ANALYSIS OF THE CUT LOCUS OF
DROSOPHILA MELANOGASTER

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ABSTRACT

Mutants of the cut (ct) locus can be divided into two classes: viable and lethal. Most of the viable alleles are characterized by varying degrees of scalloping and notching of the wings. One mutant, kinked femur, exhibits kinking of the femurs and failure of wing expansion, but no other changes in wing structure. In heterozygous combination with the other viable alleles, it exhibits complete complementation, but it fails to complement with lethal ct alleles with respect to its viable phenotype. Similarly, all of the other viable ct alleles express a mutant wing phenotype when heterozygous with lethal ct alleles. — Mapping experiments indicate that the lethal alleles, which comprise the majority of all ct mutations recovered, are confined to a small region at the right end of the locus. That this restriction is real and not an artifact imposed by the limited number of lethal mutations mapped in the locus is supported by an examination of the mutant ctJC~0, a presumptive deficiency for the left-most third of the locus. Despite its behavior as a deletion, ctJC~0 is viable, though mutant, in combination with the lethal alleles. The restriction of the noncomplementary lethals to a small part of the locus, distinct from the other ct mutants, suggests a polarity that may define a segment that functions only in cis within the complex. — Based on the comparison of the data with the prediction of several models, we suggest that the left portion of the locus, which contains the viable alleles, defines a regulatory region controlling the expression of the locus, while the segment encoding a polypeptide product is at the right end and only it is capable of mutating to a lethal state.

It is important to know whether complex loci represent clusters of genes coordinately controlled and involved in related steps in a developmental pathway, or whether they consist of a single structural element whose product interacts in several pathways, in several tissues or at several different times in development. Investigating the nature of complex loci will help us gain an understanding of how genes are regulated in eukaryotic organisms. In Drosophila, a number of complex loci are known. They are usually recognized because they exhibit allelic complementation and pleiotropy. In addition, they often appear to have large size as judged by the frequency of intragenic recombination. No clear pattern emerges concerning the organization of complex loci, despite the great amount of work that has been done with them (see JUDD 1976 for review). Noteworthy are the investigations of Lewis (1963, 1964, 1967, 1968) into the

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structure and function of the bithorax complex. The most generally accepted model for the organization of this locus postulates that it is a cluster of structural elements encoding products that affect related steps in the development of the thoracic and abdominal segments of the fly. Lewis has accumulated a great deal of evidence supporting this concept. On the other hand, the detailed genetic analyses of the Notch locus (Welshons 1965, 1974; Sellenbarger and Mohler 1975; Welshons and Keppy 1975; Keppy and Welshons 1977) indicate that it is more logically viewed as containing a single structural element. Even in the case of the rudimentary locus, which encodes three different enzymic activities in the de novo synthesis of pyrimidines (Norby 1970; Rawls and Fristrom 1975; Jarry and Falk 1975), it is not clear whether there are three separate polypeptides or only one with three active sites.

The cut locus of Drosophila melanogaster, the analysis of which we report here, is an interesting example of a complex locus. The complementation data, recombination analysis and the evaluation of the allelic interactions and pleiotropy support most strongly the concept that this complex contains a single structural element and that a major portion of the locus is devoted to the tissue- and stage-specific activation of the structural element.

**MATERIALS AND METHODS**

The cut (ct) locus is located in the X chromosome at map position 20.0. Its cytological position in the polytene chromosomes has been narrowed to 7B3–4. It is a highly mutable locus, possibly one of the most mutable loci on the X chromosome, judging from the spontaneous (Schaeft 1957) and X-ray-induced (Valencia and Muller 1949; Lefevre, unpublished) mutation rates, and it also shows a high frequency of X-ray-induced breakage (Lefevre, unpublished).

The locus exhibits a variety of phenotypic effects produced by the various viable alleles. Structures most frequently affected are wings, which typically have margins incised and the tips cut to points. Wings do not expand in some mutant types. Other effects include eye and antennal shape, leg structure, and bristle size and placement. Each viable allele has a unique phenotypic expression (Table 1). In addition to the viable alleles that affect the morphology of the adult fly, there are numerous lethal alleles, some being associated with the breakpoints of chromosomal rearrangements and some appearing cytologically normal (Table 1). Four mutants, ct^{206}, ct^{115}, ct^{112}, and ct^{R02}, obtained from G. Lefevre, were induced by treating 3-day-old wild-type males with 2000R or 3000R of X radiation. These mutants were derived from an unselected sample of sex-linked lethals and localized both on the basis of their allelism with known lethal ct alleles and by their coverage by one of the ct^{+} duplications listed in Table 1. Three mutations, ct^{149}, ct^{155}, and ct^{161}, were induced by treating 0 to 12 hr post-eclosion Canton-S males with EMS for 18 hr by the method of Lewis and Bacher (1968). Treated males were mated to In(1)sc^{S1Lsc^{R8}}, dl-49, y^{1}sc^{S1Lsc^{R8}}ct^{nsu B} homozygous females (see Lindsley and Grell 1968 for description of mutant phenotypes), and the resulting F_{1} females were placed singly in separate culture vials. They were then backcrossed to In(1)sc^{S1Lsc^{R8}}, dl-49, y^{312}sc^{S1Lsc^{R8}}ct^{nsu B} males. Backcross offspring were scored for sex-linked lethals. These lethals were tested by combining each one with one of the ct^{+} duplications. Those that survived in the presence of the duplication were tested for allelism with known lethal ct alleles.

The cytologically rearranged ct deficiencies resulted from X-ray-induced chromosome breakage, as were the duplications (Table 1).

*Recombination experiments:* Experiments designed to map various alleles within the ct locus were carried out by collecting virgin females that were heterozygous for the appropriate
markers, aging them for three days and then placing them in vials overnight with an excess of males of the appropriate genotype. The following morning, ten females and an excess of males were transferred to half-pint milk bottles containing standard cornmeal-molasses-agar Drosophila medium and maintained at 25°C. These parents were transferred to fresh food when the females were four days old (a), seven days (b), nine days (c), 12 days (d) and 15 days old, for a total of five subcultures, following the recommendations of LEFEVRE (1971). Fourteen days after the initiation of each culture, the progeny were scored. The bottles were reexamined and scored again on the 17th day and discarded.

Map distances were calculated as a percentage of recombinants among the total progeny. In an attempt to minimize the variation caused by the use of different markers and genetic backgrounds, the method of calculating intralocus distances was as follows. First, the intralocus distances were calculated as a percentage of the observed recombination in either the $cm$-$ct^6$ or $ct^s$-$sn^3$ interval. Total recombination in the $cm$-$ct^s$-$sn^3$ intervals was then adjusted to fit the standard map length of these intervals, and the intralocus distance was recalculated as a percentage of the standard interval.

**Developmental analysis:** The effective lethal period of selected mutants was examined by the following method. Four inseminated females were placed in egg-collection chambers overnight and allowed to deposit eggs on standard Drosophila medium colored with 1% activated charcoal. The following morning, the eggs were counted and transferred to fresh slabs of colored medium. The vials were then examined for the number of eggs hatched, the number of pupae formed and the number of adults emerged. The experiments were carried out in duplicate by mating wild-type males to wild-type females as controls, and to females heterozygous for one of the lethal $ct$ alleles in the experimental series. The proportion of individuals in each culture reaching successive stages in development was recorded. The percentage of individuals in the lethal-bearing cultures passing from one stage to the next was corrected for any decrease in viability as measured in the wild-type control cultures. From these observations, it was determined whether the lethal-bearing individuals die as embryos, larvae or pupae, or whether lethality is spread over more than one stage.

**RESULTS**

**Complementation among cut alleles**

Male-viable mutants of the cut locus commonly exhibit scalloped wing margins, with the tips usually cut to points. Most alleles are pleiotropic, exhibiting various combinations of aberrations of eye shape and head morphology, displaced or missing vibrissae, warped abdominal bands, reduced bristle size, deformed antennae, failure of wing expansion, kinking of the femurs, and reduced viability, in addition to the abnormal wing morphology. Most viable $ct$ alleles, however, express only one or two of these phenotypic effects. An exception is the semi-lethal allele, $ct^{d29}$ (LEFEVRE, unpublished), which exhibits rounding of the eyes, unexpanded wings, deformed femurs, missing vibrissae, deformed antennae, and reduced viability, as well as incision of the wings similar to the effects originally reported for $ct^1$ (MORGAN, BRIDGES and STURTEVANT 1925).

To establish the allelic interactions for the expression of the mutant phenotypes, complementation tests were performed by constructing heterozygous combinations of alleles. The complementation pattern among the viable alleles is shown in Table 2. The most striking observation concerns the mutant allele $k^f$. This allele, which has a fully formed, though unexpanded, wing blade, shows complete complementation with the alleles that effect wing morphology, except...
TABLE 1

<table>
<thead>
<tr>
<th>Mutant (symbol)</th>
<th>Origin</th>
<th>Phenotype</th>
<th>Cytology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable cut alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kinked femur² (kf²)</td>
<td>spontaneous</td>
<td>unexpanded wings</td>
<td>normal</td>
<td>WHITNEY and LUCCHESI (1972)</td>
</tr>
<tr>
<td>cut-notch (ctₙ)</td>
<td>heat treated flies</td>
<td>notched wings</td>
<td>normal</td>
<td>PLough and Ives (1934)</td>
</tr>
<tr>
<td>cutₖ (ctₖ)</td>
<td>spontaneous</td>
<td>scalloped wings</td>
<td>normal</td>
<td>MORGAN et al. (1925)</td>
</tr>
<tr>
<td>cut-Krivshenko (ctₖ)</td>
<td>spontaneous</td>
<td>scalloped wings fine bristles</td>
<td>normal</td>
<td>KRIVSHENKO (1956)</td>
</tr>
<tr>
<td>cut-notch of Schalet (ctₙ₟₈)</td>
<td>spontaneous in In(1)dl-49</td>
<td>like ctₙ</td>
<td>normal</td>
<td>SCHELET, unpublished</td>
</tr>
<tr>
<td>cut⁴₀²₀ (ct₄₀²₀)</td>
<td>X-ray</td>
<td>scalloped wings vibrissae missing</td>
<td>normal</td>
<td>LEFEVRE, unpublished</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unexpanded wings kinked femurs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lethal cut alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cytologically)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cut⁴₀¹₄₅ (ct₄₀¹₄₅)</td>
<td>X-ray</td>
<td>lethal</td>
<td>normal</td>
<td>LEFEVRE, unpublished</td>
</tr>
<tr>
<td>cut⁴₀¹₂₄ (ct₄₀¹₂₄)</td>
<td>X-ray</td>
<td>lethal</td>
<td>normal</td>
<td>LEFEVRE, unpublished</td>
</tr>
<tr>
<td>Lethal cut alleles (cytologically rearranged)</td>
<td>X-ray</td>
<td>lethal</td>
<td>Df(1)7A1;B8–C1</td>
<td>LEPFRE and JOHNSON (1973)</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-------</td>
<td>--------</td>
<td>----------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>cut of Johnson-4 (Df(1)ct^{4})</td>
<td>X-ray in In(1)dl.49</td>
<td>lethal</td>
<td>Df(1)7B3;C4</td>
<td>LEPFRE, unpublished</td>
</tr>
<tr>
<td>cut^{abi}(Df(1)ct^{abi})</td>
<td>X-ray</td>
<td>lethal</td>
<td>Df(1)7A4;B1 ±</td>
<td>LEPFRE, unpublished</td>
</tr>
<tr>
<td>Df(1)RF19</td>
<td>X-ray</td>
<td>lethal</td>
<td>In(1)6A1 ±; 19E8 + T(1;2) 20; 2L</td>
<td>LEPFRE, unpublished</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duplications</th>
<th>X-ray</th>
<th>cm^{+–ct^{+}},{y}^{+–ac^{+}}</th>
<th>Dp(1;2)7A8; 8A5 + In(2LR) 32C; 58E</th>
<th>JOHNSON, unpublished</th>
</tr>
</thead>
<tbody>
<tr>
<td>ct^{+}y^{+}Y^{*}</td>
<td></td>
<td>c^{+–ptg^{+}}</td>
<td></td>
<td>LEPFRE, unpublished</td>
</tr>
<tr>
<td>Dp(1;2)sn^{+72d}</td>
<td>Aneuploid of T(1;2)sn^{+72d}</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Synthesized from T(X;Y)13I (NICOLETTI and LINDSLEY 1960), using the method of Brousseau et al. (1962).
TABLE 2

Cut allele complementation patterns

<table>
<thead>
<tr>
<th>Allele</th>
<th>kř</th>
<th>ct^a</th>
<th>ct^e</th>
<th>ct^K</th>
<th>ct^{161}</th>
<th>ct^{114}</th>
</tr>
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<tbody>
<tr>
<td>kř^2</td>
<td>kinked femur</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>kinked femur</td>
<td>+</td>
</tr>
<tr>
<td>ct^a</td>
<td>cut wings</td>
<td>cut wings</td>
<td>cut wings</td>
<td>cut wings</td>
<td>cut wings</td>
<td>cut wings</td>
</tr>
<tr>
<td>ct^e</td>
<td>cut wings, vibrissae missing, antennae deformed</td>
<td>cut wings</td>
<td>cut wings, vibrissae missing, antennae deformed</td>
<td>cut wings, vibrissae missing, antennae deformed</td>
<td>cut wings</td>
<td></td>
</tr>
<tr>
<td>ct^K</td>
<td>cut wings, fine bristles</td>
<td>cut wings</td>
<td>cut wings, fine bristles</td>
<td>cut wings, fine bristles</td>
<td>cut wings</td>
<td></td>
</tr>
<tr>
<td>ct^{161}</td>
<td>kinked femur, cut wings</td>
<td>cut wings</td>
<td>kinked femur, cut wings</td>
<td>kinked femur, cut wings</td>
<td>lethal</td>
<td></td>
</tr>
<tr>
<td>ct^{114}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lethal</td>
<td></td>
</tr>
</tbody>
</table>

for ct^{1029} and the lethal alleles. Some of the ct alleles described in LINDSLEY and GRELL (1968) are reported to have unexpanded wings and reformed legs similar to those of kř^2. These mutants, as does ct^{1029}, were reported to have reduced viability. Thus, the question arises as to whether kř^2 is an allele of the cut locus that does not affect wing shape and exhibits allelic complementation with the other viable members of the locus, or whether it and those mutants that exhibit a kř-like phenotype are short, cytologically undetectable deletions that uncover two adjacent loci. This question is resolved by recombination tests reported in the next section. Tests show that kř^2 recombines with alleles that it fails to complement; thus, it is not a deletion.

Another interesting point emerges from results of the complementation tests reported in Table 2. Those alleles with abnormal wings and a variety of other defects fail to complement for the wing defect, but complement for those characteristics not common to both alleles. For example ct^a/ct^e heterozygotes have incised wings, a clear failure to complement, but vibrissae and antennae are normal, even though ct^e typically shows abnormalities in these structures.

Heterozygous combinations of the lethal ct alleles with the viable alleles of the locus, shown in Table 2, exhibit no complementation for the mutant phenotypes with the single exception of ct^{114}/kř^2, which gives complete complementation. The failure of the lethal mutations to complement any of the mutant phenotypes...
associated with the viable alleles indicates either that the lethals are null mutations or that they are deletions that remove the sites of the viable mutations. Allele ct1121 might then represent a mutation that does not delete the kf site. This question has been resolved for the most part by recombination tests in which ct1121 and the other lethal alleles map as point mutations that are positioned to the right of (proximal to) all of the viable alleles.

Recombination between cut alleles

Recombination experiments were undertaken to determine the relative positions of the mutants within the locus. Since some of the experiments required that the intralocus map distance be calculated as a percentage of either the cm-ct4 or ct6-sn5 intervals, the map of the region constructed from experiments reported here was compared to the standard map published in Lindsley and Grell (1968). A total of 200,000 flies were examined for recombination between the markers cm, ct6 and sn5 and the intergenic distances calculated. A distance of 2.1 map units, identical to that reported in Lindsley and Grell (1968), was found between cm and sn5. The distribution of recombinant events within the cm-sn5 interval differs only slightly from the standard map. Results gave distances of 1.3 map units between cm and ct6, slightly greater than the reported distance of 1.1, and 0.8 map units from ct6 to sn5, slightly less than the reported distance of 1.0.

The initial mapping within the cut locus was performed by determining the relative positions of the viable alleles kp, ctn, ctK and ctJcaO with respect to cts. Each mutant to be mapped was made heterozygous with a chromosome having the markers cm, cts and sns and the progeny of these heterozygous females were examined for ctf recombinants in the cases of ctn, ctK and ctJcaO. In the case of kf, all flies that showed recombination between the flanking markers cm and sn5 were progeny tested for the presence of kf. The map distance was calculated as a percentage of the recombination in the cm-ct6 interval for kf or ct6 and in the ct6-sn5 interval for ctK. The results are shown in Table 3. From these data, a preliminary map of the cut locus was constructed as shown in Figure 1. Additionally, it can be seen in Table 3 that cPzo failed to recombine with cts in a sample of 6,500 progeny.

To resolve the nature of ctJcaO, it was tested for recombination with kf and ctK. The results are recorded in Table 3. Based on the ctK mapping experiment, a position midway in the ct6-ctK interval is indicated for ctJcaO. If ctJcaO were a point mutant with this position, a total of 9 recombinants between ctJcaO and kf or

![Figure 1](image-url)

**Figure 1.**—A fine-structure map of the cut locus showing the relative positions of kf, ct6, ctK and ctL with respect to the lethal ct alleles, which concentrate at the right (proximal) end of the locus.
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TABLE 3
Recombination between viable cut alleles

<table>
<thead>
<tr>
<th>Heterozygote</th>
<th>Recombinants</th>
<th>Total recom.</th>
<th>Total flies observed</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm ct^e sn^3</td>
<td>cm + + (1479) + ct^e sn^3 (1304)</td>
<td>2783</td>
<td>209,798</td>
<td>cm-ct^e</td>
</tr>
<tr>
<td>cm ct^e sn^3</td>
<td>cm ct^e + (859) + + sn^3 (760)</td>
<td>1619</td>
<td>209,798</td>
<td>ct^e-sn^3</td>
</tr>
<tr>
<td>cm ct^e sn^3</td>
<td>cm + + (15)</td>
<td>30</td>
<td>46,146</td>
<td>ct^a-ct^e</td>
</tr>
<tr>
<td>cm ct^e sn^3</td>
<td>cm + + (2)</td>
<td>4</td>
<td>55,929</td>
<td>ct^n-ct^e</td>
</tr>
<tr>
<td>cm ct^e sn^3</td>
<td>+ + sn^3 (47)</td>
<td>94</td>
<td>62,582</td>
<td>ct^e-ct^n</td>
</tr>
<tr>
<td>cm kf + +</td>
<td>+ + (0)</td>
<td>0</td>
<td>6,185</td>
<td>kf-ct^n</td>
</tr>
<tr>
<td>cm ct^e sn^3</td>
<td>+ + sn^3 (0)</td>
<td>0</td>
<td>6,487</td>
<td>ct^e-ct^f</td>
</tr>
<tr>
<td>cm ct^e sn^3</td>
<td>+ + (7)</td>
<td>14</td>
<td>12,183</td>
<td>ct^f-ct^n</td>
</tr>
</tbody>
</table>

Each of the viable alleles was made heterozygous with a chromosome carrying the markers cm, sn^3 and either ct^e or ct^n. Heterozygous females were crossed to cm ct^e sn^3 males. Progeny were examined for individuals which were wild type for the cut locus and carried one of the flanking markers, cm or sn^3. The map distances were calculated using the procedure detailed in MATERIALS AND METHODS.

c^t^e would be predicted, but none was observed. It is clear that ct^f020 is associated with an inhibition of exchange within the cut locus. Although the kf^f-ct^n interval gives control frequencies of 0.25 map units, ct^f020 recombines with ct^n with a frequency of only 0.08 map units and shows no exchange with either kf^f or ct^n. This reduced frequency of recombination suggests that ct^f020 represents a cytologically undetectable rearrangement, such as an inversion or deficiency, which involves the left portion of the locus. As discussed below, a double mutant combination of kf^f and ct^n has been examined and fails to mimic the more extreme effects associated with ct^f020. Thus, it seems probable that the expression of both the kf^f and ct^n phenotypes simultaneously by ct^fzo is a result of an inactivation or deletion involving at least the kf^f and ct^n sites within the leftmost portion of the locus.

The position of kf^f in the most distal segment of the locus and the behavior of ct^f020 make the position and nature of the lethal alleles of considerable importance. To ascertain whether the lethal alleles are deletions that remove some or all of the viable mutant sites, experiments were undertaken to determine whether the lethal alleles were recombinationally separable from the viable alleles. Preliminary observations in this laboratory and by G. LeFevre (personal communication) indicated that the lethal alleles all mapped to the right of ct^n. For this reason, the lethal mutations were mapped relative to the rightmost viable allele,
Each of the lethal ct alleles was made heterozygous with a chromosome carrying the mutants ct\(^K\) and sn\(^t\). Heterozygous females were crossed to cm cte sn\(^J\) males. Progeny were examined for wild-type individuals. Map distances were calculated using the procedure detailed in MATERIALS AND METHODS.

The data are presented in Table 4 and the location of the lethal alleles within the locus is indicated in Figure 1. Because the recombination distances were calculated as percentages of the ct\(^K\)-sn\(^t\) interval, the order of the lethals with respect to one another is uncertain. It is clear, however, that all the lethal alleles are confined to a region to the right of ct\(^K\). The demonstration that the lethal ct alleles (with the exception of ct\(^{149}\)) fail to complement with k\(f^t\), although k\(f^t\) is physically separable and distinct from the lethals, clearly implicates the k\(f^t\) site as a component of the cut locus.

Additional insight into the organization of the cut locus was gained from an examination of the interaction of three deficiencies with the various cut alleles and with one another. These data are presented in Table 5. That the restriction of the lethal alleles to a small region at the right (proximal) end of the locus
is real and not an artifact of the limited number of lethal alleles identified and mapped is supported by the behavior of the semi-lethal allele, ct^lethal_0. If lethal sites are distributed throughout the locus, then ct^lethal_0 would be expected to fail to complement with the lethal ct alleles; i.e., heterozygous combinations of ct^lethal_0/ct^lethal_1 should be lethal. This is not the case. Females of such genotypes do not show the reduced viability typical of ct^lethal_0 and, more importantly, they express the same mutant characters as ct^lethal_0, except, unlike ct^lethal_0 homozygotes, such females are fertile. In agreement with these observations is the behavior of ct^lethal_0 in combination with the deletion $Df(1)ct^{1b1}$, which is believed to have one breakpoint within the cut locus at band 7B3 and to extend several bands proximally on Bridges’ (1938) map of the polytene X chromosome. The ct^{1b1}/ct^lethal_0 heterozygote has the same phenotypic characteristics as ct^lethal_0/ct^lethal_1, namely cut wings, kinked femurs and only slightly lower viability. In contrast, ct^lethal_1/ct^{1b1} heterozygotes are lethal, indicating that ct^{1b1} lacks at least one viability site and that it is not uncovered by ct^lethal_0.

$Df(1)RF19$ also casts light on the distribution of lethal sites within the locus. Cytologically, RF19 appears to lack bands 7A4-7B1, but its breakpoints cannot be determined precisely because of a superimposed inversion and an accompanying translocation of X material to the second chromosome. $Df(1)RF19$ exhibits complete complementation in heterozygous combination with ct^a, ct^e and ct^f. However, heterozygous ct^lethal_0/$Df(1)RF19$ and $k^f$/Df(1)RF19 females have normal viability and fertility, but about 20% of each show a good kinked femur-like phenotype. $Df(1)RF19/Df(1)ct^{1b1}$ and $Df(1)RF19/ct^lethal_1$ females also show good viability, and 20 to 30% of them show a kinked femur-like phenotype. It would appear that either the proximal breakpoint of RF19 exerts a position effect on the cut locus (but, if so, only through thekfregion) or that the breakpoint actually interrupts the cut locus and removes the distal portion that includes $k^f$. In either case, no lethal site appears to be affected or removed.

A curious and possibly important interaction appears in the ct^lethal_0/$Df(1)ct^{14}$ combination, which is virtually, but not completely, lethal. $Df(1)ct^{14}$ removes the entire cut locus. It appears from this result that a point mutation lethal is not the functional equivalent of the deletion of the locus. We will return to the possible significance of these results in construction of a model for the cut locus organization and function.

**Developmental analysis of lethal cut alleles**

Experiments were undertaken to determine the period of the life cycle in which lethality occurs in hemi- and homozygous lethal-bearing individuals. The point in question is whether the various lethal alleles exhibit a uniform mode and time of action. For these studies three cytologically normal, lethal alleles were used: ct^114, ct^118144, and ct^149.

The results of these experiments (Table 6) indicate that the initial period of lethality associated with lethal ct alleles commences during late embryogenesis and is completed prior to pupation. Those larvae that pupate appear to do so
successfully and give rise to pharate adults, with few exceptions. These observations do not distinguish between a monophasic pattern of the late embryonic-first instar “boundary lethal” (Hadorn 1951) type and a polyphasic pattern of lethality of the type noted by several investigators (Suzuki 1970; Shannon et al. 1972). Similarly, the lethality of lethal ct alleles during late embryogenesis and early larval life cannot be taken to define the phenocritical period (Haecker 1918). Since we are observing only the end product of the action or inaction of the cut locus, i.e., lethality, we cannot ascertain whether the phenocritical period is prior to or at the time lethality is observed to occur.

In contrast to the lethal mutations that map to the proximal portion of the locus is the mutant ctJcz0. This mutation is semi-lethal as a homozygous female (0–10% escapers, depending on culture conditions), despite its probably being an intralocus deficiency or inactivation for approximately the distal third of the cut locus. An analysis of the lethal pattern of homozygous ctJcz0 females is shown in Table 6. Such females appear to form pupae, but do not produce the expected number of adults. Examination of the pupae shows that the homozygous ctJcz0 females survive to become pharate adults, the majority of which die just prior to, during or just after eclosion.

Double mutants

The cut locus can be resolved into two regions: a recombinationally long region containing the viable alleles and a relatively short region at the proximal end of the locus where all of the lethal alleles map. Mutants in these two regions complement one another for viability, but fail to complement with respect to developmental morphology. To ascertain the interaction of the alleles in determining

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sample size (No. eggs collected)</th>
<th>Observed % eggs hatched</th>
<th>Pupae formed</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>ctJcz0</td>
<td>630</td>
<td>85 (100)</td>
<td>79 (99)</td>
<td>76 (99)*</td>
</tr>
<tr>
<td>Control</td>
<td>597</td>
<td>Observed %</td>
<td>80</td>
<td>77</td>
</tr>
</tbody>
</table>

The percentages for both wild-type and lethal ct alleles represent the observed percentage of the original sample that survive to the indicated stage of development. A corrected percent survival, shown in parentheses, was calculated by dividing the observed percent survival of the experimental class by the observed percent survival of the control class. Since 25% of the individuals in crosses involving lethal ct alleles are expected to die as a result of the lethality associated with the ct locus, the percentage of survivors in these crosses are expected to be approximately 25% less than the percentage of survivors of the wild-type controls.

* Included in these values were 2% (3%) that developed into fully formed pharate adults displaying a ctJcz0 phenotype but died before eclosion, 14.5% (19%) that eclosed, but were dead when counted, and 1.5% (2%) that were alive when scored. For all other genotypes in this column all pharate adults were able to eclose.
the expression of the locus, double mutants were constructed. Since the lethal alleles used, with the single exception of $ct^{149}$, exhibit no complementation with any of the viable or lethal alleles, it has not been possible to select individuals that can be demonstrated to carry two lethal alleles in the \textit{cis} configuration. The visible alleles, in contrast, exhibit a mixture of complementing and non-complementing phenotypic expressions. Thus, in the course of recombination experiments, it was possible to select for recombinants bearing two mutant $ct$ alleles in \textit{cis} arrangement by examining those recombinants that have exchanged appropriate outside markers. Those recombinants were progeny tested and examined for atypical interaction as heterozygotes with the viable alleles and for the distinct phenotypes as hemi- or homozygotes.

It has been possible to distinguish for double-mutant combinations of the viable alleles: $kf^*ct^6$, $kf^*ct^k$, $ct^6ct^k$ and $ct^{140}ct^k$, and one combination between a viable allele and a lethal allele, $kf^*ct^{149}$ (Table 7). Without exception, the double-mutant combinations express the sum of the component mutants, rather than acting synergistically to produce a neomorphic phenotype. Similarly, the double mutants in heterozygous combination with the other $ct$ alleles produce a phenotype that is the sum of the interactions with the component mutants.

\begin{table}
\centering
\caption{Phenotypes of double mutant combinations at the cut locus}
\begin{tabular}{lll}
\hline
Genotype & Phenotype \\
\hline
$k^fct^6$ & cut wings \\
$+ + +$ & cut wings \\
$+ + ct^{149}$ & (wild type) \\
$k^f + + +$ & kinked femur, cut wings \\
$+ ct^6 +$ & (kinked femur) \\
$k^f ct^6 + +$ & (cut wings) \\
$+ + ct^6 +$ & lethal \\
$k^f + + ct^{149}$ & (kinked femur) \\
$+ + +$ & (kinked femur) \\
$+ + ct^{149}$ & lethal \\
$+ + ct^6 +$ & (kinked femur) \\
$+ + +$ & (kinked femur) \\
$+ + +$ & (kinked femur) \\
$+ + +$ & (kinked femur) \\
\hline
\end{tabular}
\end{table}

The phenotypes in parenthesis are predicted on the basis of a model, fully described in the text, in which units I ($kf$) and II ($ct^{viable}$) are regulatory sites controlling a structural gene, unit III, mutational inactivations of which are lethal.
It appears that the presence of a second ct allele in the cis configuration does not influence the expression of the viable ct alleles in either the hemi- or homozygote, nor is there any influence on their complementation pattern with the other alleles. We are left with the distinct impression that the viable alleles are each capable of independent expression in regard to determination of the phenotype.

DISCUSSION

The results of the analysis of the cut locus by recombination, complementation and deletion mapping lead us to conclude, first of all, that the locus is large. It may not be entirely valid to extrapolate from recombination frequency to DNA content, but such calculations provide a useful framework within which other aspects of the locus organization may be viewed. LEFEVRE (1971) has estimated that each map unit represents about 380,000 nucleotide pairs (380 kb) of DNA. This figure is based on the map length of the central portion of the X chromosome and the estimate from Rudkin's (1965) data of the DNA content of that chromosome section. The recombination between cut locus alleles produces distances that add to more than 0.25 map unit between the most distal and proximal sites. It seems reasonable, therefore, that we look upon the cut locus as having great size. Whether it actually is in the range of 100 kb, as these calculations suggest, is open to further investigation.

A second attribute of the locus is the clustering of alleles with similar effects. All of the lethal alleles thus far mapped are positioned in the most proximal portion of the locus. The viable alleles affecting wing morphology map in the central segment, while the only allele affecting leg development occupies the most distal site known. The pleiotropy is of a rather interesting type. Many structures of the adult fly are affected by mutants of the cut locus, but each of the visible alleles affects only a unique subset of structures. When the pattern of pleiotropy is viewed in conjunction with the complementation pattern, it becomes evident that at least three units of distinctly different function can be identified. Unit I is characterized by the kfz mutant site; unit II contains mutant sites ct", ct" and ctK; unit III is populated by lethal alleles only. Lethals such as ct^z may actually form another unit or they could be either a part of unit II or a partial inactivation of unit III, based on their complementation pattern.

Nature of the action of the cut locus

The observation that various ct alleles exhibit changes in the head, thorax, humerus, wings and legs suggests that the locus is active in a process common in the differentiation of most, if not all, of the imaginal discs. The mechanism responsible for the change in wing shape associated with ct mutants has been studied by several investigators. Goldschmidt (1935) first noted the correlation between the wing shape of ct^* and an abnormal pattern of cell death in the wing imaginal discs during metamorphosis. This idea was supported by the more detailed examination of Blang (1942) and the work of Braun (1940), who was able to produce phenocopies of the ct^* by killing a discrete region of the wing
imaginal disc prior to pupation. Later investigators have postulated that the process of cell death is a part of normal morphogenesis and differentiation for the removal of cells not designated for inclusion in a structure (for review, see Saunders 1966). More recently, Spreij (1970) has reported the localization of degenerating cells during the metamorphosis of wild-type leg, wing, and eye-antenna discs of Calliphora erythrocephala. This points to programmed cell death, which may be regarded as a differentiation phenomenon (Saunders and Fallon 1966). These observations combined with the correlation of altered patterns of cell death in the imaginal discs with abnormal adult structures (Fristrom 1969; Spreij 1971) suggests that cell death is a major mechanism in the process of both normal and abnormal insect development. The correlation of ct6 with an abnormal pattern of cell death in the wing imaginal disc (Fristrom 1969) and the association of other ct alleles with defects in adult structures derived from other discs would tend to implicate the cut locus in a process common to the regulation of morphogenesis in several, if not all, of the imaginal discs.

The mutants of the cut locus produce similar types of changes in all of the imaginal discs affected, removing small patches of cells from the periphery of adult structures such as the wing, femurs and head that are formed by the fusion of different groups of cells. This observation has prompted Santamaria and Garcia-Bellido (1976) to suggest that the defect lies in the inability of cells to exhibit the correct properties of cell association, thereby giving rise to abnormal unions of different cell groups at their junction, followed by cell death to remove those cells not incorporated into the structure. These observations would tend to implicate the cut locus in some type of role in cell association, perhaps by a defect in some essential protein needed for adhesion or recognition.

Organization of the cut locus

Several models for the organization and function of the cut locus can be logically constructed. There are two classes, however, that appear most plausible and one of these is strongly supported by the data that were developed in this study.

The first of the two classes of models is that several polypeptides are produced by the cut complex and that each of the units of function identified by complementation tests and phenotype evaluations produces a separate, functionally distinct polypeptide or is involved in the coordinate control of the transcription of the several structural elements. The second general class of models is that there is only one structural element in the complex and that the complementation and pleiotropy must be accounted for in terms of the production of and/or the functional interactions of this single product. We favor a model of this latter class because the analysis of Df(1)RF19, Df(1)ct15, Df(1)ct16 and ct1420 appears inconsistent with the existence of several structural elements encoded in the complex.

We believe the phenotypes of ct1420, Df(1)RF19, Df(1)ct15 and Df(1)ct14 in various heterozygous combinations (Table 5) argue against a multi-element organization that assigns a structural role to units I and II, either in addition
to unit III or under the control of unit III (Figure 2). If the three units are
co-transcribed, the failure of mutants in unit III to complement mutants in
units I and II would require that mutants of unit II exert a polar effect. Evidence
is strongly suggestive that ct^{1020} is defective or deficient for parts of units I and
II. Thus, one would predict, on the basis of a co-transcription model, that ct^{1020}
and unit III mutations would have a similar effect on the function of units I
and II. Although ct^{1020} has reduced viability, there are some survivors that show
a kinked-femur, cut-wing, sterile phenotype; whereas, ct^{1020}/ct^{lethal}
heterozy-
gotes are normal for viability and fertility, though they have a kinked-femur,
cut-wing phenotype. Contrast this, however, to the virtually complete lethality
of ct^{1020} in combination with a deficiency for the entire cut locus, ct^{14}. That a
deficiency fails to complement in every respect with ct^{1020}, yet cut lethals (defec-
tive in unit III) complement ct^{1020} in viability and fertility, suggests two things.
First, a deletion of the entire cut locus and a lethal point mutation are not equiva-
lent, though they both result in lethality. Second, since units I and II are both
impaired in ct^{1020} and this impairment produces mutant morphology and
sterility but not lethality, it seems likely that units I and II are nonvital units.
Unit III is an indispensible part of the locus; therefore, it is unlikely that its
role is to regulate the activity of two nonvital elements.

In our opinion the most logical interpretation of these data is that there is a
single structural element in the cut locus complex, unit III. That the product
of this element is indispensable to normal development is an assumption that
appears justified. We propose that the remainder of the locus, units I and II,
act as tissue- and stage-specific elements regulating the activity of unit III. It is
postulated that these sequences serve for initiation of transcription in different
cell types and/or at different stages of development, or that they function in the
processing and maturation of the transcript.

Recall that mutations in units I and II complement each other completely,
indicating that these units function independently of one another. They fail to
complement mutations of unit III, however, so that it is clear they have their
effect by influencing unit III in some fashion. Heterozygous combinations I/III
and II/III produce very different phenotypes. This is precisely the result pre-
dicted if unit I regulates the activity of unit III in different tissues or at different
developmental stages than does unit II.

A significant portion of the locus, units I and II, has been suggested to be
involved in regulation; however, we can only speculate about the various mech-
anisms by which such regulation is accomplished. JOHNSON (1976) has exten-

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**Figure 2.**—A provisional interpretation of the organization of the cut locus into two regu-
larly units (I and II) and one structural unit (III).
sively examined the implications of the observed complementation patterns for various models of regulation. Two mechanisms may be mentioned. If one envisions regulation occurring by differential transcription from multiple initiation sites, it is predicted that regulatory sequences should act only in *cis*.

Phenotypes of selected double mutants can be predicted on the basis of *cis*-acting regulatory elements that complement other regulatory elements of the same locus. Table 7 lists some of these combinations. Those that have been synthesized correspond to predicted phenotypes, but three interesting combinations have not yet been recovered. If the phenotypes are as predicted when these types are synthesized, we believe that the case for *cis*-acting regulatory sequences that are independent of each other is strongly supported. Should these predictions not be realized, the argument that units I and I₁ are regulatory still appears to be well founded, but an interpretation based on *cis*-acting control by multiple sites for transcription initiation would clearly not be supported and other mechanisms of regulation would be indicated.

Alternatively, for example, we have suggested that units I and I₁ might be involved in the processing of the transcript to produce mature mRNA. Under such a scheme, fragments of RNA used in forming the mature message or used to activate other loci could be generated from transcripts made from either or both chromosomes. Such sequences would function in either *cis* or *trans* and could appear to function independently of each other. A system such as this could form the basis for an explanation of the remarkable phenomenon of transvection described by Lewis (1954).

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