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Interaction of the *Stubble-stubbloid* Locus and the *Broad-Complex* of *Drosophila melanogaster*

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ABSTRACT

The 2B5 region on the *X* chromosome of *Drosophila melanogaster* forms an early ecdysone puff at the end of the third instar. The region is coextensive with a complex genetic locus, the *Broad-Complex* (*BR-C*). The *BR-C* is a regulatory gene that contains two major functional domains, the *br* domain and the *l(1)2Bc* domain. *BR-C* mutants prevent metamorphosis, including morphogenesis of imaginal discs; *br* mutants prevent elongation and eversion of appendages and *l(1)2Bc* mutants prevent fusion of the discs. The *Stubble-stubbloid* (*Sb-sbd*) locus at 89B9-10 is best known for the effects of its mutants on bristle structure. Mutants of the *BR-C* and the *Sb-sbd* locus interact to produce severe malformation of appendages. Viable heteroallelic and homoallelic combinations of *Sb-sbd* mutants, including loss-of-function mutants, affect the elongation of imaginal disc appendages. Thus, the $Sb\text{-}sbd^+$ product is essential for normal appendage elongation. *Sb-sbd* mutants, however, do not affect eversion or fusion of discs. Correspondingly, only *BR-C* mutants deficient in *br* function interact with *Sb-sbd* mutants. The interaction occurs in deficiency heterozygotes using single, wild-type doses of the *BR-C*, of the *Sb-sbd* locus, or of both loci. These last results are formally consistent with the possibility that the *BR-C* acts as a positive regulator of the *Sb-sbd* locus. The data do not exclude other possible nonregulatory interactions between the two loci, *e.g.*, interactions between the products of both genes.

STRIKING tissue morphogenesis occurs at the onset of metamorphosis in holometabolous insects such as Drosophila. In particular, the imaginal discs, folded embryonic epithelial structures, undergo morphogenesis to form appendages, evert the appendages, and then spread and fuse to form the continuous epithelium of the head and thorax (FRISTROM and FRISTROM 1975; MILNER, BLEASBY and KELLY 1984; POODRY and SCHNEIDERMAN 1970). These developmental changes occur immediately after puparium formation during the prepupal period in response to the steroid hormone 20-hydroxyecdysone (20-HE). A major goal of our laboratory is the identification and characterization of genes that mediate imaginal disc morphogenesis. NATZLE, HAMMONDS and FRISTROM (1986) have described a molecular approach to this goal. Here we describe a genetic approach.

The *Broad-Complex* (*BR-C*), located at 2B5 on the *Drosophila melanogaster* cytogenetic map, is one of two genetically well-defined regions involved in imaginal disc morphogenesis. The other, as reported here, is the *Stubble-stubbloid* (*Sb-sbd*) locus. The *BR-C* contains two major functional domains (BELYAEVA *et al.* 1980; KISS *et al.* 1988). One, the *broad(br)* domain, acts in the elongation of appendages and in their eversion to the outside of the animal. The second, the *l(1)2Bc* domain, is primarily involved in the fusion of disc tissue to form a continuous epithelial sheet. The *BR-C* responds directly to 20-HE (ASHBURNER 1972; CHAO and GUILD 1986). The *BR-C*, as predicted by ASHBURNER (1972), appears to encode transcriptional regulators (BELYAEVA *et al.* 1980; CROWLEY, MATHERS and MEYEROWITZ 1984). Because *BR-C* mutants autonomously prevent disc morphogenesis (FRISTROM, FEKETE and FRISTROM 1981), it is possible that the *BR-C* regulates genes whose products actually mediate disc morphogenesis.

br^1, a viable allele of the *BR-C*, behaves like a classic hypomorph; its mutant character is exaggerated when br^1 is made hemizygous over a deficiency (KISS *et al.* 1988). Homozygous br^1 females and hemizygous males have wings that are slightly shorter and wider than those of wild type. Female br^1/deficiency hemizygotes exhibit a severe phenotype which we call the malformed syndrome. The wings are greatly reduced in size and the second and third legs are short, thick, and twisted (KISS *et al.* 1988). The malformed syndrome is associated with incomplete appendage elongation during the prepupal period. Furthermore, assuming the *BR-C* encodes transcriptional regulators, the malformed syndrome in br^1/deficiency hemizygotes arguably results from an underproduction of products of genes regulated by the *BR-C*. A loss-of-function mutant in one of these genes, by reducing the level of a functional product, might act as a

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dominant enhancer of br^1, *i.e.*, produce the malformed syndrome in a br^1/Y male or a br^1/br^1 female.

Prior to inducing and screening for new mutations, we conducted a survey of known mutants to attempt to identify dominant enhancers of br^1. To this end, we examined 16 autosomal mutants that affect wing and leg morphology and 25 third larval instar recessive lethals (SHEARN *et al.* 1971). With one exception, none of these mutants was a dominant enhancer of br^1. The exception was the third chromosome mutant, *Stubble*^{1}. Dominant mutants at this locus (89B9-10) are referred to as *Stubble* (*Sb*), recessive ones as *stubbloid* (*sbd*). Our studies on the interaction of the *BR-C* and the *Sb-sbd* region are described in this paper.

Before further discussing *Sb-sbd* mutants, MULLER's (1932) functional nomenclature for mutants merits review. Compared to homozygotes, the phenotypes of partial loss-of-function mutants, hypomorphs, become more extreme over deficiencies or become less extreme when multiple mutant copies are present (MULLER 1932). The effects of complete loss-of-function mutants, amorphs, do not change over deficiencies or using multiple mutant copies. Both amorphs and hypomorphs are typically recessive. Among *BR-C* mutants, $npr1^3$ is an example of an amorph, br^1 of a hypomorph (KISS *et al.* 1988). An overproducer, or hypermorph, is a mutant that functions in excess. Combination with a deficiency might ameliorate the mutant phenotype but the addition of extra wild-type copies could intensify it. An I^{Q} mutant of *Escherichia coli* that produces excess *lac* repressor is an example of a hypermorph (GILBERT and MÜLLER-HILL 1970). MULLER (1932) suggested that the Drosophila mutant *abrupt* may be a hypermorph. A neomorph is typically a dominant mutant in which expression of the mutant gene or the nature of its product differs qualitatively from normal. Addition of extra wild-type copies of the gene has no effect on the mutant phenotype. Indeed, with respect to the neomorphic function, the wild-type gene is amorphic. Consequently, phenotypes produced by neomorphic mutants may not reflect the normal function of the gene. Nevertheless, the creation of a neomorph can also cause loss of normal gene function. In cases of pleiotropy, one aspect of the phenotype could be due to neomorphy, another aspect to amorphy or hypomorphy. Although synthesized *in vitro*, the $\Delta 2,3$ *P* element transposase mutant (LASKI, RIO and RUBIN 1986), because it expresses *P* element transposase in both somatic and germ-line tissues, not just in the germ line, is an example of a neomorph in which the normal function is not lost. An antimorph is usually a dominant mutant whose effect is opposite or antagonistic to that of the normal gene. An I^D mutant of *E. coli* that encodes a functionally destructive subunit of the *lac* repressor is an antimorph (GILBERT and MÜLLER-HILL 1970). The addition of extra wild-type gene copies should counteract the effects of an antimorph.

The *Sb-sbd* locus is characterized by both recessive and dominant mutants that affect bristle structure and reduce bristle length. The recessive *sbd* mutants appear to be amorphs or hypomorphs (see RESULTS). DOBZHANSKY (1930), on the basis of studies in triploids, concluded that the dominant *Sb* mutants, to use the nomenclature of MULLER (1932), are neomorphs. LEWIS (1951) suggested that Sb^1 is an antimorph. Because the effect of Sb^1 is to reduce bristle length, *i.e.*, is opposite to normal, and because the bristle phenotype in triploids is less severe in $Sb^1/+/+$ than in $Sb^1/Sb^1/+$ (DOBZHANSKY 1930), Sb^1 may be an antimorph as LEWIS (1951) suggested. However, because two doses of a neomorph should be more extreme than one dose, DOBZHANSKY's data are equally consistent with the view that Sb^1 is neomorphic.

MATERIALS AND METHODS

The *BR-C* and *Sb-sbd* mutants, and other stocks used in this study, are described in Table 1, in KISS *et al.* (1988), or in LINDSLEY and GRELL (1968). The *BR-C* mutants (Table 1) are listed as a function of their membership in various complementation classes of the complex, the *Sb* mutants in decreasing order of the severity of the bristle phenotype. All stocks were maintained on standard cornmeal and molasses medium at 18°. Experimental crosses were made in vials with 5 males and 5 females at 25°. Eggs were collected for 6 consecutive 24-hr periods at 25° and were then incubated at 18°, 25°, or 29°. All of the progeny that emerged were scored for the malformed phenotype. Progeny were considered to have the malformed phenotype if they had only short bowed femurs or had leg defects and short misshapen wings. Viability of offspring carrying *BR-C* and *Sb-sbd* mutant alleles was estimated by comparison to the numbers of $BR\text{-}C^+$ and $Sb\text{-}sbd^+$ siblings present in each cross.

The effect of *Sb* heteroallelic combinations on prepupal morphogenesis of imaginal discs was investigated. White prepupae were recovered and maintained at 25°. Animals were dissected at different times during prepupal development to determine the degree of appendage morphogenesis. In addition, the onset of cuticle formation in Sb^1/Sb^{63b} imaginal discs was determined by electron microscopy as described by FRISTROM and LIEBRICH (1986).

RESULTS

The malformed syndrome: $br^1/npr1^3$ heterozygotes (Figure 1a) have a far more severe phenotype, here designated the malformed syndrome, than br^1/br^1 homozygotes (Figure 1b). Because $npr1^3$ acts as an amorphic allele of the *BR-C* (KISS *et al.* 1988), the malformed syndrome results from having only one dose of br^1 instead of two. A similar malformed syndrome occurs in br^1/Y; $Sb^1/+$ males and in br^1/br^1; $Sb^1/+$ females (Figure 1c). Consequently, Sb^1 acts as a dominant enhancer of br^1. Finally (see below), all heteroallelic and viable homoallelic combinations of *Sb* alleles produce the malformed syndrome by them-

TABLE 1

Mutant symbols and abbreviations

Mutant class	Current designation	Previous designation	Origin	Comments	Reference
Broad-Complex (*BR-C*)					
broad (*br*)	br^7*	lt336	EMS	Lethal	(1)
reduced bristles on palpus (*rbp*)	rbp^5*	lt376	EMS	Lethal	(1)
lethal(1)prepupal-1=	$l(1)2Bc^3$*	lt149	EMS	Lethal	(1)
lethal(1)2Bc	$l(1)2Bc^4$*	lt197	EMS	Lethal	(1)
Sb-sbd locus					
Dominant alleles					
Stubble (*Sb*)	Sb^{63b}	Sb^{63b}	Spont.	Viable	(2)
	Sb^{70}	Sb^{70}	Spont.	Viable[a]	(3)
	Sb^1	Sb^1	Spont.	Lethal	(4)
	Sb^{Spi}	Sb^{Spi}	X-ray	Viable[b]	(5)
	Sb^V	Sb^V	X-ray	T(2;3)41A-C;88-89B[c]	(6)
Recessive alleles					
stubbloid (*sbd*)	sbd^2	sbd^2	Spont.	Viable	(2)
	sbd^{201}		EMS	Viable	(9)
	sbd^{26}	sbd^{26}	X-ray	Df(3R)89B9-10;89C7-D1[d]	(7)
	sbd^{45}	sbd^{45}	X-ray	Df(3R)89B4;89B10[d]	(7)
	sbd^{105}	sbd^{105}	X-ray	Df(3R)88F9;89B9-10[d]	(8)
Other stocks	*TM6B, Tb*			Also has *e, ca, Hu*	(10)

* Based on LINDSLEY and ZIMM (1986).
[a] Presumed viable because of viability with *sbd*-associated deficiencies.
[b] Viable as originally isolated and with *sbd*-associated deficiencies.
[c] Derived from Sb^1; homozygous lethal but partially viable with sbd^{26}.
[d] Homozygous lethal.
References: (1) BELYAEVA *et al.* 1980; (2) LINDSLEY and GRELL (1968); (3) LINDSLEY and ZIMM (1982); (4) DOBZHANSKY (1930); (5) MOORE (1935); (6) LEWIS (1956); (7) SPILLMANN-FALLER (1976); (8) LEWIS (1948); (9) J. FRISTROM and L. APPEL, unpublished data; (10) CRAYMER (1984).

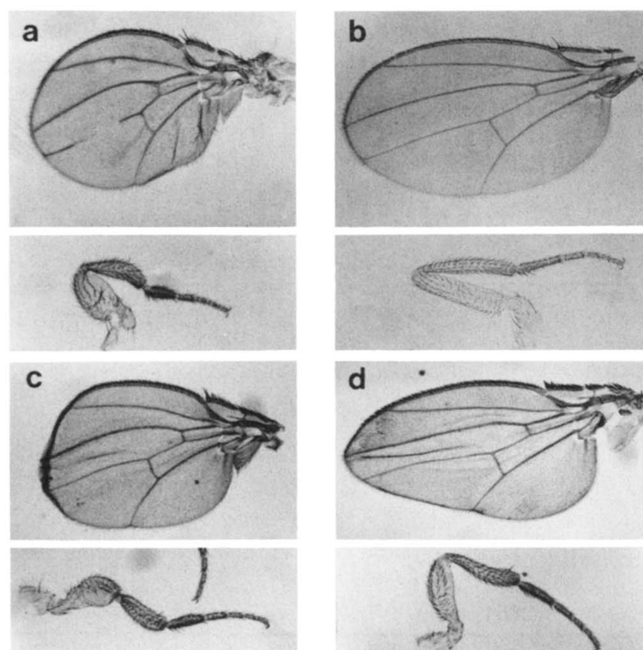

FIGURE 1.—A wing and third leg from a) $br^1/nprl^3$; +/+, b) br^1/br^1; +/+, c) br^1/Y; Sb^1/+ and d) +/Y; Sb^1/Sb^{63b}. For a comparison of br^1/Y and wild type, see KISS *et al.* (1988).

selves (*e.g.*, Figure 1d). In flies with the above genotypes, when the malformed syndrome is manifested, the third legs are always affected, the wings and second legs sometimes affected (in approximately 60% of the cases). Hence, malformed wings are typically only observed in the presence of malformed legs.

Mutants that are not dominant enhancers of br^1: In addition to *Stubble*, other mutants were tested for dominant interactions with *broad*. Fifteen of these are recessive mutants that cause defects in leg structure. These include *ad*, an^2, ap^{56f}, *app*, *cg*, *cmp*, *d*, *ds*, *dwh*, *rk*, rk^4, *fj*, *msf*, *tk* and *tkd*. None of these acted as a dominant enhancer of br^1 at 18° or 29° (data not shown). In addition, 25 third chromosome recessive lethals isolated by SHEARN *et al.* (1971) were tested for their interaction with br^1. These mutants are late larval and pupal lethals that have reasonably normal imaginal discs and include *lethals(3)m45*, v^1, v^{13}, *605*, *1711*, *2614*, *G921*, *G949*, *k60*, *m47*, *903*, *c2IL*, *e26R*, *G30R*, *g131*, *j5*, *j51*, *L36*, *m27*, *n3*, *wg89*, *1509*, *2813*, *XII-10* and *L6b*. Again, none of these lethals acted as dominant enhancers of br^1 at 18° or 29° (data not shown). Thus, the production of the malformed syndrome by intergenic interactions between br^1 and other mutants does not appear to be commonplace but may occur with only a limited set of other loci.

Phenotypic characteristics of *Sb-sbd* mutants: We have extended the investigations of DOBZHANSKY (1930) and LEWIS (1951) on Sb^1 and sbd^2 and determined the phenotypic effects of other *Sb-sbd* alleles.

TABLE 2

Phenotypes of combinations of various *Stubble* and *stubbloid* alleles

Genotypes:	Sb^{63b}	Sb^{70}	Sb^{1}	Sb^{Spi}	Sb^{V}	sbd^{2}	sbd^{201}	sbd^{26}	sbd^{45}	sbd^{105}
Sb^{63b}	Sb, mlf									
Sb^{70}	Sb, mlf	Sb, mlf								
Sb^{1}	Sb, mlf	Sb, mlf	L							
Sb^{Spi}	Sb, mlf	Sb, mlf	Sb, mlf	L						
Sb^{V}	Sb, mlf	Sb, mlf	L	ND	L					
sbd^{2}	Sb, mlf	Sb, mlf	Sb	Sb	Sb	sbd				
sbd^{201}	Sb, mlf	Sb, mlf	Sb, mlf	ND	ND	sbd	sbd, mlf			
sbd^{26}	Sb, mlf	Sb, mlf	L	Sb, mlf	E, Sb	sbd	sbd, mlf	L		
sbd^{45}	Sb, mlf	Sb, mlf	L	Sb, mlf	ND	sbd	sbd, mlf	L	L	
sbd^{105}	Sb, mlf	Sb, mlf	L	Sb, mlf	L	sbd	sbd, mlf	E, mlf	L	L

L, recessive lethal; sbd, stubbloid bristles, no malformed appendages; Sb, Stubble bristles, no malformed appendages; Sb, mlf, Stubble bristles, malformed appendages (See Figure 1); sbd, mlf, stubbloid bristles, malformed appendages. E, Sb, lethal with escapers that have Stubble bristles, normal appendages; E, mlf, lethal with escapers that have stubbloid bristles, malformed appendages (SPILLMANN-FALLER 1976). ND, not determined. sbd^{26}, sbd^{45}, and sbd^{105} are associated with deficiencies (see Table 1).

Stubble mutants are primarily known for their abnormal bristle morphology; they produce uniformly thick, short, and blunt bristles. The bristles produced in sbd^{2}/sbd^{2} flies, although shorter than those of +/+, are thinner and longer than those in *Stubble* flies and have somewhat tapered ends. Although we will regard sbd^{2} as recessive, DOBZHANSKY (1930) reported that bristles in sbd^{1}/+ flies are 1–3% shorter than those in co-isogenic +/+ flies. Deficiencies for the locus have no dominant Stubble phenotype (LEWIS 1951) and are viable and stubbloid when heterozygous with sbd^{2} (SPILLMANN-FALLER 1976). Sb^{1} and sbd^{2} are separated by 0.02 map unit (LEWIS 1951). Furthermore, the *cis* combination, Sb^{1} sbd^{2}, behaves like a recessive *sbd* allele (LEWIS 1951); it has no dominant Stubble phenotype and is stubbloid with sbd^{2}. Because sbd^{2} is a hypomorph (see below), this suggests that the expression of the Stubble phenotype in Sb^{1}/+ flies depends on sbd^{+} function. The *cis* combination, however, is lethal as a homozygote and with Sb^{1}.

Correlative effects of *Stubble* mutants on bristle structure and appendage morphogenesis: We have examined the phenotypes of most heteroallelic and heterozygous (with +) combinations of *Sb-sbd* alleles. With the exception of Sb^{1}/Sb^{V} all tested heteroallelic combinations of *Sb* alleles are viable and have the malformed syndrome (Table 2) which was first described in Sb^{Spi} homozygotes by MOORE (1935). Because all viable homoallelic and heteroallelic combinations of *Sb* alleles produce the malformed syndrome, it is clear that the phenotype results from *Sb* mutations and not from mutations located elsewhere on *Sb* chromosomes. The mutant bristle phenotype in all viable homoallelic and heteroallelic *Sb* combinations is more severe than that in the respective *Sb*/+ heterozygotes. The Stubble bristle phenotype is also intensified, compared to *Sb*/+, when a *Sb* allele is heterozygous with a *sbd* allele that is associated with a deficiency (*e.g.*, Sb^{63b}/sbd^{26}).

The severity of the effects on bristle structure varies; Sb^{63b} and Sb^{70} are the strongest alleles, Sb^{1} and Sb^{Spi} intermediate, and Sb^{V} the weakest (Table 1). When heteroallelic with sbd^{2}, the strongest *Sb* alleles (Sb^{63b} and Sb^{70}) cause the malformed syndrome, the weak alleles (Sb^{Spi}, Sb^{1} and Sb^{V}) do not (Table 2). Sb^{Spi}, when heterozygous with a *sbd*-associated deficiency (*e.g.*, Sb^{Spi}/sbd^{26}), causes the malformed syndrome. Sb^{V}/sbd^{26} escapers, however, are not malformed. Accordingly, the ability of a *Sb* allele to affect appendage structure correlates directly with the severity of its effect on bristle structure.

In addition to affecting bristle structure, *sbd* mutants affect other morphological characteristics. DOBZHANSKY (1930) found that the lengths of wings and legs, and the number of branches on aristae are slightly reduced in sbd^{1}/+ heterozygotes, and they are all substantially reduced in sbd^{1}/sbd^{1} homozygotes compared to wild type. SPILLMANN-FALLER (1976) indicated that sbd^{26}/sbd^{105} escapers exhibit the malformed syndrome. Furthermore, sbd^{201}, a new EMS-induced allele (Table 1), when hemizygous with *sbd*-associated deficiencies produces stubbloid bristles and the malformed syndrome. In addition, Sb^{1}/sbd^{201} flies, in contrast to Sb^{1}/sbd^{2} flies, express the malformed syndrome (Table 2). Although substantially more severe than sbd^{2}, sbd^{201} is apparently not amorphic because, in contrast to *sbd*-associated deficiencies, it does not intensify the bristle phenotype of sbd^{2} (as sbd^{2}/sbd^{201}, data not shown). sbd^{201}/sbd^{201} homozygotes, however, express the malformed syndrome. Thus, not only gain-of-function but loss-of-function *Sb-sbd* mutations produce the malformed syndrome. We conclude that the wild type products of the *Sb-sbd* locus are required for normal imaginal disc morphogenesis. Furthermore, the phenotypic similarities of the $br^{1}/npr1^{3}$ heterozygotes, br^{1}/Y; Sb^{1}/+ double mutants, and the Sb^{1}/Sb^{63b} heterozygotes (Figure 1) suggest that the *BR-C* and the *Sb-sbd* locus are involved

in common morphogenetic processes in imaginal discs. The sbd^{26}/sbd^{105} escapers, however, have a stubbloid, not Stubble, bristle phenotype. Thus, although the malformed and stubbloid phenotypes are caused by lack-of-function mutants, the Stubble phenotype is caused only by gain-of-function, neomorphic mutants.

Viability of *Sb* mutants: The original allele, Sb^{1} (3-58.2), behaves as a recessive lethal and is also lethal with three *sbd*-associated deficiencies that delete the 89B9-10 region (Table 2). Although $Sb^{63b}/+$ has a more severe phenotype than $Sb^{1}/+$, it is viable with *sbd*-associated deficiencies and even while exhibiting severe bristle and appendage phenotypes is partially viable as a homozygote. The Sb^{63b} chromosome as isolated behaved as a recessive lethal (J. MERRIAM, personal communication). Because full lethality was eliminated when we replaced much of the original Sb^{63b} chromosome by crossing over with a nonlethal *red* (3-53.6) and *ebony* (3-70.7) chromosome, we conclude that the lethality was associated with a mutation independent of Sb^{63b}. Sb^{Spi} was originally viable as a homozygote (MOORE 1935) but the Sb^{Spi} chromosome has apparently acquired an independent lethal because it is now lethal as a homozygote (Table 2). Sb^{63b} (as originally isolated), Sb^{70}, Sb^{Spi} and Sb^{V} are all viable or partially viable over at least one *sbd*-associated deficiency. We assume that recessive lethality of these *Sb* chromosomes, as demonstrated for the Sb^{63b} chromosome, results from lethals independent of the *Sb-sbd* locus. We have not, however, tested this assumption. Nonetheless, among five *Sb* alleles, Sb^{1} is the only one in which homozygous lethality may be causally associated with a *Sb* mutant.

One hypothetical explanation for the recessive lethality of Sb^{1} is that the four viable *Sb* alleles still retain wild-type function. This hypothesis predicts, however, that Sb^{1} is the strongest allele. This is not the case for the bristle phenotype (Table 1) nor for the production of the malformed syndrome (Table 2, also Tables 4 and 5). Moreover, the lethality of Sb^{1} homozygotes [which occurs during early larval development (A. H. BEATON, unpublished data)] appears independent of the action of *Sb* alleles in disc morphogenesis and bristle formation. Furthermore, because the *cis* combination, $sbd^{2}\ Sb^{1}$, has a stubbloid, not Stubble phenotype, it is arguable that wild-type sbd^{+} function must be maintained in all *Sb* alleles if the Stubble phenotype is to be expressed. A second explanation for Sb^{1} lethality, which we currently favor, is that the lethality is very closely associated with, but independent of, the *Sb-sbd* locus. SPILLMANN-FALLER's (1976) results with deficiencies associated with *sbd* mutations indicate that essential genes flank both sides of the *Sb-sbd* region. Inactivation of one or both of these genes could explain the homozygous lethality of the Sb^{1} chromosome, its lethality with Sb^{V} [which was derived from Sb^{1} (LEWIS 1956)], and with all *sbd*-associated deficiencies. A third but unlikely explanation is that the recessive lethality of Sb^{1} is a unique property of the neomorphic state of the Sb^{1} mutation.

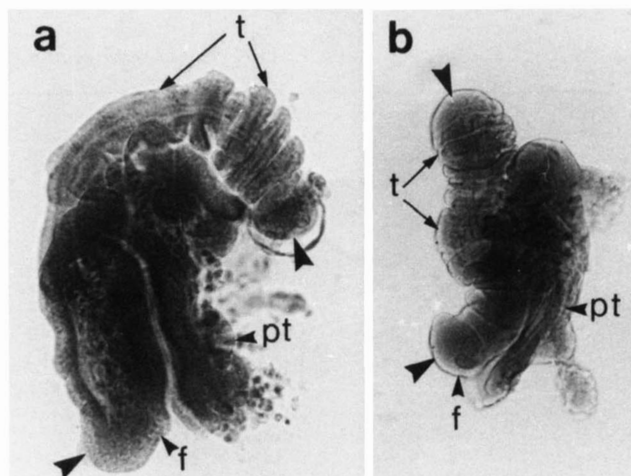

FIGURE 2.—Discs dissected from 6-hr (25°) prepupae. a) Wild type, b) Sb^{1}/Sb^{63b}. Note the marked reduction in the length of the leg (distance between arrowheads) in the Sb^{1}/Sb^{63b} animal. t, tarsus; f, femur; pt, presumptive thorax (×135).

Analysis of prepupal morphogenesis: Mutants of the *BR-C* affect elongation and eversion of appendages as well as the fusion of discs during the prepupal period (KISS *et al.* 1988). We determined whether *Sb-sbd* mutants have a comparable range of defects by dissecting prepupae 0 to 10 hr after puparium formation and observing the state of imaginal disc development. By 6 hr elongation and eversion of appendages were complete in both Sb^{1}/Sb^{63b} heterozygotes and in wild type. The Sb^{1}/Sb^{63b} legs are much less extended than those of wild type (Figure 2). Sb^{1}/Sb^{63b} discs *in vitro* fail to elongate in response to 20-HE, but do evert (data not shown). By 8 hr after puparium formation fusion of discs has occurred in both mutant and wild-type prepupae. Electron microscopic observations at 3 and 6 hr indicate that cuticle deposition follows the wild-type schedule in Sb^{1}/Sb^{63b} discs. Hence, the function of the $Sb\text{-}sbd^{+}$ gene appears to be limited during prepupal development to appendage elongation.

The interaction between mutants of the *BR-C* and of the *Sb-sbd* locus: The interactions between various *BR-C* and *Sb-sbd* alleles to produce the malformed syndrome have been further investigated to determine (1) whether the strength of the *BR-C* and *Sb-sbd* alleles correlates with the strength of their interaction, (2) whether the interaction is temperature-dependent, (3) whether the interaction is limited to particular *BR-C* complementation groups, and (4) whether both dominant and recessive *Sb-sbd* alleles participate in the interaction. These data are presented for viable *BR-C* alleles in Table 3; for lethal *BR-C* alleles in Table 4.

Viable *BR-C* alleles and *Sb* alleles: Generally, the

TABLE 3

Interactions between viable *BR-C* alleles and *Sb* alleles

BR-C genotype[a]	Temp.	Penetrance of the malformed syndrome (no. of flies) *TM6B, Tb*/+	Sb^{63b}/+	Sb^1/+	Sb^{Spi}/+
br^1/+	29°	0% (144)	4% (139)	1% (96)	
	18°	0% (342)	6% (261)	0% (158)	
br^1/Y	29°	0% (114)	32% (75)	13% (80)	
	18°	1% (238)	99% (83)	90% (117)	
br^3/+	29°	0% (165)	35% (133)	18% (66)	1% (135)
	18°	0% (297)	15% (133)	5% (64)	0% (96)
br^3/Y	29°	2% (194)	100% (54)	67% (45)	60% (109)
	18°	1% (240)	100% (78)	100% (66)	100% (74)
Binsn/+	29°		3% (180)	0% (243)	
	18°		4% (181)	0% (156)	
Binsn/Y	29°		6% (127)	0% (258)	
	18°		5% (138)	0% (182)	
+/+	29°		6% (144)		0% (122)
	18°		7% (252)		0% (114)
+/Y	29°		6% (164)		0% (121)
	18°		3% (220)		0% (88)

[a] br^x/br^x females × Sb^x/*TM6B, Tb*; *Binsn*/+ females × Sb^x/*TM6B, Tb* males; and Ore. R females *X* Sb^x/*TM6B, Tb* males (*x* equals any allele). The first number (%) is the penetrance of the malformed syndrome; the second number the number of flies examined. Viability was in excess of 90% for all crosses.

strengths of the *Sb-sbd* and the *BR-C* alleles correlate directly with the strength of the interaction between mutants of the two loci. br^3 homozygotes have a more extreme broad phenotype than br^1 homozygotes. In the presence of Sb^1 or Sb^{63b}, br^3 flies have a higher penetrance of the malformed phenotype than br^1 flies. Likewise, the strength of the *Sb* allele as judged by its effect on bristle length (Table 1, $Sb^{63b} > Sb^1 > Sb^{Spi}$) correlates directly with the penetrance of the malformed syndrome (Table 3). No effect of a *br* allele on the Stubble bristle phenotype was noted.

Temperature sensitivity: The penetrance of the malformed syndrome is temperature sensitive. In both br^1 and br^3 males penetrance of the malformed syndrome is greater at 18° than at 29°. In contrast, in heterozygous br^3/+ females penetrance is greater at 29° than at 18° (no interaction is seen with br^1/+ females). In the absence of a *br* mutant allele, the malformed syndrome is slightly penetrant but independent of temperature in Sb^{63b}/+ flies. Because temperature sensitivity is a function only of the *BR-C* genotype, we conclude that the temperature dependency involves the *BR-C*, not the *Sb-sbd* region (see below).

Lethal *BR-C* alleles and *Sb* alleles: The studies of BELYAEVA *et al.* (1980) and KISS *et al.* (1988) establish that there are at least three complementation groups in the *BR-C* (*br*, *rbp* and *l(1)2Bc*), a class of amorphic noncomplementing alleles (*npr1*), and a class of hypomorphic noncomplementing alleles (*l(1)2Bab*). The *l(1)2Bab* class is highly deficient in br^+ function but has nearly normal $l(1)2Bc^+$ function (KISS *et al.* 1988). In prepupae, amorphic *br* alleles prevent elongation and eversion of appendages; amorphic *l(1)2Bc* alleles prevent fusion of discs but allow elongation and eversion. Hypomorphic *rbp* alleles cause developmental arrest in pharate adults and have little effect on appendage elongation and fusion.

We determined which of the *BR-C* functions were critical for the interaction of *Sb* by constructing various double-mutant heterozygotes and examining them for the malformed syndrome (Table 4). All *BR-C* alleles that lack or have reduced br^+ function ($npr1^3$, $l(1)2Bab^1$, $l(1)2Bab^3$ and four *br* alleles) interact with *Sb* alleles. The interaction is stronger with Sb^{63b} than with Sb^1. The two amorphic *br* alleles (KISS *et al.*

TABLE 4

Interactions between lethal *BR-C* alleles and *Sb* alleles

BR-C genotypes	Penetrance (%) of the malformed syndrome (no. of flies) $npr1^3$/+	br^5/+	br^6/+	br^7/+	br^8/+	rbp^1/+	rbp^5/+	$2Bc^1$/+	$2Bc^3$/+	$2Bc^4$/+	$2Bab^1$/+	$2Bab^3$/+
Sb^1/+												
29°	3%	41%	2%	1%	11%	0%	0%	0%	0%	0%	7%	45%
	(95)	(51)	(43)	(82)	(102)	(67)	(84)	(144)	(34)	(46)	(74)	(33)
18°	1%	13%	0%	0%	3%	0%	0%	0%	0%	0%	6%	2%
	(54)	(38)	(46)	(100)	(90)	(86)	(80)	(74)	(45)	(110)	(72)	(62)
Sb^{63b}/+												
29°	65%	92%	98%	41%	100%	0%	2%	14%[a]	0%	0%	84%	100%
	(103)	(29)	(43)	(106)	(45)	(40)	(92)	(126)	(65)	(59)	(19)	(21)
18°	88%	67%	50%	19%	83%	6%	0%	1%	0%	0%	68%	96%
	(25)	(50)	(28)	(95)	(58)	(48)	(27)	(99)	(68)	(69)	(37)	(26)

[a] Spurious result, see text.
Mutant/Binsn females were crossed with +/*Y*; Sb^x/*balancer* males.
Crosses were at 18° or at 29° as indicated.
Viability was generally greater than 90% (br^5/+; Sb^{63b}/+ viability was lowest at 70% at 29°).
2Bab is *l(1)2Bab*; *2Bc* is *l(1)2Bc*.

1988), br^{5} and br^{8}, interact more strongly than the hypomorphic br^{6} allele. (Because br^{7} interacts weakly, we presume it is hypomorphic but we have not tested this directly.) These interactions occur without any marked effects on viability. With the exception of the $npr1^{3}$ allele, which is amorphic for all *BR-C* functions, penetrance of the malformed syndrome is greater at 29° than at 18° as was found for females heterozygous for the viable br^{3} allele. Again, no effect on the Stubble bristle phenotype was noted in these interactions.

The *rbp* and *l(1)2Bc* alleles exhibit no interaction with Sb^{63b} above the normal background of penetrance of the malformed syndrome with this strong *Sb* allele. The one apparent exception, the 14% penetrance at 29° for $l(1)2Bc^{1}/+$; $Sb^{63b}/+$ individuals (Table 4), is spurious because 11% of the non-$l(1)2Bc^{1}$ siblings were malformed (11 of 101 *Binsn*/+; $Sb^{63b}/+$ offspring). Low frequencies of the malformed phenotype are sometimes found in $Sb^{63b}/+$ flies in the absence of a *BR-C* mutant (Table 3). We did not attempt to investigate systematically the cause of occasional high frequencies (about 5–10%) of $BR\text{-}C^{+}$; $Sb^{63b}/+$ malformed flies.

In summary, *BR-C* genotypes with reduced *br* function, and so presumably with reduced ability to elongate appendages, exhibit the malformed syndrome in the presence of *Sb* alleles; those not reduced in *br* function and so presumably with full, or nearly full, capacity to elongate appendages do not exhibit the malformed syndrome in the presence of *Sb* alleles.

Interactions involving *BR-C* deficiencies and *sbd*-associated deficiencies: We also investigated whether reduction in $BR\text{-}C^{+}$ and $Sb\text{-}sbd^{+}$ products was sufficient to produce the malformed phenotype. Because deficiencies lack any gene product, we determined whether heterozygous deficiencies of the *BR-C* and of the *Sb-sbd* locus produced malformed flies. We used *Df(1)S39* because it is known to eliminate the *BR-C* region (BELYAEVA *et al.* 1987) and three *sbd*-associated deficiencies (Table 1). When heterozygous for *sbd*-associated deficiencies, males hemizygous for br^{1}, but not $br^{1}/+$ females, exhibit substantial penetrance of the malformed syndrome (Table 5). This indicates that reduction in sbd^{+} function is sufficient to cause the interaction with br^{1}. As with *Sb* alleles (see Table 3), penetrance of the malformed phenotype in br^{1} males heterozygous for *sbd*-associated deficiencies is greater at 18° than at 29°. The penetrance at 18° is comparable to that of br^{1}/Y; $Sb^{63b}/+$ and br^{1}/Y; $Sb^{1}/+$ males (Table 3). Recalling that neomorphic mutations can cause loss of normal function, this raises the possibility that loss-of-function associated with the neomorphic *Sb* alleles may play a primary role in the interaction with the *BR-C*. The results at 29° (Tables 3 and 5) do not support this view; *Sb* alleles interact more strongly with br^{1} than *sbd*-associated deficiencies (also see below). Little or no interaction is seen in br^{1} males that are heterozygous for the weak hypomorph sbd^{2} or in females heterozygous for both a lethal *BR-C* allele and sbd^{2} (data not shown). The strongly hypomorphic *sbd* allele (sbd^{201}), however, gives a clear interaction (>30% penetrance at 23°) in br^{1}/Y; $sbd^{201}/+$ males (data not shown). In all of these interactions involving *sbd* heterozygotes, the bristle phenotype was normal.

A surprising result occurred in these crosses. Namely, br^{1} males heterozygous for sbd^{2} have substantial larval lethality (60–95%) at 29°. These results will be reported in detail elsewhere (A. H. BEATON and J. W. FRISTROM, unpublished data). Otherwise, viability exceeded 80% in these crosses.

Females heterozygous for *Df(1)S39* and for a *Sb* allele exhibit the malformed syndrome (Table 5). There is no consistent temperature dependency, indicating again that temperature sensitivity is a function of the *BR-C* genotype. The strongest *Sb* allele (Sb^{63b}) causes the greatest penetrance of the malformed syndrome; the weakest allele (Sb^{Spi}), the least. The interaction also occurs in double deficiency heterozygotes. Females heterozygous for a deficiency of the *BR-C* and for a *sbd* allele associated with a deficiency exhibit, apparently in a temperature-independent fashion, 1–16% penetrance of the malformed syndrome. These results in particular demonstrate that the production of the malformed syndrome can arise as a result of the reduction of the products produced by the two loci. Again, the malformed phenotype occurred in the absence of any effect on the bristle phenotype. It is noteworthy that although the penetrance of the malformed syndrome in *Df(1)S39*/+; *sbd-deficient*/+ flies can be similar to that in *Df(1)S39*/+; $Sb^{1}/+$ and *Df(1)S39*/+; $Sb^{Spi}/+$ flies, that it is substantially lower than in *Df(1)S39*/+; $Sb^{63b}/+$ flies. This indicates, at least with the strongest *Sb* alleles, that the interaction is not merely a result of loss-of-function associated with acquisition of the *Sb* neomorphic state. The low penetrance of the malformed syndrome in *Df(1)S39*/+; $sbd^{26}/+$ flies (Table 5) is enigmatic and might suggest that sbd^{26} may retain some sbd^{+} function [recall that sbd^{26}/sbd^{105} produces some escapers (Table 2)]. This view is not supported by the results in br^{1}/Y; $sbd^{26}/+$ males (Table 5). Moreover, sbd^{26}, like other *sbd*-associated deficiencies, intensifies the bristle phenotype of sbd^{2}.

The effects of dose of $BR\text{-}C^{+}$ on the penetrance of the malformed phenotype in *Sb* flies: The interaction between the *BR-C* and the *Sb-sbd* locus could result from several causes. For example, mutants in non-*BR-C* genes (*e.g.*, hypothetically *Sb*) that regulate, or are regulated by, $BR\text{-}C^{+}$ could enhance br^{1} to produce the malformed syndrome. Also possible, however, is that

TABLE 5

Interactions between the *BR-C* and the *Sb-sbd* locus involving deficiencies

BR-C genotypes[a]	Temp.	Penetrance of the malformed syndrome (no. of flies)					
		$sbd^{26}/+$	$sbd^{45}/+$	$sbd^{105}/+$	$Sb^{1}/+$	$Sb^{Spi}/+$	$Sb^{63b}/+$
$br^{1}/+$	29°	0% (187)	4% (217)	0% (158)			
	25°	0% (153)	2% (127)	1% (96)			
	18°	0% (101)	0% (117)	1% (83)			
br^{1}/Y	29°	2% (118)	3% (144)	0% (46)			
	25°	13% (127)	2% (108)	25% (68)			
	18°	95% (111)	97% (108)	63% (83)			
Df(1)S39/+	29°	1% (367)	1% (269)	14% (206)	22% (73)	13% (46)	78% (41)
	25°	3% (372)	10% (469)	16% (200)	22% (156)	3% (239)	98% (179)
	18°	3% (249)	9% (391)	6% (246)	17% (66)	0% (20)	100% (23)

[a] br^{1}/br^{1} females were crossed to *Sb-sbd/TM2, Ubx* males or *Df(1)S39/Binsn* females were crossed to *Sb-sbd/TM2, Ubx* males.

a gene essential for appendage elongation but not regulated by $BR\text{-}C^{+}$ could, when mutant enhance br^{1} to produce malformed appendages. Genes whose products interact directly with $BR\text{-}C^{+}$ products would be included in this last category. By studying the effects of varying the dose of $BR\text{-}C^{+}$ on the expression of the malformed phenotype we sought to clarify the relationship between the two loci.

In the presence of the heteroallelic combination Sb^{1}/Sb^{Spi}, increasing the dose of $BR\text{-}C^{+}$ to four effective doses has no effect on the penetrance (Table 6) or the severity (data not shown) of the malformed syndrome. Sb^{1} and Sb^{Spi} were used in these experiments because they are moderate *Sb* alleles whose phenotypic expression might be subject to modification. Our failure in these experiments to suppress the malformed syndrome suggests that the *Sb-sbd* region does not regulate the *BR-C* because adding extra copies of $BR\text{-}C^{+}$ would have been expected to alleviate the malformed syndrome if *Sb* mutants reduced the expression of the *BR-C*.

Some transcriptional regulatory proteins, *e.g.*, the λ repressor (PTASHNE *et al.* 1980), can increase gene-specific transcription at low concentration and inhibit that transcription at high concentration. Consequently, we also investigated whether there was an interaction between extra copies of $BR\text{-}C^{+}$ in *Sb*/+ heterozygotes to produce malformed flies. To this end, using a *Y*-linked duplication of the 2B5 region ($y^{2}Y67g19.1$) (see KISS *et al.* 1988), we produced males that carried two copies of $BR\text{-}C^{+}$ in a *Sb*/+ background. Because of the presumed absence of dosage compensation (STEWART and MERRIAM 1980), this condition is equivalent to four effective copies of $BR\text{-}C^{+}$. In $Sb^{1}/+$, $Sb^{Spi}/+$, and in $Sb^{63b}/+$ flies, four effective copies of $BR\text{-}C^{+}$ did not produce significant penetrance of the malformed phenotype (Table 6). [The 4–6% penetrance of malformations in $Sb^{63b}/+$ flies is not considered above background for the strong Sb^{63b} allele. Furthermore, in flies identified as exhibiting the malformed syndrome, the legs were only slightly affected.] In $Sb^{63b}/+$ flies, depending on the cross, from 2% to 20% of the flies had misshapen wings. However, the misshapen wings, morphologically like those in Sb^{1}/Sb^{63b} flies and unlike those in br^{1}/deficiency or br^{1}/Y; $Sb^{1}/+$ flies (Figure 1), typically arose in the *absence of any effect on leg morphology* and consequently are reported in a separate category. In all other cases in which the malformed syndrome was expressed [*e.g.*, in $br^{1}/Df(1)S39$ (KISS *et al.* 1988), br^{1}/Y; $Sb^{1}/+$ and sbd^{201}/sbd^{26} flies] the third legs were always affected. In less extensive experiments, similar results to those with four copies of $BR\text{-}C^{+}$ were obtained in $y^{2}Y67g19.1/C(1)DX_{1}$; $Sb^{Spi}/+$ females carrying three copies of $BX\text{-}C^{+}$ (data not shown). Because of the difference in the pattern of morphological effects, we conclude that the interaction between extra copies of $BR\text{-}C^{+}$ and $Sb^{63b}/+$ heterozygotes that produces misshapen wings is of a different nature than that which typically produces the malformed syndrome. Furthermore, the variation in penetrance (from 2% to 20%) of misshapen wings suggests that the genetic background has an important role here, and that the interaction of four doses of $BR\text{-}C^{+}$ with $Sb^{63b}/+$ to produce the misshapen wings may be more indirect than that which produces the typical malformed syndrome. Finally, because the interaction of four doses of $BR\text{-}C^{+}$ with a *Sb* allele only occurs with Sb^{63b}, we cannot exclude the possibility that genes on the third chromosome other than Sb^{63b} have significant involvement.

In contrast, the malformed syndrome produced in $Sb^{Spi}/+$ and $Sb^{63b}/+$ metafemales ($+/C(1)DX_{1}$, Table 6) was morphologically typical (*i.e.*, the third legs were affected in all malformed examples and the wings were like those in Figure 1, a and c) and exhibited a high penetrance and a severe malformed phenotype. Only 2 (out of 64) Sb^{+}/Sb^{+} metafemales were identified that may have had a malformed phenotype. These flies did, however, exhibit the wing phenotype char-

TABLE 6

Effect of the dose of $BR\text{-}C^+$ on the penetrance of the malformed syndrome in *Sb* animals

Doses of $BR\text{-}C^+$					
Actual	Effective	Cross[a]	Genotype	*N*	Penetrance
					%
2	4	(a)	$+/YBR\text{-}C^+; Sb^1/Sb^{Spi}$	91	100
3	3	(a)	$C(1)DX_1/YBR\text{-}C^+; Sb^1/Sb^{Spi}$	63	100
2	2	(b)	$+/Binsn; Sb^1/Sb^{Spi}$	60	100
2	2	(b)	$+/Binsn; Sb^1/Sb^{Spi}$	77	100
1	1	(b)	$+/Df(1)S39; Sb^1/Sb^{Spi}$	128	100
2	4	(c)	$+/YBR\text{-}C^+; Sb^{Spi}/+$	257	1
3	2	(c)	$+/C(1)DX_1; Sb^{Spi}/+$	11	82
2	4	(d)	$+/YBR\text{-}C^+; Sb^{Spi}/+$	82	4
2	4	(e)	$+/YBR\text{-}C^+; Sb^{Spi}/+$	197	2
2	4	(f)	$+/YBR\text{-}C^+; Sb^{Spi}/+$	198	3
2	4	(c)	$+/YBR\text{-}C^+; Sb^1/+$	249	2
3	2	(c)	$+/C(1)DX_1; Sb^1/+$	1	0
2	4	(d)	$+/YBR\text{-}C^+; Sb^1/+$	68	0
2	4	(e)	$+/YBR\text{-}C^+; Sb^1/+$	194	0
2	4	(f)	$+/YBR\text{-}C^+; Sb^1/+$	142	0
2	4	(c)	$+/YBR\text{-}C^+; Sb^{63b}/+$	382	4 [20]
3	2	(c)	$+/C(1)DX_1; Sb^{63b}/+$	55	85
2	4	(d)	$+/YBR\text{-}C^+; Sb^{63b}/+$	94	12
3	2	(d)	$+/C(1)DX_1; Sb^{63b}/+$	9	67
2	4	(e)	$+/YBR\text{-}C^+; Sb^{63b}/+$	178	6 [20]
2	4	(f)	$+/YBR\text{-}C^+; Sb^{63b}/+$	177	0 [2]

All crosses were made at 25°.

[a] Crosses: (a) $C(1)DX_1$, *y w f*/$y^2Y67g19.1$; $Sb^{Spi}/TM6B$, *Tb* females × $+/y^2Y67g19.1$; $Sb^1/TM2$, *Ubx* males. (b) $Df(1)S39/Binsn$; $Sb^1/TM2$, *Ubx* females × +/*Y*; $Sb^{Spi}/TM2$, *Ubx* males. (c) $C(1)DX_1$, *y w f*/$y^2Y67g19.1$ females × +/*Y*; *Sb* allele/*TM2*, *Ubx* males. (d) $C(1)DX_1$, *y w f*/$y^2Y67g19.1$ females × +/*Y*; *Sb* allele/*TM6*, *Tb* males. (e) +/+; *Sb* allele/*TM6B*, *Tb* females × *y z*/$y^2Y67g19.1$ males. (f) +/+; *Sb* allele/*TM6B*, *Tb* females × $Df(1)S39$ $cho^2/y^2Y67g19.1$ males. In addition to $Sb^x/+$ heterozygous offspring carrying four effective doses of the $BR\text{-}C^+$ region, Sb^+/Sb^+ offspring carrying four effective doses of the $BR\text{-}C^+$ were recovered. Penetrance of the malformed phenotype in these offspring ranged for all crosses from 0% (crosses d, e, and f) to 1.4% (cross c, 7/497 offspring). Over 90% of all metafemales (3*X*,2*A*; +/$C(1)DX_1$, *y w f*) recovered exhibited scalloping on the lateral margins of the wings typical of metafemales. In addition to metafemales heterozygous for a *Sb* allele, 64 Sb^+ metafemales were also recovered; these had scalloped wings, two with malformed phenotypes. Percentages in brackets [ ] refer to flies with misshapen wings but normal legs (see text).

acteristic of metafemales (*e.g.*, slight scalloping of wing margins). Because of dosage compensation (LUCCHESI 1977; DEVLIN, HOLM and GRIGLIATTI 1988), the expression of *X*-linked genes in 3*X*;2*A* metafemales should equal that in 2*X*;2*A* females. Thus, the high penetrance and the strength of expression of the malformed syndrome in these metafemales was unexpected. Nonetheless, because the extra copy of $BR\text{-}C^+$ in these females is associated with triploidy for the entire *X* chromosome, we conclude the malformed phenotype does not arise because of three copies of $BR\text{-}C^+$ *per se* but because of changes in expression involving other genes on the *X* chromosome. It is noteworthy, however, that, although most autosomal genes are expressed normally in metafemales, the expression of the autosomal gene *LSP-1γ* is repressed (DEVLIN, HOLM and GRIGLIATTI 1988). It is tempting to speculate that the expression of the *Sb* locus may be affected in metafemales.

DISCUSSION

We have demonstrated that the *Stubble-stubbloid* locus encodes products necessary in prepupae for the normal elongation of imaginal discs to produce the appendages of the adult. Furthermore, we have identified a specific interaction between the *Sb-sbd* locus and the *Broad-Complex.* The most parsimonious hypothesis to explain the interaction holds that the *BR-C* regulates the *Sb-sbd* locus.

The role of the *Sb-sbd* locus in disc morphogenesis: DOBZHANSKY (1930) demonstrated that both sbd^1/sbd^1 and $sbd^1/+$ flies have shorter than normal legs and wings in addition to the bristle phenotype for which the locus is named. MOORE (1935) found that Sb^{Spi}/Sb^{Spi} adults had, in addition to Stubble bristles, grossly altered legs and wings, a phenotype which we refer to as the malformed syndrome. In early prepupae the legs of Sb^1/Sb^{63b} heterozygotes elongate less than those of wild type (Figure 2). Loss-of-function *sbd* alleles [*e.g.*, sbd^{201}/sbd^{26} and, according to SPILLMANN-FALLER (1976) sbd^{26}/sbd^{105} escapers] also produce the malformed syndrome presumably because they too block prepupal elongation of appendages. Consequently, the $Sb\text{-}sbd^+$ product is required for normal disc morphogenesis. Because *Sb* alleles apparently have no effect on disc eversion or disc fusion, the effects of *Sb-sbd* mutants in the prepupal period appear to be limited to appendage elongation.

The elongation of appendages is primarily a result of cell rearrangement (FRISTROM and FRISTROM 1975; FRISTROM 1976), a widespread process in epithelial morphogenesis (TRINKAUS 1984). Preliminary analyses (D. FRISTROM unpublished data) indicate that cell rearrangement is affected in Sb^1/Sb^{63b} discs. Cellular and molecular analyses of *Sb-sbd* mutants may help to elucidate the molecular mechanisms of cell rearrangement. Assuming that the *Sb-sbd* locus does not regulate the *BR-C*, a reasonable hypothesis is that the $Sb\text{-}sbd^+$ product is an apical transmembrane protein present during both cell rearrangement, and the differentiation of bristle cells. The presence of abnormal products in *Sb* mutants, or the absence of product in *sbd* mutants could have profound effects on both appendage elongation and bristle differentiation. To speculate, *Sb* mutants might affect the extracellular domain of the hypothetical transmembrane protein producing abnormal signals that are transmitted to the cytoskeleton where they cause abnormal function

or assembly of microfilaments. As a consequence, *Sb* mutants would be dominant neomorphs, not antimorphs. The recessive *sbd* mutants might affect or disable the internal domain of the hypothetical transmembrane protein to prevent the transmission of external signals to the microfilament systems and the response to that signal. Assuming such a model, a *Sb-sbd cis*-combination would produce a stubbloid phenotype.

The nature of the *Sb-sbd*, *BR-C* interaction: A regulatory function of the 2B5 region was first proposed by ASHBURNER (1972) based on the analysis of polytene chromosome puffing patterns. In addition to demonstrating the genetical coincidence of the *BR-C* and the 2B5 region, BELYAEVA *et al.* (1980, 1981) demonstrated in amorphic *npr1* mutants of the *BR-C* that early ecdysone puffs do not regress and late puffs do not form. Of particular importance, CROWLEY, MATHERS and MEYEROWITZ (1984) have found that transcription of the autosomal *Sgs-3* gene is effectively abolished in *npr1* mutants. These studies persuasively argue that the $BR\text{-}C^{+}$ products have roles in transcriptional regulation. Our studies have implicated the *BR-C* in appendage elongation. FRISTROM, FEKETE and FRISTROM (1981) reported that normal elongation and eversion is absent in $npr1^{3}$ discs. KISS *et al.* (1988) demonstrated that the *br* domain of the *BR-C* is primarily involved in the prepupal elongation and eversion of appendages.

If the *BR-C* is a regulatory complex, it must control disc morphogenesis by regulating genes whose products actually mediate morphogenesis. Consequently, in situations where *BR-C* function in limited, *e.g.*, in br^{1}/Y males, an otherwise recessive mutation in a regulated gene might enhance the br^{1} phenotype, that is produce the malformed syndrome. Because the malformed syndrome is also produced in br^{1}/deficiency females, presumably from a reduction in the amount of br^{1} transcript, enhancement of the br^{1} phenotype could also arise because of mutations in genes necessary for transcription of the *BR-C*. We presume the enhancement by *Ubl*, a mutant in the gene that encodes the major subunit of RNA polymerase II (GREENLEAF *et al.* 1980), of the broad phenotype of *Df(1)S39*/+ to produce the malformed syndrome (MORTIN and LEFEVRE 1981), is one such case. Because of the regulatory nature of the *BR-C* and the dose sensitivity of the br^{1} mutation, interactions between *BR-C* mutants and those of other genes to enhance the br^{1} phenotype are arguably likely to be regulatory.

If an interaction between two genes is regulatory, *i.e.*, the product of one gene regulates the transcription of another, one expectation is that the interaction will occur using amorphic alleles of both loci. Failure to meet such an expectation would argue against a regulatory interaction. Meeting this expectation is consistent with a regulatory interaction, but does not exclude a host of other possibilities. Because the malformed phenotype arises using no-activity mutations of both the *BR-C* and the *Sb-sbd* locus, *i.e.*, deficiency heterozygotes of both loci, a regulatory interaction is possible. If the interaction is regulatory, it is likely for two reasons that the *BR-C* regulates expression of the *Sb-sbd* locus. One, during prepupal development, *BR-C* mutants, including *br* mutants, have a wider range of phenotypic effects than *Sb-sbd* mutants, *br* mutants affect both elongation and disc eversion; *Sb-sbd* mutants only affect elongation. If the *Sb-sbd* locus regulated the *BR-C*, *Sb-sbd* mutants would also be expected to affect disc eversion. Their failure to do so suggests that the *Sb-sbd* locus does not regulate the *BR-C*. Two, if the *Sb-sbd* locus regulated the *BR-C* the addition of extra $BR\text{-}C^{+}$ gene copies, by producing more $BR\text{-}C^{+}$ product, might suppress the expression of the malformed syndrome in Sb^{1}/Sb^{Spi} flies. Such suppression, however, does not occur (Table 6). We have, nonetheless, been unable to perform an essential reciprocal experiment (CONCHA *et al.* 1988) and test whether extra copies of $Sb\text{-}sbd^{+}$ suppress the malformed syndrome produced in $br^{1}/Df(1)S39$ flies. It is important to recall that only *BR-C* alleles deficient in *br* function interact with *Sb-sbd* alleles, while *l(1)2Bc* alleles, which do not affect appendage elongation, do not interact with *Sb-sbd* alleles (Table 4). This differential specificity within the *BR-C* suggests the complex encodes products with different regulatory functions, only one of which may regulate the *Sb-sbd* locus.

To summarize, the current data on the interaction between the two loci are formally consistent with a model that hypothesizes that the *BR-C* encodes a positive transcriptional factor that regulates the transcription of the *Sb-sbd* locus. Such an hypothesis is not weakened by the results of CROWLEY, MATHERS and MEYEROWITZ (1984) which show that the amorphic $npr1^{3}$ mutant of the *BR-C* prevents transcription of the *Sgs-3* gene. Other explanations, however, cannot be excluded. For example, the same results probably would have been obtained if both loci encode transcriptional factors that are synthesized in limiting amounts and that must form dimers to regulate genes necessary for appendage elongation.

Temperature sensitivity: One somewhat remarkable observation is the inverse temperature dependence for producing the malformed syndrome in *br/Y* males and *br*/+ females (Tables 3–5). Highest penetrance of the malformed syndrome was "cold-dependent" (18°) for *br/Y* males; "heat-dependent" (29°) for *br*/+ females. Temperature sensitivity typically involves the thermal lability of proteins (SUZUKI 1970). Because no significant temperature effects were observed when only br^{+} is present (*e.g.*, in +/+; Sb^{63b}/+

or *Df(1)S39*/+ flies), we presume the switch in temperature sensitivity between, *e.g.*, br^1/Y and $br^1/+$ flies, results from the different environment in which the br^1 product exists in cells of the two genotypes. The obvious difference in the two genotypes, is that in br^1/Y flies only the br^1 product is present, in $br^1/+$ flies both br^1 and br^+ products are present. If the action of the br^1 product were independent of br^+, the same temperature-dependence should be present in hemizygotes and heterozygotes. One possible explanation for the differential temperature sensitivity is that the two products form multimers. Multimers composed only of br^1 product, only of br^+ product, or of both br^1 and br^+ could, respectively, be cold sensitive, temperature independent, and heat sensitive. Although unlikely, the differential temperature sensitivity might also stem from sex-specific effects on *br* expression, or be dependent on dosage compensation-specific effects. Supporting a possible role for multimers, KISS *et al.* (1988) have previously demonstrated that $br^1/Df(1)S39$ females are only 1% viable at 18° but are 43% viable at 29°. Thus, these females that express only the br^1 product exhibit cold-sensitivity like br^1/Y males. Because of the possible existence of allelic complementation within the *BR-C*, we have previously proposed that products of the *BR-C* exist in multimers (KISS *et al.* 1988). The current data support that proposal.

CHAO and GUILD (1986) have cloned the *BR-C* region and we are in the process of cloning the *Sb-sbd* region (L. APPEL, A. HAMMONDS and J. FRISTROM, unpublished data). Determination of the nature of both the $BR\text{-}C^+$ and $Sb\text{-}sbd^+$ products will help to elucidate the basis for the interaction between the two loci. The *Sb-sbd* locus is not the only dominant enhancer of br^1. In addition to *Ubl* on the *X* chromosome (MORTIN and LEFEVRE 1981), we have already identified another dominant enhancer of br^1 on the second chromosome (P. GOTWALS and J. FRISTROM, unpublished data). The analysis of such enhancers may ultimately help to establish a genetic hierarchy or network that governs the steroid-induced morphogenesis of imaginal discs.

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