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Special Review

The *bithorax complex:* the first fifty years#

EDWARD B. LEWIS*

Division of Biology, The California Institute of Technology, Pasadena, USA

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"The power of using abstractions is the essence of intellect, and with every increase in abstraction the intellectual triumphs of science are enhanced."
Bertrand Russell

Introduction

Genetics is a discipline that has successfully used abstractions to attack many of the most important problems of biology, including the study of evolution and how animals and plants develop. The power of genetics to benefit mankind was first recognized by the award of the Nobel Prize in physiology or medicine in 1933 to T. H. Morgan. In the 23 years that had intervened between the time Morgan introduced *Drosophila* as a new organism for the study of genetics and the award of the Prize, he and his students, especially, A. H. Sturtevant, C. B. Bridges and H. J. Muller, had vastly extended the laws of Mendel as the result of a host of discoveries, to mention only a few: that the genes (Mendel's factors) are arranged in a linear order and can be placed on genetic maps, that they mutate in forward and reverse directions, that they can exist in many forms, or alleles, and that their functioning can depend upon their position. Purely on the basis of breeding experiments, these early workers were able to deduce the existence of inver-

Abbreviations used in this paper: T, thoracic segment; A, abdminal segment; BX-C bithorax complex; HOM-C, homeotic comples; HOX, homeobox; ANT-C, antennapedia complex; COL, colinearity; COE, cis-overexpression; CIN, cis-inactivation.

***Address for reprints:** Biology Division 156-29, California Institute of Technology, Pasadena, CA 91125, USA. FAX: 626-564-9685. e-mail: lewis@cco.caltech.edu
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sions and duplications, for example, before it became possible to demonstrate them cytologically. The list of their achievements is a long one and one that has been put into historical perspective by Sturtevant in *A History of Genetics* (Sturtevant, 1965).

All of these discoveries were made with *Drosophila* by taking advantage of its small size, ease of culturing, high fecundity, short life cycle, small chromosome number, wealth of spontaneous and induced mutations, and, after their discovery in 1935, its giant salivary gland chromosomes. Of immense importance also was the existence of standard or "wild-type" strains.

That Morgan's contributions satisfied the criterion of being of benefit to mankind was evident by the remarkable extent to which the new discoveries with *Drosophila* had direct application to the understanding of the inheritance of many human traits. For example, the inheritance of colorblindness and hemophilia in human beings could be understood for the first time.

The second Nobel Prize for work in the genetics of *Drosophila* was awarded in 1946 to H. J. Muller for his discovery in 1928 that X-rays produce gene mutations and do so in direct proportion to the dose (Muller, 1927). Muller called attention to the genetic risks to the human race posed by indiscriminate use of ionizing radiations, and, prophetically, he argued that such uses would also increase the risk of cancers, if cancer is the result of somatic mutations. The implications of Muller's work were not overlooked with the advent of the atomic age. As a result, extensive genetic studies were carried out in *Drosophila* and mice to assess the relative rates of mutation in these organisms as a means of assessing the genetic risks to human beings from the use of atomic energy.

The award of the Prize in 1995 for work with *Drosophila* recognizes the growing importance of a field that has come to be called developmental genetics. The work of my co-winners, Eric Wieschaus and Christiane Nüsslein-Volhard, has identified crucial steps in the early development of the organism. Specifically, they have identified major genes involved in setting up the initial axes of the embryo and its germ layers (Nüsslein-Volhard and Wieschaus, 1980) thereby setting the stage for groups of master control genes that then program the final body plan of the organism. It is this latter group of genes with which we will be concerned here: what they do and how they came to be discovered. My part in this story began in the late 1930s and it will be first examined in relation to the concept of the gene at that time.

The gene concept

Johannsen coined the term "gene" in 1909 and it quickly replaced Mendel's "factor" (Johannsen, 1911). The concept of the gene is one of the most powerful abstractions in biology and one of great utility. For many years the gene could be satisfactorily defined as a unit within which genetic recombination, or crossing over, does not occur. The unit defined in this way tended to correspond to a unit of function, as defined by the standard phenotypic test for allelism, or the "complementation" test, to be discussed below.

In 1925, Sturtevant made two important discoveries that were eventually to lead to a re-examination of the gene concept in terms of the gene's function (Sturtevant, 1925). In analyzing the progeny of females homozygous for the unstable eye mutation, *Bar (B)*, he predicted that a rare mutation, *double-Bar (BB)*, was a tandem duplication that arose in the progeny of homozygous *B* females as the result of "unequal crossing over". He then showed that the eyes of *BB/+* females are slightly smaller than those of *B/B* and deduced that the function of a gene can depend upon its position with respect to is neighbors, the first example of the "position effect", as he named it.

Eleven years later, using the giant salivary gland chromosomes of the *Drosophila* larva, Bridges (1936) and Muller *et al.* (1936) reported that the B mutant was actually a tandem duplication of 7 bands in the X chromosome and that *BB* was a triplication for that region. Hence *BB* was arising from unequally paired duplicated regions accompanied by normal rather than "unequal" crossing over. Interestingly, Wright had predicted that *B* itself would be a duplication before it was demonstrated cytologically (Wright, 1929).

Bridges had earlier called attention to duplication-like structures in the salivary gland chromosomes of wild-type larvae (Bridges, 1935). In particular, he interpreted numerous double banded structures, or "doublets", as two duplicated bands fused along their edges. Their structure suggests that they are reverse (ABBA), rather than direct (ABAB), repeats of single bands (Fig. 1). Bridges' cytological evidence for such repeats combined with Sturtevant's demonstration of position effect suggested that multiple alleles of a given gene might in some cases be resolvable into two or more repeated genes that acted like one because of a position effect. Evidence that multiple alleles might be resolvable into separable loci began to be obtained in the late 1930s by C.P. Oliver at the University of Minnesota. He found a low frequency of revertants to wild type in the offspring of females heterozygous for two recessive *lozenge (lz)* eye mutations. Although the revertants were invariably associated with crossing over in the region, he was unable to detect a reciprocal crossover having both mutants in the same chromosome. He therefore could only suggest that the revertants could be explained as the result of "unequal crossing over or crossing over between 'repeats.'"(Oliver, 1940).

Star and *asteroid*

I was an undergraduate at that time and Oliver generously gave me a desk in his laboratory and allowed me to work on a new rough-eyed mutant that had been given to me by E. Novitski, who was then at Purdue University. [Novitski and I had begun our work with *Drosophila* in high school around 1935]. Bridges had suggested that it be called *Star-recessive (S^r)* , since it acted as an allele of a weakly dominant rough eye mutant, *Star (S)*. Thus, *S/+* flies have slightly smaller eyes that are slightly roughened; S^r/S^r flies have eyes reduced to about half their normal size and with a very roughened surface; while S/S^r flies are nearly eyeless -figured in (Lewis, 1951). Although in a preliminary test, I had found a revertant of S^r or of *S* in 3,235 offspring of S/S^r females, when flanking markers were introduced I obtained no more wild-type products among 9,294 offspring (Lewis, 1939).

In spite of these inconclusive results, I continued the study of *S* and S^r as one of Sturtevant's graduate students at Caltech, commencing in 1939. In the tradition of Morgan, Sturtevant allowed his students considerable freedom to choose their thesis research projects. Quite a risk was involved in choosing to work on *S* and its "alleles". Crossovers between them would be rare if they were to occur at all. Even if the wild-type crossover could be recovered, it was expected that it would be very difficult to detect the reciprocal, or double mutant, crossover.

To increase the resolving power of the analysis, I made use of the interchromosomal effect of rearrangements on crossing over.

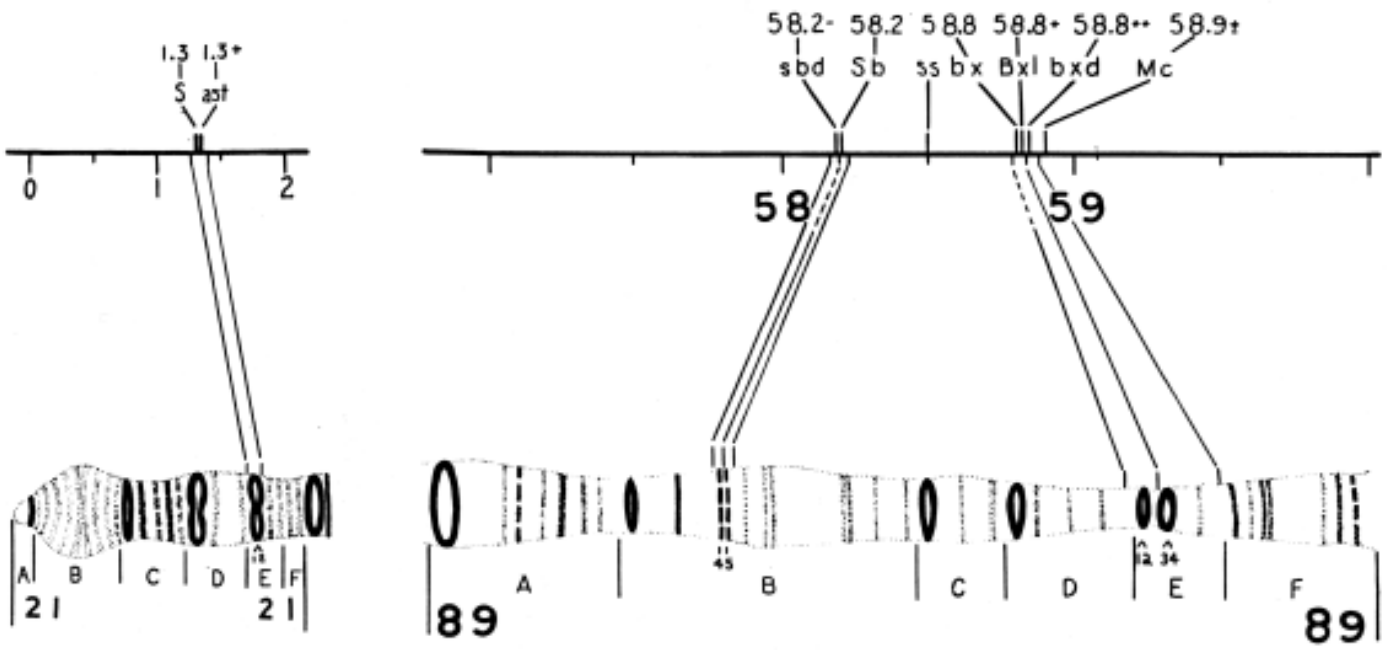

Fig. 1. A correlation of the genetic and salivary gland chromosome locations of the three sets of pseudoallelic genes studied for *cis-trans* effects. *At the left are the correspondences found near the extreme left end of the second chromosome; at the right a section from the middle of the right arm of the third chromosome is shown. The symbols ss and Mc refer to the loci spineless and Microcephalus, respectively; other symbols are described in the text. Reprinted from Lewis, 1951.*

Introduction of heterozygosity for inversions in chromosome arms other than the left arm of the second chromosome, in which *S* is located, resulted in an approximately four-fold increase in the frequency of crossing over in the vicinity of *S*. As in Oliver's work on *lz*, the revertants were invariably associated with crossing over between *S* and S^r. I renamed the latter «allele,» *asteroid (ast).*

A tandem duplication for the *S* region which I had found as an x-ray induced revertant of *ast* (Lewis, 1941) lent itself to the recovery of the *S ast* double mutant chromosome (Lewis, 1942a). A striking position effect was in evidence: whereas, *S+/+ast* is nearly eyeless, the complementary genotype, *S ast/++* , is nearly wild type, except for a slightly smaller and slightly roughened eye indistinguishable from that of *S/+* (Lewis, 1942a), figured in (Lewis, 1951).

S and *ast* proved to be localized to the 21 E 1-2 doublet of the salivary gland chromosomes (Fig. 1), the doublet which Bridges had singled out as being a representative example (Bridges, 1935). These cytogenetic studies of *S* and *ast* formed my doctor's thesis (Lewis, 1942b) published in part in 1945 (Lewis, 1945).

Comparison of the difference in phenotype between *cis* vs. *trans* genotypes is usually referred to as the *cis-trans* test, and the position effect, if present, as the *cis-trans* effect. For a history of this terminology see Hayes (1968).

Early studies of the *bithorax* mutants

In 1945, the time seemed ripe to look for more examples of the *Star-asteroid* type in the genome. An intriguing region of the third chromosome included three loci within less than one centiMorgan; namely, the bristle mutations, *Stubble (Sb)*, and *spineless (ss)* and a homeotic mutation, *bithorax (bx)* (Fig. 1). Certain useful combinations of these mutants had already been synthesized by Bridges and maintained in the Caltech stock collection. The recessive alleles of *Sb* proved to be at a separate locus, that I named *stubbloid (sbd)*, less than 0.1 centiMorgan to the left of the *Sb* locus. An especially striking position effect occurs: sbd^2*+/+Sb* flies have extremely short blunt bristles, while sbd^2 *Sb/++* flies are wild-type with no trace of the dominant short-bristle phenotype of *Sb/+* flies.

It soon became evident that the diverse array of existing mutations of the bithorax type held considerable promise of being a cluster of genes rather than a multiple allelic series. It was for this reason that they were chosen for study rather than with any belief that they would tell us something about how genes control development.

The original *bx* mutant had been found by Bridges in 1915 as a transformation of the third thoracic segment (T3) toward the second (T2), notably causing the halteres to become partially wing-like. Body segments and structures of the wild-type adult are correlated with those of the late embryo in Figure 2. *bx* was the first example of a mutant that exhibited homeosis, a term Bateson had first coined for conversion of one structure into an homologous one (discussed in Lewis, 1994). In 1919, Bridges found a somewhat similar mutant that fully complemented *bx*, so he named it *bithoraxoid (bxd)*; i.e., *bx/bxd* is wild type in phenotype. However, he later showed that bx^D, which W. F. Hollander had found, failed to complement either *bx* or *bxd* (Bridges, 1944).

Although the original *bx* mutant has 100% penetrance, it is a highly variable transformation of, as it turns out, only the anterior portion of T3 toward anterior T2. Fortunately, two other *bx*-like mutants, bx^{34e} (J. Schultz) and bx^3 (of C. Stern) had also been saved by Bridges (Bridges, 1944). These have 100% penetrance and non-variable weak and strong transformations, respectively, of anterior T3 toward anterior T2. The wing-like halter of the bx^3 homozygote is shown in Figure 3.

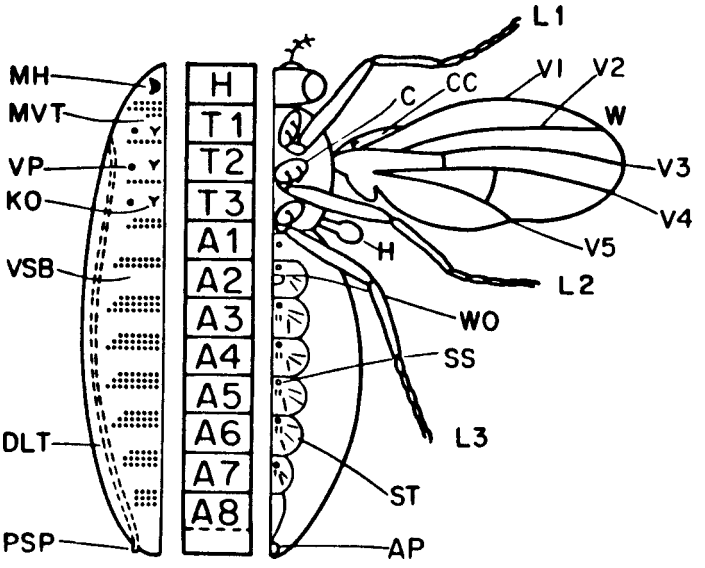

Fig. 2. Comparison of the ventral cuticular pattern of the late embryonic stage with that of the adult stage. *MH, mandibular hooks; MVT, mid-ventral tuft; VP, ventral pits; KO, Keilin's organ; VSB, ventral setal belts; DLT, dorsal longitudinal (tracheal) trunk; PSP, posterior spiracle; H, head; T, thoracic; A, abdominal; L, leg; W, wing; H, halter; C, coxa; CC, costal cell (of wing); V, vein; WO, Wheeler's organ; SS, sensillum (on segments A1 to A7, inclusive); ST, sternite; AP, anal plate. Modified from Lewis, 1982.*

Flies homozygous for *bxd* show 100% penetrance for a partial transformation of only the posterior portion of T3 toward posterior T2. The wing-like halter of a homozygote for an extreme *bxd* mutation, bxd^{100} , is shown in Figure 3. In addition, *bxd* flies also have the first abdominal (A1) segment transformed toward T3, occasionally producing tiny rudimentary T3-like legs. A *bxd* hemizygote has a well developed T3-like leg on the transformed A1 (Fig. 4D).

A crossing-over analysis showed that bx^D occupies a separate locus between the *bx* and *bxd* loci, and therefore it was first renamed *Bithorax-like (Bxl)* (Fig. 1), and later, *Ultrabithorax (Ubx)* (Lewis, 1951). This analysis provided a number of *cis* and *trans* genotypes that exhibited position effects. Examples are shown in Figure 5.

Gene evolution by tandem duplication

These early studies were viewed as supporting a simple hypothesis about how new genes arise from pre-existing genes. Based on the work of Sturtevant and Bridges, already cited above, the hypothesis proposed that new genes evolve from old genes by a two-step process: tandem gene duplication followed by one of the resulting duplicates mutating to a new function (Lewis, 1951). This "new" gene would generally not be easily established in the population unless the other, or "old" gene, was retained to carry out the old function. As a result the genome would be expected to contain clusters of closely linked and functionally related genes that superficially act like a single gene. At the Cold Spring Harbor in 1950, I reported (Lewis, 1951) on the evidence in support of this hypothesis from three studies: of other organisms; of the above mentioned *S, Sb* and *bx* regions; and of *lz* mutants by Green and Green (1949).

An early model of the *cis-trans* effect

A model (Fig. 6) was also presented at that Symposium to account for the *cis-trans* effect (Lewis, 1951). It was based on the then generally accepted biochemical dogma that genes were proteins, and that they could catalyze enzymatic reactions. The wild-type alleles of *a* and *b* were assumed to control sequential steps in a biochemical pathway in which a substrate, S, is converted into two products, A and B, that are produced at the site of the genes in the chromosome. The *a* and *b* mutants are assumed to lower production of A and B, symbolized as <A and <B, respectively (Fig. 6II). As a result, *a b/++* (Fig. 6I) is expected to produce enough B to be wild type, or nearly so. By contrast, *a+/+b* (Fig. 6II) would produce insufficient B, and therefore be mutant in phenotype (Lewis, 1951).

The model could therefore also account for polarized *cis-trans* effects. For example, when bx^3 is opposite an extreme x-ray induced *bxd* allele, such as bxd^{100}, bx^3+/+bxd^{100} flies have a very slight wing-like transformation of the posterior portion of the halteres. On the other hand, they have no trace of the *bx* phenotype, even though the latter phenotype is a more sensitive one for the detection of slight effects than is the *bxd* phenotype. Hence bx^3 appears to weakly inactivate bxd^+, but even extreme *bxd* mutants do not inactivate bx^+.

In retrospect the model is no longer compatible with our present knowledge of the structure and function of the gene. However, since no assumptions were made about the nature of the products S, A and B, the model might still be tenable if S, A and B correspond to non-coding RNA transcripts. The real value of this hypothesis was that it led to an experiment that revealed a new phenomenon of "transvection", to be discussed below.

Contrabithorax - a gain-of-function mutation

In 1954 (Lewis, 1954a), an x-ray induced mutation was found that had T2 transformed toward T3. This "gain-of-function" (Lewis, 1984) phenotype was therefore the inverse of the T3 to T2 transformation characteristic of the bx and bxd mutations. Surprisingly, mapping showed it to be a double mutation made up of a gain-

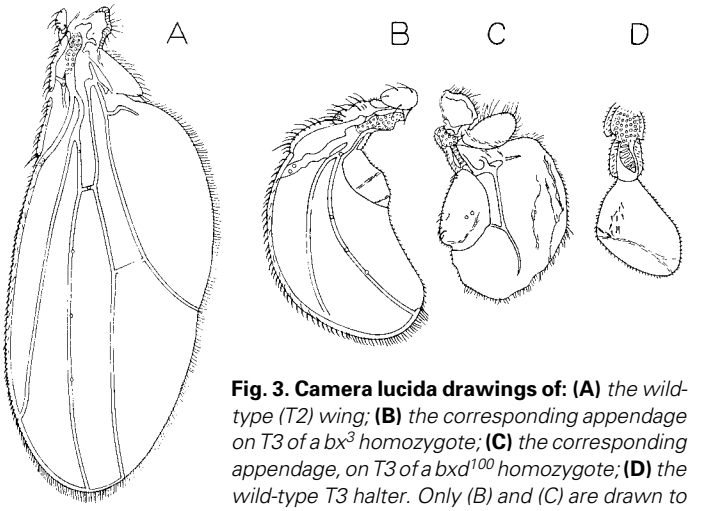

Fig. 3. Camera lucida drawings of: (A) *the wild-type (T2) wing;* **(B)** *the corresponding appendage on T3 of a bx^3 homozygote;* **(C)** *the corresponding appendage, on T3 of a bxd^{100} homozygote;* **(D)** *the wild-type T3 halter. Only (B) and (C) are drawn to the same scale. Reprinted from Lewis, 1951.*

of-function mutation, *Contrabithorax (Cbx)*, the locus of which lies between the *bx* and *Ubx* loci, and a recessive loss-of-function mutation, *postbithorax (pbx)*, that occupies a new locus distal to that of the original *bxd* mutation. Thus the map expanded to five loci, at which there were mutations with effects on one or more of the segments, T2, T3 and A1 (Lewis, 1955). This cluster of mutant loci came to be called the *Ubx* domain of a much larger cluster, the bithorax complex (BX-C) (Fig. 7). The latter name is derived from "gene complex", a term invented by Brink for a closely linked cluster of genes that he predicted would be closely related in function (Brink, 1932). Kaufman and his co-workers defined the Antennapedia-complex (ANT-C) that controls the identity of segments anterior to those controlled by the BX-C (Kaufman, 1980).

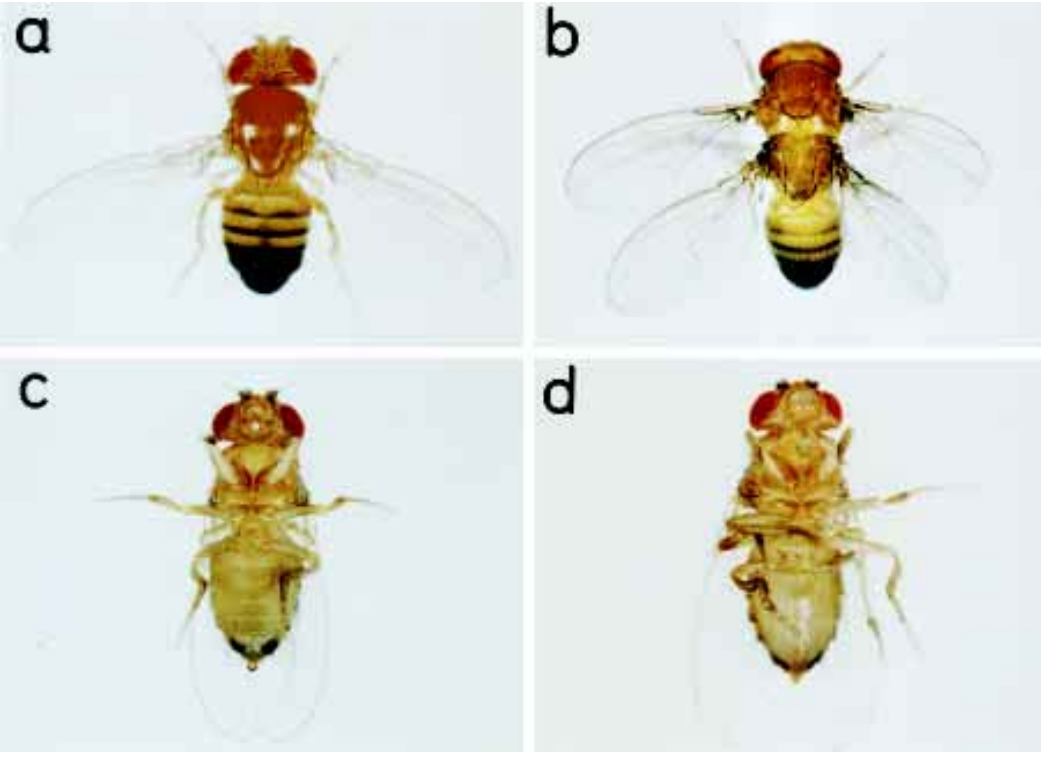

Fig. 4. Extreme segmental transformations. (a) *Wild type male.* **(b)** *abx bx^3 pbx homozygote, in which T3 is transformed toward T2.* **(c)** *Wild type female, ventral view.* **(d)** *bxd / Df-P2 female, ventral view having an extra pair of T3-like legs on A1.*

Unlike the *bxd* mutant, *pbx* has only a transformation of the posterior portion of T3 toward posterior T2. The *trans* heterozygote, *bxd+/+pbx,* shows a *pbx* phenotype but no trace of the transformation of A1 toward T3 that is typical of the *bxd* homozygote. Furthermore, *bx^3+/+ pbx* also shows, albeit weakly, a *pbx* phenotype, but no trace of a *bx* phenotype. In both of these examples the *cis*-heterozygotes are wild type. Thus, polarized inactivation of *pbx+* function can be effected in *cis* by either *bxd* or *bx^3*.

The transvection phenomenon

One of the predictions of the early model of the *cis-trans* effect (Fig. 6) was that disruption of somatic pairing might intensify the difference between *cis* and *trans* types. Specifically, heterozygosity for a chromosomal rearrangement that would disrupt pairing in an *a+/+b* individual would be expected to cause a more extreme *b* phenotype. The prediction was borne out, and a powerful new method was discovered for detecting chromosomal rearrangements in the first generation after their induction. The method was first used to measure the frequency of induction of such rearrangements in the progeny of males exposed to neutrons from an atomic bomb test (Lewis, 1954b) .

The method detects only the majority of rearrangements having one breakage point in a «critical» region of some 500 bands of the salivary gland chromosomes; namely, the region between the centromere of the third chromosome and the locus of the BX-C. Similar findings were later obtained for the *decapentaplegic (dpp)* region in 2L (Gelbart, 1982) and for the *eyes-absent (eya)* region in that arm (Leiserson *et al.,* 1994).

Although at first only *trans* genotypes showed the phenomenon, it was soon found that *Cbx Ubx/++* was also subject to transvection (Lewis, 1955). *Cbx* in this genotype was found to exert a slight gain of function of *Ubx$^+$*, chiefly expressed by spread wings and a reduced alula, when the chromosomes are paired. That effect is abolished (wings normal) when pairing is disrupted by transvection-suppressing rearrangements. As a result, it became possible to mutagenize wild type and to select rearrangements that abolished the weak *Cbx* effect of the *Cbx Ubx/++* genotype. Among the resultant rearrangements, some, as expected, had breaks within the BX-C. These breaks were unselected for any effect on function in the BX-C other than suppression of transvection. Such rearrangements, when subsequently tested over deletions of the BX-C, provided the basis for discovering additional *infra-abdominal (iab)* regions and ordering all of the known regions from *iab-2* to *iab-8,* inclusive. The *iab-9* region has been identified by means of breakpoints associated with gain-of-function mutants in that region, namely *Uab* (Lewis, 1978) and *Tab* (Celniker and Lewis, 1987) (Fig. 7).

In the process of isolating transvection-suppressing rearrangements, a sex-linked mutant was recovered in two independent cases, whose effect was to enhance the *bithorax* phenotype of *bx^{34e}/Ubx.* This mutant, originally named, *enhancer-bithorax (e-bx)*, proved to be an allele of the *zeste (z)* gene (Judd *et al.,* 1972) and to be like the *z^a*, or null, alleles of Gans (1953). It was soon found that *z$^{ae\text{-}bx}$*, as it is now symbolized, suppresses transvection not only in the case of the BX-C but also *dpp*. The *z* protein has been shown to be a DNA-binding protein that binds *in vitro* to the *Ubx* gene as well as to other genes (Benson and Pirrotta, 1987). Benson and Pirrotta suggest that "transvection effects are a by-product of normal intragenic *z* action" (Benson and Pirrotta, 1988).

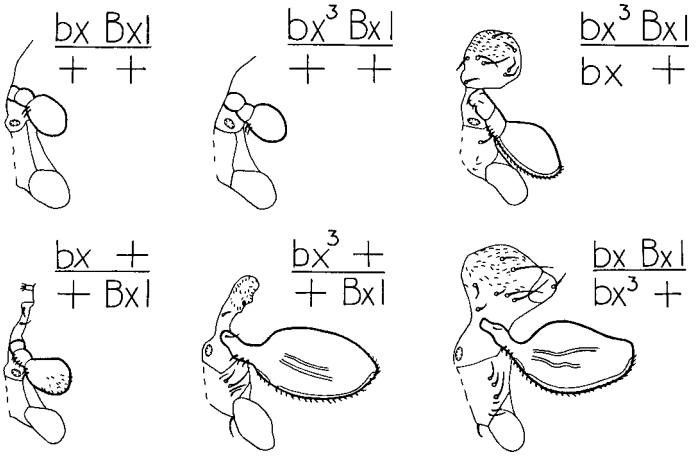

Fig. 5. *Cis-trans* effects involving the *bithorax (bx)* and *Ultrabithorax* (here designated *Bxl)* mutants, illustrated by camera lucida drawings of the dorsal and lateral region of T3 of the adult fly. *The pair of genotypes in each vertical column are identical except for the way in which the alleles are distributed between homologous chromosomes. Reprinted from Lewis, 1951.*

Remarkably, tandem duplications for the BX-C region act as powerful suppressors of transvection, when placed opposite the *Cbx Ubx* chromosome (Lewis, unpublished). Evidently, the duplicate regions pair intrachromosomally with one another and prevent the *Cbx* mutant from gaining access to the Ubx^+ regions. In organisms which lack somatic pairing between homologous chromosomes, such as the vertebrates, intrachromosomal pairing of tandem repeats may still occur. In that event, transvection may prove to be a general phenomenon applicable to tandemly repeated regions in all organisms.

Mobile elements in the *bithorax complex*

In 1932 Bridges reported (Bridges, 1932) the discovery of one of the first suppressor mutants in *Drosophila*. He named it *suppressor-of-Hairy wing* [now symbolized *su(Hw)*] and found that it acted as a recessive suppressor of certain alleles of a number of other genes. Although the bx^3 mutation had been saved as a balanced stock, when I used it in 1946 the homozygote appeared wild type in phenotype, as if the mutant had reverted. In fact, the stock had acquired a suppressor that mapped to the same locus as that of Bridges' *su(Hw)*. His mutant had been lost, but the new occurrence, named su^2*-Hw*, suppressed the same group of specific alleles as was reported for *su(Hw)*. In addition, we found that it not only suppressed bx^3, bx^{34e} and *bxd*, but also specific alleles of many other genes (Lewis, 1986).

The mechanism by which su^2*-Hw* suppresses specific alleles proved elusive until many years later, when it was shown that such alleles are the result of an insertion of the mobile element, gypsy, almost invariably in the non-coding portion of the gene (Modolell *et al.,* 1983). The wild-type su^2*-Hw* gene codes for a DNA-binding protein (Parkhurst *et al.,* 1988) that is assumed to bind to specific sequences in the gypsy element, thereby lowering the rate of transcription of the gene containing that element (Parkhurst and Corces, 1986). Hence, in the su^2*-Hw* homozygote, failure of the mutant protein to block transcription of that gene would restore the wild type phenotype.

In retrospect, it now seems extremely fortunate that the early mapping of mutants in the Ubx domain was carried out using mutations that were insertions or deletions. Thus, bx^3 and *bxd* are gypsy insertions (7 kb in length), *Ubx* is a *«Doc»* mobile element (Bender *et al.,* 1983), and *pbx* and *Cbx* are a deletion and insertion, respectively, of a 17 kb segment of DNA (Bender *et al.,* 1983). Had they been true point mutations, they might then have been subject to gene conversion, a phenomenon first discovered in fungi and characterized by high negative interference over short map regions and aberrant segregation of alleles in a meiotic tetrad (reviewed by Holliday, 1964)]. As a result, unambiguous ordering of mutants in the *Ubx* domain would probably not have been possible.

Half-tetrad mapping of the *Ultrabithorax* domain

The great diversity of phenotypes represented by mutants at the five known loci of the *Ubx* domain made it relatively easy to derive double mutants and, in turn, higher multiples, including the quintuple mutant, bx^3 *Cbx Ubx bxd pbx*. Although flanking marker recombination provided unambiguous ordering of these loci, the possibility of gene conversion was of sufficient concern that I undertook a half-tetrad analysis of that domain.

Attached autosomal arms had been synthesized, partly to be able to perform such an analysis, by I. Rasmussen and E. Orias, working in my laboratory (Lindsley and Grell, 1968). Females were constructed with the quintuple mutant combination in one of the attached arms and the corresponding five wild-type alleles in the other arm, along with appropriate flanking markers (Fig. 8); their phenotype was indistinguishable from that of *Ubx/+* (Lewis, 1967). Among approximately 221,000 female offspring, 19 were the result of exchanges in the regions between the loci of bx^3 and *pbx*. Reciprocal crossovers were recovered simultaneously from four out of five of the regions and were easily detected by their having strong *cis-trans* effects when compared with the maternal *Ubx/+* phenotype. None of the half-tetrads showed evidence of gene conversion. As one possible explanation it was suggested that

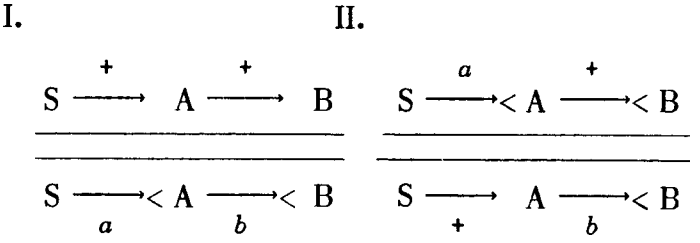

Fig. 6. An early model to explain *cis-trans* effects. *Paired homologous chromosomes are diagrammed by the long horizontal lines. Two adjacent loci are shown with either wild-type (+) or mutant (a or b) alleles. The genes at these loci are assumed to catalyze the reaction of the substrate S into product A, and product A to product B. The A product is assumed to remain in the vicinity of the locus where it is produced. The cis configuration* **(I)** *produces sufficient B to give a nearly wild-type phenotype. The trans configuration* **(II)** *produces insufficient B resulting in a mutant phenotype. Reprinted from (Lewis, 1951).*

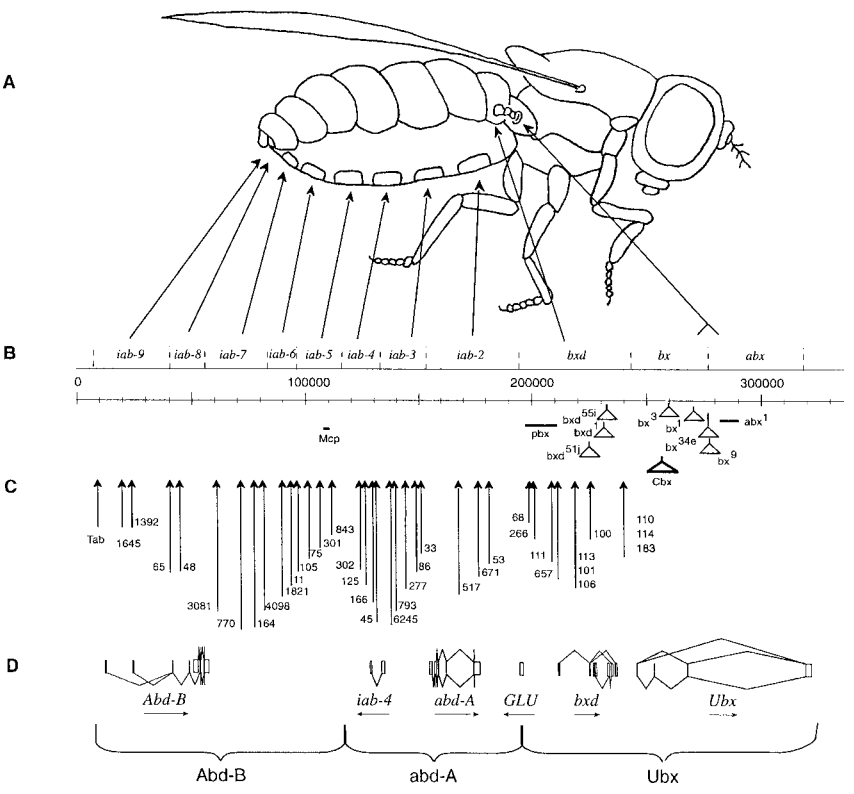

Fig. 7. Genetic and molecular maps of the BX-C. (A) *Adult female, showing the segments affected by BX-C mutations.* **(B)** *Regulatory regions aligned to the DNA map which covers 338 kb (Martin et al., 1995).* **(C)** *Mutant lesions. Insertions are indicated by triangles, deletions by horizontal bars, and rearrangement breakpoints by vertical arrows.* **(D)** *Transcription units within the three domains, AbdB, abdA and Ubx. Alternate promoters and alternate splicing patterns are indicated. GLU marks a sequence predicted to encode a homolog of a mammalian glucose transporter protein; the fly sequence has no apparent function in segmental specification (Martin et al., 1995). The iab-4 and bxd transcription units do not encode proteins (see text). The iab-9 through iab-5 regulatory regions control expression patterns of Abd-B; iab-4, iab-3, and iab-2 regions control abd-A; and the bxd, bx, and abx regions control Ubx.*

"one or more of the mutants are associated with minute rearrangements which have precluded the occurrence of intragenic recombination" (Lewis, 1967).

I had earlier used attached-X females to perform half-tetrad analyses of the *white (w)* eye mutant and its "allele", *white-apricot (w^a)* (Lewis, 1952). Exchanges between w and *apricot (apr)*, as I renamed w^a, were detected in the progeny of *w+/+apr* attached-X females carrying closely linked flanking markers. Reciprocal crossover products of such exchanges were recovered simultaneously in several daughters. Whereas, *w +/ + apr* females flies have a pale pink eye color, *w apr/++* females have the red eye color of wild type. Flanking markers indicated that *apr* lies to the right of *w*, the map distance being about 0.01 centiMorgan. No evidence of gene conversion was detected.

The *bithorax complex* and its organization

Duncan has provided a comprehensive and thorough review of the complex (Duncan, 1987). I have recently given a brief historical review of work on the homeotic clusters in a number of organisms (Lewis, 1992). The following sections will be concerned chiefly with the organization and function of the BX-C.

By generating somatic mosaics for the *bx* phenotype, I was able to show that the effects of the *bx* mutants are highly autonomous (Lewis, 1963). Thus, when cells mutant for the bx^3 function arise from induced somatic crossing over in $bx^3/+$ animals, the cells express the expected mutant phenotype, namely T2-type bristles on T3, which normally lacks any bristles. Morata and García-Bellido provided additional examples and showed that the mutant tissue could arise from exchange events induced as late as the last larval instar (Morata and García-Bellido, 1976). Thus, the wild-type products of at least the *Ubx* domain are not diffusible to any appreciable extent, and such products continually regulate the development of cuticular structures of T3 into late larval life.

In 1964, borrowing from the then-prevailing biochemical dogma based on the operon model, I interpreted the function of the genes of the BX-C to be to "repress certain systems of cellular differentiation and thereby allow other systems to come into play" (Lewis, 1964). Clearly, that function could also be to activate other systems, as García-Bellido later pointed out (García-Bellido, 1975).

Early studies of the BX-C had reached an impasse until homozygotes for deletions of parts, or of all, of the complex were found to have striking effects on cuticular structures of the late embryo. Simple preparations of late embryos cleared in a drop of lactic acid permitted the study of many embryonic lethal phenotypes.

It became evident that the BX-C included genetic material that programmed the development of not only T3 and A1, but also all of the remaining abdominal segments from A2 through A9, inclusive (Lewis, 1978). Thus, animals lacking the entire BX-C, as the result of being homozygous for deletions that removed all of the 89E1-4 bands, were found to die at the end of embryonic development and to have a striking transformation of the first seven abdominal segments toward the T2 segment. The cuticular structures involved include anterior spiracles, ventral pits, Keilin organs and other sense organs. The A8 and A9 segments transform even more anteriorly toward a head segment, based on their developing tiny rudiments of the mandibular hooks (Fig. 2).

It is always dangerous to deduce the wild type function of a gene from a loss-of-function mutations, especially for genes which affect morphology. The wild-type function of major regions of the BX-C could be inferred by adding them, to a homozygous deletion of the BX-C *(Df-P9)* (Lewis, 1978). For example, a duplication, $Dp(3)bxd^{100}$, that includes a wild-type copy of the *Ubx* domain proximal to the *bxd* region, restores the longitudinal tracheal trucks in all segments from T2 to A8, inclusive. The genes of the BX-C control the development of specific structures and organs of the segments rather than segmentation *per se*. The

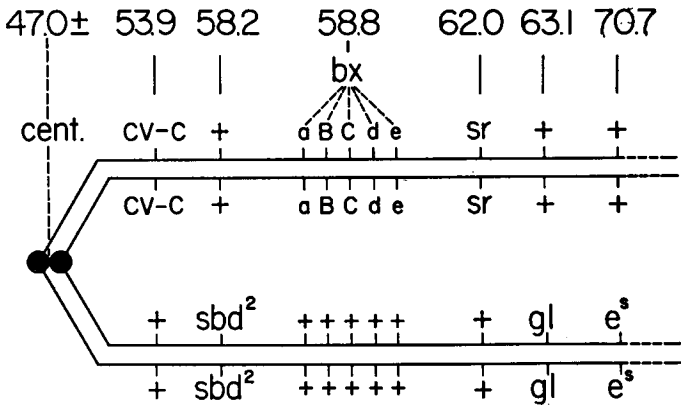

Fig. 8. Diagram of the genetic constitution of attached 3R chromosomes heterozygous for a quintuple bithorax mutant combination and for closely linked marker genes. *The symbols are: cent., centromere; cv-c, crossveinless-c; +, wild type allele; bx, the BX-C; a, bithorax-3; B, Contrabithorax; C, Ultrabithorax; d, bithoraxoid; e, postbithorax; sr, stripe; sbd², stubbloid-2; gl, glass; e^s, ebony-sooty. The standard map locations are shown above the mutant symbols, in centiMorgan units. Reprinted from (Lewis, 1967).*

particular segments in which a given BX-C gene is expressed is determined by the combined action of *trans*-regulatory genes.

The analysis of the functions of *cis*-regulatory regions located distal to the *Ubx* domain, made use of chromosomal rearrangements having breakpoints in those regions. Loss-of-function mutations in those regions were designated as *infra-abdominal (iab)* mutations; for example, a loss-of-function in the *iab-2* region, associated with the transposition, *Tp(2;3)P10*, causes A2 to transform toward A1. By 1978, three *iab* regions had been identified, *iab-2,-3*, and *-8,* and a fourth, *iab-5,* was inferred from an analysis of revertants of a dominant gain-of-function mutation, *Miscadastral pigmentation (Mcp)* by M. Crosby (Lewis, 1978). Subsequently, the regions of the BX-C controlling abdominal development were divided into two domains, *abdominal-A (abd-A)* and *Abdominal-B (Abd-B)*, based on lethal complementation studies (Sanchez-Herrero *et al.,* 1985; Tiong *et al.,* 1985).

Rules governing cis-regulation of the BX-C

The BX-C is regulated in *cis* and in *trans*. Rules governing its *cis*-regulation are considered first and were deduced from genetic analysis. Many of the rules are highly unusual and possibly unique. It seems likely that their molecular analysis will reveal hitherto unsuspected regulatory mechanisms.

Colinearity

The rule of colinearity (COL) states that the order of the BX-C loci in the chromosome parallels the order in which the units at those loci are expressed along the antero-posterior axis of the body. Two types of gradients had been invoked to explain this rule: "an antero-posterior gradient in repressor concentration along the embryo and a proximo-distal gradient along the chromosome in the affinities for repressor of each gene's *cis*-regulatory element" (Lewis, 1978).

Molecular studies confirmed the rule and extended it to all of the abdominal *cis*-regulatory regions from *iab-2* to *iab-8*, inclusive (Karch *et al.,* 1985). Associated with the COL rule is the strong tendency for the proteins of the BX-C, once expressed to continue to be expressed more posteriorly in the body except for the terminalia. This is elegantly shown in Figure 9, for the *Ubx*, *abd-A* and *Abd-B* proteins visualized by the use of immunostaining with antibodies specific to each.

Cis-inactivation

The second rule of *cis*-inactivation (CIN) states that loss-of-function mutations in a given *cis*-regulatory region tend to inactivate the next more distal region of the complex. Examples have already been cited for the polar inactivation of the *pbx*$^+$ function by *bx*^{3} and by *bxd*. Other examples were later found in analyzing rearrangement breakpoints in the *iab* regions of the BX-C (Lewis, 1986). It has not been possible to establish whether there are CIN effects between major domains of the BX-C.

Cis-overexpression

The third rule of *cis*-overexpression (COE) is a quite surprising one. The rule states that the loss of function associated with a given *cis*-regulatory region tends to be accompanied by an overexpression of the function associated with the *cis*-regulatory region that lies immediately proximal to it. In the abdominal domains, rearrangements with breaks in the *iab-3* region, for example, not only have a loss-of-function *iab-3* phenotype (A3 transformed toward A2) but a gain-of-function of the *iab-2*$^+$ region that is manifested as a transformation of the A1 segment toward A2. Other examples have been described (Lewis, 1986).

COE effects are known not only for breakpoints of chromosomal rearrangements but for gypsy insertions. An important one is a COE effect of *bx*^{3}. Flies homozygous for *bx*^{3} have a reduction in the extreme anterior region of T2. This effect is dominant since it is not suppressed by duplications that totally suppress the recessive *bx*^{3} transformation of T3 toward T2. An x-ray induced mutant, *anterobithorax (abx)*, was discovered that had a weak bithorax-like phenotype. It is located just proximal to *bx*, and *abx bx*^{3} double mutants lack the COE effect on T2 seen in the *bx*^{3} single mutant genotype. Until a *abx* had been found, it was not possible to achieve a full transformation of T3 toward T2; i.e., the *bx*^{3} *pbx* double mutant homozygote fails to transform the most anterior portion of T3. Flies homozygous for the triple mutant, *abx bx*^{3} *pbx* , were constructed and proved to have virtual complete transformation of the wing and cuticular structures of T3 transformed toward those of T2, resulting in a four-winged fly (Fig. 4).

Negative trans-regulation of the *bithorax complex*

In 1947, a remarkable x-ray induced dominant mutant, *Polycomb (Pc)*, was found by P. H. Lewis (Lewis, 1949) . It had sex combs on not only on the first, but the 2nd and 3rd pair of legs, and rudimentary antennal to leg transformations resembling those of *Antennapedia (Antp)* mutants. It also had effects that were only later realized to be gain of function of genes in the *Ubx* domain; namely, reduction in the extreme anterior region of T2 and reduction in the wing similar to that of weak *Cbx* phenotypes, such as in *Cbx Ubx/+*. It was nearly 30 years before it was realized that *Pc* is a mutation in a gene that acts as a negative regulator of the BX-C, and of the ANT-C complex as well. Thus, the homozygous *Pc* embryo has the three thoracic and the first seven abdominal segments all transformed toward A8, presumably as the result of

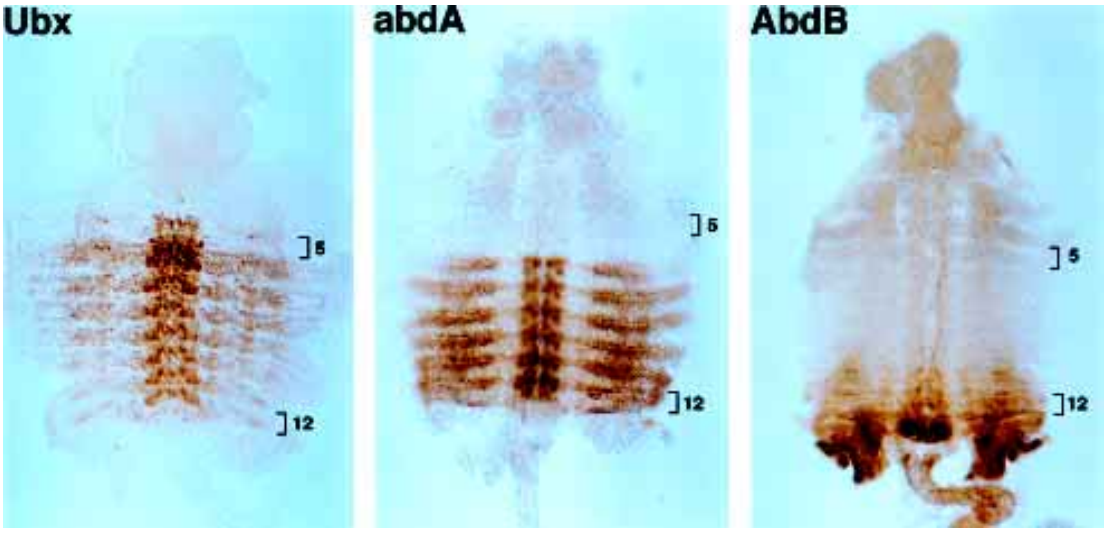

Fig. 9. Embryos stained with monoclonal antibodies to the protein products of the BX-C. *Preparations are of 10-12 h embryos, split along their dorsal midlines and flattened. Brackets indicate parasegments 5 and 12, which correspond approximately with the third thoracic and seventh abdominal segments, respectively. Ubx protein appears in parasegments 5-13, abd-A protein in 7-13, and Abd-B protein in 10-13 (from W. Bender).*

derepression of the *Abd-B* domain (Lewis, 1978) (figured in Duncan, 1982).

Duncan found a second mutant of the *Polycomb* type, *Polycomb-like (Pc-l)* (Duncan, 1982). *Pc* and *Pcl* have proved to be but two of a family of genes that act as negative regulators (Jurgens, 1985). That the *Pc* protein is involved in binding to the BX-C and the ANT-C regions (as well as to other regions) has been elegantly shown by immunostaining of salivary gland chromosome with an antibody to that protein (Zink, 1989). Since the *Pc* protein is a non-histone chromosomal protein, rather than a DNA-binding protein (Paro and Hogness, 1991) its binding specificity may reside in its complexing with proteins of other genes of the *Pc* family, some of which first bind specifically to BX-C and ANT-C.

Positive trans-regulation of the *bithorax complex*

Positive *trans*-regulators were also found, such as *Regulator of bithorax (Rg-bx)*. An analysis of this mutant, and of deficiencies which include the locus, indicate that the wild-type gene is a positive regulator of the BX-C (Lewis, 1981). A partial loss-of-function allele, *trithorax (trx)*, was then found by Ingham (Ingham and Whittle, 1980). The *trx* gene has been cloned and is a DNA-binding protein of the zinc finger category (Kuzin *et al.,* 1994; Stassen *et al.,* 1995). More recently, Kennison and Tamkun have identified a family of genes like *trx* that act when mutated as enhancers of *bx* phenotypes (Kennison and Tamkun, 1988).

Additional classes of *trans*-regulators of the BX-C have come from the studies of Nüsslein-Volhard and Wieschaus (Nüsslein-Volhard and Wieschaus, 1980). For example, the gap gene, *hunchback (hb)*, is involved in establishing major subdivisions of the body regions. It encodes a zinc finger protein and acts as a negative regulator of the BX-C, keeping the complex turned off in the anterior regions of the body, presumably by the binding of the *hb* protein to at least one specific motif in the *Ubx* gene (Qian and Pirrotta, 1988). A dominant mutant, *Regulator of postbithorax (Rg-pbx)*, is now known to be a gain-of-function mutation in the *hb* gene (Bender *et al.,* 1987). It produces variable *pbx*-like transformations of the halter (Lewis, 1968).

Another example is the *Krupple (Kr)* gene of Gloor (1950). It is also a gap gene and encodes a DNA-binding protein (Rosenberg *et al.,* 1986; Schuh *et al.,* 1986). One motif to which it binds is in the *iab-2* region and, on two independent occasions, a mutation in a single specific base pair of that motif has resulted in a dominant *Hyperabdominal (Hab)* phenotype (Schimel *et al.,* 1994). These gain-of-function mutants have poor penetrance, but in some crosses *Hab/+* flies occasionally have only four legs and no halteres owing to T3 being transformed toward A2 (Lewis, 1978).

Molecular analysis of the *bithorax complex*

Molecular analysis of the *Ubx* domain of the BX-C was initiated by D. Hogness and co-workers in 1978 and they soon identified the major features of that region. The *bx* mutants, *Ubx*, and several *bxd* mutants all proved to be insertions of transposable elements (Bender *et al.,* 1983). Molecular studies revealed a single transcription unit coding for proteins in the *Ubx* domain (O'Connor *et al.,* 1988; Kornfeld *et al.,* 1989). The embryonic distribution of the UBX protein products was determined by White and Wilcox (1984) and by Beachy *et al.* (1985); see also Figure 9. The transcription unit and protein product of the second domain, *abdominal-A*, were characterized by Karch *et al.,* (1990). The third domain, *Abdominal-B,* produces at least four transcripts (DeLorenzi *et al.,* 1988; Kuziora and McGinnis, 1988; Sanchez-Herrero and Crosby, 1988; Celniker *et al.,* 1989; Zvortink and Sakonju, 1989) and two *Abd-B* proteins (Celniker *et al.,* 1989,1990; Boulet *et al.,* 1991).

Surprisingly, the *cis*-regulatory regions are transcriptionally active, as first shown for the *bxd* region of the BX-C (Lipshitz *et al.,* 1987). This region produces a large (26.5 kb) primary transcript, that is then spliced to yield a family of non-protein coding RNAs (i.e., containing multiple stop codons). Similar non-coding transcription units are known for the *iab-4* region (Cumberledge *et al.,* 1990).

The Transabdominal mutation

King and Wilson (1975) called attention to the possible importance in evolution of creating novel phenotypes solely by rearrangements involving *cis*-regulatory sequences. A striking example was our discovery of an X-ray induced dominant mutation, *Transabdominal (Tab)*. *Tab/+* flies have a sexually dimorphic pattern of pigmented bands in the dorsal thorax of T2 (Fig. 10).

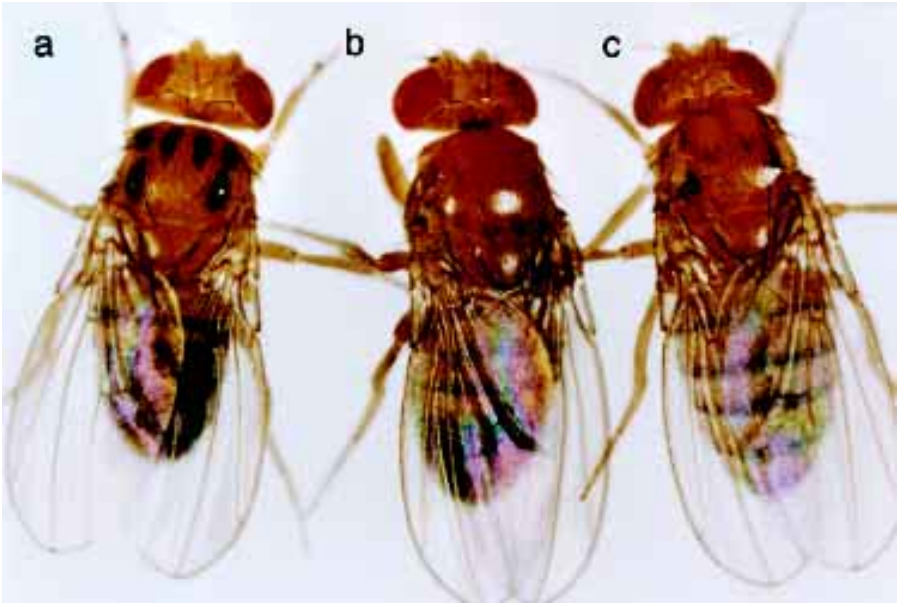

Fig. 10. *Transabdominal,* a sexually dimorphic mutant of the *Abdominal-B* domain. (a) *Tab/+male.* **(b)** *Wild type male.* **(c)** *Tab/+female. Thoracic transformations are described in the text.*

Unlike the great majority of dominant gain-of-function phenotypes, *Tab /+* has 100% penetrance and complete expressivity. Molecular and morphological studies (Celniker and Lewis, 1987) indicate that the pigmentation pattern of the bands resembles that normally found in the tergites of segments A5 and A6. Thus, the pigmented bands in the *Tab/+* male dorsal thorax are broad as in the A5 and A6 male tergites; whereas, in the *Tab/+* female they are narrow as in the corresponding female tergites. The *Tab* mutant is associated with an inversion having one breakpoint in the *iab-9 cis*-regulatory region (Fig. 7) and the other near the *stripe (sr)* locus in 90D which codes for an early growth-response transcription factor (Lee *et al.,* 1995). *In situ* studies (Celniker and Lewis, 1993) of the dorsal thoracic disc of T2, which gives rise to the dorsal thorax, show cells in *Tab/+* animals that express the ABD-BII protein and its RNA. These cells correspond to the sites of the bands in the *Tab/+* adult thorax and appear to be the sites of attachment for certain thoracic muscles. Our studies of the RNA and protein distributions in embryos and imaginal discs indicate that the *Tab* mutation represents a case in which *cis*-regulatory regions of a gene involved in defining the development of muscle attachment sites is now driving *Abd-B* protein expression (Lee *et al.,* 1995). Other minor disturbances in the abdominal tergites of *Tab/+* flies are believed to involve ectopic expression of the *Abd-B* protein in such attachment sites for abdominal tergite muscles.

Control of somatic gonad development in *Drosophila* and *Bombyx*

As early as 1943, Itikawa reported (Itikawa, 1943) on a mutant designated E^N whose phenotype when homozygous parallels closely that of the homozygous deficiency for the BX-C in Drosophila (*Df-P9*). Itikawa's discovery that certain mutants of the "E" series lacked gonads led me to examine a dominant mutant, *Ultra-abdominal*^{4} *(Uab*^{4}*)*, which is associated with a recessive *iab-3* phenotype. Internally, the *Uab*^{4} hemizygote was found to lack gonads (Lewis, 1978). Subsequently, I found that rearrangements with breakpoints in the *iab-4* region of the BX-C, when viable as homozygotes appear virtually wild type, but internally they lack gonads (Lewis, 1986). Loss of gonads in *iab-2* and *iab-3* mutant animals results from *cis*-inactivation of the *iab-4* region [Lewis, unpublished]. Since the gonad is of mesodermal origin, its loss was one of the first indications that the BX-C phenotypes were not limited to ectodermal tissues.

A comparative molecular analysis of the *iab-4 cis*-regulatory with regions controlling gonad formation in *Bombyx* and other animals may show how the homeotic genes control the development of a specific structure. Thus, since some of the more primitive non-segmented animals, such as the nematode, have somatic gonads, it is likely that control of the initiation of their development will have common features. Of great interest will be the target genes in *Drosophila* that accomplish such initiation. A promising approach to understanding the process in human beings can be expected to come from analyzing molecularly the basis of inherited defects in the human gonad.

The homeobox and tandem gene duplication

Molecular support for the assumption that tandem gene duplication was responsible for at least the coding portions of the BX-C and the ANT-C complex finally came with the discovery of the homeobox in 1984, by McGinnis *et al.* (1984) and Scott and Weiner (1984) who independently showed that the proteins encoded by the *Ubx* and *Antp* genes contain a remarkably conserved group of amino acids, known as the homeodomain. The DNA sequence encoding the homeodomain was named the homeobox (McGinnis *et al.,* 1984). The homeobox sequence is conserved to a remarkably high degree throughout the animal kingdom and it was used to probe for homologs of the BX-C and ANT-C in many other organisms, including vertebrates as well as invertebrates (Gehring and Hiromi, 1986). Most of these organisms have the homologs of both the BX-C and the ANT-C in a single complex known as the homeotic complex (HOM-C) (Beeman, 1987).

In unsegmented organisms like Caenorhabditis (Kenyon and Want, 1991) there are apparently only a few *HOM-C* genes. Insects such as the silkworm, *Bombyx* (Tazima, 1964) and the flour beetle, *Tribolium* (Beeman *et al.,* 1993) have larger clusters as in *Drosophila*. The most primitive vertebrates represented by the lancelet, *Amphioxus* (García-Fernandez and Holland, 1994; Holland *et al.,* 1994) also have a single large HOM-C. However, higher vertebrates have four semi-redundant copies of the HOM-C. In the mouse and human, each copy is on a different chromosome. This redundancy makes it difficult to dissect the function of a given gene in any one of the sets. Remarkable progress is being made by using gene knock-out techniques in mice, to study the role of the *HOM-C* genes in development. *HOM-C* gene expression in the mouse,

as in *Drosophila*, obeys the rule of colinearity (reviewed by Lewis, 1992). Their segmental expression limits are also regulated in *trans* by genes that are remarkably parallel to those of the *Pc* Group and *trx* Group (reviewed by Simon, 1995). *HOM-C* genes are now regarded as master control genes whose proteins bind to the *cis*-regulatory regions of target genes. The latter then activate or repress systems of cellular processes that accomplish the final development of the organism. Even minor mutant lesions in *HOM-C* genes may be expected to have global effects on such systems. An example is a targeted gene-disruption of the mouse *HOX A3* gene (formerly *HOX 1.5*) that leads to defects in the thyroid glands and surrounding tissues (Chisaka and Capecchi, 1991). The resultant group of defects resembles those seen in the congenital DiGeorge syndrome of human beings.

Complete sequence of the *bithorax complex*

The DNA sequence of the BX-C has now been completely determined (Martin *et al.,* 1995) and a preliminary analysis made of it (Lewis *et al.,* 1995). The protein coding regions comprise only 2% of the entire sequence. The other 98% is expected to contain a diverse group of motifs to which *trans*-regulatory proteins bind, thereby conferring the specific spatial and temporal expression of the protein products of each domain. There may also be a regulatory role for non-coding RNA's of the type identified in the *bxd* and *iab-4* regions.

The next fifty years

Only three of the many future challenges will be outlined: (1) molecular and genetic approaches are needed to determine the immediate target genes that are turned on or off by the genes of the HOM-C; (2) since the genes of the HOM-C have tended to remain tightly linked and colinear with their expression patterns along the body axis, it will be exciting to discover the underlying mechanisms that have kept them together and; (3) comparative DNA sequence analysis of the HOM-C among many different organisms may provide evidence that the *cis*-regulatory regions have evolved by tandem duplication. Ultimately, comparisons of the HOM-C throughout the animal kingdom should provide a picture of how the organisms, as well as the genes of the HOM-C, have evolved.

Conclusions

Basic research concerned with testing a simple hypothesis about how new genes arise from old genes led after many circuitous routes to the discovery of the homeotic complex (HOM-C). This cluster of master control genes programs much of the development of all higher animal organisms. Each of the genes contain a homeobox, a remarkably conserved DNA sequence that provides molecular support for the hypothesis that the complex itself arose by a process of tandem gene duplication. The high degree of conservation of the HOM-C, itself, between vertebrates and invertebrates indicates that it arose from an ancestral complex over 500 million years ago, the estimated time of separation of these two great groups of organisms.

It is likely that mutations within the HOM-C's of human beings are the cause of certain genetically based abnormalities that arise at various stages of human development. Somatic mutations in genes of the HOM-C may conceivably be involved in the generation of tumors. Meanwhile, future genetic and molecular studies of the HOM-C in lower creatures that have but one set of the complex promise to advance our understanding of its role as a master regulator of development.

Much has been learned about the role of the HOM-C in development, and about its molecular products. Nevertheless, we are still unable to make sense of much of the DNA sequence of the bithorax complex (BX-C) or to explain how the that complex is itself regulated. Progress will still need to be driven by the logic of genetics, and by further increases in abstraction.

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References

BEACHY, P.A., HELFAND, S.L. and HOGNESS, D.S. (1985). Segmental distribution of Bithorax Complex proteins during *Drosophila* development. *Nature 313:* 545-551.

BEEMAN, R.W. (1987). A homeotic cluster in the red flour beetle. *Nature 327:* 247-249.

BEEMAN, R.W., STUART, J.J., BROWN, S.J. and DENELL, R.E. (1993). Structure and function of the homeotic gene-complex (Hom-C) in the beetle, Tribolium-Castaneum. *Bioessays 15:* 439-444.

BENDER, M., TURNER, F.R. and KAUFMAN, T.C. (1987). A developmental genetic analysis of the gene regulator of po*stbithorax* in *Drosophila melanogaster*. *Dev. Biol. 119:* 418-432.

BENDER, W., AKAM, M., KARCH, F.A., BEACHY, P.A., PEIFER, M., SPIERER, P., LEWIS, E.B. and HOGNESS, D.S. (1983). Molecular genetics of the Bithorax Complex in *Drosophila melanogaster. Science 221:* 23-29.

BENSON, M. and PIRROTTA, V. (1987). The product of the Drosophila-ze*este* gene binds ot specific DNA-Sequences in *white* and *Ubx. Eur. J. Mol. Biol. 6:* 1387-1392.

BENSON, M. and PIRROTTA, V. (1988). The Droso*phila* zeste protein binds cooperatively to sites in many gene regulatory regions: implications for transvection and gene regulation. *Eur. Mol. Biol. Org. J. 7:* 3907-3915.

BOULET, A.M., LLOYD, A. and SAKONJU, S. (1991). Molecular definition of the morphogenetic and regulatory functions and the *cis*-regulatory elements of the *Drosophila Abd-B* homeotic gene. *Development 111:* 393-405.

BRIDGES, C. (1932). Specific Suppressors in Drosophila. *Proc. Int. Congr. Genet.* (6th) *2:* 12-14.

BRIDGES, C.B. (1935). Salivary chromosome maps with a key to the banding of the chromosomes of *Drosophila melanogaster*. *J. Hered. 26:* 60-64.

BRIDGES, C.B. (1936). The Bar 'gene': a duplication. *Science 83:* 210-211.

BRIDGES, C.B. (1944). *The Mutants of Drosophila Melanogaster*. Carnegie Institution of Washington Pyblication 552, (Ed. D.S. Brehme). The Lord Baltimore Press. Baltimore. pp. 1-257.

BRINK, R.A. (1932). Are the chromosomes aggregates of groups of physiologically interdependent genes? *Am. Nat. 66:* 444-451.

CELNIKER, S.E. and LEWIS, E.B. (1987). Transabdominal: a dominant mutant of the Bithorax Complex produces a sexually dimorphic segmental transformation in *Drosophila. Genes Dev. 1:* 111-123.

CELNIKER, S.E. and LEWIS, E.B. (1993). The molecular basis of Transabdominal-a novel sexually dimorphic mutant of the bithorax complex of *Drosophila. Proc. Natl. Acad. Sci. USA 90:* 1566-1570.

CELNIKER, S.E., KEELAN, D.J. and LEWIS, E.B. (1989). The molecular genetics of the bithorax complex of Drosophila: characterization of the products of the *Abdominal-B* domain. *Genes Dev. 3:* 1425-1437.

CELNIKER, S.E., SHARMA, S., KEELAN, D. and LEWIS, E.B. (1990). The molecular genetics of the bithorax complex of *Drosophila* cis-regulation in the *Abdominal-B* domain. *Eur. Mol. Biol. Org. J. 9:* 4277-4286.

CHISAKA, O. and CAPECCHI, M.R. (1991). Regionally restricted developmental defects resulting from targeted disrution of the mouse homeobox gene *Hox-1.5*. *Nature 350:* 473-479.

CUMBERLEDGE, S., ZARATZIAN, A. and SAKONJU, S. (1990). Characterization of 2 RNAs transcribed from the cis-regulatory region of the Abd-A domain within the *Drosophila* Bithorax complex. *Proc. Natl. Acad. Sci. USA 87:* 3259-3263.

DELORENZI, M., ALI, N., SAARI, G., HENRY, C., WILCOX, M. and BIENZ, M. (1988). Evidence that the *Abdominal-B* r element function is conferred by a *trans*-regulatory homeoprotein. *Eur. Mol. Biol. Org. J. 7*: 3223-3231.

DUNCAN, I. (1982). Polycomblike: A gene that appears to be required for the normal expression of the bithorax and Antennapedia gene complexes of *Drosophila melanogaster. Genetics 102:* 49-70.

DUNCAN, I. (1987). The Bithorax Complex. *Annu. Rev. Genet. 21:* 285-319.

GANS, M. (1953). Genetic and physiological study of mutant Z of Drosophila melanogaster. *Bull. Biol. Fr. Belg. 28:* 1-90.

GARCÍA-BELLIDO, A. (1975). Genetic control of wing disc development in *Drosophila*. (Brenner S, ed.). Associated Scientific Publishers, New York. Cell Patterning, *Ciba Found. Symp.*

GARCÍA-FERNANDEZ J. and HOLLAND, P.W.H. (1994). Archetypal Organization of the Amphioxus Hox Gene Cluster. *Nature 370:* 563-566.

GEHRING, W.J. and HIROMI, Y. (1986). Homeotic genes and the homeobox. *Annu. Rev. Genet. 20:* 147-173.

GELBART, W.M. (1982). Synapsis-Dependent Allelic Complementation at the Decapentaplegic Gene-Complex in Drosophila-Melanogaster. *Proc. Natl. Acad. Sci. USA 79:* 2636-2640.

GLOOR, H. (1950). Arch. Julius Klaus-Stift. Vererbungsforsch. *Sozialanthropol. Rassenhyg. 25:* 38-44.

GREEN, M.M. and GREEN, K.C. (1949). Crossing-over between alleles at the lozenge locus in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA 35:* 586-591.

HAYES, W. (1968). *The Genetics of Bacteria and Their Viruses:* S*tudies in Basic Genetics and Molecular Biology*. (2nd ed.) John Wiley and Sons Inc. New York.

HOLLAND, P.W.H., GARCIA-FERNANDEZ, J., HOLLAND, L.Z., WILLIAMS, N.A. and HOLLAND, N.D. (1994). The Molecular Control of Spatial Patterning in Amphioxus. *J. Mar. Biol. 74:* 49-60.

HOLLIDAY, R. (1964). A mechanism for gene conversion in fungi. *Genet. Res. 5:* 282-304.

INGHAM. P. and WHITTLE, J.R.S. (1980). *Trithorax:* A new homeotic mutation of *Drosophila melanogaster* causing transformations of abdominal and thoracic imaginal segments. *Mol. Gen. Genet. 179:* 607-614.

ITIKAWA, N. (1943). Genetical and embryological studies of a dominant mutant, the «new additional crescent» of the silworm, *Bombyx mori. Jpn. J. Genet. 19:* 182-188.

JOHANNSEN W. (1911). The Genotype Conception of Heredity. *Am. Nat.* [XLV(March)]: 129-159.

JUDD, B.H, SHEN, M.W. and KAUFMAN, T.C. (1972). The anatomy and function of a sement of the X chromosome of *Drosophila melanogaster. Genetics 71:* 139-156.

JURGENS, G. (1985). A group of genes controlling the spatial expression of the bithorax complex in *Drosophila. Nature 316:* 153-155.

KARCH, F., BENDER, W. and WEIFFENBACH, B. (1990). abd-A expression in *Drosophila* embryos. *Genes Dev. 4:* 1573-1587.

KARCH, F., WEIFFENBACH, B., PEIFER, M., BENDER, W., DUNCAN, I., CELNIKER, S., CROSBY, M. AND LEWIS, E.B. (1985). The abdominal region of the Bithorax Complex. *Cell 43:* 81-96.

KAUFMAN, T.C., LEWIS, R. and WAKIMOTO, B. (1980). Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster:* the homeotic gene complex in polytene chromosomal interval 84A,B. *Genetics 94:* 115-133.

KENNISON, J.A. and TAMKUN, J.W. (1988). Dosage-dependent modifiers of Polycomb and Antennapedia mutations in *Drosophila. Proc. Natl. Acad. Sci. 85:* 8136-8140.

KENYON, C. and WANT, B. (1991). A cluster of *Antennapedia*-class homeobox genes in a nonsegmented animal. *Science 253:* 516-517.

KING, M. and WILSON, A.C. (1975). Evolution at Two Levels in Humans and Chimpanzees. *Science 188:* 107-116.

KORNFELD, K., SAINT, R.B., BEACHY, P.A., HARTE, P.J., PEATTIE, D.A. and HOGNESS, D.S. (1989). Structure and expression of a family of Ultrabithorax mRNAs genrated by alternative splicing and polyadenylation in *Drosophila. Genes Dev. 3:* 243-258.

KUZIN, B., TILLIB, S., SEDKOV, Y., MIZROKHI, L. and MAZO, A. (1994). The Drosophila tr*ithorax* gene encodes a chromosomal protein and directly regulates the region-specific homeotic gene *fork head. Genes Dev. 8:* 2478-2490.

KUZIORA, M.A. and MCGINNIS, W. (1988). Different transcripts of the Drosophila Abd-B gene correlate with distinct genetic sub-functions. *Eur. Mol. Biol. Org. J. 7:* 3233-3244.

LEE, J.C., VIJAYRAGHAVAN, K., CELNIKER, S.E. and TANOUYE, M.A. (1995). Identification of a Drosophila Muscle Development gene with structural homology to mammalian early growth-response transcription factors. *Proc. Natl. Acad. Sci. USA 92:* 10344-10348.

LEISERSON, W.M., BONINI, N.M. and BENZER, S. (1994). Transvection at the *eyes absent* gene of *Drosophila. Genetics 138:* 1171-1179.

LEWIS, E.B. (1939).Star-recessive, a spontaneous mutation in Drosoph*ila melanogaster. Proc. Minnesota Acad. Sci. 7:* 23-26.

LEWIS, E.B. (1941). Another case of unequal crossing over in Droso*phila Melanogaster. Proc. Natl. Acad. Sci. USA 27:* 31-34.

LEWIS, E.B. (1942a). The Star and *asteroid* loci in *Drosophila melanogaster. Genetics 27:* 153-154.

LEWIS, E.B. (1942b). *A Genetic and Cytological Analysis of a Tandem Duplication and its Included Loci in Drosophila melanogaster [Ph.D.].* California Institute of Technology.

LEWIS, E.B. (1945). The relation repeats to position effect in *Drosophila melanogaster. Genetics 30:* 137-166.

LEWIS, E.B. (1949). su2-Hw: suppressor-2-Hairy-wing. *Drosophila Information Service 23:* 59-60.

LEWIS, E.B. (1951). Pseudoallelism and gene evolution. *Cold Spring Harbor Symp. Quant. Biol. 16:* 159-174.

LEWIS, E.B. (1952). The pseudoallelism of white and apricot in Drosophila *melanogaster. Proc. Natl. Acad. Sci. USA 38:* 953-961.

LEWIS, E.B. (1954a). Pseudoallelism and the gene concept. *Proc. Int. Congr. Genet. (9th)1:* 100-105.

LEWIS, E.B. (1954b).The theory and application of a new method of detecting chromosomal rearrangements in *Drosophila melanogaster. Am. Nat. 88:* 225-239.

LEWIS, E.B. (1955). Some aspects of pseudoallelism. *Am. Nat. 89:* 73-89.

LEWIS, E.B. (1963). Genes and developmental pathways. *Am. Zool. 3:* 33-56.

LEWIS, E.B. (1964). Genetic control and regulation of developmental pathways. In *Role of Chromosomes in Development.* (Locke M, ed.). Academic Press Inc. New York. pp. 231-252.

LEWIS, E.B. (1967). Genes and gene complexes. In *Heritage from Mendel.* (Brink RA, ed.). University of Wisconsin Press. Madison. pp. 17-47.

LEWIS, E.B. (1968). Genetic control of developmental pathways in *Drosophila melanogaster. Proc. Int. Congr. Genet.,* (12th). Tokyo, Japan: Science Council of Japan: 96-97.

LEWIS, E.B. (1978). A gene complex controlling segmentation in *Drosophila. Nature 276:* 565-570.

LEWIS, E.B. (1981). Developmental genetics of the bithorax compelx in *Drosophila.* In *Developmental Biology Using Purified Genes.* (Brown DD, Fox CF, ed.). ICN-

UCLA Symposia on Molecular and Cellular Biology. Academic Press. Keystone, Colorado. pp.189-208.

LEWIS, E.B. (1982). Control of body segment differentiation in *Drosophila* by the bithorax gene complex. In *Embryonic Development, Part A: Genetic Aspects.* (Burger MM, Weber R, ed.). Alan R. Liss, Inc. New York. pp.269-288.

LEWIS, E.B. (1984). Regulation in Cis and Trans of the Bithorax Gene Complex in Drosophila. *J. Cell. Biochem. (Suppl.) 8B:6.*

LEWIS, E.B. (1986). Regulation of the genes of the bithorax complex in *Drosophila. Cold Spring Harbor Symp. Quant. Biol. 50:* 155-164.

LEWIS, E.B. (1992). Clusters of Master Control Genes Regulate the Development of Higher Organisms. *J. Am. Med. Assoc. 267:* 1524-1531.

LEWIS, E.B. (1994). Homeosis: the first 100 years. *Trends Genet. 10:* 341-343.

LEWIS, E.B., KNAFELS, J.D., MATHOG, D.T. and CELNIKER, S.E. (1995). Sequence analysis of the *cis*-regualtory regions of the bithorax complex of *Drosophila. Proc. Natl. Acad. Sci. USA 92:* 8403-8407.

LEWIS, P.H. (1949). Pc: Polycomb. *Drosophila Information Service 21:* 69.

LINDSLEY, D.L. and GRELL, E.H. (1968). *Genetic Variations of Drosophila melanogaster.* Carnegie Institution of Washington, vol Publication 627. Washington D.C.

LIPSHITZ, H.D., PEATTIE, D.A. and HOGNESS, D.S. (1987). Novel transcripts from the *Ultrabithorax* domain of the Bithorax Complex. *Genes Dev. 1:* 307-322.

MARTIN, C.H., MAYEDA, C.A., DAVIS C,A., ERICSSON, C.L., KNAFELS, J.D., MATHOE, D.R., CELNIKER, S.E., LEWIS, E.B. AND PALAZZOLO, M.J. (1995). Complete Sequence of the bithorax complex of *Drosophila. Proc. Natl. Acad. Sci. USA. 92:* 8398-8402.

MCGINNIS, W., LEVINE, M., HAFEN, E., KUROIWA, A. and GEHRING, W.J. (1984). A conserved DNA sequence in homeotic genes of the *Drosophila* Antennapedia and Bithorax complexes. *Nature 308:* 428-433.

MODOLELL, J., BENDER, W. and MESELSON, M. (1983). Drosophila-melanogaster mutations suppressible by the suppressor Hairy-wing are insertions of a 7.3-kilobase mobile element. *Proc. Natl. Acad. Sci. USA 80:* 1678-1682.

MORATA, G. and GARCIA-BELLIDO, A. (1976). Developmental analysis of some mutants of the Bithorax System of Drosophila. *Roux Arch. Dev. Biol. 179:* 125-143.

MULLER, H.J. (1927). Artificial transmutation of the gene. *Science 66:* 84-87.

MULLER, H.J., PROKOFYEVA-BELGOVSKAYA, A.A. and KOSSIKOV, K.V. (1936). Unequal crossing-over in the Bar mutant as a result of duplication of a minute chromosome section. *C.R. (Doklady) Acad. Sci. URSS 1:* 87-88.

NÜSSLEIN-VOLHARD, C. and WIESCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila. Nature 287:* 795-801.

O'CONNOR, M.B., BINARI, R., PERKINS, L.A. and BENDER, W. (1988). Alernative RNA products from the *Ultrabithorax* domain of the bithorax complex. *Eur. Mol. Biol. Org. J. 7:* 435-445.

OLIVER, C.P. (1940). A reversion to wild-type associated with crossing over in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA 26:* 452-454.

PARKHURST, S.M. and CORCES, V.G. (1986). Mutations at the suppressor of forked locus increase the accumulation of Gypsy-encoded transcripts in *Drosophila-Melanogaster. Mol. Cell. Biol. 6:* 2271-2274.

PARKHURST, S.M., HARRISON, D.A., REMINGTON, M.P., SPANA, C., KELLEY, R.L. COYNE, R.S. and CORCES, V.G. (1988). The Drosophila su(Hw) gene, which controls the phenotypic effect of the gypsy transposable element, encodes a putative DNA-binding protein. *Genes Dev. 2:* 1205-1215.

PARO, R. and HOGNESS, D.S. (1991). The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of Drosophila. *Proc. Natl. Acad. Sci. USA 88:* 263-267.

QIAN, S.M.C. and PIRROTTA, V. (1991). The bx region enhancer, a distant cis-control element of the *Drosophila Ubx* gene and its regulation by *hunchback* and other segmentation genes. *Eur. Mol. Biol. Org. J. 10:* 1415-1425.

ROSENBERG, U.B., SCHRODER, C., PREISS, A., KIENLIN, A., COTE, S., RIEDE, I. and JACKLE, H. (1986). Structural homology of the product of the *Drosophila Kruppel* gene with *Xenopus* transcription factor IIIA. *Nature 319:* 336-339.

SANCHEZ-HERRERO, E. and CROSBY, M.A. (1988). The Abdominal-B gene of Drosophila melanogaster: overlapping transcripts exhibit two different spatial distributions. *Eur. Mol. Biol. Org. J. 7:* 2163-2173.

SANCHEZ-HERRERO, E., VERNOS, I., MARCO, R. and MORATA, G. (1985). Genetic organization of *Drosophila* Bithorax Complex. *Nature 313:* 108-113.

SCHUH, R., AICHER, W., GAUL, U., COTE, S., PREISS, A., MAIER, D., SEIFERT, E., NAUBER, U., SCHRODER, C., KEMLER, R. and JACKLE, H. (1986). A Conserved Family of Nuclear Proteins Containing Structural Elements of the Finger Protein Encoded by Kruppel, a Drosophila Segmentation Gene. *Cell 47:* 1025-1032.

SCOTT, M.P. and WEINER, A.J. (1984). Structural relationships among genes that control develpment: Sequence homology between the Antennapedia, Ultrabithorax, and fushi tarazu loci of *Drosophila. Proc. Natl. Acad. Sci. USA 81:* 4115.

SHIMELL, M.J., SIMON, J., BENDER, W. and O'CONNOR, M.B. (1994). Enhancer point mutation results in a homeotic transformation in *Drosophila. Science 264:* 968-971.

SIMON, J. (1995). Locking in stable states of gene expression:Transcriptional control during Drosophila development. *Curr. Opin. Cell Biol. 7:* 376-385.

STASSEN, M.J., BAILEY, D., NELSON, S., CHINWALLA, V. and HARTE, P.J. (1995). The Drosophila-trithorax proteins contain a novel variant of the nuclear receptor-type DNA-binding domain and an ancient conserved motif found in other chromosomal proteins. *Mech. Dev. 52:* 209-223.

STURTEVANT, A.H. (1925). The effects of unequal crossing over at the Bar locus in *Drosophila. Genetics 10:*117-147.

STURTEVANT, A.H. (1965). *A History of Genetics.* Harper and Row. New York

TAZIMA, Y. (1964). *The Genetics of the Silkworm.* Logos. London.

TIONG, S., BONE, L.M. and WHITTLE, J.R. (1985). Recessive lethal mutations within the Bithorax Complex in Drosophila. *Mol. Gen. Genet. 200:* 335-342.

WHITE, R.A.H. and WILCOX, M. (1984). Protein products of the Bithorax Complex in Drosophila. *Cell 39:* 163-171.

WRIGHT, S. (1929). The Dominance of Bar over Infra-Bar in Drosophila. *Am. Nat. 63:* 479-480.

ZAVORTINK, M. and SAKONJU, S. (1989). The morphogenetic and regulatory functions of the *Drosophila Abdominal-B* gene are encoded in overlapping RNAs transcribed from separate promoters. *Genes Dev. 3:* 1969-1981.

Zink, B. and PARO, R. (1989). In vivo binding pattern of a trans-regulator of homeotic genes in Drosophila melanogaster. *Nature 337:* 468-471.