y z w^sp-3	—	—	—	—	—	—	—	—
z^p69a	—	—	—	—	—	—	—	..

+ = phenotype enhanced.
— = phenotype not changed.

There are two other exceptions to the enhancement of *bx* by z^a and these are bx^{H1} and bx^T. Both of these alleles are not enhanced by any combination of *z*, z^a or *w* and, as will be remembered, neither of these alleles is affected by the Ubx^{1so} chromosome.

Finally, *z*, z^a and z^{11G3} were tested for their ability to enhance different combinations of *bx* alleles as outlined in Cross 3, Figure 1. The results of this study are given in Table 7. Some of the results are equivocal, especially those concerning bx^1; but this can be explained by its unique interaction in the previous experiment. The only other unclear result is that obtained with homozygous bx^3. Since this is the more extreme allele used, the most extremely transformed and, therefore, mutant individuals may have been lethal; and, indeed, many dead pupae cases were found which, upon dissection, proved to contain extremely mutant adult flies. Here again, as in the previous experiment, bx^{H1} proves to be a solid exception. In no case was bx^{H1}, when either hetero- or homozygous, enhanced by z^a over its normal mutant condition. Unfortunately, bx^T, the other exceptional

TABLE 7

Effect of selected zeste alleles on the inter se *combinations at the bithorax locus*

	Ki p^p bx sr e^s	*bx³*	*sbd² ss bx³⁴ᵉ*	*bx^H1*	*D Sb bx^H2*	*bx^H3*	*Sb bx^H4*
Ki p^p bx sr e^s	—	±	±	—	+	+	—
bx³	..	±	+	—	+	+	+
sbd² ss bx³⁴ᵉ	..	..	+	—	+	+	—
bx^H1	..	..	..	—	—	—	—
D Sb bx^H2	..	..	..	..	0	+	0
bx^H3	..	..	..	..	..	+	+
Sb bx^H4	..	..	..	..	..	..	0

+ = phenotype enhanced by z^a and z^{11G3}.
— = phenotype not enhanced by z^a and z^{11G3}.
± = variability in phenotype overlaps both enhanced and unenhanced phenotype.
0 = not tested due to homozygous lethality of *Sb*.

case previously discussed, has not been characterized with the other *bx* alleles in regard to enhancement as of yet.

From these results we can see that like the *z* locus, the *bx* locus can be subdivided first into three categories with regard to phenotypic interactions and second into two categories with regard to z^a enhancement. These categories are not mutually exclusive, as the two bithorax alleles which are not enhanced are both classified as "weak" *bx* mutants; however, bx^1 is placed in the same category (i.e., "weak") but is enhanced by both z^a and Ubx^{1so}. The most striking difference between the alleles at *bx* which are enhanced and those which are not seems to be in their origin. Both alleles, bx^{H1} and bx^T, which are not enhanced, were induced by EMS (see Table 1), whereas all of the remaining alleles are spontaneous in origin.

Cellular autonomy: The results of the crosses used to obtain gynandromorphs are given in Table 8. Not many total adult flies were scored, as the fertility of homozygous ca^{nd} females is extremely poor; this, coupled with the fact that so many recessive markers were used, resulted in a very low progeny yield. The yield of gynandromorphic flies is also quite low when compared to that obtained with the unstable ring *X* method. However, enough individuals were obtained to determine the cellular autonomy of enhancement. All of the 44 putative *X/O* males showed excellent enhancement of both the bx^{34e}/bx^{34e} and bx^{34e}/Ubx phenotypes; therefore, the *Y* chromosome apparently has no effect on the expression of z^a. Of the nineteen gynandromorphs, six were bilateral mosaics of the dorsal metathorax. In all six cases, the side exhibiting the *y*, w^a, *spl* or sn^3 markers also showed a definite increase in mesothoracic tissue between the scutellum and first abdominal segment. The contralateral side, however, showed no such increase. When, as in the thirteen other flies, the dorsal metathorax was not involved, there was no increase (enhancement) whether the *X/O* tissue occurred in the head, pro-, mesothorax or abdomen. Therefore, enhancement appears to be autonomous and is not repressed by surrounding non-enhanced tissue.

TABLE 8

Results of the cross used to obtain gynandromorphs

Genotype of progeny		Number of progeny
sbd ss bx^{34e} *e+ ca+*	♀♀	53
	♂♂	61
sbd ss bx^{34e} e^s ca^{nd}	*X/0* ♂♂	21
	Gynandromorphs	10
sbd Sb+ ss bx^{34e} *e+ ca+*	♀♀	94
	♂♂	70
sbd Sb ss+ Ubx e^s ca^{nd}	*X/0* ♂♂	23
	Gynandromorphs	9
		341
Gynandromorphs 5.57%		
X/0 males 12.9 %		

Temperature shift studies: It has been known for quite some time (VILLEE 1945) that the bx^{34e} allele of *bx* is temperature-sensitive. However, due to the variability of the penetrance of its phenotype, it was rather difficult to get an exact localization of the tsp. We have found that through the use of the z^a enhancer gene, this variability can be overcome. When a fly of the genotype $z^a;bx^{34e}/Ubx$ is grown at 17°, there is no mesothoracic tissue formed between the scutellum and first abdominal segment; the halter phenotype stays the same. If this same genotype is grown at 28°, a moderately wide band of tissue is present and at 22° an intermediate amount is present. We know this effect to be due to bx^{34e}, for if any other *bx* allele is substituted, the temperature under which the flies are raised has no effect on the phenotype. The same temperature sensitivity can also be observed in homozygous bx^{34e} flies. The differences in phenotype at the two extreme temperatures (17° and 28°), however, are not as pronounced. Therefore, the heterozygote was employed for ease in scoring. With this clear-cut difference in phenotype, we set out to delineate the tsp of bx^{34e}. The results of the shift studies are given in Figure 4. If cultures are maintained at a temperature of 17° (permissive) for a period of up to 156 hours after egg deposition (this corresponds to approxi-

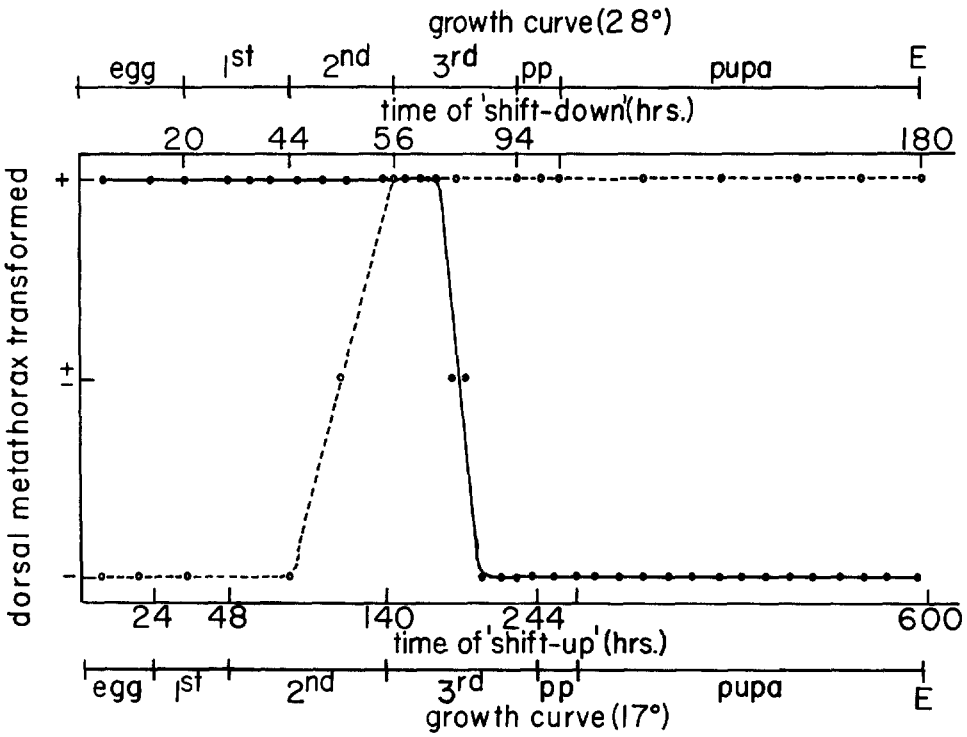

FIGURE 4.—Results of shift studies to delineate the tsp of bx^{34e}. Closed circles are from shift up experiments (17°–28°); open circles are from shift down experiments (28°–17°). + indicates formation of a wide band of mesothoracic tissue. ± indicates an intermediate amount. — indicates little or no mesothoracic transformation.

mately mid third larval instar) and are then shifted to 28° for the remainder of the life cycle, the resultant adults show the restrictive phenotypic (i.e., wide band of mesothoracic tissue). If, however, the shift in temperature is made after this point in development, there is a decreasing amount of mesothoracic tissue until at 192 hours after egg deposition there is none at all. This, then, localizes the end of the tsp to the mid third larval instar with a transitional period of about 48 hours in which all of the flies show an intermediate phenotype as if they had been raised at 22° (Figure 5).

Conversely, if the cultures are maintained at 28° and then shifted down to 17°, it is found that any shift down 48 hours after egg deposition yields adults with a restrictive phenotype. A shift earlier than this results in a permissive phenotype (no mesothoracic tissue). This result places the beginning of the tsp in the mid to

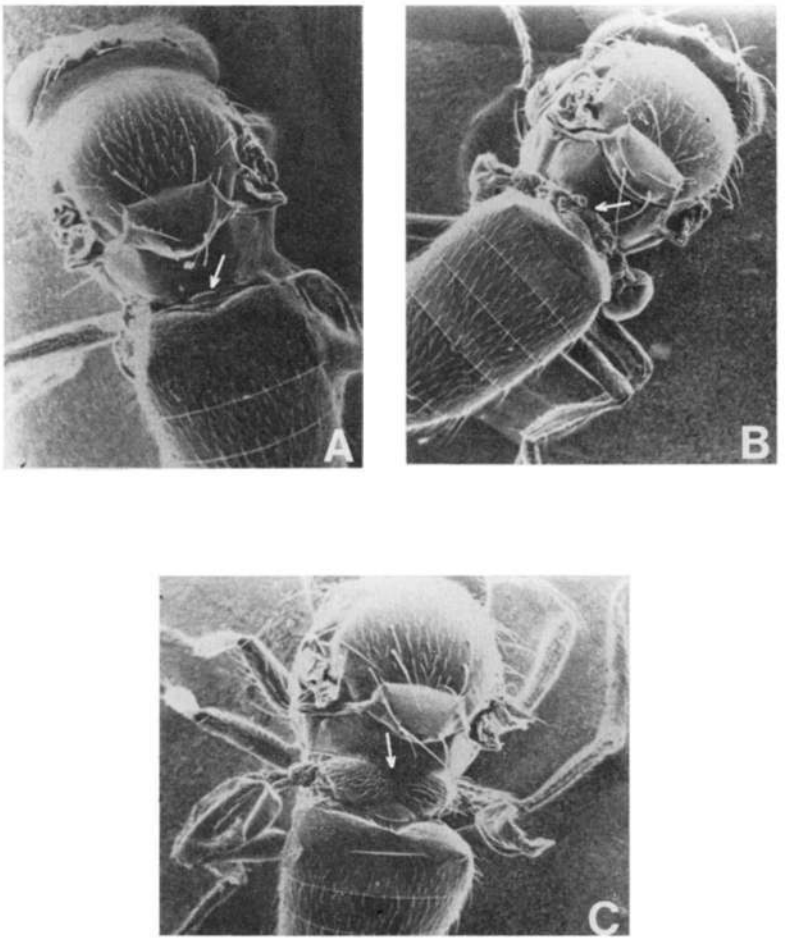

FIGURE 5.—Scanning electron micrographs of *y* z^a w^{sp-3};*sbd ss* bx^{34e}/*sbd Sb Ubx* ♂ ♂ grown at three different temperatures. A. 17° B. 22° C. 28°. Arrows indicate area of dorsal metathorax transformed into dorsal mesothorax. Mag. 70X.

late second instar. Here again we see a transitional period of approximately 24 hours in which all the flies have an intermediate phenotype. Therefore, the temperature-sensitive period of the bx^{34e} gene appears to be in the developmental interval from the mid to late second instar larva to the middle of the third instar.

Further studies were conducted to determine if only the length of the period at the permissive or non-permissive temperature was important or if there was some definite process which, once initiated, resulted in the change in phenotype. To this end, cultures were maintained at the non-permissive (restrictive) temperature (28°) up to a point just before the previously determined initiation of the tsp or until just after. At one of the two aforementioned times, cultures were successively shifted to the permissive temperature (17°) and then again at successive intervals, the cultures were shifted back to the non-permissive temperature (28°). The results showed that if a culture is shifted to the permissive temperature before the beginning of the tsp, it must spend at least 180 hours before a shift back to the non-permissive temperature will reverse the temperature effect. If, however, the culture is shifted after the initiation of the tsp, the culture may remain at the permissive temperature until after the tsp would normally be over and still give the non-permissive phenotype. In other words, once the temperature-sensitive process has begun, even a period of time at the permissive temperature, which includes the remainder of the tsp, is insufficient to reverse the initial effect. The converse experiment shows that if a sufficient period is spent at the non-permissive temperature, even after the previously determined initiation point is past, the resultant flies will be restrictive in phenotype. That is to say, even if the temperature-sensitive process is begun at the permissive temperature, the ability of the lower temperature to "suppress" the formation of dorsal mesothoracic tissue can be overcome by keeping the cultures at the non-permissive temperature throughout the remainder of the tsp.

DISCUSSION

From the results of the complementation at the zeste locus and the ability of its alleles to produce a mutant eye phenotype, there appear to be at present four subtypes of mutant. The first type (z and z^{58g}) is the same as that originally discovered by GANS (1953); both alleles of this type are spontaneous in origin. The second type is thus far unique (z^{p69a}) in that it produces an intermediate eye color phenotype. By far the largest group is the z^a type allele with seven members which can be either spontaneous or induced in origin. At this point, it is interesting to note that GELBART (1971) recovered only this type of allele in his screen for mutants at the zeste locus. The fourth and final type has only one member, z^{11G3}. In many ways it is similar to the z^a type of allele and differs only in that three doses of the white locus are needed to allow it to express a mutant phenotype when heterozygous for z.

The zeste locus can again be divided with regard to its interactions with the bx locus. In this case, there are only two types: those alleles which enhance and those which do not. This subdivision of the locus falls along the same lines as that

revealed by the complementation analysis previously mentioned. Those mutants in subgroups 1 and 2 above (i.e., z, z^{58g} and z^{p69a}) which are all capable of producing a pigment change in the eyes of homozygous females do not show enhancement, whereas the 3rd and 4th subgroups do not change eye color but do enhance *bx*. This property of the pigment changing alleles doe not appear to be due to an amorphic change at the locus, as z^a/z does not enhance whereas z^a/z^- does. Therefore, it appears that z is having an antimorphic effect on the ability of z^a to affect enhancement.

A second possible indication of the nature of the *z*:*bx* interaction lies within the interaction itself. We have found that the *bx* locus can be divided into two distinct groups: those which are enhanced by z^a and show an increase in mutant phene with the Ubx^{130} chromosome and those which are not affected by either z^a or Ubx^{130}. As has been mentioned above, this dichotomy is related to origin, as both of the non-enhanced alleles were induced by EMS whereas those which are enhanced were all spontaneous in origin. This type of division by origin is not a new phenomenon. It has been shown that the vermilion (v) locus (GREEN 1954) and the forked (f) locus (GREEN 1952) yield the same result when they are tested for suppression by the two suppressor genes, *su(s)* for v and *su(f)* for f.

The temperature shift experiments indicate that the tsp for bx^{34e} extends from the mid second larval instar to the mid third larval instar. Apparently the first 24 hours of the tsp is critical in that exposure of the culture to 28° during that interval is sufficient to induce a mutant phenotype. Later in the tsp, a more prolonged exposure to 28° is required for the mutant phenotype. Since the effect is autonomous, the *z* locus is likely to be acting in these cells at approximately the same time. If this is true, then the *bx*-enhancing activity of the zeste locus occurs at a different time from its effects on eye pigment formation. BECKER (1960) has shown that the zeste locus acts in the eye anlagen at the end of the first larval instar at a time when there are approximately twenty cells. This effect on eye pigmentation is autonomous and shows a normal cell lineage pattern. The temporal and spatial separation of zeste locus "action" can be interpreted in two ways. The locus itself may be showing two different times of activity, one in the eye and the other in the haltere anlagen. On the other hand, the zeste locus may be active at all times but this "activity" is only seen through its interaction with two separate morphogenetic systems, i.e., eye pigmentation and metathoracic determination, which themselves have differential times of activity. Since we are surmising the "activity" of the zeste locus through its interaction with a temperature-sensitive allele of *bithorax*, the time of activity in the haltere is by necessity inferred. Nevertheless, it can be still stated that products of the zeste locus are having an effect in two distinct tissue type autonomously at two distinct times during development.

The time of activity of bx^{34e}, as revealed by these results, compares rather favorably with those of OUWENEEL (1969) who found an almost identical tsp for for the mutant *loboid-ophthalmoptera* (a transformation of eye to wing). However, the tsp's of two other mutants, ss^{a40a} (GRIGLIATTI and SUZUKI 1971) and *pb* (VILLEE 1944) (both of these mutants transform antennae→tarsus or proboscis→

tarsus) were found to be somewhat later in development and to follow and partially overlap the tsp's of bx^{34e} and loboid-ophthalmoptera. These correlations fit rather nicely with the known sequence of developmental events as detected morphologically in the anlagen. The wing disc is one of the earliest to separate itself and become innervated, while the leg anlagen do not segregate to separate entities until later in development (AUERBACH 1936). Therefore, when a tissue (disc) is transformed toward wing and the mutation causing the effect is temperature-sensitive, the earlier development of the wing disc (tissue) seems to be reflected in the earlier tsp.

At this point in the discussion, we believe that a comment is warranted. We have already (see above) compared our results with an "enhancer" gene with those of two "suppressor" genes. However, we believe that this comparison is valid and these two phenomena could conceivably be acting through similar if not identical mechanisms and indeed we have found several parallels between the two. With this premise in mind, we can make some of the following observations and conclusions about the possible nature of the *z*: *bx* interaction.

The phenomenon of suppression has been well worked out and is well understood in prokaryotic organisms (for a review, see GORINI and BECKWITH 1966). These two authors (*loc. cit.*) have proposed three major classes of suppression: (a) indirect or metabolic suppression, (b) intragenic suppressors or second site reversions, and (c) informational suppressors (e.g., amber and ochre t-RNA suppressors). It is our belief that most if not all *su* and *e* genes thus far isolated in Drosophila fall into the first class (a) and we will present evidence to support this thesis.

If one surveys LINDSLEY and GRELL (1968) and lists all enhancer and suppressor loci and the alleles on which they act, some interesting generalizations appear. First, the origin of the *su* or *e* gene is itself unimportant—e.g., Table 1, of the eight z^a-type enhancer of bithorax alleles, one is spontaneous, one is X-ray induced, one is gamma ray-induced and five are EMS-induced. The origin of the affected locus is, on the other hand, quite important. They are, with only four exceptions out of 81 total spontaneous in origin. This is perhaps best exemplified by the *su(Hw)* gene which has been tested with the largest number of genes. In every case but one at any given locus tested, only those alleles of spontaneous origin are suppressed. The inverse, however, is not true, as there are both spontaneous and induced alleles which are not suppressed.

One of the most extensively studied *su* genes is the *su(s)* locus. There are at present eight alleles of this locus; one X-ray-induced, five spontaneous and one EMS-induced. This locus suppresses alleles at the vermilion (*v*) locus, the speck (*sp*) locus, the purple (*pr*) locus and the sable (*s*) locus—all suppressible alleles being spontaneous in origin. The most intensively studied of the interactions of the *su(s)* locus is its relationship to the *v* locus. GREEN (1952) has shown that the *v* locus is divisible into suppressible and non-suppressible alleles and of 22 tested (GREEN 1954), only 3 of spontaneous origin are suppressible, whereas 3 others of spontaneous origin, as well as 16 induced alleles, are not suppressible. The factor which makes this system so amenable to analysis is that the gene product

of the *v* locus is known, isolatable and analyzable. The *v* locus is the structural gene for tryptophan pyrrolase and the functionality of this enzyme has been characterized in *v* hemi-, homo- and heterozygotes with and without *su(s)* (MARZLUF 1965 and TARTOF 1969). It has further been shown that the *su(s)* gene is responsible in some manner for the production of one of two chromatographically separable species of t-RNA^{tyr}. This species of t-RNA^{tyr} binds to tryptophan pyrrolase and inhibits the enzymatic activity (JACOBSON 1971; TWARDZIK, GRELL and JACOBSON 1971). It has further been shown that asparaginyl-, aspartyl- and histidyl-t-RNA are also affected (i.e., are missing separable peaks) in *su(s)* stocks, which is interpreted as meaning that a conversion enzyme is necessary for the production of these separable species of t-RNA from the remaining species (WHITE *et al.* 1973). It can, therefore, be concluded that in the case of the *su(s)* locus, the method of suppression falls into the (a) category of GORINI and BECKWITH (1966).

With regard to our original thesis, at this point it is interesting to note the results of studies involving the feeding of certain chemicals to Drosophila and their effect on certain mutations. It has been shown that the addition of certain amides, the most effective being acetamide (KAJI 1954; DE MARINIS 1966) to the medium, has the effect of increasing facet number in *Bar* eye strains of Drosophila. In a further study, it was found that double amides, specifically glutaramide (DE MARINIS and SHEIBLEY 1963), have an even stronger effect. Finally, it was found that when double amides were joined into a ring, the effect was still present but was not as intense (i.e., feeding of Uracil) (DE MARINIS 1966, 1967; DE MARINIS and SHEIBLEY 1960). The feeding of urea, methylurea and ethylurea not only had a suppressive effect on the Bar locus but also suppressed certain forked alleles and spineless (DE MARINIS 1969). On the other hand, there also exist compounds which have an antagonistic effect on these same loci; nitromin (KAJI and HIROSE 1967) and 5FU and 5BU (GEHRING 1963; DE MARINIS 1966) have an inhibitory effect on facet formation in both Bar and wild-type strains.

KAJI and OGAKI (1953) have shown that the phenocritical period of the feeding effect is at ca. 70 hours of development. Furthermore, at this point ^{3}H-acetimide is incorporated maximally into the anlagen of the eye (HIROSE and KAJI 1968); however, the injection of lactamide at this period of development does not result in the same effect as feeding (LUCE 1963). It can, therefore, be concluded that these compounds must be digested and metabolized for them to become active in the suppression of the Bar phenotype.

With the results of these feeding studies in mind, we can refer back to our original list of *su* and *e* genes and note that the loci affected by the feeding experiments also have both enhancer and suppressor genes which affect them. We believe this to be more than just a fortuitous coincidence. The results of the z^a:*bx* interaction show that a locus involved in pteridine synthesis (i.e., *z*) can act as an enhancer. Therefore, apparently one can alter the phenotype of certain genes in Drosophila by the addition or removal of certain heterocyclic compounds, whether externally or internally (i.e., by exogenous food supply or by gene mutation).

This alteration in metabolic balance could conceivably occur, not at the level of transcription or translation, but rather at the level of enzyme function as seems to be the point of interaction in the *su(s)* system. If this is, indeed, the type of system which is operative (i.e., pteridine interacting with enzyme molecule), the heterocyclic compound, if normally present, could be used *in vivo* as an inhibitor or cofactor in some enzyme system. The lack of this compound would then release a mutant enzyme with a low specific activity (inhibitor) or would not allow a low level of activity by not being present to release the enzyme (cofactor). This model would predict that only a very special class of mutants would be affected by the *su* and *e* genes—which is indeed the case; only certain spontaneous alleles are affected. The specific change at the molecular level is unknown at present except that the change does seem to be site-specific at the *v* locus (GREEN 1954). The data thus far, however, do not rule out a level of interaction at translation or transcription and a more definitive scheme awaits further developments.

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Corresponding Editor: B. JUDD