Three Neighboring Genes Interact With the *Broad-Complex* and the *Stubble-stubbloid* Locus to Affect Imaginal Disc Morphogenesis in Drosophila

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ABSTRACT

The *Broad-Complex* (*BR-C*) is a complex regulatory locus at 2B-5 on the *X* chromosome of *Drosophila melanogaster*. The wild-type *BR-C* products are apparent transcription factors necessary for imaginal disc morphogenesis. Alleles of the *Stubble-stubbloid* (*Sb-sbd*) locus at 89B9-10 act as dominant enhancers of *broad* alleles of the *BR-C*. *Sb-sbd* wild-type products are necessary for appendage elongation. We report, here, on three new loci implicated in imaginal disc morphogenesis based on their genetic interactions with both *BR-C* and/or *Sb-sbd* mutants. *Enhancer of broad* (*E(br)*) was identified as a dominant enhancer of the br^1 allele of the *BR-C* and is a recessive lethal. Mapping of *E(br)* has led to the identification of two loci, *blistered* and *l(2)B485*, mutants of which interact with *E(br)* and the *Sb-sbd* locus. *Blistered*, but not *l(2)B485*, interacts strongly with the *BR-C*. Alleles of the *blistered* locus are viable and disrupt proper wing disc morphogenesis independent of genetic interactions. All three loci map within the 0.6-map unit interval between the genetic markers *speck* and *Irregular facets* and to the cytological region 60C5-6; 60E9-10 at the tip of chromosome *2R*. Genetic evidence is consistent with the view that the *BR-C* regulates *blistered*.

MANY aspects of embryogenesis and organogenesis require dramatic morphogenetic changes in epithelial cells and tissues. Despite recent advances in the molecular analysis of cell-cell and cell-matrix interaction (see BERNFIELD 1989 and DAMSKY 1989), our understanding of the morphogenesis of epithelial cells and tissues *in vivo* remains poor.

Imaginal discs of *Drosophila melanogaster*, small epithelial sacs that give rise to much of the adult integument, are a convenient model for studying general epithelial morphogenesis. They are easily isolated, undergo development *in vitro*, and their development is autonomously disrupted by known mutants. Discs undergo striking morphogenesis in response to the steroid hormone 20-hydroxyecdysone (ecdysone) at the onset of metamorphosis. Disc morphogenesis can be divided into three distinct phases; appendage elongation, eversion, and fusion (FRISTROM *et al.* 1987). Initially, each appendage elongates within the lumen of the disc. The elongated appendage is then everted from within the sac to the outside of the animal to resemble the adult structure. Finally, the cells residing around the periphery of the everted disc spread and fuse with the cells of neighboring discs to form a continuous imaginal epidermis. These morphogenetic events occur between pupariation and pupation, which we refer to as the prepupal period. We are interested in identifying those genes and their products that underlie the cellular mechanics of disc morphogenesis. Below, we introduce a genetic approach to this problem; screening for dominant enhancers of a viable *Broad-Complex* (*BR-C*) mutant. To characterize mutant alleles, we will follow the nomenclature of MULLER (1932), as discussed in BEATON *et al.* (1988).

The *BR-C* is a complex, regulatory locus in the 2B-5 region of the *X* chromosome (BELYAEVA *et al.* 1980). It is coextensive with an ecdysone-induced salivary gland puff and genetically has at least two major functional domains that affect imaginal disc morphogenesis, the *broad* (*br*) domain and the *l(1)2Bc* domain (KISS *et al.* 1988). In particular, mutants of the *broad* domain prevent appendage elongation and eversion. Recent sequence evidence (P. R. DiBello, D. A. Withers, C. A. Bayer, J. W. Fristrom and G. M. Guild, manuscript submitted for publication) indicates that the *BR-C* encodes three or more DNA-binding proteins produced by alternative splicing. Furthermore, mutants in the *BR-C* also affect the transcription of third larval instar genes (CROWLEY, MATHERS and MEYEROWITZ 1984). It is likely that the *BR-C* directly regulates genes whose products are necessary for disc morphogenesis.

A recessive viable, hypomorphic allele of the *BR-C*, br^1, causes homozygous females and hemizygous males to have shorter and wider wings than wild-type. When br^1 is made hemizygous over a deficiency, these females exhibit an exaggerated malformed (mlf) phenotype (KISS *et al.* 1988). In the most extreme cases, the second and third legs are gnarled and twisted and the wings are crumpled. FRISTROM *et al.* (1987) have shown that this syndrome is, in part, due to improper appendage elongation during the prepupal period,

implicating the wild-type *broad* product in this developmental process. The malformations seen in flies that have a single dose of br^1 may result from the underexpression of genes, regulated by the *BR-C*, whose products are needed for disc morphogenesis. A loss-of-function mutant in one of these genes might act as a dominant enhancer of br^1, generating the mlf syndrome in br^1 homozygous females and br^1 hemizygous males. A survey of 16 autosomal mutants that affect wing and disc morphology and of 25 third instar recessive lethals identified the *Sb-sbd* locus, located at 89B9-10 on the third chromosome, as one such enhancer (BEATON *et al.* 1988).

Mutants of the *Sb-sbd* locus have shortened, tapered bristles. This locus is characterized both by recessive *sbd* mutations that behave as amorphs or hypomorphs and dominant *Sb* mutations that behave as neomorphs (DOBZHANSKY 1930; LEWIS 1951). The *Sb-sbd* locus acts as a dominant enhancer only of the *broad* alleles of the *BR-C*. Likewise, the wild-type *Sb-sbd* product is necessary for appendage elongation. The observation that deficiencies for the *BR-C* and the *Sb-sbd* locus dominantly interact is compatible with the view that the *BR-C* regulates the *Sb-sbd* locus (BEATON *et al.* 1988).

To identify new dominant enhancers of br^1, we used a single generation screen for EMS-induced mutants that interacted with br^1 to generate mlf flies. This screen identified a novel recessive lethal mutation, *Enhancer of broad* (*E(br)*), which maps to the tip of the right arm of the second chromosome and acts as a dominant enhancer of *BR-C* alleles and alleles of the *Sb-sbd* locus. In the course of mapping *E(br)*, we have identified two deficiencies, *Df(2R)Px2* and *In(2LR)Px4*, which uncover two independent loci, *blistered* and *l(2)B485*, that interact with *E(br)* to produce malformed flies. The *blistered* locus is defined by a set of viable alleles that disrupt proper imaginal wing disc morphogenesis resulting in a blistered adult wing. All three loci lie within the 0.6-map unit interval between *speck* and *Irregular facets* and within the cytological boundaries 60C5-6; 60E9-10 near the tip of chromosome *2R*. Consequently, we have identified a set of clustered, but independent, genes that are involved in imaginal disc morphogenesis.

MATERIALS AND METHODS

Mutant stocks and crosses: Mutants and stocks used in this study are described in Table 1 (BEATON *et al.* 1988; KISS *et al.* 1988). All stocks were maintained on standard cornmeal and molasses medium at 18°. All crosses and scoring of progeny were performed according to BEATON *et al.* (1988). Specific genotypes of flies used in crosses is given in the table legends. Progeny were raised at either 18° or 25°. The criteria used to score progeny as mlf are described in RESULTS.

Mutagenesis and isolation of *E(br)*: br^1 males were mutagenized with EMS (LEWIS and BACHER 1968) and mass mated at 23° to br^1/br^1 females. Progeny (G_1) that exhibited the malformed phenotype were recovered and mated back to normal br^1 males or to br^1/br^1 females. mlf male progeny (G_2) were mated to normal br^1/br^1 females. mlf sibs (G_3) from this cross were mated to each other to establish lines of mlf flies. Among approximately 19,000 G_1 progeny (based on wet weight), 98 flies were recovered (56 males, 42 females) carrying possible dominant enhancers of br^1, of which 54 (35 males, 19 females) produced G_2 progeny. From these, 17 mlf lines were established from G_3 flies that had an initial mlf penetrance ≥20%. Of these, one line was lost and two (*E(br)* and sbd^{201}) carried dominant enhancers that were assignable, using balancers, to the second and third chromosomes, respectively. These mutations were both recovered in males and their original G_4 penetrance at 23° was 50% for *E(br)* and 36% for sbd^{201}. Both chromosomes, as originally isolated, however, contained recessive lethals linked to, but independent of the enhancers. $br^1/br^1;sbd^{201}/sbd^{201}$ flies lacking the linked lethal are viable and exhibit 100% penetrance of an extreme mlf phenotype. *E(br)*, however, is, itself, a recessive larval lethal. The dominant enhancing activity in the remainder of the lines was not assignable, using balancers, to a single chromosome and presumably was due to multigenic effects. These stocks were discarded.

Recombination mapping: *E(br)* was initially mapped to the tip of the right arm of the second chromosome using the marker chromosome *al* (0.01), *dp* (13.0), *b* (48.5), *pr* (54.5), *c* (75.5), *px* (100.5), *sp* (107). More precise mapping, using the marker chromosome *al b pr cn sp If* (107.6), placed *E(br)* within 0.1 map unit centromere-proximal to *If*. Recombinant chromosomes were always tested for interaction with br^1 and Sb^{63b}, and for lethality with the original *E(br)* chromosome. In no case was the interaction with Sb^{63b} or the homozygous lethality separated from the interaction with br^1.

RESULTS

The malformed syndrome: The phenotype of hypomorphic br^1 homozygotes is intensified when br^1 is placed in *trans* to $npr1^3$, a complete loss of function allele of the *BR-C* (see Figure 1 of BEATON *et al.* 1988), resulting in the malformed (mlf) syndrome. mlf flies show a range of partially penetrant phenotypes. In the most severe cases, flies exhibit twists or gnarls in the femurs and tibias of the second and third legs and the wings are often round and crumpled. Typically, wing malformations occur only in the presence of leg defects. The mildest expression of the mlf syndrome, a dent in the femur of the third leg, is, however, considered diagnostic of the mlf syndrome. The mlf phenotype can be produced by a dominant genetic interaction between two unlinked loci, the *BR-C* and the *Sb-sbd* locus (BEATON *et al.* 1988). Here, we introduce three additional genes that behave as intergenic noncomplementing loci to produce the mlf phenotype. For our purposes, a genetic interaction refers solely to this nonallelic, noncomplementing behavior. Some interacting alleles are partially dominant with respect to the mlf phenotype, independent of any genetic interaction. For instance, about 5% of $Sb^{63b}/+$ flies exhibit the mlf syndrome, while 1–2% of *E(br)*/+ flies

TABLE I

Mutant symbols and abbreviations

Mutant class	Designation	Origin	Comments	Reference[a]
Broad Complex				
broad function	br^1	Spontaneous	Viable	1
	br^3	Spontaneous	Viable	1
	br^5	EMS	Lethal	1
l(1)2Bc function	$l(1)2Bc^1$	EMS	Lethal	1
	$l(1)2Bc^2$	EMS	Lethal	1
Noncomplementing	$npr1^3$	EMS	Lethal	1
	$l(1)2Bab^1$	EMS	Lethal	1
Sb-sbd locus				
Sb dominants	Sb^{63b}	Spontaneous	Viable	2
	Sb^{70}	Spontaneous	Viable	2
	Sb^1	Spontaneous	Lethal	2
	Sb^{spi}	X-ray	Viable	2
	Sb^v	X-ray	T(2:3)41A-C;88-89B	2
sbd recessives	sbd^{201}	EMS	Viable	2
	sbd^1	Spontaneous	Viable	2
	sbd-Sb	Synthetic	Viable	2
Enhancer of br^1	*E(br)*	EMS	Lethal	3
blistered locus	bs^2	Spontaneous	Viable	4
balloon	bs^{ba}	Spontaneous	Viable	4
A48	bs^{A48}	EMS	Viable	5
Deficiencies	*Df(1)S39*	X-ray	Df(1)1E1-2;2B5-6	1
	Df(2R)SB1	X-ray	Df(2R)60E10-F1;60F5	6
	Df(2R)IIX62	EMS	Df(2R)60E9-10;60F1-2	7
	Df(2R)ES1	EMS	Df(2R)60E6-8;60F1-2	8
	Df(2R)Px2	X-ray	Df(2R)60C5-6;60D9-10	6
	In(2LR)Px4	Synthetic	Deficient for 60B-D1; Duplicated for 21D1-22A3	6
	sbd^{45}	X-ray	Df(3R)89B4;89B10	2
Other stocks	$In(2LR)bw^{VI}$	X-ray	In(2LR)21C8-D1;60D1-2 + In(2LR)40F;59D4-E1	6
	$B3t^5$	EMS	Lethal β3-tubulin mutation	5
	l(2)B485	EMS	Lethal	5
	l(2)D424	DEB	Lethal	5

[a] (1) KISS *et al.* (1988); (2) BEATON *et al.* (1988); (3) this work; (4) LINDSLEY and ZIMM (1985); (5) KIMBLE, DETTMAN and RAFF (1990); (6) LINDSLEY and ZIMM (1987); (7) NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING (1984); (8) CÔTÉ *et al.* (1987).

are malformed. We consider a genetic interaction to be significant when penetrance of the mlf phenotype is at least 5 times that of the background penetrance for either of the interacting alleles in populations of progeny typically equal to or greater than 50 flies.

Figure 1 displays the prepupal wing discs, the adult wings and the adult legs produced by two combinations of these interacting loci, $br^1/Y;E(br)/+$ and $E(br)/+;Sb^{63b}/+$. Wings of mlf flies produced by genetic interactions involving *E(br)* often exhibit a gathering or notching of tissue in the posterior region of the wing blade. Prepupal wing discs derived from stocks of mlf flies show a notch in a corresponding position of the presumptive wing blade (Figure 1, e and h). This observation supports the view that the mlf syndrome seen in adults results from improper prepupal disc morphogenesis. Evidence of abnormal twisting in the presumptive femur and tibia of the prepupal leg disc is also seen, but more difficult to document in photographs.

Interactions between *E(br)* and *BR-C* alleles: *E(br)* was initially identified as a mutation that enhances br^1 to produce the mlf syndrome. Because the *BR-C* is a complex locus exhibiting both complementing and noncomplementing sets of alleles, we characterized the interaction between *E(br)* and representative *BR-C* alleles. Females carrying different *BR-C* alleles were crossed to *E(br)* heterozygotes and the progeny were scored for the mlf phenotype (Table 2).

Flies, homozygous for the hypomorphic br^1 or br^3 alleles have wings that are shorter and wider than wild type. *E(br)* interacts with both of these alleles in hemizygous males to a similar extent (Table 2a). *E(br)* does not interact above background with either of these alleles in heterozygous females. Moreover, *E(br)* does not interact above background with br^5, an amorphic, homozygous lethal allele, in heterozygous females (Table 2b). Although the interaction between *E(br)* and *BR-C* depends on loss of *broad* function, one wild-type *br* allele is sufficient to prevent the interaction

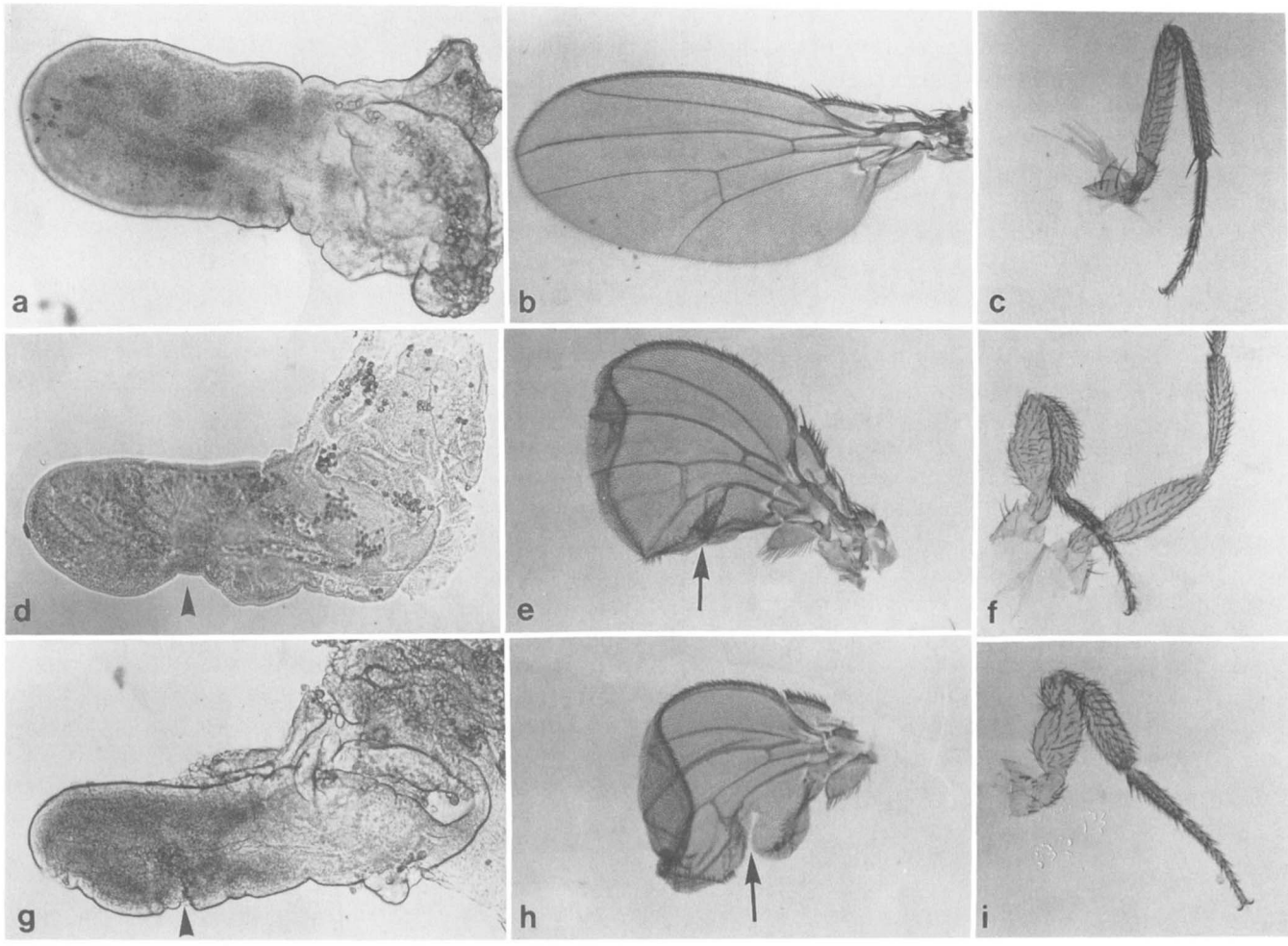

FIGURE 1.—Prepupal wing discs, adult wings and legs from: (a, b, c) wild type; (d, e, f) $br^1/Y;E(br)/+$; (g, h, i) $E(br)/+^{\bullet 63b}/+$. Arrows identify a gap or tissue gathering in the posterior region of the wing blade and the corresponding position in the prepupal wing disc. Prepupal wing discs were dissected 6 hr after pupariation.

TABLE 2

Interactions between *E(br)* and the *Broad-Complex*: Percent penetrance of the malformed syndrome (no. of flies)

a. Viable *BR-C* alleles

Genotypes[a]	T[b]	$br^1/+$	br^1/Y	$br^3/+$	br^3/Y
E(br)/+	18	2 (144)	38 (120)	2 (135)	23 (101)
	25	2 (101)	16 (128)	1 (196)	10 (183)
Df(2R)ES1/+	18	<3 (35)	<3 (39)	ND	ND

b. Lethal *BR-C* alleles

Genotypes[c]	T	$npr1^3/+$	$2Bab^1/+$	br^5 +/+ $2Bc^1$	$br^5/+$	$2Bc^1/+$	$2Bc^2/+$
E(br)/+	18	18 (61)	28 (36)	34 (50)	<3 (69)	<1 (132)	<2 (58)
	25	15 (145)	12 (127)	27 (22)	<1 (90)	4 (123)	<1 (74)

Abbreviations: ND, not determined; T, temperature.

[a] br^n/br^n females were crossed to either *al dp b pr cn E(br)/SM1* or *Df(2R)ES1/CyO* males. *Df(2R)ES1* is deficient for the *E(br)* region.

[b] In all crosses, progeny were raised at either 18° or 25°.

[c] Females carrying lethal *BR-C* alleles were crossed to *al dp b pr cn E(br)/SM1* males. Lethal *BR-C* alleles were maintained over the balancers *FM6l* or *Binsn*.

between *E(br)* and these loss-of-function *broad* alleles.

The noncomplementing allele $npr1^3$ behaves as an amorphic allele for the entire *BR-C* region while $l(1)2Bab^1$ behaves as a hypomorphic allele for the entire region. They result in a loss or partial loss of function of both the *broad* and *l(1)2Bc* domains. Because both of these alleles are homozygous lethal, their interaction in hemizygous males cannot be examined. In heterozygous females, *E(br)* interacts with these alleles at a penetrance of 18–28% at 18° (Table

2b). One wild-type copy of the entire *BR-C* is not sufficient to prevent the interaction between *E(br)* and these noncomplementing alleles.

The observation that *E(br)* interacts more strongly with $npr1^3$ than with br^5 suggests that loss of *broad* function is not solely responsible for the interaction between *E(br)* and the *BR-C* alleles. There is, however, no significant interaction between *E(br)* and $l(1)2Bc^1$/+ or $l(1)2Bc^2$/+ (Table 2b), lethal, amorphic *l(1)2Bc* alleles. This shows that the interaction between *E(br)* and the *BR-C* alleles is not primarily due to improper *l(1)2Bc* function. One explanation for the observed interaction between *E(br)* and $npr1^3$ or $l(1)2Bab^1$ is that the *BR-C* contains redundant functional domains. In heterozygous females, loss of *l(1)2Bc* function may be partially restored by wild-type *broad* function. Conversely, wild-type *l(1)2Bc* function may compensate for defective *broad* function. Loss of both functional domains might, however, lead to a dominant interaction with *E(br)*. The observation that *E(br)* interacts with the heteroallelic combination br^5 +/+ $l(1)2Bc^1$, which presumably removes the same amount of *BR-C* function as $npr1^3$/+, is consistent with this view (Table 2b). This explanation is also supported by the fact that the *BR-C* encodes multiple pairs of conceptual DNA-binding zinc fingers (P. R. DiBello, D. A. Withers, C. A. Bayer, J. W. Fristrom and G. M. Guild, manuscript submitted for publication). If a mutation in one pair disrupts one functional domain, another wild-type pair may compensate.

The interaction between *E(br)* and all the *BR-C* alleles is temperature dependent. Flies reared at 18° show a higher penetrance of mlf phenotype than those reared at 25° consistent with the temperature sensitivity of *broad* function alleles (Kiss *et al.* 1988).

Interactions between *E(br)* and the *Stubble-stubbloid* locus: During imaginal disc morphogenesis, the *Stubble-stubbloid* locus is predominantly involved with appendage elongation. Correspondingly, *Sb-sbd* mutants only interact with *BR-C* alleles defective in *broad* function. Because *E(br)* interacts with *broad* alleles, we tested whether *E(br)* also interacts with the *Sb-sbd* locus to generate the mlf phenotype. *Sb-sbd* mutants were crossed to *E(br)* heterozygotes and the progeny were scored for mlf phenotype. The data in Tables 3, a and b, shows that *E(br)* interacts to some degree with all dominant *Sb* alleles, but interacts much less, if at all, with the recessive *sbd* alleles examined.

The dominant *Sb* mutations of the *Sb-sbd* locus represent a set of neomorphic alleles varying in strength. Sb^{63b} and Sb^{70} are the strongest, exhibiting very short, stocky bristles of uniform length. Sb^1 is more moderate and Sb^{spi}, the weakest, exhibits slightly longer and more tapered bristles than Sb^1. Sb^v is associated with a translocation generated from a Sb^1 stock. In general, the intensity with which *E(br)* interacts with these alleles reflects the strength of the *Sb-sbd* allele. At 25°, *E(br)* interacts with Sb^{63b} and with Sb^{70} at 100% penetrance. In contrast, at the same temperature, *E(br)* interacts with Sb^1 at 50% penetrance and with Sb^{spi} at 25% penetrance. Sb^v behaves similarly to Sb^1.

We also examined the interactions between *E(br)* and representative recessive *sbd* alleles (Table 3b). These homozygous recessive mutations generate a range of phenotypes including short, tapered bristles and malformed appendages (Beaton *et al.* 1988). sbd^1, a hypomorphic allele, fails to interact as a heterozygote with *E(br)*. Similarly, the *cis* combination sbd^2-Sb^1, which in other genetic tests behaves solely as a *sbd* allele, fails to interact. An apparent deficiency for the locus, sbd^{45}, interacts weakly with *E(br)*, but the penetrance is lower than that with even the weakest *Sb* allele. sbd^{201}, however, behaves differently from the other recessives. This allele was identified in the same screen that generated *E(br)* (MATERIALS AND METHODS) and mapped to the *Sb-sbd* locus (L. Appel and J. W. Fristrom, unpublished results). In *trans* to other *Sb-sbd* alleles, it behaves as a strong hypomorphic or amorphic allele, yet at 25°, sbd^{201} dominantly interacts with *E(br)* at a penetrance of 20–26% (Table 3b). The sbd^{201} allele may express a gene product which has little or no wild-type function. However, this defective gene product may interact with the *E(br)* gene product to generate a more severe phenotype. This explanation would account for the observation that sbd^{201} interacts more severely than an apparent deficiency. Our data suggest that the interaction between *E(br)* and the *Sb-sbd* locus does not necessarily depend on the dominant *Sb* mutations, but that expression of an aberrant *Sb-sbd* product may be necessary for a strong interaction. Temperature does not dramatically affect the interaction between *E(br)* and the *Sb-sbd* locus.

Mapping of *E(br)* identifies new interacting regions: *E(br)* was initially mapped to the tip of the right arm of the second chromosome (see METHODS AND MATERIALS). To define the location of this locus cytologically with respect to the salivary polytene chromosomes, we crossed heterozygous *E(br)* females to heterozygotes carrying various chromosomal deficiencies near the tip of chromosome *2R*. *Df(2R)SB1* (60E10-F1;60F5) (Lindsley and Zimm 1987), *Df(2R)IIX62* (60E9-10;60F1-2) (Nüsslein-Volhard, Wieschaus and Kluding 1984), and *Df(2R)ES1* (60E6-8; 60F1-2) (Côté *et al.* 1987) constitute a set of overlapping deficiencies that extend from the tip of *2R* to 60E6-8, just proximal to *If* (Figure 2). *E(br)* is lethal in *trans* to *Df(2R)ES1*, but fully viable with the other two deficiencies (data not shown). This places the lethal associated with the *E(br)* locus between 60E6-8, the proximal break point of *Df(2R)ES1*, and 60E9-10, the proximal break point of *Df(2R)IIX62*.

TABLE 3

Interactions between *E(br)* and the *Sb-sbd* locus: percent penetrance of malformed syndrome (no. of flies)

a. The *Sb* dominant alleles

Genotypes[a]	T	S	Sb^{63b}/+	Sb^{70}/+	Sb^{1}/+	Sb^{sPr}/+	Sb^{v}/+
E(br)/+	18	F	90 (49)	100 (62)	55 (56)	57 (47)	31 (48)
		M	75 (45)	88 (43)	29 (51)	43 (69)	46 (24)
	25	F	100 (48)	100 (33)	52 (54)	24 (67)	48 (41)
		M	100 (50)	100 (49)	35 (46)	25 (64)	39 (33)
Df(2R)ES1/+	25	F	<3 (38)	ND	ND	ND	ND
		M	<3 (36)				

b. The *sbd* recessive alleles

Genotypes[a]	T	S	sbd^{45}/+	sbd^{201}/+	sbd^{2}-Sb^{1}/+	sbd^{1}/+
E(br)/+	18	F	<1 (96)	3 (70)	<2 (66)	2 (58)
		M	11 (104)	5 (76)	5 (66)	<2 (63)
	25	F	2 (134)	20 (41)	<2 (55)	<2 (67)
		M	8 (140)	26 (53)	2 (55)	<2 (66)

Abbreviations: ND, not determined; T, temperature; S, sex; M, male; F, female.

[a] *Sb-sbd* lethals were maintained over the balancers TM6 or TM6B. Flies carrying selected *Sb-sbd* alleles were crossed to *al dp b pr cn E(br)/SM1* or *Df(2R)ES1/CyO* flies.

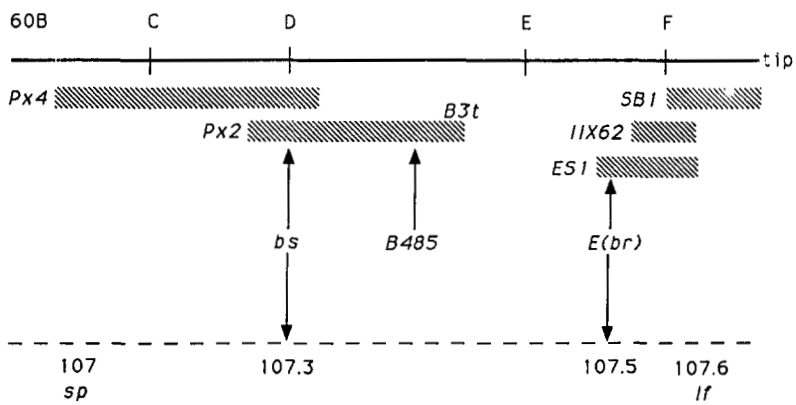

FIGURE 2.—Genetic and cytological maps of interacting loci on the tip of chromosome *2R*. The solid black line illustrates the 60B-F region of a polytene salivary gland chromosome. The hatched boxes represent chromosomal deficiencies which uncover *bs*, *l(2)B485* and *E(br)*. See Table 1 for deficiency break points. The distal breakpoint of *Df(2R)Px2* lies in the 5′ region of the β3-tubulin gene (*B3t*). The dashed line illustrates the genetic map associated with the cytological region. The map positions for *bs* and *E(br)* are given as well as the map position of two markers (*sp* and *lf*) used to map *E(br)*.

Df(2R)Px2 (60C5-6;60D9-10, here referred to as *Px2*) and *In(2LR)Px4* (deficient for 60B;60D1-2, here referred to as *Px4*) uncover 60B to 60D9-10. When *E(br)* is crossed to stocks carrying these deficiencies, progeny of the genotype *E(br)/deficiency* are fully viable, but exhibit the mlf phenotype at a penetrance of 14–35% (Table 4). This formally suggests that the mutation generating the enhancing phenotype could be uncovered by *Px2*, while an associated recessive lethal could be uncovered by *Df(2R)ES1*.

To address this issue, we mapped the two phenotypes associated with the *E(br)* locus with respect to a β3-tubulin mutation, $B3t^{5}$, a homozygous lethal mutation that is partially rescued by the β3-tubulin gene introduced by *P* element-mediated transformation (KIMBLE, DETTMAN and RAFF 1990). The distal break point of *Px2* lies within the 5′-flanking region of the *Drosophila* β3-tubulin transcript (KIMBLE, DETTMAN and RAFF 1990), allowing us to use $B3t^{5}$ as a genetic marker for the distal break point of *Px2*. Interestingly, the β3-tubulin gene is transcribed in imaginal discs (KIMBLE, INCARDONA and RAFF 1989) and is also induced by ecdysone in both Kc cells (MONTPIED *et al.* 1988) and imaginal discs (P. J. GOTWALS and J. W. FRISTROM, unpublished results), implicating it in disc morphogenesis. None of the five tested β3-tubulin mutants, however, interacts with loci examined in this report.

An *E(br)* chromosome marked by *sp* was placed in *trans* to a $B3t^{5}$ chromosome marked with *lf*. Females of this genotype were crossed to males homozygous for the mutations *al b pr cn sp lf*. Male progeny were scored and recombinants between *sp* and *lf* were crossed to both br^{1} and $B3t^{5}$. Of 5626 males scored,

TABLE 4

Complementation groups under *Px2* and *Px4* interact with *E(br)*, the *BR-C* and the *Sb-sbd* locus: percent penetrance of malformed syndrome (no. of flies)

Genotypes[a]	T	S	*Px2*/+	*l(2)B485*/+	*l(2)D424*/+	*Px4*/+	bs^2/+	bs^{ba}/+	bs^{A48}/+
br^1/Y	18	F	NA	NA	NA	NA	NA	NA	NA
		M	28 (87)	2 (53)	<1 (81)	20 (55)	36 (59)	<1 (71)	<3 (35)
	25	F	NA	NA	NA	NA	NA	NA	NA
		M	1 (64)	<2 (58)	<1 (75)	<1 (79)	<1 (99)	<2 (52)	<2 (50)
E(br)/+	18	F	1 (92)	4 (57)	3 (60)	50 (55)	20 (64)	<4 (25)	4 (49)
		M	1 (98)	7 (60)	<1 (80)	27 (63)	18 (76)	<3 (35)	<4 (25)
	25	F	35 (99)	14 (59)	13 (68)	16 (90)	25 (48)	20 (59)	<2 (56)
		M	22 (110)	7 (54)	5 (76)	14 (92)	11 (61)	9 (35)	<3 (29)
Sb^{63b}/+	18	F	<1 (77)	2 (48)	<1 (44)	<2 (57)	44 (107)	50 (28)	36 (56)
		M	5 (76)	2 (44)	<4 (25)	<2 (50)	18 (101)	16 (32)	11 (45)
	25	F	11 (150)	70 (33)	20 (65)	<2 (61)	30 (123)	47 (17)	33 (36)
		M	26 (167)	20 (25)	16 (69)	<2 (61)	19 (116)	46 (26)	7 (32)

Abbreviations: NA, not applicable; T, temperature; S, sex; M, male; F, female.

[a] br^1/br^1, *al dp b pr cn E(br)*, or $Sb^{63b}/TM6B$ females were crossed to *Df(2R)Px2/Cyo*, *l(2)B485/Cyo*, *l(2)D424/CyO*, *In(2LR)Px4/Cyo*, bs^2/bs^2, bs^{ba}/bs^{ba}, or bs^{A48}/bs^{A48} males.

24 *sp If* chromosomes and 10 sp^+ If^+ recombinants were recovered. This recombination frequency agrees with the published map distance of 0.6 map unit between *sp* and *If*. Of the 16 *sp If* flies that produced progeny, none carried *E(br)* as revealed by lack of interaction with br^1, while 8 were lethal with $B3t^5$. Of the 8 sp^+ If^+ flies that produced progeny, 7 carried *E(br)*, while all 8 were lethal with $B3t^5$. The recessive lethality and the dominant enhancer associated with *E(br)* map just proximal to *If*. These data reveal that in 15 of 24 possibilities, $B3t^5$ recombined with the *E(br)* locus. This clearly shows that *E(br)* is separate from and maps distal to the β3-tubulin gene and, therefore, is not uncovered by *Px2*. Moreover, in none of 41 tested recombinants recovered between *sp* and *If* has the enhancing phenotype of *E(br)* ever been separated from the recessive lethal. We conclude that the two phenotypes result from a single mutation that is uncovered by *Df(2R)ES1*, and that *Px2* and *Px4* must contain one or more interacting loci.

***Px2* and *Px4* uncover two interacting loci:** To identify possible loci uncovered by *Px2* that interact with *E(br)*, we obtained mutations isolated under this deficiency (KIMBLE, DETTMAN and RAFF 1990), crossed representative males to heterozygous *E(br)* females and scored the progeny for the mlf phenotype. One complementation group, represented by two alleles, *l(2)B485* and *l(2)D424*, interacts with *E(br)* (Table 4), although not nearly as strongly as *Px2*. These mutations also interact weakly with Sb^{63b}, but they do not enhance br^1. There is an additional noteworthy observation with respect to these mutations. *Px2* presumably acts as a null mutation for this locus. *l(2)D424* is probably a small deficiency based on the observation that it uncovers two complementation groups (KIMBLE, DETTMAN and RAFF 1990). One would expect these two mutations to behave similarly. *Px2*, however, in contrast to both *l(2)D424* and *l(2)B485*, acts as a dominant enhancer of br^1 (Table 4). It also interacts more strongly with *E(br)* than either of the other mutants. The locus represented by *l(2)B485* may not solely account for the interaction between *Px2* and *E(br)*. The observation that *Px4*, which complements *l(2)B485*, also interacts with *E(br)* (Table 4) suggests that the overlap region between these two deficiencies might contain another interacting locus.

Blistered (*bs*; 107.3), and *balloon* (*ba*; 107.4) are two mutations, identified by BRIDGES and MORGAN (1919), that lie in the region of overlap between *Px2* and *Px4*. These mutations generate blistered wings and exhibit venation defects (LINDSLEY and ZIMM 1985). However, *bs* and *ba* have not been separated by recombination. Another mutation, *A48* (107.3; data not shown) which as a homozygote results in blistered wings was identified in a screen for lethal mutations uncovered by *Px2* (KIMBLE, DETTMAN and RAFF 1990). These three mutations may be allelic (see below). For simplicity, we will refer to them as members of the *blistered* (*bs*) locus, renaming *ba* as bs^{ba} and *A48* as bs^{A48}. After pupation, the dorsal and ventral wing surfaces separate in wild-type flies. According to WADDINGTON (1940), mutations at the *bs* locus result in the subsequent improper apposition of these epithelial sheets. Our data, however, suggest that *blistered* may affect dorsal-ventral flattening of the wing during prepupal morphogenesis (see below).

We crossed bs^2, bs^{ba}, and bs^{A48} males to *E(br)* females and scored the progeny for mlf phenotype at both 18° and 25°. bs^2, a strong allele of the *bs* locus,

interacts with *E(br)* and Sb^{63b} at both temperatures (Table 4). bs^{ba} interacts with *E(br)* at 25° and with Sb^{63b} at both temperatures while bs^{A48} interacts only with Sb^{63b}, primarily in females. Furthermore, both *Px4* and bs^2 act as relatively strong dominant enhancers of br^1 at 18°, while bs^{ba} and bs^{A48}, as heterozygotes, do not interact with br^1.

The observation that *Px2* also uncovers the *bs* locus resolves certain discrepancies among the interaction data concerning *Px2*, *l(2)B485*, and *l(2)D424*. First, bs^2 acts as a dominant enhancer of br^1. This would account for the fact that *Px2* interacts with br^1, but *l(2)B485* and *l(2)D424* do not. Second, loss of both the bs^2 and *l(2)B485* loci could be additive with respect to their interaction with the *E(br)* locus. This would account for the observation that *Px2* interacts more strongly with *E(br)* than either *l(2)B485* or *l(2)D424* alone.

The dominant interaction between *Px2*, *Px4* or bs^2 with br^1 is observed only at 18°. This is not unexpected because br^1 expression is cold sensitive. Conversely, the penetrance of the interactions between *Px2*, *l(2)B485*, *l(2)D424* and Sb^{63b} or *E(br)* is higher at 25° than at 18°. For example, the penetrance of the interaction in *l(2)B485/+;*Sb^{63b}*/+* females is 70% at the higher temperature and a negligible 2% at the lower temperature (Table 4). This is much more extreme than the slight temperature effect seen in the interaction between *E(br)* and the more severe *Sb* alleles. Temperature has little effect at all on the interaction between the mutations in the overlap region between the *Px* deficiencies and *E(br)* or Sb^{63b}.

Genotypic and phenotypic characterization of the *blistered* locus: Virtually nothing has been reported concerning the genetics of the *bs* locus since its original identification by BRIDGES and MORGAN (1919). In an initial study, we addressed the allelism of mutations in the *bs* region. We also determined various adult phenotypes associated with *bs* mutations.

Allelism: Because bs^{ba} was first described as a locus separate from *bs* (see *balloon*, BRIDGES and MORGAN 1919; LINDSLEY and ZIMM 1985), the first question we addressed was whether bs^2, bs^{ba}, and bs^{A48} are alleles of the same gene or mutations in very closely linked genes involved in the same developmental process. All three mutations disrupt proper wing blade apposition, map to the *Px2-Px4* overlap region, interact with the *Sb-sbd* locus and partially complement. This suggests that they are allelic. They all behave differently, however, with respect to br^1 and *E(br)*. bs^2 acts as a dominant enhancer of br^1, and interacts with *E(br)* (Table 4). bs^{ba} interacts with *E(br)*, but has only a recessive enhancing effect on br^1 (Table 5b). bs^{A48}, which has the most severe homozygous phenotype of the three mutations, does not interact with *E(br)* and acts only as a mild recessive enhancer of br^1. Moreover, while bs^{A48} and bs^2 clearly fail to complement, bs^{ba} appears to fully complement both bs^{A48} and bs^2 at 25° and partially complement these mutations at 18° (Table 5a). The interpretation of this complementation test is tenuous because bs^{ba} is such a weak allele as a homozygote. It is possible that the dominant irregular venation phenotype of *Px2* and *Px4* is due to a loss of a number of genes involved in the same process and that bs^2, bs^{ba} and bs^{A48}, are mutations in more than one gene. Alternatively, they could be mutations in distinct, but related functional domains of a single gene. The incomplete penetrance and variability of these phenotypes would make data from fine structure mapping experiments ambiguous. Pending molecular characterization and clarification, we will consider these three mutations *bs* alleles.

Phenotypes: A range of adult phenotypes (Figure 3) is generated by various genetic combinations between *Px2*, *Px4*, bs^{ba}, bs^2, and bs^{A48} (Table 5). We have subdivided these wing phenotypes into four classes in order of ascending severity (weak plexate < plexate < blistered < balloon). Weak plexate (Figure 3a) involves slightly abnormal venation defects in which a small extra vein often derives from and runs perpendicular to the posterior crossvein. Small free veins also occasionally appear in the intervein region. Plexate (Figure 3b) is characterized by severe venation defects. Veins IV and V often end in deltas and give rise to extraneous veins. Free veins occur at a much higher frequency than in weak plexate. Blistered (Figure 3c) results not only in the venation defects described above, but also in complete blisters forming in the posterior region of the wing blade. These blisters are sometimes filled with fluid in newly eclosed adults, but generally dry out within 1 hr. Balloon (Figure 3d) results in a blister which covers the entire surface of the wing blade leaving only the wing margins intact. Veins are absent in balloon wings because apposition of the dorsal and ventral surfaces is eliminated (see GARCIA-BELLIDO 1977). These balloon wings contain both fluid and air pockets in newly eclosed adults and often fail to dry out.

Homozygous bs^{A48} prepupal wing discs, dissected 6 hr after puparium formation, exhibit numerous folds in the dorsal and ventral epithelia (Figure 4a), suggesting that these two cell layers are not as closely apposed as in wild type. These mutant discs give rise to wings with severe blisters (Figure 4b). Presumably the molecules necessary to either generate and/or maintain the apposition of the dorsal and ventral epithelial layers of the wing are missing during both prepupal wing disc morphogenesis and during wing blade expansion after eclosion.

bs^{ba}, as a homozygote, exhibits a very weak plexate phenotype (Table 5a). This is a weaker phenotype than that described by LINDSLEY and ZIMM (1985).

TABLE 5

Genetics of the *blistered* locus

a. Heteroallelic combinations generate different phenotypes

Genotypes[a]	*Px2*	*Px4*	bs^{ba}	bs^{2}	bs^{A48}
bs^{ba}	Blistered	Blistered	Weak plexate		
bs^{2}	Blistered	bs/ba; 35% mlf[b]	wk px/+[c]	Plexate	
bs^{A48}	Balloon	ba, 30% mlf	wk px/+[c]	px/bs	px/bs
Wild type	Plexate	px/bs	+	+[d]	+[d]

b. br^{1} enhances alleles of the *blistered* locus

Genotypes[a]	bs^{ba}/bs^{ba}		bs^{2}/bs^{2}		bs^{a48}/bs^{a48}	
	wing	*leg*	*wing*	*leg*	*wing*	*leg*
Wild type	wk px	+	Plexate	+	px/bs	+
br^{1}/br^{1}	Balloon	50 (83)[e]	px/bs	100 (65)[e]	px/bs	50 (87)[e]

Abbreviations: wild type (+); weak plexate (wk px); plexate (px); blistered (bs); balloon (ba).
[a] Development at 18°
[b] mlf refers only to the leg phenotype.
[c] bs^{ba} fully complements both bs^{2} and bs^{A48} at 25°; see text.
[d] Occasional free vein effect; see BRIDGES and MORGAN (1919).
[e] Percent penetrance of malformed syndrome (no. of flies).

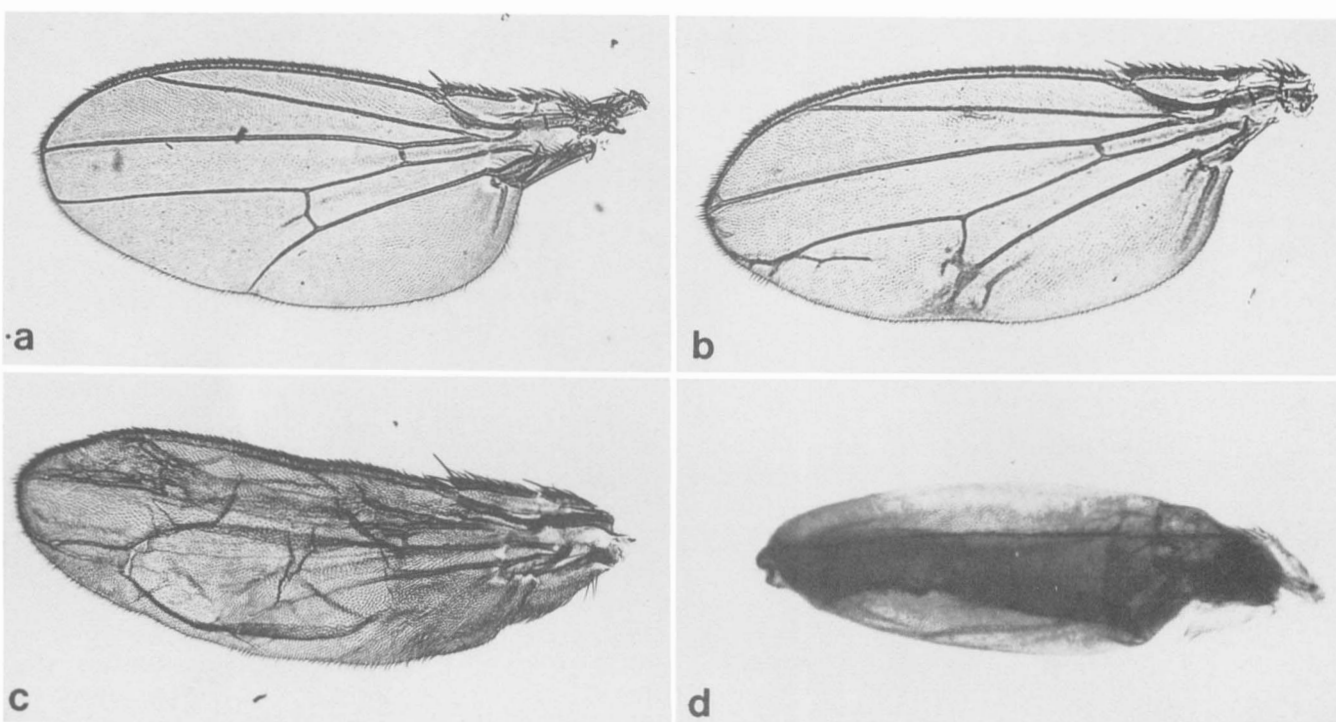

FIGURE 3.—Wing phenotypes associated with the *blistered* locus. (a) Weak plexate (bs^{ba}/bs^{ba}); (b) plexate (bs^{2}/bs^{2}); (c) blistered ($bs^{2}/Df(2R)Px2$); (d) balloon ($bs^{2}/In(2LR)Px4$).

This description, however, is based on BRIDGES and MORGAN (1919) and selection for a weaker phenotype may have occurred in the intervening years. bs^{2} is somewhat more severe, revealing the plexate phenotype as a homozygote. bs^{A48}, the strongest of the examined *bs* alleles exhibits a very strong blistered phenotype. Heteroallelic combinations of these mutations, in contrast, show less severe phenotypes; bs^{ba}/bs^{2} or bs^{ba}/bs^{A48} adult wings show only a semipenetrant weak plexate phenotype at 18°, while bs^{2}/bs^{A48} adult wings generally exhibit fewer blisters than bs^{A48}/bs^{A48} wings. *Px2* and *Px4* both show dominant plexate phenotype. Wings of adults that carry any *bs* allele in *trans* to *Px2* or *Px4* are always blistered or balloon and legs of bs^{2} or $bs^{A48}/Px4$ adults often exhibit the malformed phenotype.

We have already noted that bs^{2} acts as a dominant enhancer of br^{1}. As the three alleles of the *bs* locus are homozygous viable, we have also examined their behavior as homozygotes in a homozygous or hemizygous br^{1} background (Table 5b). br^{1} dramatically enhances the wing phenotype of the bs^{ba} allele. $br^{1}/$

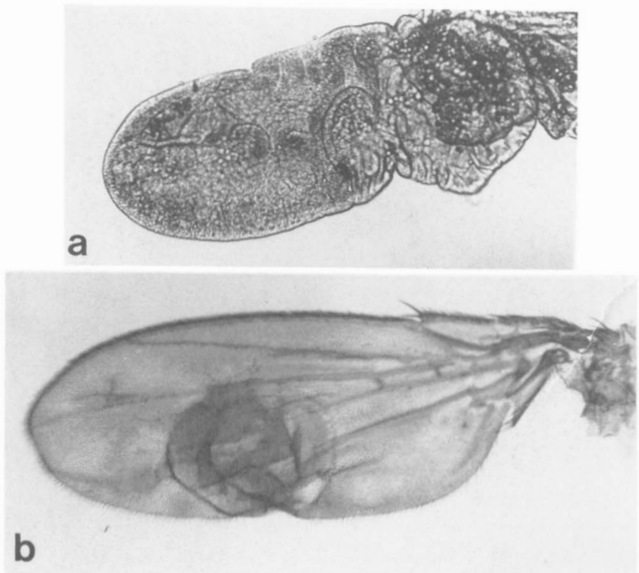

FIGURE 4.—(a) bs^{A48}/bs^{A48} wing disc, disected 6 hr after pupariation. Note the abnormal folds in epithelium, which are apparently due to improper dorsal-ventral flattening of the wing surfaces during wing disc elongation. Compare to wild-type wing disc (Figure 2b); (b) bs^{A48}/bs^{A48} adult wing.

$br^1;bs^{ba}/bs^{ba}$ adults have balloon wings while bs^{ba}/bs^{ba} wings are phenotypically weak plexate. Moreover, the legs of $br^1/br^1;bs^{ba}/bs^{ba}$ adults are often malformed. Similarly, $br^1/br^1;bs^2/bs^2$ legs are always malformed and the wings show extreme plexate phenotype or blisters. Unexpectedly, the number of wing blisters and balloons displayed by $br^1/br^1;bs^{A48}/bs^{A48}$ adults is almost identical to those in bs^{A48}/bs^{A48} adults. Legs of $br^1/br^1;bs^{A48}/bs^{A48}$ adults are, however, often mlf. The observation that all the *bs* alleles interact with br^1 to disrupt both wing and leg development suggests that the *bs* locus plays a general role in prepupal imaginal disc morphogenesis. It is not specific to wing disc development.

Genetic characterization of the interacting loci: A comparison between interacting alleles and the deficiencies that uncover them reveals that the most penetrant interactions depend on the presence of a mutant gene product. Interaction is not primarily a result of loss of gene function (Table 6). Double deficiency heterozygotes generated from *Df(1)S39*, *Px2*, *Px4*, *Df(2R)ES1*, or sbd^{45} almost never interact (Table 6a), while putative point mutations interact with a wide range of penetrance (Table 6b). For example, bs^2 interacts with Sb^{63b} at a penetrance of 44%, while *Px2* or *Px4* fail to interact with sbd^{45}. The interaction between putative point mutations is also much stronger than between a deficiency and a point mutant. *E(br)* offers a dramatic example of this observation. *E(br)* acts as a dominant enhancer of br^1 and interacts with Sb^{63b} at a penetrance of 100%. *Df(2R)ES1*, in contrast, while lethal in combination with *E(br)*, does not interact with either br^1 or Sb^{63b} (Tables 2a and 3a). Conversely, all the dominant *Sb* alleles interact with *E(br)*, but a deficiency for the *Sb-sbd* locus does not interact much above background (Table 3). This suggests that production of aberrant gene products is needed from both *E(br)* and the *Sb-sbd* locus to generate the most penetrant phenotype.

Analysis of the loci uncovered by *Px2* and *Px4* is more problematic. *Px2* uncovers at least two loci, *l(2)B485* and *bs*, involved in imaginal disc morphogenesis. We, therefore, cannot resolve how a null for a single locus behaves. Indeed, none of the mutations uncovered by *Px2* show the dominant plexate phenotype of the deficiency alone. *l(2)B485*, however, interacts more strongly with Sb^{63b} than either *Px2* or *l(2)D424*. Furthermore, *Px4* shows no interaction with Sb^{63b} while bs^{ba}, bs^2 and bs^{A48} interact with a penetrance up to 50%. These data again suggest that aberrant gene products are needed for the most severe interaction. All the mutations in this study, then, with the exception of those in the *BR-C* and *l(2)D424*, which is presumably a small deficiency, appear to behave as either antimorphs or neomorphs. A distinction between the two is determined by dosage experiments. Extra wild-type copies of the gene in question suppress antimorphic, but not neomorphic behavior. Unfortunately, the only available duplications of the tip of chromosome *2R* carry *bs*, *l(2)B485*, and *E(br)*. The use of these chromosomal duplications would lead to uninterpretable results. We believe a molecular analysis of these loci will more readily resolve this question.

DISCUSSION

We have identified three loci at the tip of chromosome *2R* implicated in imaginal disc morphogenesis based on their dominant genetic interactions with the *BR-C* allele br^1 and with alleles of the *Sb-sbd* locus to generate the mlf phenotype. *E(br)* was identified in a screen for dominant enhancers of the br^1 mutation and interacts with *BR-C* alleles deficient for *broad* function and with *Sb-sbd* alleles. Deficiency mapping of the *E(br)* locus revealed interactions between this locus and both *Px2* and *Px4*. Closer examination of genes uncovered by these deficiencies revealed that two loci, *l(2)B485* and *blistered*, can account for all the observed genetic interaction between *E(br)* and the *Px* deficiencies. Although alleles of these three loci interact to generate a similar phenotype, genetic and deficiency mapping experiments of recessive lethality demonstrate that they are not allelic. *E(br)* lies to the right of the distal break point of *Px2* and is uncovered by *Df(2R)ES1*, *l(2)B485* is uncovered by *Px2* alone, and *bs* lies within the overlap region of *Px2* and *Px4*.

The role of the *BR-C* in gene interactions: Both genetic and molecular evidence indicates that the *BR-C* is a transcriptional regulator necessary for a wide variety of events during metamorphosis. It encodes a

TABLE 6

Comparison of the interaction between deficiency heterozygotes and between putative point mutants: percent penetrance of malformed syndrome (no. of flies)

a. Double deficiency heterozygotes fail to interact

Genotypes[a]	*Df(1)S39/+*	*Df(2R)Px2/+*	*In(2LR)Px4/+*	*Df(2R)ES1/+*	*sbd*^{45}*/+*
Df(1)S39/+		<1 (89)	9 (120)[b]	<1 (84)	10 (469)[c]
Df(2R)Px2/+			Lethal	<1 (74)	0 (250)
In(2LR)Px4/+				<1 (102)	<1 (114)
DF(2R)ES1/+					<1 (120)

b. Putative point mutants interact

Genotypes[d]	*br*^{1}*/Y*	*bs*^{2}*/+*	*l(2)B485/+*	*E(br)/+*	*Sb*^{63b}*/+*
br^{1}*/Y*		36 (59)	2 (53)	38 (120)	99 (83)[c]
bs^{2}*/+*			<2 (42)	25 (48)	44 (107)
l(2)B485/+				14 (59)	70 (33)
E(br)/+					100 (50)

[a] *Df(1)S39/Binsn*, *Df(2R)Px2/CyO*, *In(2LR)Px4/CyO*, *Df(2R)ES1/CyO* and *sbd*^{45}*/TM6B* were crossed pairwise.

[b] The mlf phenotype in this class is very mild and may be due to a non-specific dosage effect. We do not regard this interaction as significant.

[c] See BEATON *et al.* (1988) for discussion of this interaction.

[d] For simplicity, the data presented reflects the developmental temperature and sex of flies exhibiting the highest penetrance of the mlf phenotype. See Tables 2, 3 and 5 for complete data.

set of apparent transcription factors produced by alternative splicing, each of which contains one of three pairs of zinc-finger domains (P. R. DiBello, D. A. Withers, C. A. Bayer, J. W. Fristrom and G. M. Guild, manuscript submitted for publication). Loss of these functional domains directly affects the transcription of other third instar larval genes. *br*^{1}*/npr*^{1} animals presumably exhibit the malformed phenotype due to a reduction in the wild-type *br* product. Reduction of the wild-type *br* product may limit the expression of effector genes, transcriptionally regulated by the *BR-C*, that are necessary for morphogenesis. Therefore, enhancement of the *br*^{1} phenotype could result from a mutation in a gene that is necessary for transcription of the *BR-C*, in a gene whose product acts with the wild-type *br* product to regulate transcription, or in a downstream effector gene. BEATON *et al.* (1988) have argued that the *BR-C* regulates the *Sb-sbd* locus because: (1) The phenotypic effects of *Sb-sbd* mutations, unlike those of the *BR-C*, are limited to the adult epidermis and suggestive of defects in cytoskeletal function. (2) Reduction of gene dosage in double deficiency heterozygotes (*BR-C*$^{+}$*/def;Sb-sbd*$^{+}$*/def*) produces a mlf interaction indicating that protein-protein interactions between abnormal gene products are not required to produce the mlf phenotype. (3) Extra copies of the *BR-C* do not suppress the mlf phenotype of *Sb-sbd* mutants, as would be expected if the *Sb-sbd* mutants acted by reducing *BR-C* transcription. Our view is that the *BR-C* also regulates *bs* function. First, deficiency heterozygotes that uncover *bs* interact with *br*^{1}, suggesting that a change in the expression of the remaining wild-type *bs* gene product results in the mlf phenotype. An abnormal *bs* gene product is not needed to reveal the interaction. Second, many alleles of the *BR-C* are lethal and prevent metamorphosis while all known *bs* alleles are viable and specifically effect imaginal disc morphogenesis. If the *bs* wild-type product were necessary for the transcription of the *BR-C*, homozygous *bs* mutations should be lethal or affect a variety of processes associated with metamorphosis. Genetic evidence indicates that *E(br)* does not regulate the *BR-C*. Four effective copies of the *BR-C* do not suppress the interaction between *E(br)* and *Sb*^{1} (data not shown), indicating that *E(br)* does not interact with *Sb-sbd* mutations by reducing wild-type *BR-C* expression. The larval lethality of *E(br)*, however, suggests more general functions of the wild-type *E(br)* product in development than those of the *Sb-sbd* and *bs* loci. Thus, although we favor the view that *E(br)* is an effector gene regulated by the *BR-C* we cannot exclude the possibility that the *E(br)* encodes a transcriptional factor that acts in concert with the *BR-C* product to mediate disc morphogenesis.

The nature of the *Sb-sbd*, *E(br)*, and *bs* interactions: *E(br)*, *bs*, and the *Sb-sbd* locus form a set of interacting loci. The observation that deficiencies for these loci fail to interact suggests that gene expression is necessary for the most penetrant phenotype. Although this behavior could be due to either gene misexpression or the expression of abnormal gene products, the study of mutations in genes that encode structural proteins provide a compelling precedent for the latter view.

Screens for intergenic or unlinked noncomplementing mutations have been used successfully in a number of organisms to study functional protein-

protein interactions. A number of investigators have shown that mutations in the *Caenorhabditis elegans* muscle heavy chain myosin gene dominantly interact with mutations in the paramyosin gene, which encodes a protein that interacts molecularly with the heavy chain myosin (reviewed by WATERSTON 1988). Using this approach to study microtubules in both *Saccharomyces cerevisiae* and *D. melanogaster*, screens for mutations that fail to complement mutations in β-tubulin loci resulted in the identification of mutations in the alpha-tubulin locus which makes a gene product known to interact molecularly with β-tubulin subunits (HAYS *et al.* 1989; STEARNS and BOTSTEIN 1988). This approach has been extended in an attempt to identify genes encoding accessory proteins involved in microtubule function. REGAN and FULLER (1988) have identified an allele of the *haywire* locus whose dominant interaction with mutations in a β-tubulin gene requires the presence of the mutant *haywire* gene product.

The *C. elegans sqt-1* locus encodes one of the collagens critical for organismal morphogenesis (KRAMER *et al.* 1988). Mutations in this locus that result in a phenotype depend on abnormal gene expression. Animals homozygous for null alleles of this locus are wild type, but those carrying dominant mutations exhibit a variety of gross morphological defects (KUSCH and EDGAR 1986). Collagens interact molecularly and presumably most amino acid substitutions that affect the tertiary structure of this molecule disrupt morphology, while loss of the gene product can be compensated by one of the many (50–150) other collagen genes.

The *sqt-1* locus mutates to dominance at a high frequency, a phenomenon also seen in a number of *C. elegans* muscle genes (WATERSTON 1988). The *Sb-sbd* locus also exhibits this behavior. It is defined, in part, by four spontaneous dominant mutations which are not due to haploinsufficiency. These *Sb* dominant alleles may be expressing a defective polypeptide.

The nature of the phenotypes associated with the interacting loci: *E(br)*, *bs*, and the *Sb-sbd* locus are implicated in proper imaginal disc development because they interact to generate the mlf phenotype. Independent of genetic interactions, mutations in the *bs* locus generate wing blisters and mutations in the *Sb-sbd* locus affect appendage elongation and bristle development.

The evidence suggesting that both cytoskeletal and adhesion molecules play an important role during epithelial morphogenesis is overwhelming (FRISTROM 1988). Ecdysone induced changes in membrane proteins point to a major role of the cell surface in disc morphogenesis (RICKOLL and FRISTROM 1983). CONDIC, FRISTROM and FRISTROM (1991) have shown that imaginal leg disc elongation is primarily due to changes in cell shape, necessitating corresponding changes in the distribution of the cytoskeleton as well as molecular changes at the cell surface. Mutations in the *Sb-sbd* locus disrupt these cell shape changes (CONDIC, FRISTROM and FRISTROM 1991) suggesting a possible defect in either a cytoskeletal or adhesion molecule.

Mutations in the α or β subunits of Drosophila integrin, encoded by the *inflated* (*if*) and *l(1)myospheroid* (*mys*) loci respectively, generate a blistered wing phenotype similar to the phenotype of the *blistered* locus (BROWER and JAFFE 1989; WILCOX, DIANTONIO and LEPTIN 1989; ZUSMAN *et al.* 1990). Mutations in *if*, *mys* and *bs* may interfere with the apposition of the dorsal and ventral wing surfaces and/or the maintenance of the apposed surfaces. In vertebrates, integrins are heterodimeric cell surface receptors involved in both cell-cell and cell-matrix interactions (HYNES 1987). Although these molecules are not well characterized in Drosophila, it is clear that they are necessary not only for the maintenance of close apposition of the wing blade during metamorphosis, but also are associated with muscle attachment sites (BOGAERT, BROWN and WILCOX 1987). Only two α subunits have been identifed in Drosophila. Vertebrates contain multiple α subunits. It is speculative, but conceivable that the *blistered* locus encodes an α subunit other than that encoded by *inflated* or a polypeptide that interacts with an integrin.

In summary, the *BR-C* encodes an apparent transcriptional regulatory molecule. Genetic data are consistent with the view that the *BR-C* regulates the expression of *bs* and the *Sb-sbd* products. *E(br)* appears not to regulate the *BR-C*. Two lines of evidence suggest that *E(br)*, *bs*, and the *Sb-sbd* locus may encode cell surface or cytoskeletal molecules. First, the phenotypes associated with mutations at these loci suggest the disruption of structural molecules. Second, the genetic behavior of these interacting loci mimics the behavior of interacting mutations in known structural molecules. Although only a full molecular analysis of these genes and their respective products will reveal the true nature of these genetic interactions, it is clear from investigations of the *Drosophila* integrins that analysis of mutations which disrupt imaginal disc morphogenesis can identify molecules involved in cell-cell or cell-matrix interactions.

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