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 II. From *cis-trans* Test to the Genetic Control of Development

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As discussed in Part I of this *Perspectives* (see the April issue of Genetics), E. B. Lewis's early studies of the BX-C revealed a series of five bithorax complex (BX-C) "pseudoalleles" separable by recombination and by function, with the map order: *bx Cbx Ubx bxd pbx*. Lewis found pronounced *cis-trans* position effects among these pseudoalleles. He interpreted these position effects in terms of a model in which the BX-C pseudoalleles define separate genes and in which these genes function to control sequential biochemical reactions occurring along the chromosome.

THE BX-C AND THE GENE CONCEPT

"The original problem of defining the unit of heredity, which almost 50 years ago was designated 'the gene,' has not yet been solved," wrote M. Demerec in his introduction to the 1951 Cold Spring Harbor symposium volume containing Lewis's first comprehensive description of the BX-C system (Demerec 1951). Only 2 years later, Watson and Crick's model for the double-helical structure of DNA initiated a revolution in the gene concept. With this molecular model in mind, Benzer embarked on his fine structure analysis of the *rII* region of phage T4. This work demonstrated that recombination occurs within genes and led to the conclusion: "The classical 'gene,' which served at once as the smallest element of genetic recombination, of mutation, and of function, is no longer adequate. These units require separate definition" (Benzer 1957, p. 70). The first two units Benzer called the "recon" and "muton"; each of these could be resolved to one or a few DNA nucleotides. The unit of function was much larger and "can be defined genetically, independent of biochemical information, by means of the elegant *cis-trans* comparison devised by Lewis." Benzer called this unit the "cistron"; molecular biologists came to view the cistron as the nucleotide sequence encoding a single polypeptide.

Thus, Lewis's interpretation of the *cis-trans* test was turned on its head. In the new model, the BX-C mutations no longer necessarily defined separate but functionally interacting genes. Molecular biologists began to tell Lewis that "I was simply dealing with missense and nonsense mutants *within* a protein and that all we were doing was mapping sites within a single protein coding unit!" (quoted in Lawrence 1992). A sense of the difficulties Lewis encountered in this new scientific climate can be gauged from a 1963 letter by Max Delbrück to a former member of Lewis's laboratory (Rhoda Grell). Delbrück wrote: "I then plunged into the bithorax saga for which Lewis very kindly sent me his latest manuscript. . . . I must say I am puzzled by the apparent agreement in his analysis between the two ways of subdividing this gene complex, to wit by recombination on the one hand and by function on the other hand. I strongly suspect that there is something wrong here in the analysis."

In fact, there was nothing wrong with Lewis's analysis. What was lacking was an understanding of the complex, modular organization of *cis*-regulatory and coding regions making up many metazoan genes. The genetic interactions that struck some of Lewis's contemporaries as bizarre oddities can now be seen as manifestations of the elaborate, long-range, *cis*-regulatory mechanisms governing animal development. This realization would have to wait until the 1980s. In the meantime, there were compelling reasons for Lewis to reject the single-protein-coding models presented to him by molecular biologists. One was the recovery of rearrangements broken between *Ubx* and *bxd* (see above), which had full function of bx^+ and Ubx^+, but appeared almost completely defective for *bxd* and *pbx*. Such rearrangements were readily understood in terms of the Lewis model, but were difficult to rationalize in terms of the single-

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gene model. A second problem was the discovery of pairing-dependent complementation.

A key feature of the sequential reaction model was that the reaction products generated by the BX-C pseudoalleles are produced at or very close to the gene loci involved. This localized production was the basis for Lewis's explanation of the *cis-trans* position effects seen: the product of one gene was freely available to an adjacent gene *in cis*, but not to the same gene *in trans*. Since homologous chromosomes are intimately paired in somatic cells in the Diptera, it seemed likely to Lewis that some leakage of products, or "cross-feeding," could occur between homologs. Such cross-feeding would be expected to result in weak pairing-dependent complementation between pseudoallelic mutations.

To test this prediction, Lewis investigated the effects of heterozygosity for chromosome rearrangements that disrupt pairing in the vicinity of the bithorax series. The first case of pairing dependence found involved *Ubx* and the weak *bx* allele bx^{34e} (E. B. Lewis 1954b). Remarkably, Lewis found that the degree of dorsal transformation of T3 in the + *Ubx*/bx^{34e} + heterozygote is very sensitive to pairing. By selecting for enhancement of this transformation after X irradiation, Lewis recovered a large number of chromosome rearrangements that disrupt pairing of the bithorax region when heterozygous. All of these rearrangements had at least one breakpoint between the centromere and the bithorax genes (located in the middle of the 3R chromosome arm), suggesting that somatic pairing is initiated at the centromere and propagated more distally.

Lewis gave the name "transvection" to this pairing-dependent complementation. This name conveyed well his idea that its basis was the leakage of immediate gene products *in trans* from one homolog to the other. At the same time, he coined the term "cisvection," which he pictured as resulting from the movement of gene products *in cis* from one gene to the next. In practice, transvection is a position effect revealed by disrupting chromosome pairing, whereas cisvection is a position effect revealed by *cis-trans* tests. Subsequently, Lewis found transvection in three additional heterozygotes: bx^{3} +/+ *pbx*, bx^{3} +/+ *Ubx*, and the *cis*-arrangement *Cbx Ubx*/+ +. The latter genotype shows a weak transformation of wing to haltere when pairing is allowed, but complete suppression of this transformation when pairing is disrupted.

In one of the first practical uses of Drosophila genetics, Lewis used the transvection effect to measure fast neutron levels released during atomic bomb tests. Males homozygous for bx^{34e} were placed at different distances from detonations, and the transvection method was used to measure chromosome rearrangement frequencies in their offspring. By comparing the results to rearrangement frequencies induced by known doses of fast neutrons, Lewis was able to derive good estimates of the doses received. Lewis had planned to conduct these experiments himself, but was denied security clearance, perhaps because he had been seen in the company of a left-leaning scientist who was under FBI surveillance. Fortunately, Lewis was able to enlist the assistance of a geneticist with government connections, George Beadle, who is acknowledged at the end of the paper for transporting "the flies to and from one of the nuclear detonation sites."

Lewis's discovery of transvection is a perfect example of the hypothetico-deductive method. From his sequential reaction model, Lewis predicted an unprecedented phenomenon: pairing-dependent complementation. It must be stressed that this really was a prediction and not a prior observation that went into formulation of the model. That this prediction was fully confirmed argued strongly against the "single-protein" model and must have given Lewis great confidence that he was on the right track with the sequential reaction model. As he wrote in his first report of transvection (E. B. Lewis 1954a), "These rearrangement studies are at variance with the speculation that the whole region occupied by a pseudoallelic series acts as a functional unit; instead, they demand a particulate interpretation in which genes at individual loci of the pseudoallelic series are distinguishable from one another not only by crossing over but by function, as well."

Subsequent observations presented additional, seemingly insurmountable, problems for the single-gene model. A particularly important experiment involved *Tp*(*3;3*) bxd^{110}, a transposition in which a region of some 12 polytene bands (from 91EF to 92A) is inserted between *Ubx* and *bxd*. Like other *bxd* rearrangements, this insertion causes an essentially complete inactivation of bxd^{+} and pbx^{+}, but retains bx^{+} and Ubx^{+} function. The inserted region in bxd^{110} contains the wild-type allele of the gene *Delta*. By irradiating bxd^{110} and selecting for new *Delta* mutations, a number of deletions of the inserted material were recovered (E. B. Lewis and N. A. Shaw, unpublished results cited in Judd 1976). One of these deletions was restricted to the inserted region and removed all but one or two bands of the insertion. Subsequent tests showed that this derivative had partially regained the function of bxd^{+} and pbx^{+}, even though Ubx^{+} and bxd^{+} remained noncontiguous. This result seemed to rule out the possibility that the mutations of the bithorax series defined sites within a single gene. Lewis's finding that pbx^{+} retains weak, pairing-dependent activity in a second *bxd* rearrangement, *Tp*(*3;3*) bxd^{100} (E. B. Lewis, unpublished results cited in Duncan 1987), was also very difficult to reconcile with the single-gene model.

Nevertheless, during the 1950s and early 1960s the idea of position pseudoalleles fell out of favor. This was due largely to Benzer's work and to the demonstration by Chovnick and his co-workers of crossing over within the *rosy* gene of Drosophila. Further, the details of gene function that emerged at this time were not easily recon-

ciled with Lewis's sequential reaction model. In particular, the finding that protein synthesis occurs in the cytoplasm and not in the nucleus seemed incompatible with the strictly local gene-controlled reactions he postulated.

In 1961, Jacob and Monod published their operon model for the coordinate regulation of the *lac* genes of *E. coli* (Jacob and Monod 1961). The similarities between the *lac* operon and the bithorax pseudoalleles were striking. Both appeared to be sets of closely linked and functionally integrated genes, and within each cluster similar polarized *cis-trans* position effects were present. Within the bithorax series, polarity was from left to right, with *Ubx* mutations showing polar *cis*-inactivation of bxd^+ and pbx^+ and *bxd* mutations showing polar *cis*-inactivation of pbx^+. Correspondingly, in the *lac* operon, some mutations in *lacZ* cause polar *cis*-inactivation of the *lacY* and *lacA* genes, and some alleles of *lacY* cause *cis*-inactivation of *lacA*. A particularly striking feature of the *lac* operon was the *operator-constitutive* (o^c) class of mutation. The *Cbx* mutation of the bithorax series had very similar properties; just as the o^c mutations must be *in cis* to $lacZ^+$ to show constitutive β-galactosidase expression, so Lewis had shown that *Cbx* must be *in cis* to Ubx^+ to cause the transformation of T2 to T3.

Because of these similarities, Lewis adopted the operon as an alternate framework for interpreting the bithorax series. In his first paper after the appearance of the operon model, Lewis presented a model in which Ubx^+, bxd^+, and pbx^+ encode different products that are coordinately regulated by the Cbx^+ operator element (E. B. Lewis 1963). Lewis postulated that Ubx^+ (and possibly bx^+) encodes a product [S1] that promotes an anterior T3 level of development from a basal anterior T2 level, pbx^+ encodes a substance [S2] that promotes a posterior T3 level, and bxd^+ encodes a substance [S3] that promotes an A1 level of development. To explain how coordinate regulation of these three products specifies the identities of T3 and A1, Lewis suggested that the operator is controlled by a posterior-anterior gradient of inducer, so that all three genes would be repressed in T2, but active in increasing levels in T3 and A1. To explain the difference between T3 and A1, Lewis assumed that sufficient quantities of S3 would not be made until A1 is reached: "Once having been made, it is necessary to suppose that S3 takes precedence over S1 and S2." The operon model accounted quite well for the *cis-trans* position effects seen in the bithorax series and had the major advantage of providing an interpretation that was consistent with protein synthesis occurring in the cytoplasm. The operon model allowed Lewis to retain a scheme in which the pseudoallelic loci were separate genes responsible for the synthesis of different substances: "These substances might be messenger RNA molecules, polypeptides, or products of the enzyme activity of such polypeptides" (E. B. Lewis 1964). Indeed, the close formal similarity between the bithorax pseudoalleles and the *lac* genes served strongly to reinforce this view. The only serious deficiencies of the operon model were that it provided no explanation for the weak functioning of *bxd* and *pbx* when separated from *Ubx* or for the transvection effect.

THE BX-C AND THE GENETIC CONTROL OF DEVELOPMENT

Lewis's 1963 article is the first in which it becomes clear that Lewis's focus is shifting away from the questions of gene evolution that originally motivated him and toward exploring the role of the BX-C in development. Consistent with this new focus, Lewis describes in this article the first studies of flies mosaic for the bithorax pseudoalleles. In mosaics generated by loss of a ring chromosome during cleavage, bx^+, Ubx^+, and pbx^+ were all found to function cell autonomously. In a second report (E. B. Lewis 1964), Lewis explored the temporal requirements for bx^+. The phenotypes of homozygous mutant mitotic recombination clones produced by X irradiation at different times revealed that bx^+ is required continuously and autonomously until late in development.

More importantly, however, it is in this period of the early 1960s that Lewis developed or reinforced three key concepts that were extraordinarily influential in setting the basic framework for our current understanding of the genetic control of development. These concepts were: (1) the BX-C genes are expressed locally within certain Drosophila body regions and are both necessary and sufficient for specifying their morphological identities; (2) the spatial expression of the BX-C genes is controlled by *cis*-regulatory elements that are defined by a specific class of mutations; and, more speculatively, (3) these *cis*-regulatory elements could be pictured as responding to an anterior-posterior gradient of a morphogen present early in development.

The *Cbx* mutation was pivotal in the formulation of these ideas. This mutation, wrote E. B. Lewis (1964, p. 249), "represents a dominant gain of function in the sense that it acts as if it produces an excessive amount of the bithorax substances in [T2]".[3] The suppression of the *Cbx* dominant phenotype by *Ubx* "loss of function" mutations *in cis* indicated that "an excessive amount of S1 [the hypothetical Ubx^+ substance] only is all that is needed to bring about the . . . metathoracic-like modification of the mesothorax" (Lewis 1964, p. 250). Thus, Cbx^+ came to be viewed as a *cis*-regulatory element that functions to keep Ubx^+ repressed in segments anterior

[3] Lewis appears to have been the first to use the terms "dominant gain of function" and "recessive loss of function" to describe allelic types. Although Jacob and Monod (1961) used the terms "recessive gain of function" and "dominant loss of function" to refer to the repressor mutants i^- and i^s, respectively, this usage differed fundamentally from that of Lewis in denoting the effects of these mutations on the operon structural genes rather than on the repressor gene itself.

to T3. Critically, the observation that in the *Cbx* mutant ectopic activation of Ubx^+ in T2 causes a transformation to T3 argued that Ubx^+ is not only necessary, but also sufficient for specifying T3 identity. In considering how the Cbx^+ regulatory element is controlled in normal development, Lewis invoked an anteroposterior gradient of some molecule (in modern terms, a morphogen), which, like the inducer of the *lac* operon, binds to and inactivates a repressor protein.

At the XII International Congress of Genetics in Tokyo in 1968, Lewis reported the first evidence that the bithorax series extends beyond the region from bx^+ to pbx^+ (E. B. Lewis 1968). This was the discovery of a dominant gain-of-function mutation that causes T3 to develop as an abdominal segment. This transformation, which is weak and variable, was initially interpreted as a transformation to A1. Since this is opposite to the transformation caused by *bxd*, the mutant was first named *Contrabithoraxoid* (*Cbxd*). Lewis mapped *Cbxd* close to the right of *pbx*. The presumption was that *Cbxd*, like *Cbx*, was a *cis*-regulatory mutation that causes ectopic expression of one of the bithorax pseudoalleles in T3. However, when Lewis made double mutants of *Cbxd* with each of the mutants bx^3, *Ubx*, *bxd*, and *pbx*, the results were surprising. None of these mutations suppressed the transformation of T3 when *in cis* to *Cbxd*. Subsequently, using a special stock to enhance the phenotype of *Cbxd*, Lewis showed that in fact *Cbxd* causes a transformation of T3 and A1 to A2 (see Figure 2 in Part I, Duncan and Montgomery 2002). He renamed the mutant *Hyperabdominal* (*Hab*) and inferred that it causes ectopic expression of a new gene in the bithorax series that normally functions to define the identity of A2. This gene he named *infra-abdominal-2* (*iab-2*).

At the same time, Lewis reported the discovery of the first candidate *trans*-regulatory mutation of the BX-C, called *Regulator-of-postbithorax* (*Rg-pbx*). *Rg-pbx* is a dominant mutation that causes a *pbx*-like transformation of incomplete penetrance and expressivity. Dosage studies indicated that *Rg-pbx* behaves like the "super-repressor" mutants (i^s) found in the *lac* operon. In flies with only one dose of pbx^+, penetrance of the *Rg-pbx* transformation increases; conversely, in flies with extra doses of pbx^+, penetrance decreases, as if extra copies of pbx^+ "titrate" out the *Rg-pbx* super-repressor. Lewis concluded his 1968 report: "It is hoped that the analysis of such mutants will throw light on the mechanism by which during normal development the wild-type bithorax genes apparently are repressed in the mesothoracic regions and become selectively derepressed in the metathorax and abdominal regions."

Lewis would not publish another word on the BX-C for 10 years. Yet this was a decade of dramatic progress in his studies of the BX-C, documented in the annual reports of the Caltech Biology Division and communicated by Lewis in occasional seminars. By the mid 1970s it was clear to many Drosophilists that Lewis had a most remarkable story to tell. Peter Lawrence conveyed this sentiment to an editor of *Nature*, Miranda Robertson, who invited Lewis to submit a paper on his work. When Lewis telephoned Lawrence to ask what kind of paper *Nature* wanted, Lawrence remembers encouraging him to "use this opportunity to put your opinions, ideas, and facts all in one article."

Lewis seems to have taken Lawrence's advice very much to heart. The famous review he published in December 1978, "A gene complex controlling segmentation in *Drosophila*" (E. B. Lewis 1978), is a scientific epic compressed into six pages. Data and model are often as intricately interlinked as the genetic interactions Lewis is tracing. This presents a real challenge to the reader and is very much a reflection of Lewis's genius and approach to science. The 1978 review presents a mass of pioneering observations, but, just as importantly, it uses these data to "build a picture of ideas" (Lawrence and Locke 1997) about BX-C functions.

The most important advance made in the decade between publications was the isolation in 1973 of a deficiency (*Df P9*) that removes the entire bithorax cluster. This deficiency had an astonishing phenotype when homozygous: homozygotes died as first instar larvae that showed transformations of segments from T3 through A8 toward T2. These transformations could be seen easily in the external cuticle of the larva, but also affected the tracheal system and internal organs such as the ventral nerve cord. The phenotype of *Df P9* larvae indicated that the bithorax cluster was far more extensive than anyone had imagined, containing genes that specify the identities of all of the abdominal segments as well as T3. To convey the apparent size of the bithorax cluster, Lewis began calling it the bithorax complex, or BX-C. It may seem surprising that complete deletions for the BX-C were not isolated much sooner. There is a very good reason for this: deletions for the entire complex cause dominant sterility in both sexes. This is due to the haplo-insufficiency of a gene (*Abd-B*) at the right end of the cluster. By chance, Lewis isolated the *P9* deficiency in a heterozygote with *Microcephalus*, an eye mutation associated with a tandem duplication of *Abd-B* that suppresses this haplo-insufficiency.

The discovery of *Df P9* added a new dimension to the analysis of the bithorax genes. To define new functions within the BX-C, Lewis adopted a novel strategy. The usual approach taken by developmental geneticists—an approach in which Lewis was a pioneer—is to infer gene function by comparing the phenotypes of animals mutant for a gene to wild type. Now Lewis took the opposite approach. He tested the effects of wild-type alleles of BX-C genes by adding back fragments of the complex to zygotes otherwise homozygous for *Df P9*. This allowed him to make a direct test of the wild-type function of specific BX-C genes. The experiments revealed four key features of BX-C gene function. First, Lewis found that the order along the chromosome of the BX-C genes

was the same as the order along the body of the segments in which each becomes active. Second, he found that once a BX-C gene becomes active in a segment, it remains active in all more posterior segments. Third, Lewis found that to some extent the BX-C genes overlap in function. For example, several of the BX-C genes could restore continuity to the tracheal trunks. Fourth, Lewis found that segment identity is controlled in a mosaic fashion. The presence of a particular type of sense organ (the ventral pits), for example, is controlled solely by *bxd*$^+$. In a *bxd* mutant, all trunk segments develop this organ regardless of which other segmental attributes they might have.

In the 1978 paper, Lewis also described several new gain-of-function mutations that affect abdominal segment identities in the adult. One of these, *Mcp*, was particularly important because it was the first mutation in the part of the complex controlling posterior abdominal development that could be mapped by recombination. *Mcp* was found by Madeline Crosby, then a technician in Lewis's lab, and causes a transformation of A4 to A5. In the male, this results in a striking phenotype, since A5 and A6, but not A4, are black in wild type, whereas A4, A5, and A6 are black in the mutant. Initially, Crosby called this mutation *Male chauvinist pigmentation*, but after some pressure from Lewis she gave in and renamed it *Miscadestral pigmentation* (*Mcp*). Mapping revealed that *Mcp* lies midway between *Hab* and *Microcephalus*, a location consistent with the colinearity of BX-C genes and segments. Crosby then tested the hypothesis that *Mcp* is a *cis*-regulatory mutation that causes a BX-C gene (*iab-5*$^+$), normally expressed only in A5 and more posterior segments, to become active in A4. She recovered a mutation that reverted *Mcp* and then separated it from *Mcp* by recombination. The crossovers recovered indicated that the reverting mutation was located close to the right of *Mcp*. On its own, this mutation caused A5 to transform toward A4, the phenotype expected for a mutation in *iab-5*. A6 and A7 also transformed to A4, which was interpreted as resulting from a polar *cis*-inactivation of *iab-6*$^+$ and *iab-7*$^+$.

Ideally, an epic should end with a dramatic revelation or, as in Homer's *Odyssey*, with the hero's return to his wife. In the conclusion to his 1978 review, Lewis managed both at once. Thirty years before, Lewis's own Penelope, his wife Pamela (see Figure 1), had discovered a mutant to which she gave the name *Polycomb* (*Pc*) (P. H. Lewis 1949). Now in 1978 Lewis reported the very exciting discovery that larvae homozygous for null alleles of *Pc* show extreme transformations of segments in the head, thorax, and anterior abdomen to posterior abdominal segments. These transformations are opposite in direction to the posterior-to-anterior transformations seen in *Df P9* homozygotes and were those predicted to occur if all of the BX-C genes were to become expressed in all segments. Indeed, the transformations require the presence of at least one dose of the BX-C in the genome; that is, "*Pc*^{3} *Df-P9* homozygotes closely resemble *Df-P9* homozygotes." Lewis concluded that *Pc*$^+$ "in all likelihood is coding for a repressor of BX-C" (E. B. Lewis 1978, p. 569).

Lewis further proposed that during normal development an anteroposterior gradient in concentration of the *Pc*$^+$ repressor controls the differential expression of BX-C genes along the body axis. Consistent with this hypothesis, animals carrying only one dose of *Pc*$^+$ show weak transformations that mimic BX-C dominant gain-of-function phenotypes (Duncan and Lewis 1982). In an animated movie Lewis made himself during this period (with initial guidance from a professional Hollywood animator), Lewis depicted the *cis*-regulatory elements of BX-C genes as containing "hooklets," representing *Pc*$^+$ repressor binding sites, whose number progressively increased in more distally located genes. The number of binding sites would define the affinity of a *cis*-regulatory element for *Pc*$^+$ repressor. Thus, the colinear expression of BX-C genes was pictured as resulting from "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" (E. B. Lewis 1978, p. 565).

The picture that Lewis painted in the 1978 review was that the BX-C contained one gene for each segment in the posterior thorax and abdomen and that these genes were colinear with the segments each controls. However, the BX-C elements controlling abdominal segment identities remained poorly defined. Thus, a major goal after 1978 was to identify new mutations in the abdominal region of the complex. A variety of screening methods were employed. One of the most elegant and productive was to screen for new rearrangements that disrupt transvection in *Cbx Ubx*/+ + heterozygotes. With normal sequence chromosomes, this screen is quite inefficient, as it leads to the recovery of rearrangements that have breakpoints located anywhere between the centromere and the middle of the 3R chromosome arm, where the BX-C is located. To increase the efficiency of this method, Lewis screened in a background homozygous for an inversion that places the BX-C close to its centromere. This had the advantages of dramatically reducing the size of the "critical region" and of inverting the BX-C so as to include the abdominal region of the complex within this critical region. Gradually a set of mutations that allowed definition of each "*iab*" region was acquired. As expected, these regions proved to be colinear with the segments each controls (Figure 2).

MOLECULAR STUDIES OF THE BX-C

About the time Lewis's review appeared in *Nature*, cloning of the BX-C was initiated in David Hogness's lab at Stanford. An entry into the complex was provided by a *Ubx* rearrangement whose second breakpoint was

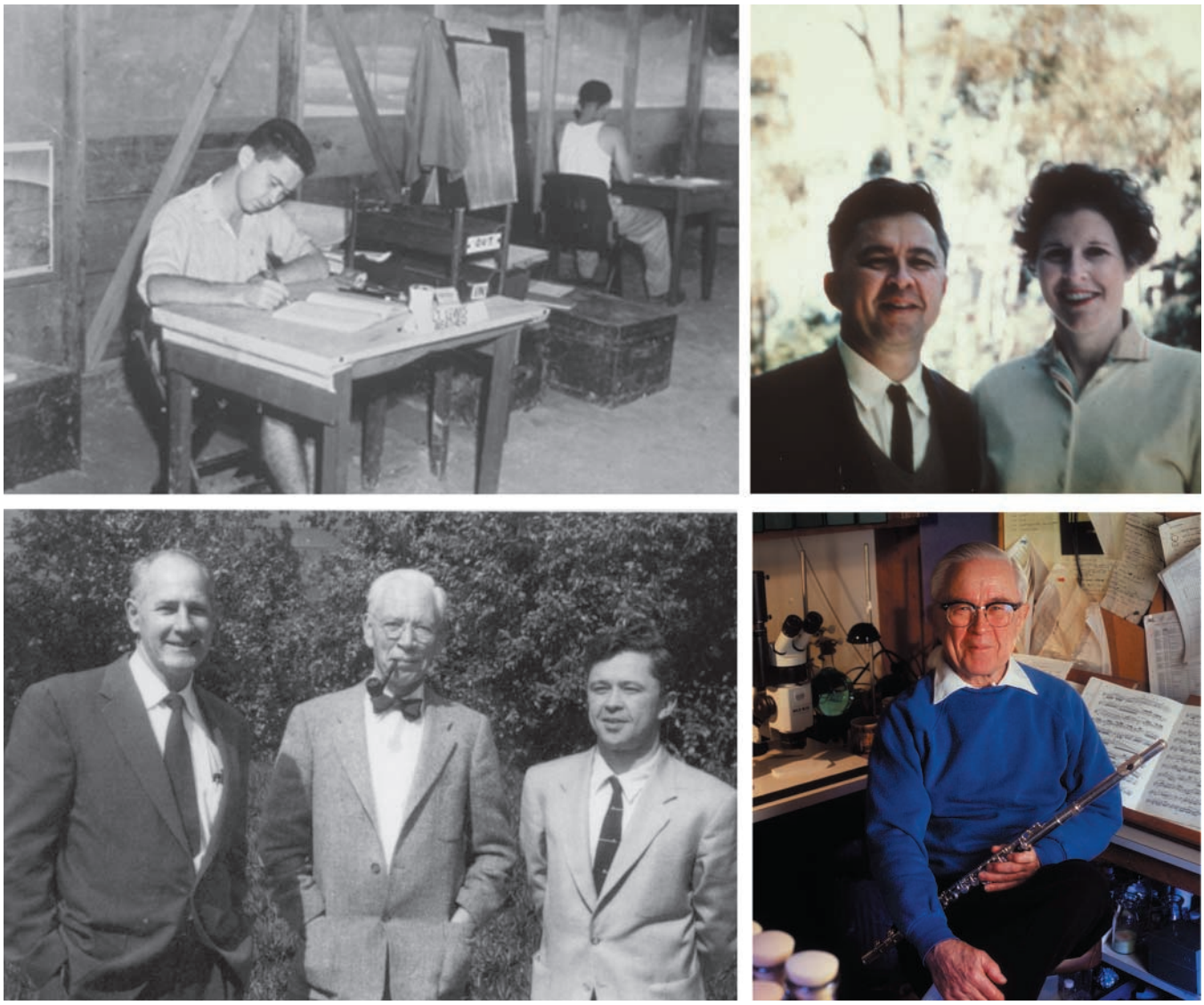

Figure 1.—Ed Lewis at different times in his career. Top left: Lewis at his desk on Okinawa in 1945. Lewis trained as a cadet in the US Army Air Force program in meteorology at Caltech and served as the weather officer in the US Tenth Army G2 section. The sign on the desk reads "Lt. Lewis—Weather"(courtesy of E. B. Lewis). Bottom left: Lewis (right) with Alfred Sturtevant (middle) and George Beadle (left) circa 1960 (courtesy of the California Institute of Technology Archives). Top right: Ed and Pam Lewis, circa 1960 (courtesy of E. B. Lewis). Bottom right: Lewis in his office in 1996 (courtesy of Harold Sweet).

located in a region that had already been cloned. From this entry point, Welcome Bender proceeded to walk through the complex, mapping mutants from Lewis's collection as he went. The complex was eventually revealed to be huge, about 315 kb in length, with each segment-specific region occupying 15–30 kb (Bender *et al.* 1983; Karch *et al.* 1985). Early reports presented a bewildering series of biochemical puzzles. The *Ubx* transcription unit, for instance, was found to have the largest intron ever discovered and to be processed into multiple spliceforms (Beachy *et al.* 1985). For a time, members of the Hogness laboratory pursued a variation of the Lewis "many-developmental-substances" model, in which, for example, the function of *bx* was to control the processing of a specific *Ubx* RNA.

Clarity came with convergent findings from several sources. In 1980, Thom Kaufman and his colleagues defined genetically the Antennapedia complex (ANT-C), a set of homeotic genes that controls the identities of segments in the head and anterior thorax (Kaufman *et al.* 1980, 1990; for history see Denell 1994). Using molecular methods pioneered in the Hogness laboratory, the ANT-C was cloned by Kaufman's group (Scott *et al.* 1983) and by Walter Gehring's group (Garber *et al.* 1983). Shortly afterward came the discovery of the homeobox, a sequence of 180 bp conserved among ANT-C and BX-C genes (McGinnis *et al.* 1984a; Scott and Weiner 1984; for history see McGinnis 1994). Lewis's hypothesis that these genes had evolved by gene duplication and divergence was central in motivating

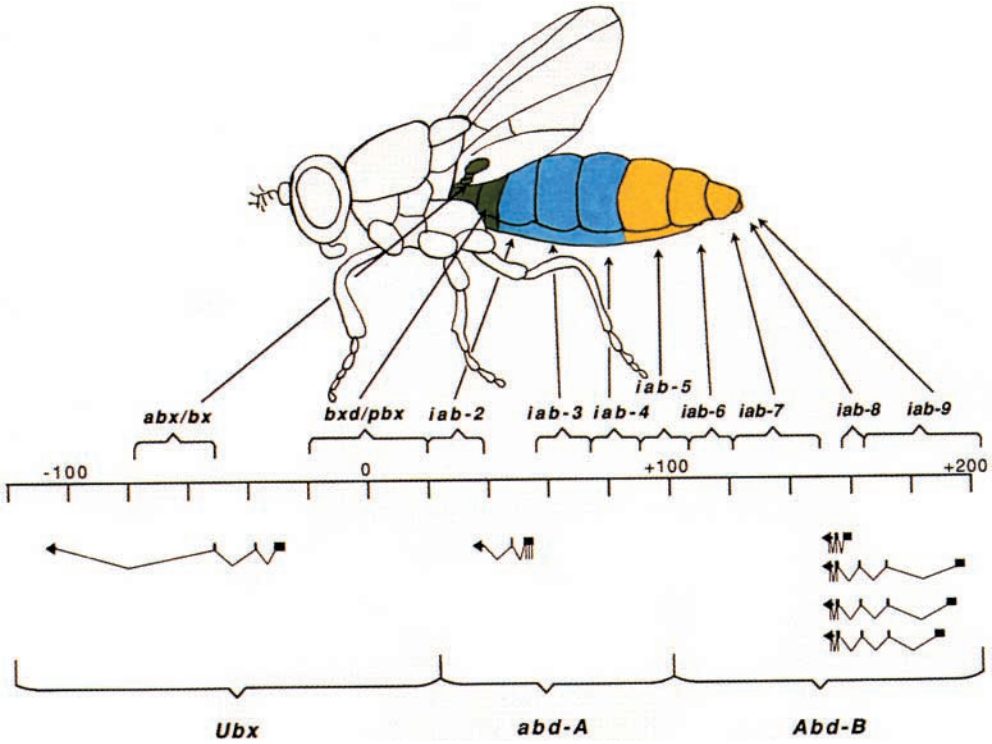

FIGURE 2.—Schematic diagram showing the colinearity of body segments with the genes and regulatory regions of the BX-C. The fly is color coded to indicate the segments whose identities are controlled by *Ubx* (green), *abd-A* (blue), and *Abd-B* (orange). Taken from Engineering & Science, Publication of the Caltech Alumni Association, 1996.

these groups to look for conserved sequences. The homeobox was found to lie in the coding regions of the genes that contained it and to encode a DNA-binding domain. Remarkably, although each of the ANT-C genes had one homeobox, the entire BX-C had only three (REGULSKI *et al.* 1985). This observation was published at about the same time that Morata's group (SÁNCHEZ-HERRERO *et al.* 1985) and TIONG *et al.* (1985) published genetic studies that dramatically changed our view of the BX-C. These groups screened for EMS-induced lethals located within *Df P9* and found only three lethal complementation groups within the BX-C. When examined in mosaics, alleles from each complementation group caused transformations of multiple segments, and in aggregate their effects accounted for the phenotype of *Df P9* homozygotes. One of the complementation groups identified in the lethal screens was *Ubx*. The others were named *abdominal-A* (*abd-A*), which controls the identities of A2–A4, and *Abdominal-B*, which controls the identities of A5–A9. The correspondence between the presence of three homeoboxes and three lethal complementation groups within the BX-C suggested that the BX-C might contain only three protein-coding genes.

Decisive evidence came from the visualization of *Ubx* expression patterns. Hogness's lab isolated *Ubx* cDNAs, which contained a homeobox, and encoded a homeodomain protein (BEACHY *et al.* 1985). Antibody probes were made for this protein and used in immunofluorescence experiments to determine the pattern of *Ubx* expression. As expected, *Ubx* was found to be expressed at high levels in T3 and A1. *Ubx* is also expressed in more posterior segments, as inferred by Lewis, although at lower levels. In 1985 a series of reports (BEACHY *et al.* 1985; CABRERA *et al.* 1985; WHITE and AKAM 1985; WHITE and WILCOX 1985) appeared, demonstrating that the *bx*, *bxd*, and *pbx* mutations all cause loss of *Ubx* expression in the regions where these mutations had their effects: *bx* mutants showed a loss of *Ubx* expression in the anterior haltere imaginal disc, *pbx* mutants lost *Ubx* expression in the posterior haltere, and *bxd* mutants caused a reduction in *Ubx* expression in A1 and posterior T3. These observations indicated that *bx*, *pbx*, and *bxd* are all *cis*-regulatory regions that function to control the expression of *Ubx*. Importantly, *Cbx* was shown to cause ectopic expression of *Ubx* in the wing imaginal disc, indicating that *Ubx* protein is both necessary and sufficient for specifying T3 identity. Subsequent work revealed that the *iab* regions also function as segment-specific *cis*-regulatory regions and control the expression of the *abd-A* and *Abd-B* proteins. Lewis's laboratory made important contributions to the molecular characterization of these *iab* regions and of the *Abd-B* protein-coding region (CELNIKER *et al.* 1989, 1990).

Thus, to Lewis's surprise, the segment-specific subfunctions of the BX-C were found not to be separate "genes" encoding separate "substances." Rather, they were complex *cis*-regulatory regions that are loaded with multiple enhancers and other specialized *cis*-elements. The *cis-trans* position effects studied for so long by Lewis were finally understood to reflect the requirement that the *bx*, *bxd*, and *pbx* regions be *in cis* to their target, the *Ubx* promoter, for full function. The polar effects of *bxd* mutations on pbx^+ were found to result either from transposable element insertions that block enhancer-

promoter interactions or from chromosome breaks that separate the *pbx*$^+$ enhancers from *Ubx*. Moreover, the fly embryo was found not to behave with the elegant simplicity predicted by Lewis's *Polycomb* gradient model. It turns out that the initial patterns of BX-C gene expression are set by the products of the segmentation genes, which were systematically identified by his co-Nobelists Nüsslein-Volhard and Wieschaus. The "super-repressor" mutation Lewis reported in 1968, *Rg-pbx*, was found to be an allele of the gap gene *hunchback*, which does in fact function as the major embryonic repressor of the *Ubx* domain and is thought to fulfill some of the morphogen gradient functions Lewis ascribed to *Polycomb* (Struhl *et al.* 1992). As Lewis had predicted in the 1978 paper, "the *Hab* mutation presumably damages a regulatory element adjacent to *iab-2*$^+$ [*abd-A*] in such a way as to reduce its affinity for repressor"—but the repressor is not encoded by *Polycomb* but by the gap gene *Krüppel* (Shimmel *et al.* 1994). Once the initial pattern of BX-C gene activities is laid down by transient expression of the segmentation genes, *Polycomb* and related genes function to maintain these patterns through later development by mechanisms currently under intensive investigation.

Yet, prior to molecular studies, it is difficult to conceive how anyone could have foreseen the immense complexity of BX-C regulation. The vast majority (98.6%) of the BX-C is composed of noncoding regions that are packed with regulatory elements capable of operating over tremendous distances. For example, the *iab-5* region identified by Crosby somehow must act over a distance of about 60 kb to influence expression of *Abd-B*. This ability to act over great distance explains why in Lewis's work the *bxd*$^+$ and *pbx*$^+$ regions appeared to have some function when separated from *Ubx*$^+$. It turns out that these regions are able to act on the *Ubx* promoter *in trans* when homologs are synapsed or *in cis* even when a large cytologically visible insertion separates them from the *Ubx* promoter. Who could have imagined such mechanisms?

In fact, Lewis came close. It turns out that the *Cbx* mutation, which arose simultaneously with *pbx*, is an insertion of the *pbx* region into an intron of *Ubx*. Apparently this causes the *pbx* region to become active in T2, which in turn drives high-level expression of *Ubx* in T2. Lewis suggested that *Cbx* might "represent an insertion of the wild-type allele of the [*pbx*] gene between the [*bx*] and [*Ubx*] loci, with accompanying escape of that gene from repression" (E. B. Lewis 1968). In addition, he concluded that *Cbx* regulates *Ubx*$^+$ in a *cis*-dominant fashion (E. B. Lewis 1978). Both of these inferences are true and if combined might have led to the idea that *pbx*$^+$ in its normal location is also *cis*-regulatory on *Ubx*. However, Lewis never made this last step. Lewis also foresaw the wide conservation of homeotic genes like those of the BX-C. In 1981, for the "Significance" section of an NIH proposal, he wrote: "The results of the proposed research are expected to have direct significance in terms of human health in the sense that the human embryo, like the embryo of the fly, is expected to use sets of master regulatory genes of the bithorax-complex type to control the differentiation of its head, thoracic and abdominal regions. Mutations within such genes are expected to result in profound developmental defects and to be the basis of some types of congenital abnormalities. Once genes which produce homeotic mutant alleles are cloned, as is already the case for some bithorax complex genes, there is a possibility of using them as probes to seek for homologous genes across species, or hopefully, more remote barriers."

Not only were "genes of the bithorax-complex type" found to be present in humans (McGinnis *et al.* 1984b), but in 1989 biologists were astonished by reports that entire Hox gene clusters containing colinearly arranged homologs of both BX-C and ANT-C genes were present in the mouse (Duboule and Dollé 1989; Graham *et al.* 1989). This and subsequent work indicated that an essentially complete Hox complex was present in the common ancestor of the bilaterian animals. Thus, evolutionary changes in arthropod segmentation do not appear to have been caused by the appearance of new BX-C genes, as long envisioned by Lewis. Rather, these morphological changes must have resulted either from changes in the regulation of the Hox genes or from changes in the response of target genes downstream of the Hox genes (Warren *et al.* 1994).

No description of Ed Lewis's contributions would be complete without mention of his broader importance to the Drosophila field. Many of the tools that are used daily by Drosophila geneticists were developed in his lab. For example, most of the balancer chromosomes in frequent use were produced by him. Compound autosomes were also first generated in his lab. The importance of Lewis's contributions to "fly infrastructure" is well illustrated by his article that for many years had the highest citation index: his protocol for EMS mutagenesis (Lewis and Bacher 1968). Lewis discovered that EMS could be fed to males, rather than injected, which greatly simplified mutant screens, including those of his co-Nobelists. Lewis also kept the Drosophila stock center going at Caltech through the "dark years" when prokaryotes were ascendant and Drosophila appeared to have a dim future. Two major figures in the renaissance of Drosophila, David Hogness and Antonio Garcia-Bellido, spent important transitional periods of their careers in Lewis's lab in the late 1960s. It was there that Hogness became acquainted with the BX-C system and that Garcia-Bellido learned of the intrasegmental transformations displayed by the *engrailed* mutation (Garcia-Bellido 1998). All of us working on flies owe Lewis a tremendous debt of gratitude. Few Drosophilists are aware that for many years Lewis has also been concerned with radiation hazards to humans. His 1957 paper in Science (E. B. Lewis 1957), which statistically analyzed the incidence of leukemia in Hiroshima and Nagasaki, helped shape a national debate that led eventually to

the banning of atmospheric testing of nuclear weapons. Given that the primary source of funding in Lewis's laboratory in the 1950s was the Atomic Energy Commission, these studies must have taken considerable courage to pursue.

Of course, Ed Lewis will always be known for his work on the BX-C. However, viewing Lewis's work from a modern perspective, it is easy to lose sight of a more basic contribution. Lewis changed how developmental biologists think. Most importantly, he introduced the idea that bodies are formed by regulatory genes acting in specific regions of the animal. This key revelation of modern developmental biology dates to Lewis's early models for the differential functioning of the BX-C genes in T3 and A1 and now encompasses a wide variety of developmental control genes. What remains unique about the Hox gene cluster is the parallel between its genetic organization and its functions along the body axis. The basis of this colinearity, discovered by Lewis in the BX-C, is still a mystery.

Although now emeritus, Lewis continues his studies of the BX-C with the same high enthusiasm, in the same Caltech office he has occupied for a half century. On the wall across from his desk is an old genetic map of the *Ubx* domain of the BX-C; below this map, in sheets of computer printout, is the complete DNA sequence of the *Abd-B* domain, with sequences highlighted and annotated by hand. Appropriately, the BX-C was the first large region of the Drosophila genome to be sequenced (Martin *et al.* 1995). Since the early 1990s, Lewis has been using his background in biostatistics to search the vast noncoding regions of the BX-C for novel *cis*-regulatory motifs (E. B. Lewis *et al.* 1995), an effort that places him at the frontiers of the new field of computational genomics. In these pages a few years ago (E. B. Lewis 1995), Lewis said of his mentor: "For Sturtevant, science must have been an exciting and rewarding journey into the unknown." Ed Lewis's own journey of exploration of the BX-C—a journey that began in genetics' late classical period, spanned the revolution in molecular genetics, and has now entered the postgenomic era—surely ranks among the most remarkable in the history of biology.

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. The quote from Max Delbrück and the photograph of Beadle, Sturtevant, and Lewis are courtesy of the California Institute of Technology Archives. We also thank Engineering & Science (Caltech Alumni Association) and Harold Sweet for permission to reproduce photographs and Dianne Duncan for preparation of the figures. Finally, we are very grateful to Jennifer Brisson, Susan Celniker, Dianne Duncan, Peter Lawrence, Howard Lipshitz, William McGinnis, and Joanne Topol for critical reading of the manuscript.

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