Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

E. B. Lewis and the Bithorax Complex: Part I

Ian Duncan*,[1] and Geoffrey Montgomery†,[2]

**Department of Biology, Washington University, St. Louis, Missouri 63130 and †Brooklyn, New York 11231*

IN 1995, Edward B. Lewis shared in the Nobel Prize in Physiology or Medicine for his work on the bithorax complex (BX-C), a gene cluster that controls the identities of body segments in the abdomen and posterior thorax of Drosophila. Over a period of some 50 years, Lewis defined the functions and genetic organization of the BX-C. Much of his work appeared for the first time in a review published in 1978 (Lewis 1978). This landmark article presented Lewis's vision of a gene cluster in which fundamental mysteries of development, evolution, and the organization of metazoan genes all converge. As a commentator on the article would later say, Lewis's work revealed "the most illuminating genetic system yet discovered in complex organisms" (Lawrence 1993). Lewis's analysis of the BX-C set the stage for molecular studies that led to the identification of the homeobox and, ultimately, to the realization that Hox gene clusters like the BX-C control the specialization of body regions in most, perhaps all, animal forms.

Surprisingly, Lewis's early work on the BX-C was not motivated by any desire to tackle the problem of how fertilized eggs shape themselves into animal bodies. This problem seemed to him intractable. Rather, Lewis was drawn to the bithorax genes because they appeared particularly promising as material for testing a model of how new genes evolve from old ones by duplication and functional divergence. Beginning in 1939 as a graduate student at Caltech, Lewis pioneered two fundamental genetic approaches that were of key importance throughout his career. These were fine-structure mapping and the "*cis-trans*" test. The major purpose of this article is to trace how Lewis's remarkable insights into the genetic logic underlying animal development grew out of the application of these approaches to the genes of the BX-C.

We first sketch the context in which Lewis developed fine-structure mapping and the *cis-trans* test and describe his early findings in the BX-C. We then focus on two long-standing issues that emerged from this analysis. First, did the bithorax series of mutations define sites in a single gene or instead a cluster of separate but functionally integrated genes? Second, how did Lewis come to picture the developmental control functions and the spatial regulation of these genetic elements? Finally, we briefly review how molecular studies of the BX-C have addressed the genetic and developmental conundrums with which Lewis grappled. For recent historical reviews by Lewis himself, see Lewis (1992, 1994, 1995).

FINE-STRUCTURE MAPPING AND THE *cis-trans* TEST

There were two major influences on Lewis's early work. The first was an old idea, that new genes arise by duplication of an ancestral gene, followed by divergence in function. Lewis was particularly influenced in this regard by Bridges's interpretation of certain salivary gland chromosome bands (Bridges 1935). In many cases, prominent bands that are adjacent appear to associate along their edges so as to produce a symmetric capsule. Bridges interpreted such capsules as tandem duplications and called them doublets, a term that has been retained despite a lack of molecular evidence that they represent repeats. The view at the time was that gene duplications were probably rather frequent occurrences. Metz (1937, 1947) described several polymorphisms in the fly *Sciara ocellaris* in which a single band is present at a locus in some strains, while a doublet is present in others. One possibility he considered was that the single-band types were ancestral and that the doublet forms arose by duplication.

The second major influence on Lewis's thinking was the discovery by Sturtevant of the position effect at *Bar* (Sturtevant 1925). As is well known, *Bar* is a tandem duplication for a few bands on the Drosophila X chromosome that causes loss of anterior eye tissue. Females

[1] *Corresponding author:* Department of Biology, Washington University, 1 Brookings Dr., St. Louis, MO 63130.
E-mail: duncan@biology.wustl.edu

[2] *Present address:* 217 Union St., Brooklyn, NY 11231.
E-mail: montgog@mail.rockefeller.edu

homozygous for *Bar* produce wild-type and more extreme *Bar* chromosomes at a frequency of about 1 in 1600. Sturtevant showed that these new types arise by unequal crossing over, with the wild-type derivative having one copy of the *Bar* region and the more extreme type having three copies. In the course of this work, Sturtevant found that females heterozygous for the triplication and a normal chromosome show greater reduction in eye size than females homozygous for the *Bar* duplication. This is despite the fact that both genotypes carry the same number of copies of the *Bar* region and of the duplication breakpoint, which actually causes the eye reduction. This "position effect" demonstrated that the functioning of genes can depend on their neighbors *in cis* and suggested that duplicate genes in particular may work better when located adjacent to one another than when separated. To account for such position effects, Sturtevant suggested that genes produce unstable products that diffuse away from the genes that produce them. As he explained in his textbook with Beadle (Sturtevant and Beadle 1939, p. 226), "If the two substances interact, the amount of their joint product will depend on the distance that lies between the two genes concerned. If the joint product influences the course of development, we have all that is needed to picture a possible mechanism for the position effect."

These two ideas, the evolution of new genes by gene duplication and the interaction of genes *in cis*, had far-reaching implications. They suggested that one could be badly misled by complementation data and that many instances described as multiple allelism could be cases in which more than one gene is present, but in which each gene requires the others to be adjacent for normal functioning. Much of Lewis's early work was devoted to identifying such cases.

Lewis began his undergraduate training at Bucknell College, where he was supported by a music scholarship (he played the flute in his high school orchestra and with the Wilkes-Barre Symphony). However, in 1937, after just one year, Lewis transferred to the University of Minnesota, largely because it had one of the lowest out-of-state tuition fees of any major state university. At Minnesota, Lewis worked in the laboratory of C. P. Oliver, who had been a student of H. J. Muller. Oliver appears to have had a major influence on Lewis's career.

At the time, Oliver had discovered that "reversions to wild-type" occur in females heterozygous for the *glossy* and *spectacle* alleles of *lozenge* and showed that these reversions occur in association with crossing over (Oliver 1940, 1941). We now know that these "reversions" were produced by crossing over between different alleles of a single gene, *lozenge*. However, at that time the dogma was that genes were indivisible by recombination and by chromosome rearrangement. Therefore, Oliver suggested two explanations for the reversions. First, he thought they could be unequal crossovers that duplicated the *lozenge* gene. A normal phenotype would result from cooperation between the alleles in the duplication. The second possibility considered was that the two mutations were in adjacent genes and that the wild-type reversions resulted from crossing over between them. According to this model, the two mutations failed to complement because the wild-type alleles of these genes must be *in cis* to function properly. Oliver summarized these models: "The condition can be a case of unequal crossing over; but it can as likely be a case which involves the repeat hypothesis developed by Bridges . . ." (Oliver 1940, p. 454). A critical test of these models would be to recover the complementary crossovers. In the first case, these would be deleted for *lozenge*, whereas in the second they would be the double mutant. Despite considerable effort, Oliver was unable to identify the complementary crossover type.

Oliver gave Lewis a desk in his laboratory and allowed him freedom to pursue his own project. Lewis decided to work on a new rough-eye mutant that had been found and sent to him by his friend Edward Novitski, then an undergraduate at Purdue University. Lewis and Novitski had gone to high school together in Wilkes-Barre, Pennsylvania, where they began working with flies after hours in the school biology laboratory. Both would become luminaries of Drosophila genetics. In Wilkes-Barre, Novitski had discovered a mutant he called *held-out*. At Purdue he set out to map *held-out* and entered into correspondence with Bridges, who sent him several marker stocks, including the second chromosome mutant *Star* (*S*). The new rough-eye mutant arose spontaneously in a bottle of *S* flies. In correspondence with Novitski, Bridges suggested that the rough-eye mutation be called *Star-recessive* (S^r), as it acted as an allele of *S*. Thus, $S/+$ flies have slightly smaller eyes that are slightly roughened, S^r/S^r flies have strongly reduced eyes that are very roughened, and S/S^r flies are nearly eyeless. To test allelism, Lewis crossed S/S^r females to S^r/S^r males and scored for wild-type crossover progeny. In an initial cross, which was scored in his parents' kitchen while Lewis was on break at home in Wilkes-Barre, 1 wild-type fly was recovered among 3235 progeny from this cross. Unfortunately, no outside markers were present to confirm that this was a crossover. When such markers were incorporated into subsequent crosses, no wild-type progeny were recovered among 6059 additional progeny. In his first article (Lewis 1939), published in the Proceedings of the Minnesota Academy of Science, Lewis concluded that, with the exception of one possible crossover, his data provided strong evidence that S^r is an allele of *S*.

Lewis graduated with a B.Sc. degree in biostatistics after only two years at the University of Minnesota. With Oliver's help, he was awarded a teaching fellowship at Caltech and began graduate work in August 1939. He became one of A. H. Sturtevant's students and decided to continue his work on *S* and S^r. When tests using

outside markers were made on a larger scale, Lewis was soon able to identify a number of wild-type crossovers between *S* and S^r. The exchange of outside markers indicated that S^r lies to the right of *S*. These experiments were very much facilitated by the use of inversions on other chromosomes to increase crossing over in 2L, where *S* is located. However, to Lewis the identification of these wild-type crossovers left the allelic relationship between *S* and S^r ambiguous. As discussed by Oliver for *lozenge*, reversions to wild type associated with crossing over could be due to unequal crossing over, crossing over between duplicate genes, or perhaps some event similar to mutation. The key to resolving these possibilities was to recover the complementary crossover.

Initially, Lewis assumed that *S* S^r/+ + flies would resemble *S* +/+ S^r flies and scored for progeny that were more extreme than *S*/+ in the cross of *S*/S^r females to +/+ males. However, no such progeny were identified. Lewis then adopted a remarkably inventive strategy for identifying the double mutant (Lewis 1942, 1945). He made use of a tandem duplication for the *S* region he had isolated as a revertant of S^r (Lewis 1941). As initially recovered, this duplication carried S^r in each element. By crossing over, Lewis introduced *S* into the left-hand element of the duplication. Using parentheses to indicate its two elements, this duplication can be designated (*S*) (S^r). Lewis then crossed females heterozygous for this duplication and S^r [*i.e.*, (*S*) (S^r)/S^r females] to +/+ males. The large majority of progeny were phenotypically wild type. However, a few were like *S*/+, being produced as crossovers that remove *S* from the duplication. Lewis then tested five of these *S* crossovers to determine whether they also carried S^r. For all five, Lewis was able to extract S^r by crossing over, confirming them as double mutants. The isolation of these double mutants showed unequivocally that crossing over can take place between *S* and S^r and ruled out unequal crossing over or other mutation-like events to explain the origin of wild-type crossovers between them. Since the dogma was that crossing over does not take place within genes, Lewis gave S^r a new name, *asteroid* (*ast*), to indicate that it is not allelic to *S*. Although Lewis's role as a pioneer of fine-structure mapping is not widely recognized, his recovery of reciprocal crossovers between *S* and *ast* provided the first demonstration of crossing over between mutations that otherwise behave as alleles.

Having the double mutant allowed Lewis to compare the *cis*- and *trans*-mutant types. A striking position effect was apparent: the *cis* form *S ast*/+ + shows a slight reduction and roughening of the eye indistinguishable from that seen in *S* +/+ +, whereas the *trans* form *S* +/+ *ast* is almost eyeless. Lewis's interpretation of this *cis-trans* position effect was that the *S* and *ast* genes arose by tandem duplication of an ancestral locus and that each requires the wild-type allele of the other to be *in cis* for normal functioning. As discussed above, this interpretation was based on the *Bar* position effect and on Bridges's idea that polytene chromosome doublet bands represent tandem duplications. Particularly persuasive to him was the fact that *S* and *ast* are located within the 21E1-2 polytene chromosome doublet (Figure 1), the very bands Bridges had singled out as a representative example of his duplication hypothesis (Bridges 1935).

The *cis-trans* position effect of *S* and *ast* was only the second such position effect to have been reported. The first was the *cis-trans* position effect of the *Bar* duplication (Sturtevant 1925). However, the position effect involving *S* and *ast* was very different from that found at *Bar* in that no chromosome rearrangement was present. Therefore, unlike the *Bar* position effect, the *cis-trans* position effect of *S* and *ast* was indicative of interactions occurring between genes or genetic elements that are normally adjacent. Thus, the *cis-trans* test as it is generally employed was invented by Lewis. This test became critically important in Lewis's later work on the BX-C.

Lewis coined the term "position pseudoalleles" to designate the relationship between *S* and *ast* (Lewis 1948, 1951). The term "pseudoallelism" was first used simply to indicate the failure of deficiencies to complement recessive alleles of more than one gene (Morgan *et al.* 1938). The term was later restricted by McClintock (1944) to designate cases of closely linked genes having similar effects. Lewis went a step further and used the term "position pseudoallelism" to designate a form of position effect in which "the activity of a gene is altered when its relation with respect to a specified allele in a neighboring gene is changed" (Lewis 1951). That is, he used position pseudoallelism to indicate cases in which closely linked genes of similar function show pronounced *cis-trans* position effects. With time, this definition came to apply generally to the term pseudoallelism.

In 1946, Lewis began a deliberate search for additional cases of position pseudoallelism. His motivation was to test the idea that a common way that new genes arise in evolution is by gene duplication. As he stated (Lewis 1951, p. 159), "Our underlying thesis will be that in those instances of pseudoallelism in which there is evidence for close functional similarity among the component genes we may come close to seeing the direct results of a process which produces new genes." A second case of pseudoallelism was soon identified. It involved the dominant mutation *Stubble* (*Sb*), which truncates the bristles when heterozygous with wild type, and a closely linked recessive mutation, originally called *Sb-recessive* (Sb^r), which has a similar phenotype when homozygous. Lewis showed that these mutations are separable by crossing over and renamed Sb^r as *stubbloid* (*sbd*; Lewis 1948, 1951). *Sb* and *sbd* show pronounced *cis-trans* position effects. Flies of genotype *Sb* sbd^2/+ + have normal bristles, whereas *Sb* +/+ sbd^2 have extremely short stubby bristles. Again, Lewis's interpretation was that *Sb* and *sbd* were separate genes that had

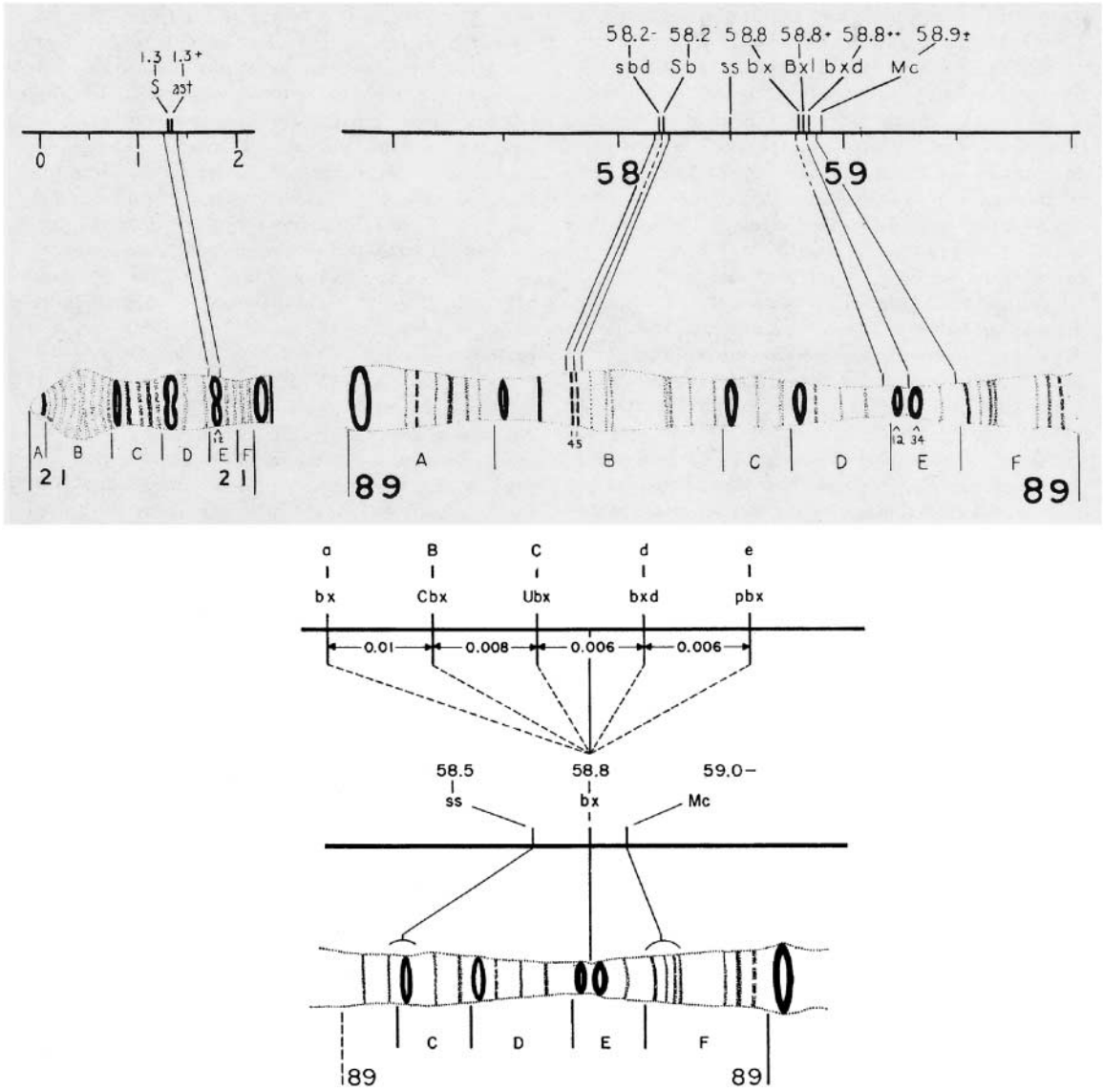

FIGURE 1.—(Top) Genetic and polytene maps showing the locations of *S/ast*, *Sb/sbd*, and the bithorax complex pseudoalleles *bx*, *Ubx* (*Bxl* in the figure), and *bxd* (from LEWIS 1951). (Bottom) Map of the bithorax complex including the loci of *Cbx* and *pbx* (from LEWIS 1963).

arisen from a common ancestor by gene duplication and that required one another to be adjacent for normal function. Although the cytological evidence was not as striking as for *S* and *ast*, the bands in which *Sb* and *sbd* were located could also reasonably be interpreted as a repeat (Figure 1).

To explain why S^+ and ast^+ or Sb^+ and sbd^+ must be *in cis* for normal function, Lewis suggested that these genes function by controlling sequential reactions at or very near to the site of the genes in the chromosome (LEWIS 1951). This model was derived from the idea of interacting immediate gene products proposed by Sturtevant to explain the *cis-trans* position effect at *Bar*. In the following diagram (taken from LEWIS 1951) the gene a^+ controls the conversion of some substrate S to A, and an adjacent gene b^+ controls the conversion of A to B:

$$\mathrm{S} \xrightarrow[a^+]{} \mathrm{A} \xrightarrow[b^+]{} \mathrm{B}$$

If it is assumed that the substances A and B do not readily diffuse from one chromosome to the next, then, as shown below, mutations in these genes would be expected to show pronounced *cis-trans* position effects:

$$\textit{cis}: \quad \begin{array}{llll} & \mathrm{S} & \xrightarrow[a]{+} & \mathrm{A} \xrightarrow[b]{+} \mathrm{B} \\ & \mathrm{S} & \xrightarrow[a]{} & {<}\mathrm{A} \xrightarrow[b]{} {<}\mathrm{B} \end{array}$$

$$\textit{trans}: \quad \begin{array}{llll} & \mathrm{S} & \xrightarrow[+]{a} & {<}\mathrm{A} \xrightarrow[b]{+} {<}\mathrm{B} \\ & \mathrm{S} & \xrightarrow[+]{} & \mathrm{A} \xrightarrow[b]{} {<}\mathrm{B} \end{array}$$

If the phenotype were to depend on the amount of

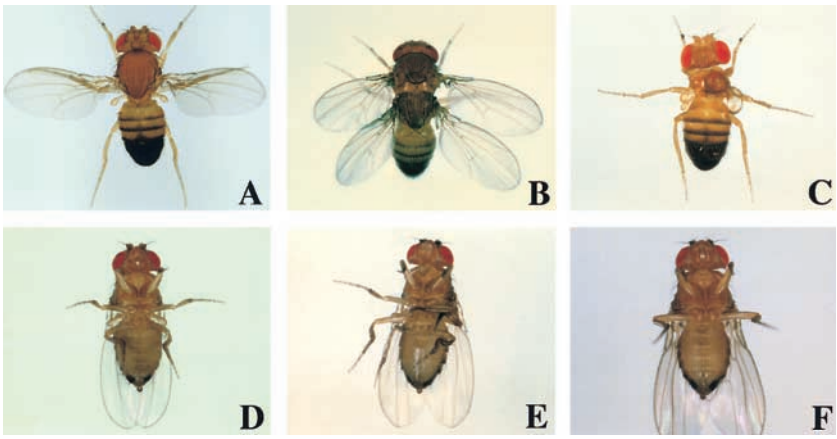

FIGURE 2.—Phenotypes of bithorax complex mutations. (A) Dorsal view of a wild-type male. The T2 segment produces the single pair of wings as well as almost all of the dorsal thorax. Dorsally, T3 produces only the halteres, small club-shaped organs located posterior to the wings. (B) The famous four-winged fly, in which T3 is transformed to T2. This male is hemizygous for the triple-mutant combination *abx bx*^{3} *pbx*. The *abx* pseudoallele has effects similar to *bx* mutations, but causes a stronger transformation of the very anterior portion of T3. (C) A *Cbx* male showing transformation of T2 toward T3. Generally, *Cbx* transforms only the posterior portion of T2, as seen on the right side of this fly. Occasionally, all of T2 is affected, as occurs on the left side. (D) Ventral view of a wild-type female. Each of the thoracic segments produces a pair of legs. (E) Ventral view of a *bxd* hemizygous female. An extra pair of legs is present as a result of the transformation of A1 toward T3. (F) Ventral view of an *Hab* female. The third pair of legs is lost because T3 is transformed toward A2. (B–F courtesy of E. B. Lewis.)

substance B, then it can be seen that the *trans*-type will show a mutant phenotype, whereas the *cis*-type will produce half the normal amount of B and appear as wild type if dominance is complete. Lewis described an appealing mechanism by which such interacting genes could evolve (LEWIS 1951). By analogy with enzymatic reactions, he assumed that gene-controlled reactions were reversible. Therefore, each gene would have an affinity for both the substrate and product of the reaction it controls. If a new duplicate gene were to mutate so as to change the reaction it controls, but retain affinity for the product of the old gene, then a new step would be added to the sequential reaction series. This suggested to Lewis that cases of pseudoallelism would almost of necessity involve evolutionarily related genes. Of course, these models were developed before there was any understanding of the molecular mechanisms of gene expression. Nevertheless, they were very influential in guiding Lewis's subsequent work on the BX-C.

EARLY STUDIES OF THE BX-C

Lewis began working with mutations of the bithorax region because the diversity of existing allelic types and the complementation behavior of these alleles strongly suggested that they might define a new pseudoallelic series. The prototype mutant of the region, *bithorax* (*bx*), was discovered by Bridges in 1915 and was the first homeotic mutation identified. Homozygotes show a transformation of the metathorax (T3) toward mesothorax (T2). This was initially described by Bridges as involving all of T3, including a transformation of the balancer organs (halteres; see Figure 2) toward wings. The *bithoraxoid* (*bxd*) mutation was discovered in 1919, also by Bridges. The phenotype was described as being similar to that of *bx*, but weaker and with inflated rather than flat and elongated halteres. Because of the similar phenotypes and map locations of *bx* and *bxd*, BRIDGES and MORGAN (1923) were very surprised to find that the two mutations complement. They wrote (p. 226): "This is the most striking case that we have met of the danger of judging allelomorphism from somatic characters or from linkage relations or from both." A third type of mutation was discovered in 1934 by W. F. Hollander. This mutation had been given several names, including *bx*^{D}, *bxd*^{D}, and *Bxl*, but was renamed *Ultrabithorax* (*Ubx*) by Lewis in the early 1950s. *Ubx*/+ animals show slightly swollen halteres, which result from a weak transformation toward wing, whereas *Ubx* homozygotes die as larvae. Bridges made the important observation that the *Ubx* mutation fails to complement both *bx* and *bxd* mutations. That is, *Ubx*/*bx* animals resemble *bx* homozygotes, and *Ubx*/*bxd* animals resemble *bxd* homozygotes. These observations suggested to Lewis that the *bx*, *Ubx*, and *bxd* mutations were likely to define another pseudoallelic gene cluster.

The results of fine-structure mapping confirmed this suspicion; *bx* was found to lie 0.02 units to the left of *Ubx* and *bxd* about 0.01 units to the right of *Ubx*. These three loci are depicted in Lewis's first published map of the bithorax region (Figure 1), presented at the 1951 Cold Spring Harbor Symposium (LEWIS 1951). Consistent with the idea that pseudoalleles arise by duplication, Lewis found that the bithorax mutations lie within two polytene chromosome doublets, which are located in 89E. To Lewis, the bithorax pseudoalleles presented a key advantage over *S*/*ast* and *Sb*/*sbd* for studying gene evolution. As he noted in his 1951 paper, the developmental activities of *S* and *ast* or *Sb* and *sbd* could not be differentiated because in both cases the phenotypes of the component pseudoalleles are very similar. Therefore, it seemed difficult if not impossible to determine how these pseudoalleles may have diverged in function after their postulated duplication. In the bithorax series, the situation was very different.

Lewis made the key observation that the *bx* and *bxd* mutations cause transformations of mutually exclusive portions of the body (LEWIS 1949). He discovered that

bx mutations, now represented also by the extreme allele bx^{3} of Stern and the weaker allele bx^{34e} of Schulz, transform only the anterior portion of T3. This is most dramatically illustrated by the haltere, but holds for all of the T3 segment. In bx^{3} homozygotes, the anterior half of the haltere is transformed to an almost full anterior half wing, whereas the posterior half of the haltere is unaffected. The *bxd* mutation, on the other hand, has no effect on anterior T3, but transforms the posterior part of this segment to posterior T2. Lewis found that *bxd* also causes a transformation of the first abdominal segment (A1) toward a thoracic state, an effect that Bridges did not describe. The A1 tergite is invariably lost in *bxd* mutants, and legs and wing-like halteres occasionally develop in A1 (see Figure 2). The view presented in the 1951 paper was that bx^{+} and bxd^{+} had arisen by gene duplication followed by divergence. The products of these genes were seen to promote anterior T3 and A1 levels of development, respectively, from a T2 level that was viewed as both a developmental and evolutionary "ground state." The view that fundamental changes in body segment morphology resulted from the evolution of new BX-C genes is woven through almost all of Lewis's subsequent work and is summarized in the first paragraph of his 1978 review:

> Flies almost certainly evolved from insects with four wings instead of two and insects are believed to have come from arthropod forms with many legs instead of six. During the evolution of the fly, two major groups of genes must have evolved: "leg-suppressing" genes which removed legs from abdominal segments of millipede-like ancestors followed by "haltere-promoting" genes which suppressed the second pair of wings of four-winged ancestors. If evolution indeed proceeded in this way, then mutations in the latter group of genes should produce four-winged flies and mutations in the former group, flies with extra legs. In Drosophila, not only have both types of mutation been observed, they have been shown to involve a single cluster of pseudoallelic genes known as the bithorax complex (BX-C). During evolution a tandem array of redundant genes presumably diversified by mutation to produce this complex (Lewis 1978, p. 565).

When Lewis applied his *cis-trans* test to the newly resolved *bx*, *Ubx*, and *bxd* mutations, he found pronounced *cis-trans* position effects between *bx* and *Ubx* and between *Ubx* and *bxd* (Lewis 1951). Thus, the *trans*-type *bx* +/+ *Ubx* shows a strong transformation of anterior T3 to anterior T2, whereas the *cis*-type *bx Ubx*/+ + shows only a swollen haltere phenotype identical to that seen in *Ubx*/+ heterozygotes. Similarly, the *trans*-type *Ubx* +/+ *bxd* shows a strong transformation of posterior T3 and A1, whereas the *cis*-type *Ubx bxd*/+ + shows only the dominant effect of *Ubx*. Lewis interpreted these *cis-trans* effects, just as he had the *S/ast* and *Sb/sbd* position effects, as manifestations of sequentially interacting immediate gene products. In 1951, he proposed that the three wild-type genes of the bithorax series control successive reaction steps as indicated,

$$\text{S} \xrightarrow{bx^{+}} \text{A} \xrightarrow{Ubx^{+}} \text{B} \xrightarrow{bxd^{+}} \text{C}$$

where substance C is responsible for promoting the A1 level of development, and substances A or B promote the T3 level. (Lewis was unable to clearly distinguish the effects of *bx* and *Ubx* and was uncertain of the order of the first two steps.) This scheme readily explained the *cis-trans* position effect of *bx* and *Ubx* and of *Ubx* and *bxd*. However, the lack of a position effect for *bx* and *bxd* was a problem; since both the *bx* and *bxd* mutations should cause a reduction in C, one would expect the *trans*-type to show some effect of the *bxd* type. Although not seen for the original *bxd* mutation, such an effect was found for more extreme *bxd* alleles Lewis isolated after X irradiation. Most of these extreme alleles were chromosome rearrangements broken within the bithorax doublets. These rearrangements behave as if they are bx^{+} and Ubx^{+}, but are severely defective for *bxd*. When heterozygous with bx^{3}, they show a weak transformation of posterior haltere to wing, consistent with a weak reduction in levels of C by bx^{3}.

The *bxd* rearrangement alleles provided additional strong support for the sequential reaction model. Lewis interpreted these rearrangements as having breakpoints between Ubx^{+} and bxd^{+}. According to the model, such rearrangements would be expected to have full bx^{+} and Ubx^{+} function, as they do. However, bxd^{+} function should be severely impaired or absent in such rearrangements because the newly located bxd^{+} gene would not be exposed to the product (B) of the *Ubx* gene and could not, therefore, produce any product C.

Lewis's 1951 article is remarkable in foreshadowing several future developments. A striking example is his suggestion, based largely on the phenotype of the *bxd* mutant, that "The posterior portions of the mesothoracic segments and metathoracic segments . . . appear, in the presence of the mutants, to behave as though they were embryologically related to the anterior portion of the segment that follows, rather than precedes, them . . ." (Lewis 1951, p. 167). This presaged our current understanding that parasegments, units composed of the posterior of one segment and the anterior of the adjacent segment, are the fundamental units of insect segmentation (Martinez-Arias and Lawrence 1985). A second example is Lewis's report that homozygotes for *Ubx* mutations or the *bx bxd* double mutant show a T2-like modification of T3 and A1 in the cuticle of the third instar larva. This is one of the earliest cases in which the larval cuticle was utilized for scoring mutant phenotypes. Many years later, the larval cuticle became a central focus for work in fly development. Indeed, Nüsslein-Volhard and Wieschaus shared the Nobel Prize with Lewis for their large-scale screens for mutations affecting the cuticular pattern of the first instar larva. A third example is Lewis's introduction of the term "level of development," which he first used in

connection with his study of the *bx bxd* double mutant. Lewis was struck by the fact that *bx bxd* homozygotes show the effects of the *bx* mutation not only in anterior T3, but also in anterior A1; the latter region is transformed to anterior T3 in the *bxd* mutant and to anterior T2 in the double mutant. This observation demonstrates that the functioning of *bx* is not restricted to T3. Rather, it suggested that bx^+ is better understood as promoting a change from a T2 level of development to a T3 level regardless of anatomical location. The term *level of development* later became widely used, and its introduction in the 1951 article indicates that almost from the outset Lewis understood the abstract nature of the control of segmental identities by homeotic genes.

At the IX International Congress on Genetics at Bellagio in 1953, Lewis reported the discovery of two more loci in the bithorax series (Lewis 1954). This discovery "came about in a curious way." In August 1949, Lewis's technician, Lyle Bacon, discovered a small-wing mutant while screening for X-ray-induced changes of *Sb*. Close examination revealed that the wing reduction in this mutant resulted from a transformation to haltere, and initial mapping suggested that the mutation was located within the BX-C. Strangely, fine-structure mapping revealed that the mutation was associated with two BX-C mutations that had been induced simultaneously. One was responsible for the dominant effects of the new mutation and occupied a locus between *bx* and *Ubx*. The other was recessive and occupied a locus just to the right of *bxd*. Remarkably, these two mutations cause inverse transformations. The recessive mutation, which is called *postbithorax* (*pbx*), causes a very strong transformation of posterior T3 to posterior T2. Conversely, the dominant mutation, named *Contrabithorax* (*Cbx*), transforms posterior T2 to posterior T3; occasionally the transformation extends to anterior T2. Significantly, *pbx* is almost entirely suppressed by *Cbx*; that is, while + *pbx*/+ *pbx* animals show an essentially complete transformation of posterior T3, *Cbx pbx*/+ *pbx* animals have a nearly normal T3 segment.

After the discovery of *Cbx* and *pbx*, Lewis quickly extended his *cis-trans* tests to include all pairwise combinations of these mutations with *bx*, *Ubx*, and *bxd* (Lewis 1954, 1955). From these tests, Lewis found that *pbx* fits quite well into the sequential reaction model that he had devised to account for *cis-trans* comparisons of the three previously identified BX-C loci. Strong position effects were found for *Ubx* and *pbx* and for *bxd* and *pbx*. For these, the *trans*-type showed a strong transformation of posterior T3 to posterior T2, whereas the *cis*-types were normal (for *bxd pbx*/+ +) or showed only the weak dominance of *Ubx* (for *Ubx pbx*/+ +).

The discovery of *pbx* led Lewis to add a step to his sequential reaction model (Lewis 1955):

$$S \xrightarrow{bx^+} A \xrightarrow{Ubx^+} B \xrightarrow{bxd^+} C \xrightarrow{pbx^+} D$$

In this scheme, substances A or B promote a T3, as opposed to T2, level of development in anterior T3; substance D has a similar function in posterior T3; and substance C functions to promote the A1 level of development. To explain why the BX-C genes are active in T3 and A1, but inactive in T2, Lewis invoked an anterior-posterior gradient of the substrate S in the early embryo (Lewis 1955, 1964). He suggested that in T2 the levels of S were too low to produce sufficient amounts of substances A–D, whereas in T3 and A1 increasing amounts of these substances were produced. To explain the difference between T3 and A1, Lewis assumed that only in A1 were sufficient levels of C produced to have an effect.

The wild-type *Cbx* locus could not be fitted easily into Lewis's sequential reaction models. However, *cis-trans* comparisons revealed a moderately strong position effect between *bx* and *Cbx* and a strong position effect between *Cbx* and *Ubx*; for these the *cis*-types (*bx Cbx*/+ + and *Cbx Ubx*/+ +) showed transformations of posterior T2 much weaker than those of the corresponding *trans*-types. Thus, Lewis suggested that the *Cbx* mutation might act by causing the excess accumulation of bx^+ or Ubx^+ products (substances A or B).

No *cis-trans* position effect was found for *bx* and *pbx*, the *bx pbx*/+ + and *bx* +/+ *pbx* genotypes both being wild type. However, when the bx^3 *pbx* double mutant was made homozygous, the *bx* and *pbx* transformations fitted together like two pieces of a jigsaw puzzle and caused an almost complete transformation of T3 to T2. The result was Lewis's four-winged fly (Figure 2), perhaps the most famous mutant in modern genetics. The first published photograph of this phenotype appeared in an article by Lewis in the November 1957 issue of Engineering & Science, an alumni publication of the California Institute of Technology (Lewis 1957). The New York Times picked up on the story, and in their November 27, 1957, issue published the same picture in an article headed "4-Wing Flies Bred in Study of Genes; Throwback of Million Years May Explain Mechanics of Human Heredity" (Plumb 1957).

The second part of this article will appear in the May issue of Genetics.

We are greatly indebted to Ed Lewis for graciously hosting us during a week-long visit to his laboratory in July 2001, and to the Caltech Division of Biology for supporting this visit. We also thank Ed Lewis for permission to reproduce photographs, Dianne Duncan for preparation of the figures, and Jennifer Brisson, Susan Celniker, Dianne Duncan, Peter Lawrence, Howard Lipschitz, William McGinnis, and Joanne Topol for critical reading of the manuscript.

LITERATURE CITED

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., and T. H. Morgan, 1923 *The Third-Chromosome Group*

of Mutant Characters of Drosophila melanogaster. The Carnegie Institution, Washington, DC.

Lawrence, P. A., 1993 Outstanding paper in biology: selected and introducted by Peter A. Lawrence, p. 93 in *Outstanding Papers in Biology*, edited by R. Palmer. Current Biology, London.

Lewis, E. B., 1939 *Star-recessive*, a spontaneous mutation in *Drosophila melanogaster*. Proc. Minn. Acad. Sci. **7:** 23–26.

Lewis, E. B., 1941 Another case of unequal crossing-over in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **27:** 31–34.

Lewis, E. B., 1942 The *Star* and *asteroid* loci in *Drosophila melanogaster*. Genetics **27:** 153–154.

Lewis, E. B., 1945 The relation of repeats to position effect in *Drosophila melanogaster*. Genetics **30:** 137–166.

Lewis, E. B., 1948 Pseudoallelism in *Drosophila melanogaster*. Genetics **33:** 113.

Lewis, E. B., 1949 A study of adjacent genes. Heredity **3:** 130.

Lewis, E. B., 1951 Pseudoallelism and gene evolution. Cold Spring Harbor Symp. Quant. Biol. **16:** 159–174.

Lewis, E. B., 1954 Pseudoallelism and the gene concept. Proc. IX Int. Congr. Genet., Caryol. **Suppl:** 100–105.

Lewis, E. B., 1955 Some aspects of position pseudoallelism. Am. Nat. **89:** 73–89.

Lewis, E. B., 1957 Two wings or four?, pp. 19–21, in *Engineering and Science*, Vol. 21, No. 2. Caltech, Pasadena, CA.

Lewis, E. B., 1963 Genes and developmental pathways. Am. Zool. **3:** 33–56.

Lewis, E. B., 1964 Genetic control and regulation of developmental pathways, pp. 231–252 in *Role of Chromosomes in Development*, edited by M. Locke. Academic Press, New York.

Lewis, E. B., 1978 A gene complex controlling segmentation in *Drosophila*. Nature **276:** 565–570.

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., 1995 The bithorax complex: the first fifty years, pp. 232–260 in *Les Prix Nobel*, edited by T. Frängsmyr. Almqvist & Wiksell International, Stockholm.

Martinez-Arias, A., and P. A. Lawrence, 1985 Parasegments and compartments in the *Drosophila* embryo. Nature **313:** 639–642.

McClintock, B., 1944 The relation of homozygous deficiencies to mutations and allelic series in maize. Genetics **29:** 478–502.

Metz, C. W., 1937 Small deficiencies and the problem of genetic units in the giant chromosomes. Genetics **22:** 543–556.

Metz, C. W., 1947 Duplication of chromosome parts as a factor in evolution. Am. Nat. **81:** 81–103.

Morgan, T. H., C. B. Bridges and J. Schultz, 1938 Constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Year Book **37:** 304–309.

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

Oliver, C. P., 1941 Crossing over between two alleles of lozenge in Drosophila melanogaster. Genetics **26:** 163.

Plumb, R. K., 1957 4-wing flies bred in study of genes, p. 33 in the November 27, 1957, issue of the New York Times.

Sturtevant, A. H., 1925 The effects of unequal crossing over at the *Bar* locus in *Drosophila*. Genetics **10:** 117–147.

Sturtevant, A. H., and G. W. Beadle, 1939 *An Introduction to Genetics*. Saunders, Philadelphia (reprinted in 1962 by Dover, New York).