The Genetic and Molecular Organization of the *Dopa decarboxylase* Gene Cluster of *Drosophila melanogaster*

Dean G. Stathakis,[1] Ellen S. Pentz,[2] Mark E. Freeman,[3] John Kullman,[4] Gerald R. Hankins,[5] Nathaniel J. Pearlson and Theodore R. F. Wright

Department of Biology, University of Virginia, Charlottesville, Virginia 22903-2477

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ABSTRACT

We report the complete molecular organization of the *Dopa decarboxylase* gene cluster. Mutagenesis screens recovered 77 new *Df(2L)TW130* recessive lethal mutations. These new alleles combined with 263 previously isolated mutations in the cluster to define 18 essential genes. In addition, seven new deficiencies were isolated and characterized. Deficiency mapping, restriction fragment length polymorphism (RFLP) analysis and *P*-element-mediated germline transformation experiments determined the gene order for all 18 loci. Genomic and cDNA restriction endonuclease mapping, Northern blot analysis and DNA sequencing provided information on exact gene location, mRNA size and transcriptional direction for most of these loci. In addition, this analysis identified two transcription units that had not previously been identified by extensive mutagenesis screening. Most of the loci are contained within two dense subclusters. We discuss the effectiveness of mutagens and strategies used in our screens, the variable mutability of loci within the genome of *Drosophila melanogaster*, the cytological and molecular organization of the *Ddc* gene cluster, the validity of the one band-one gene hypothesis and a possible purpose for the clustering of genes in the *Ddc* region.

IN prokaryotes and fungi operons group enzymes for a metabolic pathway or component proteins for a complex structure to facilitate control of individual gene expression. However, the clustering of genes with related biochemical or structural functions is the exception rather than the rule in higher eukaryotes. Thus, genes encoding enzymes in the same metabolic pathways (MACINTYRE and O'BRIEN 1976), elements of common developmental pathways (GAUL and JACKLE 1990) and genes encoding interacting myofibrillar components (BERNSTEIN *et al.* 1993) are dispersed throughout the genome. However, gene clustering is still found in higher eukaryotes with extremes ranging from a single duplication event generating two adjacent homologous genes to an extensive repetitive grouping where hundreds of identical genes lie in a tandem array.

In *Drosophila melanogaster*, gene clusters can be classified into three categories: reiterated gene clusters, gene complexes, and dense gene clusters. Reiterated gene clusters contain multiple copies of a tandemly repeated transcription unit that encompass large expanses of DNA with lengths over 500 kb being common. The transcription unit itself may contain only a single gene or several genes. While intergenic spacers are common, the transcription unit is largely devoted to the coding regions of the genes themselves. Therefore, these regions exhibit high transcriptional activity. In addition, while polymorphic changes do exist, the products produced by reiterated gene clusters are essentially the same, if not identical. The repetition of these genes is almost certainly due to the need for large amounts of their product. The multiple copies of the genes encoding the histone proteins at 39DE (LIFTON *et al.* 1977), the 18S and 28S ribosomal RNA genes located in the nucleolus organizers on the *X* and *Y* chromosomes (LONG and DAWID 1980) and the 5S rRNA genes clustered at 56F (TSCHUDI and PIRROTTA 1980) are examples of reiterated gene clusters.

A gene complex is a group of functionally related genes defined by their complex pattern of intergenic complementation. Gene complexes also span large regions of DNA, usually from 100 to 300 kb, but very little of this region is used by the actual transcription unit encoding the protein. Instead, most of this region contains complex arrays of *cis*-acting regulatory elements, some found well beyond the transcription units themselves. These regulatory elements are necessary for the sophisticated expression patterns required by these genes during development. Mutations in the regulatory elements are most likely responsible for the complex

Corresponding author: Theodore R. F. Wright, Department of Biology, University of Virginia, Gilmer Hall, Charlottesville, VA 22903-2477. E-mail: trw3j@faraday.clas.virginia.edu.

[1] *Present address:* Developmental Biology Center, University of California, Irvine, Irvine CA 92717-2275.

[2] *Present address:* Department of Pediatrics, University of Virginia Health Sciences Center, Charlottesville, VA 22903.

[3] *Present address:* Pharmacia Biotech, Inc., 800 Centennial Ave., Piscataway, NJ 08855-1327.

[4] *Present address:* Boehringer Mannheim Corporation, 9115 Hague Rd., Indianapolis, IN 46250-0414.

[5] *Present address:* Department of Radiation Oncology, Duke University Medical Center, Durham, NC 22710-3914.

genetic interactions observed in these genes. Well documented examples of gene complexes are the Bithorax Complex at 89E (DUNCAN 1987; PEIFER *et al.* 1987), the Antennapedia Complex at 84AB (KAUFMAN *et al.* 1990), the Achaete-Scute Complex at 1B (ALONSO and CABRERA 1988) and the Enhancer of Split Complex at 96F (SCHRONS *et al.* 1992; KNUST *et al.* 1992).

By far the most common type of gene clustering involves a dense spatial grouping of genes within a small defined region. These gene clusters usually encompass no more than 20 kb of DNA. Although most of the genes within these clusters are functionally and structurally related, dense clusters containing genes that do not share structural similarity also exist. While dense gene clusters of functionally and structurally related genes most likely arose from gene duplication events, it is not known how or why unrelated genes became clustered. Some examples of dense gene clusters include the heat shock protein gene cluster at 67B (AYME and TISSIÈRES 1985), the *serendipity-janus* gene cluster at 99D (VINCENT *et al.* 1985; YANICOSTAS *et al.* 1989), the salivary gland-specific gene cluster at 71E (RESTIFO and GUILD 1986), the larval cuticle protein gene cluster at 44D (SNYDER *et al.* 1982; SNYDER and DAVIDSON 1983), the cuticle protein gene cluster contained within the Antennapedia Complex at 84AB (PULTZ *et al.* 1988; KAUFMAN *et al.* 1990), the *cecropin* gene cluster at 99E (KYLSTEN *et al.* 1990), the z600-*gonadal*-Eip28/29 gene cluster at 71CD (SCHULZ and BUTLER 1989), the two chorion protein gene clusters at 7F and 66D (SPRADLING *et al.* 1980; SPRADLING 1981) and the salivary gland glue protein gene cluster at 68C (MEYEROWITZ and HOGNESS 1982; CROWLEY *et al.* 1983).

The *Dopa decarboxylase* (*Ddc*) gene region is arbitrarily defined by *Df(2L)TW130,* 37B9-C1,2;D1-2, an 8–12 band deletion near the proximal end of the left arm of the second chromosome (WRIGHT *et al.* 1981). The clustering of functionally related genes within this region was originally proposed when genetic and biochemical evidence suggested that two closely linked loci, *Ddc* and *amd,* were both involved in catecholamine metabolism (WRIGHT *et al.* 1976b). Subsequent work identified a total of 18 genetic complementation groups within this region: *hook* (*hk*), *l(2)37Ba* (*Ba*), *l(2)37Bb* (*Bb*), *l(2)37Bc* (*Bc*), *l(2)37Bd* (*Bd*), *l(2)37Be* (*Be*), *Dox-A2* (formerly designated *l(2)37Bf*), *l(2)37Bg* (*Bg*), *amd, Ddc, l(2)37Ca* (*Ca*), *l(2)37Cb* (*Cb*), *l(2)37Cc* (*Cc*), *l(2)37Cd* (*Cd*), *l(2)37Ce* (*Ce*), *brat* (formerly designated *l(2)37Cf*), *l(2)37Cg* (*Cg*), *fs(2)TW1* (WRIGHT 1987a). Mutations in most of these loci result in surprisingly similar morphogenetic defects of the cuticle or catecholamine-related abnormalities. Fifteen loci affect cuticle formation, cuticle sclerotization or cuticle melanization, with 10 of these same loci (including *Ddc* and *amd*) forming melanotic psueudotumors in mutant larvae or pupae, a symptom suggesting abnormal catecholamine metabolism (WRIGHT 1995). HPLC studies on seven of these psueudotumor-forming mutations revealed that all exhibited aberrant catecholamine pool levels during prepupal and pupal development (T. HOMYK and T. R. F. WRIGHT, unpublished data; reviewed in WRIGHT 1995). Therefore, the *Ddc* gene cluster may represent a large cluster of functionally related genes involved in catecholamine metabolism. Extensive work on specific loci supports this assertion. First, the considerable work done on *Ddc* and *amd* demonstrated their enzymatic involvement in catecholamine metabolism (reviewed in WRIGHT 1987b, 1995). Second, studies on *Dox-A2* shows this locus encodes the structural gene for the A2 component of the phenol oxidase enzyme complex (PENTZ *et al.* 1986; PENTZ and WRIGHT 1986). This enzyme complex converts all known catecholamines to their respective quinones (substrate precursors of cuticle formation) using either the sclerotization or melanization pathways (HOPKINS and KRAMER 1992). The genes within the *Ddc* region, therefore, may represent an unusual example in higher eukaryotes of a gene cluster whose enzymatic products are required for a common biochemical pathway. By virtue of the fact the *Ddc* locus was the first gene in this cluster whose function was identified, this region is referred to as the *Ddc* gene cluster.

From the beginning, it was realized that the loci within this region were organized in a tightly compact arrangement (WRIGHT *et al.* 1981). The objective of this report is to provide a fine-scale molecular and cytological map of the loci within the *Ddc* gene cluster. We provide a complete physical and genetic description of a 162.5-kb region of cloned genomic DNA that contains the entire *Ddc* gene cluster, and we describe a number of new complementation groups and chromosomal rearrangements and the complete mapping of the 18 known loci and three unmutated transcription units. The most striking feature of this cluster is the location of the majority of these genes within two subclusters. The distal subcluster consists of six loci and two transcription units contained in no more than 27.4 kb, while the proximal subcluster contains eight loci and one transcription unit within 23.0 kb. In addition, the detailed mapping of this region allows us to assign more precisely specific genes to specific chromomeres. These assignments clearly demonstrate that many of the chromomeres within the 37B10-C7 contain more than two genes each. Furthermore, our *P*-element-mediated germline transformation experiments indicate each locus within this gene cluster can function independently of its neighbors, suggesting global coordinate control of gene expression for loci within the *Ddc* gene cluster is unlikely.

MATERIALS AND METHODS

Strains, stock maintenance and cultures: Except as described below, all *D. melanogaster* mutations, aberrations and balancer chromosomes used were previously described

(WRIGHT *et al.* 1976a,b, 1981, 1982; LINDSLEY and ZIMM 1992). The wild-type stock used was an Oregon-R strain originally obtained from the Johns Hopkins stock collection in 1965 and maintained since at the University of Virginia. Table 4 indicates mutants kindly provided by G. BEWLEY, J. BRITTNACHER, R. HODGETTS, D. LINDSLEY, C. NÜSSLEIN-VOLHARD and T. SCHÜPBACH. Chromosomal deficiencies used in this study are listed in Table 5. Unless otherwise noted, all other mutant and genetically recombined strains used in this study were generated in this lab using standard genetic procedures (ASHBURNER 1989a,b). All *D. melanogaster* second chromosome lethals and deficiencies were maintained as balanced lethal stocks with *CyO* as the balancer chromosome. Most crosses were made in shell vials on a yeast-agar-dextrose vial food medium (CARPENTER 1950).

Deficiency screens: Table 1 summarizes the screens producing deficiencies within the *Ddc* region. The "*Ddc* escaper" deficiency screens are based on the observation that most *Ddc* heteroallelic complementing heterozygotes and temperature-sensitive hemi- and homozygotes raised at partially restrictive temperatures exhibit an adult escaper phenotype (WRIGHT *et al.* 1976b, 1982). These adult escapers are very lightly pigmented, exhibit significantly reduced DDC activity and have a developmental time that is protracted by at least 4 days. This distinctive adult mutant phenotype easily distinguishes escapers from their heterozygous siblings. Thus newly induced *Ddc* deficiencies and point mutations (WRIGHT *et al.* 1982) are recovered as escapers over Ddc^{ts2} that must then survive long enough to produce progeny. Males heterozygous for lethal-free *cn bw* or *pr* second chromosomes were irradiated with 4000 r of γ-rays or fed 5 mM diepoxybutane (DEB) and mated (Day 0) at 22° *en masse* to Ddc^{ts2} homozygous virgin females. From Day 15 to Day 21 all progeny were inspected for the *Ddc* escaper phenotype.

Genetic screens: Tables 2 and 3 summarize the screens producing recessive lethal and female sterile mutations within the *Ddc* gene cluster, respectively. Mutagenesis screening procedures for the E, 200, 300A, 300B, 400, 500, 600, 7000/7100, 7300/7400, 7600, and 7800 *Ddc* escaper screens were previously reported (WRIGHT *et al.* 1976b, 1981, 1982).

Bacteriophage λ library screening, DNA isolation and stocks: The four bacteriophage λ-*Drosophila* hybrids (λLL18, λCS6.12, λCS2.20 and λCS2.27) covering 25.5 kb proximal to λ16 were isolated from the Canton-S DNA library (MANIATIS *et al.* 1978) using the chromosomal walking technique (BENDER *et al.* 1983). Bacteriophage λ growth, purification and DNA isolation followed standard procedures (SAMBROOK *et al.* 1989) as described previously (PENTZ and WRIGHT 1986). Isolation of the λ-series and λRS-series of bacteriophage λ-*Drosophila* hybrids were previously reported (GILBERT *et al.* 1984; STEWARD *et al.* 1984; PENTZ and WRIGHT 1986). DNA coordinates for clones are as follows: λRS25 −95.7:−78.6, λRS24 −82.1:−67.3, λRS23 −75.2:−62.3, λRS22 −72.7:−56.1, λRS21 −65.4:−49.4, λ20 −57.5:−44.1, λ11 −46.6:−32.7, λ7 −36.8:−21.1, λ5 −23.3:−8.8, λ1 −15.6:+1.0, λ2 −6.4:+9.3,λ4 −2.1:+9.4, λ3 −1.2:+12.4, λ6 +0.7:+15.0, λ13 +14.0:+29.7, λ16 +27.1:+41.3, λCS6.12 +35.8:+50.8, λLL18 +39.6:+54.0, λCS2.20 +46.4:+59.7 and λCS2.27 +51.6:+66.8.

Plasmid DNA extraction, purification and cloning procedures: Isolation of plasmid DNA, purification, restriction enzyme digestion and gel electrophoresis and other common molecular biology methods followed standard procedures (SAMBROOK *et al.* 1989). Desired fragments were isolated from gels using the GeneClean method under conditions specified by the supplier (Bio 101, La Jolla, CA). Preparation and transformation of competent *Escherichia coli* strains and identification of bacterial colonies containing recombinant plasmids followed standard molecular biology protocols (SAMBROOK *et al.* 1989).

Restriction fragment length polymorphism (RFLP) analysis: Isolation of total genomic DNA from parental and mutant stocks, restriction enzyme digestion, gel electrophoresis, DNA transfer and probe hybridization followed conditions described previously (PENTZ and WRIGHT 1986).

***P*-element-mediated germline transformation analysis:** Isolated genomic fragments were subcloned into one of the following vectors: pCaSpeR (PIROTTA *et al.* 1985), pUChsNEO 7.2, pUChsNEO 5.2 (STELLER and PIROTTA 1985) or pW8 (KLEMENZ *et al.* 1987). *P*-element-mediated germline transformation experiments followed standard injection procedures (SPRADLING 1986) into homozygous *y* $w^{67c23(2)}$ (STELLER and PIROTTA 1985) (pCaSpeR and pW8) or Oregon-R (pUChsNEO) flies. The plasmid construct pπ25.7wc was used as the source of transposase (KARESS and RUBIN 1984). Transformed flies were identified by the appearance of red eyes (pCaSpeR or pW8) or survival on food containing 600–700 μg/ml neomycin derivative G418 (pUChsNEO). Transformed lines were tested for the ability to rescue mutants by crossing transformant flies carrying a *CyO*-balanced second chromosome with selected *CyO*-balanced mutants.

cDNA library screening and isolation: A pNB40 12–24 hr embryonic library (BROWN and KAFATOS 1988), two λgt10 embryonic libraries (3–12 hr and 12–24 hr) (POOLE *et al.* 1985), a λgt10 crawling third instar larval library (WHARTON *et al.* 1985) and a λgt10 24–48 hr pupal library (WHARTON *et al.* 1985) were screened, and clones were isolated using standard methods (SAMBROOK *et al.* 1989).

RNA isolation and Northern blot analysis: Total RNA extraction, gel electrophoresis, RNA transfer and hybridization followed procedures described previously (PENTZ and WRIGHT 1986).

RESULTS

Generation of overlapping deficiencies in the *DOPA decarboxylase* region: A total of 38 deletions with breakpoints in or close to this region were obtained and used in this study (Table 5). We recovered 29 deficiencies from a series of 20 screens conducted to isolate overlapping deficiencies within the *Ddc* region (Table 1). More than 550,000 chromosomes were screened after treatment with six different mutagenic agents: X-rays, γ-rays, EMS, formaldehyde (F), diepoxybutane (DEB) and *P*-element-induced hybrid dysgenesis. Screening strategies included the recovery of chromosomes lethal over *Df(2L)TW50* or *Df(2L)TW130*, the reversion of the dominant marker *Tufted* (*Tft*, 2-53.6, 37A3-6) or w^+ [contained in transposable element TE37 (formerly TE42) inserted into the 37C3-4 region], the uncovering of three recessive visible mutations flanking the *Ddc* region: *reduced ocelli* (*rdo*, 2-53, 36E4-F1), *hook* (*hk*, 2-53.9, 37B10) and *purple* (*pr*, 2-54.5, 38B1-2), and the recovery of *Ddc* escapers (see MATERIALS AND METHODS).

Nine other deletions used in this study were generously provided by other investigators. We obtained *Df(2L)hk-UC1*, *Df(2L)hk-UC2* and *Df(2L)NST* from J. L. MARSH, *Df(2L)hk39*, *Df(2L)Sd37*, *Df(2L)Sd57* and *Df(2L)-Sd77* from B. GANETZKY, *Df(2L)TE37B7* from J. G. BRITTNACHER, and *Df(2L)OD15* from D. CONTAMINE.

Summary of *Df(2L)TW130* F_2 lethal screens: Five sep-

TABLE 1

Summary of deficiency screens in the *Ddc* region

Screen series[a]	Parent chromosome	Mutagen[b]	Screening markers: Reverted	Screening markers: Uncovered[d]	Chromosomes screened	Deficiencies recovered[c]	Recovery rate (%)	Deficiency designation[d]
DF 1[1]	*Tft l(2)74i*	X-ray	*Tft*	*rdo hk pr*	~25,000	5	0.020	*TW1, TW2, TW3, TW9, TW12*
DF 2[1]	*Tft l(2)74i*	X-ray	*Tft*	*rdo hk pr*	~25,000	3	0.012	*TW50, TW65, TW84*
DF 3[1]	*cn bw*	X-ray	—	*rdo hk pr*	~50,000	6[1]	0.012	*TW119, TW130, TW137, TW150, TW158, TW161*
DF 4[1]	*rdo hk pr*	EMS	—	*TW50* lethal	4,000	3	0.074	*E53, E55, E71*
DF 5	*b Tft l(2)74i*	γ-ray	*Tft*	*rdo hk pr*	Unknown	2	Unknown	*TW201, TW202*
DF 6[2]	*Sco TE42, w*$^+$	F/γ-ray	*w*$^+$	*rdo hk pr*	~100,000	1	0.001	*TE42-1*
DF 7	*nub b TE42, w*$^+$	F/γ-ray	*w*$^+$	*rdo hk pr*	~1,000	0	0	
DF 8[2]	*al dp b Tft* / *Sco TE42, w*$^+$	γ-ray	Tft, w$^+$	*rdo hk pr*	~31,000	1	0.003	*TW203*
DF 9	Oregon-R	EMS/F	—	*TW130* lethal	4,603	1	0.020	*TW330*
DF 10	*cn bw*	γ-ray	*Ddc*$^+$	*Ddc*^{ts2}	7,820	0	0	
DF 11	*cn bw*	γ-ray	*Ddc*$^+$	*Ddc*^{ts2}	6,029	0[2]	0	
DF 12	*cn bw*	γ-ray	—	*rdo hk pr*	~75,000	15	0.020	*VA1, VA2, VA3, VA4, VA5, VA6, VA7, VA8, VA10, VA11, VA12, VA13, VA14, VA15, VA16*
DF 13	*pr*	γ-ray	*Dcd*$^+$	*Ddc*^{ts2}	~40,000	2[3]	0.005	*VA17, VA18*
DF 14	*pr*	EMS/γ-ray	—	*TW130* lethal	4,029	2	0.050	*VA19, VA22*
DF 15	*pr*	γ-ray	—	*TW130* lethal	1,093	0	0	
DF 16	*pr*	DEB	—	*TW130* lethal	3,250	0	0	
DF 17	*pr*	DEB	*Ddc*$^+$	*Ddc*^{ts2}	~55,000	2[4]	0.004	*VA20, VA21*
DF 18	*pr*	DEB	*Ddc*$^+$	*Ddc*^{ts2}	~40,000	2	0.005	*VA23, VA24*
DF 19	*pr*	DEB	*Ddc*$^+$	*Ddc*^{ts2}	~45,000	0[5]	0	
DF 20	*cn bw*	*Harwich P*	*Ddc*$^+$	*Ddc*^{ts2}	~40,000	1	0.003	*VA25*
Totals					~550,000	46	0.008	

[a] Published information on these deficiencies are found in the following: 1, WRIGHT *et al.* (1976a); 2, WRIGHT *et al.* (1981).

[b] X-ray, young adult males (0–48 hr) were exposed to 4000 r X-rays; EMS, young adult males (0–48 hr) were fed 25 mM EMS (LEWIS and BACHER 1968); γ-ray, young adult males were exposed to 4000 r γ-rays; F/γ-rays, larvae were fed 100 mM formaldehyde (F) as first instars (AUERBACH 1956) and then exposed to 4000 r γ-rays as young adults; F/EMS, larvae were fed formaldehyde (F) as first instars and 25 mM EMS as young adults; EMS/γ-ray, young adult males were fed 25 mM EMS and then exposed to 4000 r γ-rays; DEB, young adult males were fed 5 mM diepoxybutane (DEB) (SHUKLA and AUERBACH 1980; OLSEN and GREEN 1982).

[c] The following alleles were isolated from the indicated deficiency screens: 1, *hk*^{127} and *hk*^{131}; 2, *Ddc*^{17} and *Ddc*^{18}; 3, *Ddc*^{27}, *Ddc*^{28} and *Ddc*^{29}; 4, *Ddc*^{35}; 5, *Ddc*^{40}, *Ddc*^{41} and *Ddc*^{42}.

[d] For simplicity, all deficiencies are abbreviated without the *Df(2L)* designator, i.e., *Df(2L)TW130* is *TW130*.

arate F_2 mutagenesis screens (7000/7100, 7300/7400, 7800, 7900 and 8000) were run in an ongoing effort to isolate recessive lethal mutations within the *Df(2L)-TW130* region (Table 2). The 7800-series screen was run on chromosomes previously examined for *Ddc* escapers (WRIGHT *et al.* 1982). These five screens resulted in the acquisition of 77 additional lethal mutations. To date this investigation has screened over 33,000 chromosomes using various mutagenesis schemes to isolate recessive lethal mutations (Table 2). From these 12 screens, we isolated 239 *Df(2L)TW130* nonconditional lethal mutations, 19 conditional lethal mutations, two female sterile mutations and two recessive visible mutations. In addition, 11,739 of these same chromosomes were also screened for female sterile mutations in the *Ddc* region, resulting in the isolation of 13 female sterile mutations (Table 3). Of the 258 nonconditional and conditional lethal mutations, 129 were induced with EMS as the sole mutagen, three with γ-rays and two with DEB. Seventy-two of these mutations were recovered in chromosomes exposed to both EMS and γ-rays and 52 in chromosomes treated with both EMS and formaldehyde (Table 2). Of the 14 female sterile mutations isolated, eight were induced with EMS as the sole mutagen, five with both EMS and γ-rays and one with EMS and formaldehyde (Tables 2 and 3). Lastly, the two EMS-γ-ray-induced, recessive visible mutations were from the *hk* locus (Table 2).

In addition to the recessive lethal mutagenesis screens, a number of other mutations were also available for study. First, our laboratory previously isolated 11 *amd* alleles (SPARROW and WRIGHT 1974; WRIGHT *et al.* 1976b) and one *Ddc* allele (BISHOP and WRIGHT 1987). Second, our deficiency screens resulted in the recovery of nine *Ddc* and two *hk* alleles (Table 1). Third, 28 EMS-induced *Df(2L)TW130* lethals, two nonconditional lethals, nine female steriles and two *hk* alleles were kindly provided to us by other laboratories (Table 4). Lastly, one lethal allele was spontaneous in origin (*Bc*^{10}). In total, 339 mutations were available for study: 289 nonconditional lethals, 21 conditional lethals, 23 sterile mutations and six *hk* mutations.

Genetic loci within the *Ddc* gene cluster: Intergenic complementation was done to assign all newly isolated

TABLE 2

Summary of lethal screens over *Df(2L)TW130*

Screen series[a]	E	200	300A	300B	400	500	600	7000/7100	7300/7400	7800[f]	7900	8000	Totals
Parent chromsome	*rdo hk pr*	*pr*	Oregon-R	Oregon-R	Oregon-R	Oregon-R	Oregon-R	*pr*	*pr*	*pr*	*pr*	*or*	
Mutagen[b]	EMS	EMS	EMS/F	EMS/F	EMS/F	EMS/γ-ray	EMS	γ-ray	EMS/γ-ray	DEB	γ-ray	EMS/γ-ray	
Temperature	25°	30°	25°	30°	30°	25°	25°	25°	25°	25°	25°	25°	
Mosaics[c]	Yes	No	No	Yes	No	No	No	No	No	No	No	No	
Chromosomes screened	4,060	3,916	4,603	[3,916][e]	1,157	88	4,456	194	4,247	2,064	1,093	3,213	33,007
Lethals recovered[d]	29 (1/1)	47 (4/1)	29 (5)	18 (4)	5	2	54 (3)	2	45 (2)	2	1	26	261 (19/2)
Mutation rate ($\times 10^{-3}$)	7.1	12.0	6.3	4.6	4.3	22.7	12.1	10.3	10.8	1.0	0.9	8.1	7.9
						Number of alleles[g]							
Locus													
hook	0	0	0	0	0	0	0	0	1	0	0	1	2
l(2)37Be	0	0	0	0	0	0	2*	0	2	0	0	1	5
l(2)37Bc	4	6 (1)	1	3	0	0	5	0	3 (1)	0	0	3	25 (2)
l(2)37Ba	0	1	1	0	0	1	3	1	0	0	0	2	9
l(2)37Bb	4	4	0	1	0	0	1	0	1	0	0	0	11
Dox-A2	0	0	0	0	0	0	0	0	3	0	0	1	4
l(2)37Bg	0	0	0	0	0	0	0	0	1	0	0	0	1
l(2)37Bd	0	1	2	1	1	0	1	0	1	0	0	0	7
l(2)amd	2	4	5	0	0	0	6	0	6	0	0	5	28
l(2)Ddc	8	3 (1)	5 (1)	1	1	0	10 (3)	0	5	0	0	2	35 (5)
l(2)37Cc	0	5	1 (1)	2 (1)	0	0	2	0	5	1	0	2	18 (2)
l(2)37Cb	2	4 (1)	0	4 (1)	0	1	8	0	7	0	0	0	26 (2)
l(2)37Cd	0	1	4 (1)	0	0	0	2	1	2	0	0	1	11 (1)
l(2)37Ca	3	11 (1)	6	3 (1)	3	0	8	0	5 (1)	0	1	2	42 (3)
l(2)37Ce	2	1	0	1	0	0	1	0	0	0	0	2	7
l(2)37Cg	0	0	1 (1)*	0	0	0	1*	0	1	0	0	1	4 (1)
l(2)brat	4 (1/1)	5	3 (1)	2 (1)	0	0	4	0	3	1	0	3	25 (2/1)
fs(2)TW1	0	1 (0/1)	0	0	0	0	0	0	0	0	0	0	1 (0/1)

[a] We previously reported data from the E series screen over *Df(2L)TW50* (WRIGHT *et al.* 1976b) but included it again for completeness. We also updated the data from the 200, 300A, 300B, 400, 500 and 600 series screens reported earlier (WRIGHT *et al.* 1981) to include alleles previously unassigned.

[b] EMS, young adult males (0–48 h) were fed 25 mM EMS (LEWIS and BACHER 1968); F/EMS, larvae were fed 100 mM formaldehyde (F) as first instars (AUERBACH 1956) and 25 mM EMS as young adults; EMS/γ-ray, young adult males were fed 25 mM EMS and then exposed to 4000 r γ-rays; γ-ray, young adult males were exposed to 4000 r γ-rays; DEB, young adult males were fed 5 mM diepoxybutane (DEB) (SHUKLA and AUERBACH 1980; OLSEN and GREEN 1982).

[c] An extra generation was included in screen to resolve for mosaics (EPLER 1966; JENKINS 1967).

[d] Figures in parenthesis indicate alleles that are conditional lethals/sterile mutations.

[e] Figure in brackets indicates the number of chromosomes tested for lethality over *Df(2L)TW130* both before (300A) and after (300B) being passed through the extra generation to resolve for mosaics.

[f] These chromosomes were originally generated from *Ddc* escaper screens (WRIGHT *et al.* 1982) that were subsequently screened for lethals over *Df(2L)TW130.*

[g] Numbers with asteriks (*) indicate alleles that were previously unassigned (WRIGHT *et al.* 1981).

TABLE 3

Summary of female sterility screens over *Df(2L)TW130*

Screen series[a]	300A	600	7500/7600	Totals
Parent chromosome	Oregon-R	*pr*	*pr*	
Mutagen[b]	EMS/F	EMS	EMS/γ-ray	
Temperature	25°	25°	25°	
Chromosomes screened	3,555	4,385	3,800	11,739
Steriles recovered	1	7	5	13
Mutation rate ($\times 10^{-3}$)	0.3	1.6	1.3	1.1
		Number of alleles		
Locus				
l(2)37Bc	0	0	2	2
l(2)37Ba	1	0	0	1
Dox-A2	0	0	1	1
brat	0	1	0	1
fs(2)TW1	0	6	2	8

[a] See footnote [a] Table 2.
[b] See footnote [b] Table 2.

lethal alleles recovered from the five 7000–8000 series screens to complementation groups (WRIGHT *et al.* 1976b, 1981, 1982). Ten of the 77 new lethal mutations complemented all previously described groups and further testing resulted in their being assigned to four new complementation groups. These new groups were designated as *l(2)37Be, l(2)Dox-A2, l(2)37Bg* and *l(2)37Cg*. In addition, four previously unassigned lethals (WRIGHT *et al.* 1981) fell into these new complementation groups, two belonging to the *Be* locus and two to the *Cg* locus (Table 2). All 339 mutations were genetically assigned to one of 18 loci by intergenic complementation and all are reported in Table 4, including those alleles obtained through other screens or sources.

Mutations in 16 of the 18 loci are lethal with the vast majority of the 318 mutant alleles being lethal as hemizygotes over *Df(2L)TW130*. One of the genes, *fs(2)TW1*, is a recessive female sterile with all 15 mutant alleles having no effect on zygotic viability. Interestingly, five recessive lethal loci also have alleles that are female sterile: *Bc, Ba, Dox-A2, Cc,* and *brat* (Tables 3 and 4). One of these, Cc^{13} is a dominant female sterile mutation, while another, $Dox\text{-}A2^{mfs1}$, is both male and female sterile. The other nonessential gene is the recessive visible mutation *hk* in which all six alleles examined produce a hooked bristle phenotype.

Genetic mapping of loci within the *Ddc* gene cluster: All loci were assigned to ten distinct intervals by crossing representative mutant alleles from each of the 18 loci to the overlapping deficiencies (Table 6). However, this mapping only determined the linear order of five loci with respect to adjacent loci (*hk, Bg, Cb, brat* and *fs(2)TW1*), with the remaining 13 loci falling into intervals containing two, three or four loci each. All of these loci were subsequently and unequivocally ordered by means of RFLP analysis or *P*-element-mediated germline transformation experiments (see below).

Cloning of DNA from the *Ddc* gene cluster: *Ddc* was the first gene cloned from this region (HIRSH and DAVIDSON 1981). Clones covering 60 kb distal and 40 kb proximal to *Ddc* were isolated, and the breakpoints of *Df(2L)VA12, Df(2L)VA13, Df(2L)VA17* and *Df(2L)VA18* mapped within this 100-kb region (GILBERT and HIRSH 1981; GILBERT *et al.* 1984). From our deficiency mapping data, it was clear that the walk did not include DNA covering the distal genes *hk, Be, Bc, Ba, Bb* and *Dox-A2* or the proximal genes *brat* and *fs(2)TW1*. The λRS phage walk extended the cloned region an additional 38 kb distally (STEWARD *et al.* 1984) and included the proximal breakpoints of *Df(2L)OD15* and *Df(2L)-TW203* (Table 5). Since *Df(2L)TW203* uncovers *hk* (Table 6), this distal walk isolated cloned DNA covering the distal subcluster. Beginning from the proximal end of λ16, the proximal walk was extended a further 25 kb and included the distal breakpoints of *Df(2L)Sd37, Df(2L)Sd57* and *Df(2L)Sd77*. Since these deficiencies define the proximal extent of the *Ddc* gene cluster (Table 6), the genomic DNA for *brat* and *fs(2)TW1* was isolated by this walk. This combined effort resulted in the isolation of 162.5 kb of contiguous DNA that contain all 16 recessive lethal loci, *fs(2)TW1* and the recessive visible gene *hook* (Figures 1 and 7).

Mapping deficiency breakpoints within λ phage clones: The breakpoints of all newly generated deficiencies used to genetically subdivide the *Ddc* gene cluster were mapped. Breakpoints were localized by screening genomic Southern blots with probes synthesized from whole phage clones, followed by using appropriate subfragments from that λ clone as probes (data not shown). DNA coordinates for deficiency breakpoints are given in Table 5 and Figures 2–6.

RFLP analysis of mutants from *Ddc* gene cluster loci: To map specific gene locations within the DNA subregions, alleles were examined for aberrations by screen-

TABLE 4

Loci of the *Ddc* gene cluster

Locus[a]	Allele[b]	Screen series	Comments[c]
hk (6)	1	Mohr	Previously reported.[13]
	2	Bridges	Previously reported.[13]
	127, 131	DF 3	Previously reported.[2]
	492	7400	Isolated as visible mutant. Contains a 7- to a 8-kb insertion.
	C1	8000	Isolated as visible mutant. Contains an aberration that eliminates an *Eco*RI site.
l(2)37Be (5)	1, 2	600	
	3, 4	7400	
	5	8000	Has an 800-bp intragenic deletion.
l(2)37Bc (29)	1, 2, 3, 4	E	Initially designated as *l(2)E13, l(2)E59, l(2)E130* and *l(2)E132*, respectively.[3]
	5, 6, 7, 8, 9, ts1	200	
	10	Spontaneous	Discovered during population studies.[4]
	11	300A	
	12, 13, 14	300B	Bc^{12} contains an aberration that eliminates an internal *Pst*I site.
	15, 16, 17, 18, 19	600	
	20, 21	7400	
	22	Lindsley	Recovered as *l(2)M1559* over *Df(2L)TW158.*
	23, 24, 25	8000	
	cs1, fs1	7600	Bc^{cs1} initially designated Bc^{fs2} before discovery of cold-sensitive phenotype.
	cs2	Schüpbach	Originally identified as female sterile *fs(2)OT50.*[12]
l(2)37Ba (12)	1	200	
	2	300A	
	3	500	
	4, 6, 7	600	
	8	7100	
	9, 10	Lindsley	Recovered as *l(2)M584* and *l(2)M283* over *Df(2L)TW50*, respectively.
	11, 12	8000	
	fs1	7600	
l(2)37Bb (11)	1, 2, 3, 4	E	Initially designated as *l(2)E58, l(2)E138, l(2)E152* and *l(2)E154*, respectively.[3]
	5, 6, 7, 8	200	
	9	300B	
	10	600	
	11	7400	Has a 400-bp intragenic deletion.
Dox-A2 (5)	1, 2, 3	7400	Previously reported[9]. *Dox-A2*^{1} and *Dox-A2*^{2} have intragenic deletions of 100 and 1100 bp, respectively.
	5	8000	
	mfs1	7600	Previously reported.[9]
l(2)37Bg (2)	1	7400	
	2	Lindsley	Recovered as *l(2)M564* over *Df(2L)TW50.*
l(2)37Bd (9)	1	200	
	2, 3	300A	Bd^{2} has a 2300-bp intragenic insertion.
	4	300B	
	5	400	
	6	600	
	7	7400	
	8, 9	Lindsley	Recovered as *l(2)M1440* and *l(2)M2032* over *Df(2L)TW158*, respectively.
amd (40)	1, 2, 3, 4, 5, 6, 7, 8	αMD	Initially designated as amd^{H1}, amd^{H7}, amd^{H14}, amd^{H45}, amd^{H82}, amd^{H89} and amd^{H121}, respectively.[1]
	9, 10	H1 lethal	Originally designated as amd^{H60} and amd^{H122}, respectively.[3]

TABLE 4

Continued

Locus[a]	Allele[b]	Screen series	Comments[c]
amd (40)	12	Hodgetts	Originally designated as amd^{HX1}.[3]
	8, 11	E	Originally designated as amd^{H8} and amd^{H149}.[3]
	21, 22, 23, 24	200	Previously reported.[6]
	25, 26, 27, 28, 29	300A	Previously reported.[6]
	30, 31, 32, 33, 34, 35	600	Previously reported.[6]
	36	7300	Previously reported.[6]
	37, 38, 39, 40, 41	7400	Previously reported.[6] amd^{37} has a 750-bp intragenic insertion and amd^{40} contains an aberration that eliminates an internal *Bgl*II site.[11]
	42	Schüpbach	Originally identified as *l(2)WK26.*
	43, 44, 45, 46, 47	8000	
Ddc (51)	1, 2, 3, 4, 5, 6, 7, 8	E	Previously reported.[3]
	9, 10, ts1	200	Previously reported.[6]
	11, 12, 13, 14, ts2	300A	Previously reported.[6]
	15	300B	Previously reported.[6]
	16	400	Previously reported.[6]
	17, 18	DF 11	Previously reported.[6]
	19, 20, 21, 22, 23, 43, lo1, ts3, ts4, ts5	600	Previously reported.[6] Dcd^{43} was initially designated as Ddc^{b1}.
	24, 25, 26	Nüsslein	Previously reported.[6]
	27, 28, 29	DF 13	Previously reported.[6] Ddc^{27} contains 2200-bp intragenic deletion.[7]
	30, 31, 32, 33, 34	7400	Previously reported.[6]
	35	DF 17	Previously reported.[6]
	36, 37, 38, 39	Brittnacher	Previously reported.[6]
	40, 41, 42	DF 19	
	44, 45	8000	
	DE1	*Ddc* escaper	Previously reported.[10]
l(2)37Cc (25)	1, 2, 3, 4, 5	200	
	ts1	300A	
	6, ts3	300B	
	7, 8	600	
	9, 10, 11, 12, 13	7400	Cc^{13} is a dominant female sterile, recessive lethal mutation.
	14, 15, 16, 17	Lindsley	Cc^{14}, Cc^{16} and Cc^{17} recovered as *l(2)M947, l(2)M546* and *l(2)M5* over *Df(2L)TW50.* Cc^{15} recovered as *l(2)M1683* over *Df(2L)TW158.*
	18, 19	Brittnacher	Originally identified as *l(2)L4* and *l(2)L13,* respectively.
	20	7800	Has a 100 bp intragenic deletion.
	21, 22	8000	
	fs1	Schüpbach	Originally identified as female sterile *fs(2)HH32.*[12]
l(2)37Cb (32)	1, 2	E	Initially designated as *l(2)E104* and *l(2)E118,* respectively.[3]
	3, 4, 5, ts1	200	
	6, 7, 8, ts2	300B	
	9	500	
	10, 11, 12, 13, 14, 15, 16, 17	600	
	18, 19, 20, 21, 22, 23, 30	7400	Cb^{18} has a 180-bp intragenic deletion.
	24, 25, 26	Lindsley	Cb^{25} and Cb^{26} recovered as *l(2)M569* and *l(2)M586* over *Df(2L)TW50.* Cb^{24} recovered as *l(2)M1652* over *Df(2L)TW158.*
	27, 28, 29	Brittnacher	Originally identified as *l(2)L1, l(2)L12* and *l(2)L16,* respectively.
l(2)37Cd (11)	1	200	
	2, 3, 4, ts1	300A	
	5, 6	600	
	7	7000	

TABLE 4

Continued

Locus[a]	Allele[b]	Screen series	Comments[c]
l(2)37Cd (11)	8, 9	7400	
	10	8000	
l(2)37Ca (44)	1, 2, 3	E	Initially designated as *l(2)E11, l(2)E56* and *l(2)E125,* respectively.[3]
	4, 5, 6, 7, 8, 9, 10, 11, 12, 13, ts1	200	
	14, 15, 16, 17, 18, 21	300A	
	19, 20, ts3	300B	
	22, 23, 24	400	
	25, 26, 27, 28, 29, 30, 31, 38	600	
	32	7300	
	33, 34, 39, ts2	7400	
	35	Brittnacher	Originally identified as *l(2)L2.*
	36	7900	
	37	Hall	Originally identified as *l(2)P3.*
	40, 41	8000	
l(2)37Ce (8)	1, 2	E	Initially designated as *l(2)E62* and *l(2)E111,* respectively.[3]
	3	200	
	4	300B	
	5	600	
	6	Brittnacher	Originally identified as *l(2)L18.*
	7, 8	8000	
l(2)37Cg (4)	1	7400	
	2	600	Initially designated as *l(2)37Ba*[5].
	3	8000	
	ts1	300A	
brat (30)	1, 2, ts1, fs1	E	Initially designated as *l(2)E60, l(2)E127, l(2)E29* and *fs(2)E60,* respectively.[3]
	3, 4, 5, 6, 7	200	
	8, 9, 10	300A	*brat*^{10} was lost.
	11, ts2	300B	
	12, 13, 14, 15	600	
	16, 17, 18	7400	*brat*^{18} has a 1400-bp intragenic deletion.
	19, ts3	Lindsley	*brat*^{19} recovered as *l(2)M1725* over *Df(2L)TW50.* *brat*^{ts3} recovered as *l(2)M899* over *Df(2L)TW158.*
	20	7800	
	21, fs2, fs3	Schüpbach	Originally identified as *l(2)RS53, fs(2)PI13* and *fs(2)PM43,* respectively.[12]
	22, 23, 24	8000	
fs(2)TW1 (15)	1	200	
	2, 3, 4, 5, 6, 14	600	
	7, 8	7600	
	9, 10	Lindsley	*TW1*^{9} recovered as *fs(2)M11* over *Df(2L)TW50.* *TW1*^{10} recovered as *fs(2)M1111* over *Df(2L)TW158.*
	11, 12, 13, 15	Schüpbach	Originally identified as *fs(2)PL10, fs(2)RU34, fs(2)HL2* and *fs(2)RS42,* respectively.[12]

[a] Figures in parenthesis indicate number of alleles isolated to date.

[b] Underlined alleles were sent to the Bowling Green or Bloomington stock centers.

[c] All information is from this study, unless otherwise noted. All loci are referenced in LINDSLEY and ZIMM (1992). Published information on these loci is found in the following: 1, SPARROW and WRIGHT (1974), 2, WRIGHT *et al.* (1976a); 3, WRIGHT *et al.* (1976b); 4, BEWLEY (1978); 5, WRIGHT *et al.* (1981); 6, WRIGHT *et al.* (1982); 7, GILBERT *et al.* (1984); 8, MARSH and WRIGHT (1986); 9, PENTZ *et al.* (1986); 10, BISHOP and WRIGHT (1987); 11, BLACK *et al.* (1987); 12, SCHUPBACH and WIESCHAUS (1989); 13, LINDSLEY and ZIMM (1992).

TABLE 5

Location of deficiency breakpoints within or near the *Ddc* gene cluster

Deficiency designation	Cytological breakpoints[a]	DNA coordinates[b]	Comments[c]
Df(2L)TW50	36E4-F1; 38A6-7		Breakpoints previously identified and originally named *Df(2L)50*.[1]
Df(2L)TW158	37B2-8; 37E2-F4		Breakpoints previously identified and originally named *Df(2L)158*.[1]
Df(2L)VA24	*37B2-8; 37E2-F4*		
Df(2L)pr-A16	*37B2-12*; 38D2-5		Breakpoints previously identified and originally named *Df(2L)A16*.[2]
Df(2L)Sd68	37B3-7; 38E3-5		Breakpoints previously identified and originally named *Df(2L)SD-Mad*^{R68}.[4]
Df(2L)TW137	36C2-4; 37B9-C1		Breakpoints previously identified and originally named *Df(2L)137*.[1,7]
Df(2L)TW130	37B9-C1; 37D1-2		Breakpoints previously identified and originally named *Df(2L)130*.[1]
Df(2L)VA23	37B9-C1; **37D5**	+16.2:+17.0[d]	Breakpoints previously identified.[8]
Df(2L)TW203	36E4-F1; ***37B9-C1***	−85.4:−83.2	Coordinates formerly reported as −79.4:−77.6[6,7]
Df(2L)OD15	*36F7-9;* ***37B9-C1***	−67.4:−67.3	Coordinates formerly reported as −67.3:−68.3.[6]
Df(2L)hk-UC1	*37B2-8;* **37B9-C1**	−56.8:−54.9	
Df(2L)hk18	36E4-6; **37B12-C1**	−52.2:−50.6	Coordinates formerly reported as −53.0:−51.4.[5]
Df(2L)NST	***37B9-C1;*** *37C6-D1*	−36.8:−31.6	
Df(2L)VA17	**37C1-2;** 37F5-38A11	+2.8:+3.5	Breakpoints initially described as 37B9-C1; 37F5-38A1.[5]
Df(2L)VA18	36C4-D1; **37C2-5**	+6.1:+6.7	Coordinates formerly reported as +5.1:+5.6.[5,7]
Df(2L)hk-UC2	*37B2-8;* ***37C2-5***	+10.3:+11.0	Coordinates formerly reported as +8.2:+11.0.[9]
Df(2L)TE37B7	***37C2-5;*** *37C6-D1*	+24.5:+27.1	Originally named *Df(2L)TE42B7* (J. G. BRITTNACHER, unpublished data).
Df(2L)TE37C-1	**37C2-5;** 38F5-39A1	+26.5:+29.1	Breakpoints previously identified and originally named *Df(2L)TE42-1*.[3] Coordinates formerly reported as +25.5:+28.1.[5]
Df(2L)VA12	**37C2-5;** 38B2-C1	+26.5:+29.1	Coordinates formerly reported as +25.5:+28.1.[5]
Df(2L)VA21	36D1-F1:**37C2-D1**	+28.0:+30.8	
Df(2L)VA19	**37C2-D1;** 38A6-B1	+30.8:+31.8	
Df(2L)VA13	**37C2-5;** 38C2-D1	+31.7:+36.4	Coordinates formerly reported as +30.7:+35.4.[5]
Df(2L)Sd57	***37C6-D1;*** 38C1-2	+44.7:+46.3	Breakpoints previously identified and originally named *Df(2L)SD-Roma*^{R57}.[4] Coordinates formerly reported as +38.6+40.6.[9]
Df(2L)Sd77	***37C6-D1;*** 38C1-2	+49.2:+51.4	Breakpoints previously identified and originally named *Df(2L)SD-Mad*^{R77}.[4] Coordinates formerly reported as +47.1:+52.6.[9]
Df(2L)TE37B7	*37C2-5;* ***37C6-D1***	+51.4:+60.1	Originally named *Df(2L)TE42B7* (J. G. BRITTNACHER, unpublished data).
Df(2L)Sd37	***37C6-D1;*** 38A6-B2	+60.1:+66.8	Breakpoints previously identified and originally named *Df(2L)SD-5*^{R37}.[2]
Df(2L)E55	*37C6-1;* 37F5-38A1		Breakpoints previously identified.[1]
Df(2L)VA6	*37C6-D1;* 38F2-39A1		
Df(2L)E71	36F2-6; 37D1-2		Breakpoints previously identified.[1]
Df(2L)VA14	*37A1-B8;* 37D1-2		
Df(2L)VA16	*36F7-9; 37D1-2*		
Df(2L)hk39	36F6-37A1; 37D1-2		Breakpoints previously identified and originally named *Df(2L)SD-Roma*^{hk39}.[4]
Df(2L)VA25	*37A1-B8; 37D1-2*		
Df(2L)VA8	*37C6-D1;* 38F5-39A1		
Df(2L)TW330	36E4-F1; 37D1-2		Breakpoints previously identified.[7]
Df(2L)VA22	36E4-F7; 37D1-2		
Df(2L)pr-A14	*37C6-D1;* 39A4-7		Breakpoints previously identified and originally named *Df(2L)A14*.[2]
Df(2L)VA15	*36F-37A1;* 37D5		
Df(2L)VA20	37B2-8; **37D5**	+16.2:+17.0[d]	Breakpoints previously identified.[8]

[a] Deficiency breakpoints are ordered distal to proximal relative to the *Ddc* region. Italicized breakpoints indicate that information is inferred from genetic data and not by cytological investigation. Bold-face breakpoints delineate the DNA coordinates given in the third column.

[b] DNA coordinates are relative to the *Hpa*I restriction site near the 3′ end of the *Ddc* gene.

[c] All information is from this study unless otherwise noted. All deficiencies are referenced in LINDSLEY and ZIMM (1992). Published information on these deficiencies are found in the following: 1, WRIGHT *et al.* (1976a); 2, GANETZKY (1977); 3, WRIGHT *et al.* (1981); 4, BRITTNACHER and GANETZKY (1983); 5, GILBERT *et al.* (1984); 6, PENTZ and WRIGHT (1986); 7, STEWARD and NÜSSLEIN-VOLHARD (1986); 8, NOLAN *et al.* (1991); 9, LINDSLEY and ZIMM (1992).

[d] DNA coordinates are relative to the 5′ end of the *Top2* gene (NOLAN *et al.* 1991).

TABLE 6

Deficiency mapping analysis

	Deficiency[a]																	
Locus	*TW137*	*TW130*	*TW203*	*0D15*	*hkUC1*	*VA18*	*hkUC2*	*VA21*	*NST*	*VA17*	*TE37B7*	*VA12*	*VA19*	*Sd57*	*Sd77*	*Sd37*	*VA23*	*E55*
hook	+	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+
l(2)37Be	+	–	+	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+
l(2)37Bc	+	–	+	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+
l(2)37Ba	+	–	+	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+
l(2)37Bb	+	–	+	+	–	–	–	–	+	+	+	+	+	+	+	+	–	+
Dox-A2	+	–	+	+	–	–	–	–	+	+	+	+	+	+	+	+	–	+
l(2)37Bg	+	–	+	+	+	–	–	–	+	+	+	+	+	+	+	+	–	+
l(2)37Bd	+	–	+	+	+	–	–	–	–	+	+	+	+	+	+	+	–	+
amd	+	–	+	+	+	–	–	–	–	+	+	+	+	+	+	+	–	+
Ddc	+	–	+	+	+	–	–	–	–	–	+	+	+	+	+	+	–	+
l(2)37Cc	+	–	+	+	+	–	–	–	–	–	+	+	+	+	+	+	–	+
l(2)37Cb	+	–	+	+	+	+	–	–	–	–	+	+	+	+	+	+	–	+
l(2)37Cd	+	–	+	+	+	+	+	–	–	–	+	+	+	+	+	+	–	+
l(2)37Ca	+	–	+	+	+	+	+	–	–	–	+	+	+	+	+	+	–	+
l(2)37Ce	+	–	+	+	+	+	+	–	–	–	+	+	+	+	+	+	–	+
l(2)37Cg	+	–	+	+	+	+	+	–	–	–	+	+	+	+	+	+	–	+
brat	+	–	+	+	+	+	+	+	–	–	–	–	–	–	+	+	–	+
fs(2)TW1	+	–	+	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+

[a] For simplicity, all deficiencies are abbreviated without the *Df(2L)* designator, i.e., *Df(2L)TW130* is *TW130* etc.

ing genomic Southern blots probed with either whole phage or genomic subclones from regions identified by deficiency breakpoints. In most cases, alleles thought likely to contain a chromosomal aberration (*i.e.,* those generated with formaldehyde, γ-rays or DEB) were analyzed for RFLPs. However, for some genes, all alleles were examined including ones produced solely with EMS (Table 7). Since all lethals are maintained as balanced stocks, restriction enzyme fragments from heterozygotes were examined and their patterns compared to both the parental and *CyO* digestion patterns. In total 211 or 62.1% of all mutant strains were analyzed and 14 RFLPs detected, establishing the linear order of 13 loci with respect to adjacent loci (Table 7, Figures 2–6). In the distal subcluster only the exact location of *Ba* was not revealed (Table 7). However, RFLP analysis

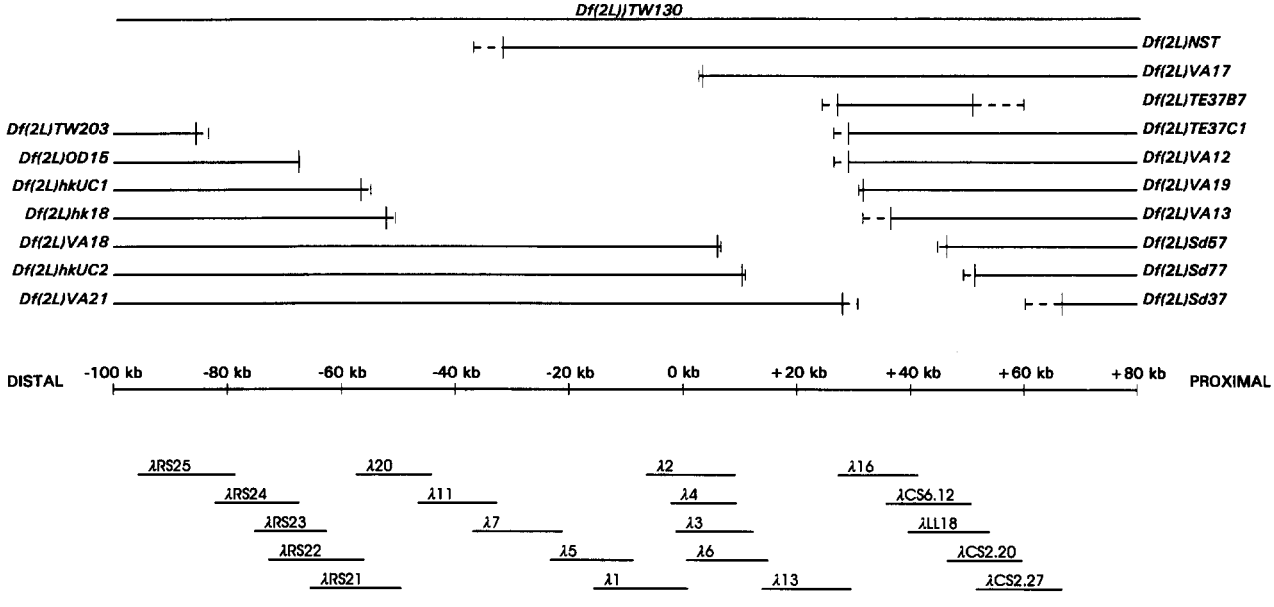

FIGURE 1.—Location of deficiency breakpoints and bacteriophage λ clones mapping within the *Ddc* gene cluster. Deficiency breakpoints located within the *Ddc* gene cluster are shown in relation to the DNA coordinate system used in this region. ———, the extent of a deletion; |---|, the region of mapping uncertainty for the breakpoint. Distal and proximal are relative to the centromere. The zero coordinate corresponds to the *Hpa*I restriction endonuclease site near the 3′ end of the *Ddc* gene, and the coordinates are positive in the direction of the centromere and negative in the direction of the telomere. Individual *D. melanogaster* bacteriophage λ clones are shown below the coordinate scale with grid coordinates given in MATERIALS AND METHODS. The diagram is drawn to scale.

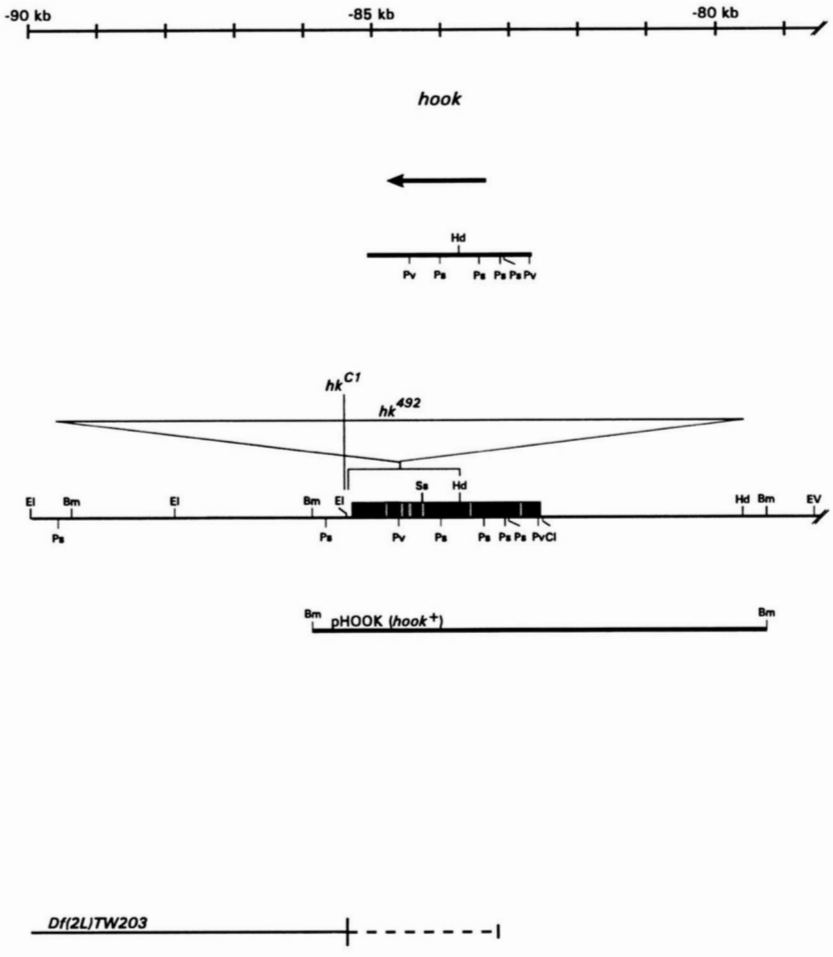

FIGURE 2.—Molecular organization of the *hook* region from the *Ddc* gene cluster. The DNA coordinate system is at the top (see Figure 1 for description). Below the coordinate scale is the gene name, the direction of its transcription (line with arrowhead) and a restriction endonuclease map of the genome region derived from isolated phage clones. Only relevant sites are shown. Restriction enzyme symbols are as follows: Bm, *Bam*HI; Cl, *Cla*I; EI, *Eco*RI; EV, *Eco*RV; Hd, *Hind*III; Ps, *Pst*I, Pv, *Pvu*II; Ss, *Sst*II. This genomic map portrays the location of both *hook* RFLPs determined in this study (Table 7). The genomic map includes the deduced position and structure of *hook* (M. PHISTRY and H. J. KRÄMER, personnal communication). ▨, exons; □, introns. Table 9 contains additional information on *hook*. Below the genomic map, a heavy solid line represents the genomic fragment used in *P*-element-mediated germline transformation experiments of *hook* (M. PHISTRY and H. J. KRÄMER, personnal communication). At the bottom, *Df(2L)TW203* is represented by —, showing the extent of the deletion. |---|, the region of mapping uncertainty for the breakpoint. The diagram is drawn to scale.

of the proximal subcluster was not as successful since the gene order of *Cd, Ca, Cg* and *Ce* could not be determined (Table 7). RFLPs found in *Bd* and *brat* alleles confirmed the genetic mapping analysis.

P-element-mediated germline transformation of genes within the *Ddc* gene cluster: *P*-element germline transformation studies were initiated to localize and determine if these closely clustered genes could function when transformed individually into ectopic sites. Based on the results gathered from the deficiency mapping and RFLP analysis, selected fragments of DNA were subcloned into suitable vectors and subjected to germline transformation. One or more transformed fragments rescued mutant alleles of all 18 genes with the exception of *Bd* and *fs(2)TW1* (Table 8). In addition, fertility for *Bc, Ba, Dox-A2, Cc,* and *brat* sterile alleles is restored by the same transformants of wild-type DNA that rescue lethality for the respective mutant.

In the distal subcluster, transformation of eight different genomic fragments confirmed the gene order from distal to proximal as *hk, Be, Bc, Ba, Bb, Dox-A2* (Figures 2 and 3), while in the proximal subcluster transformation of 14 different genomic fragments determined the gene order from distal to proximal as *amd, Ddc, Cc, Cb, Cd, Ca, Ce* and *Cg* (Figure 5). The scattered B gene *Bg* was rescued by pB8, which defines its location to within 5 kb distal to *Bd* (Figure 4). *Bd* alone was never rescued. However, before the *Bd* RFLP was identified, the 7.4-kb *Eco*RI fragment (pB9) covering −17.4 to −10.0 was determined not to rescue the mutation (Table 8). A 5.8-kb *Cla*I fragment (Table 8) (pC15) rescued *brat* alleles, indicating the extent of this gene is confined to +45.4 to +51.2 (Figure 6). This fragment did not rescue *fs(2)TW1* mutations.

Isolation of cDNAs and structural analysis of genes within the *Ddc* gene cluster: To deduce the gene structure, direction of transcription and message size cDNAs were isolated for all the loci with the exception of *fs(2)TW1,* which was not attempted, and *Bg* and *Cg,* for which screens were unsuccessful (Table 9). Libraries were probed using genomic DNA fragments identified by RFLP analysis and *P*-element germline transformation experiments (Table 9). Comparison of cDNA and genomic restriction maps, Southern blot analysis of digested genomic DNA using cDNA probes and/or Northern blot analysis using genomic fragments and cDNA inserts as probes allowed the precise mapping of 15 genes and three unmutated transcription units and inference of their exon/intron structure (Table 9, Figures 2–6). The use of directionally cloned cDNA libraries and/or Northern blot analysis using single-strand

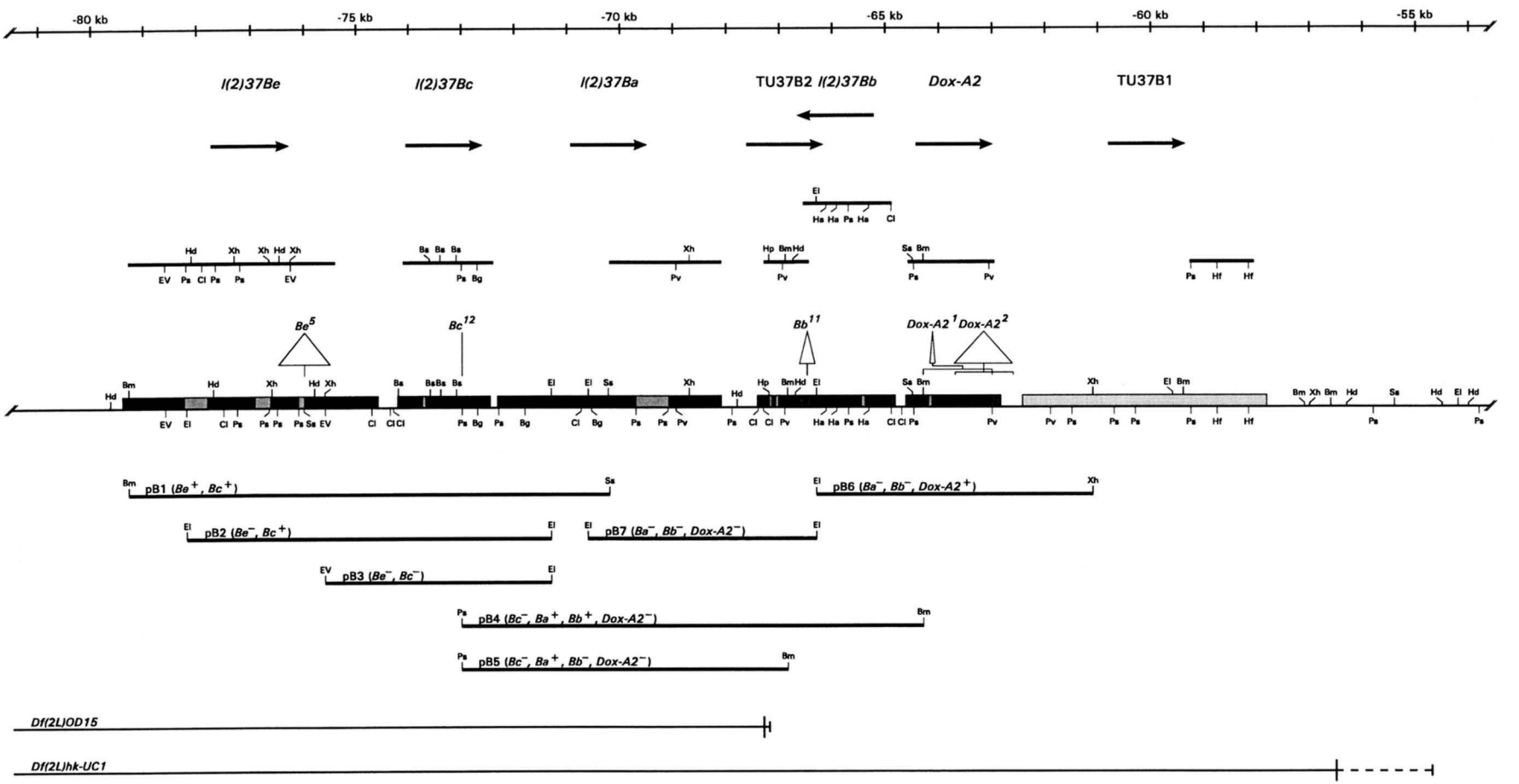

FIGURE 3.—Molecular organization of the distal subcluster region from the *Ddc* gene cluster. The DNA coordinate system is at the top (see Figure 1 for description). Below the coordinate scale is the gene name, the direction of its transcription (line with arrowhead), if known, and a restriction map of its corresponding cDNA (heavy solid line), if isolated. Below this is a restriction endonuclease map of the genome region derived from isolated phage clones. Only relevant sites are shown. Restriction enzyme symbols are as follows: Bg, *Bgl*II; Bm, *Bam*HI; Bs, *Bst*XI; Cl, *Cla*I; EI, *Eco*RI; EV, *Eco*RV; Ha, *Hae*I; Hd, *Hind*III; Hf, *Hinf*I; Hp, *Hpa*I; Pv, *Pvu*II; Ps, *Pst*I; Sl, *Sal*I; Ss, *Sst*II; Xh, *Xho*I. The genomic map portrays the location of all RFLPs determined in this study or reported previously, *Dox-A2*^{1} and *Dox-A2*^{2} (Table 7) (PENTZ and WRIGHT 1986). The map also includes the deduced position and structure of all known genes and transcription units within this region. ▨, exons; light gray rectangles, introns; medium gray rectangles, leader and trailer regions, if known. TU37B1 is portrayed as a light grey rectangle since its exon/intron structure was not determined. Table 9 contains additional information on each gene. Below the genomic map, a heavy solid line represents the genomic fragments used in *P*-element-mediated germline transformation experiments. Genes in parentheses represent mutants tested for rescue with that genomic fragment. Table 8 contains additional information on these constructs. At the bottom, *Df(2L)OD15* and *Df(2L)hk-UC1* are represented by —, showing the extent of the deletion; |---|, the region of mapping uncertainty for the breakpoint. The diagram is drawn to scale.

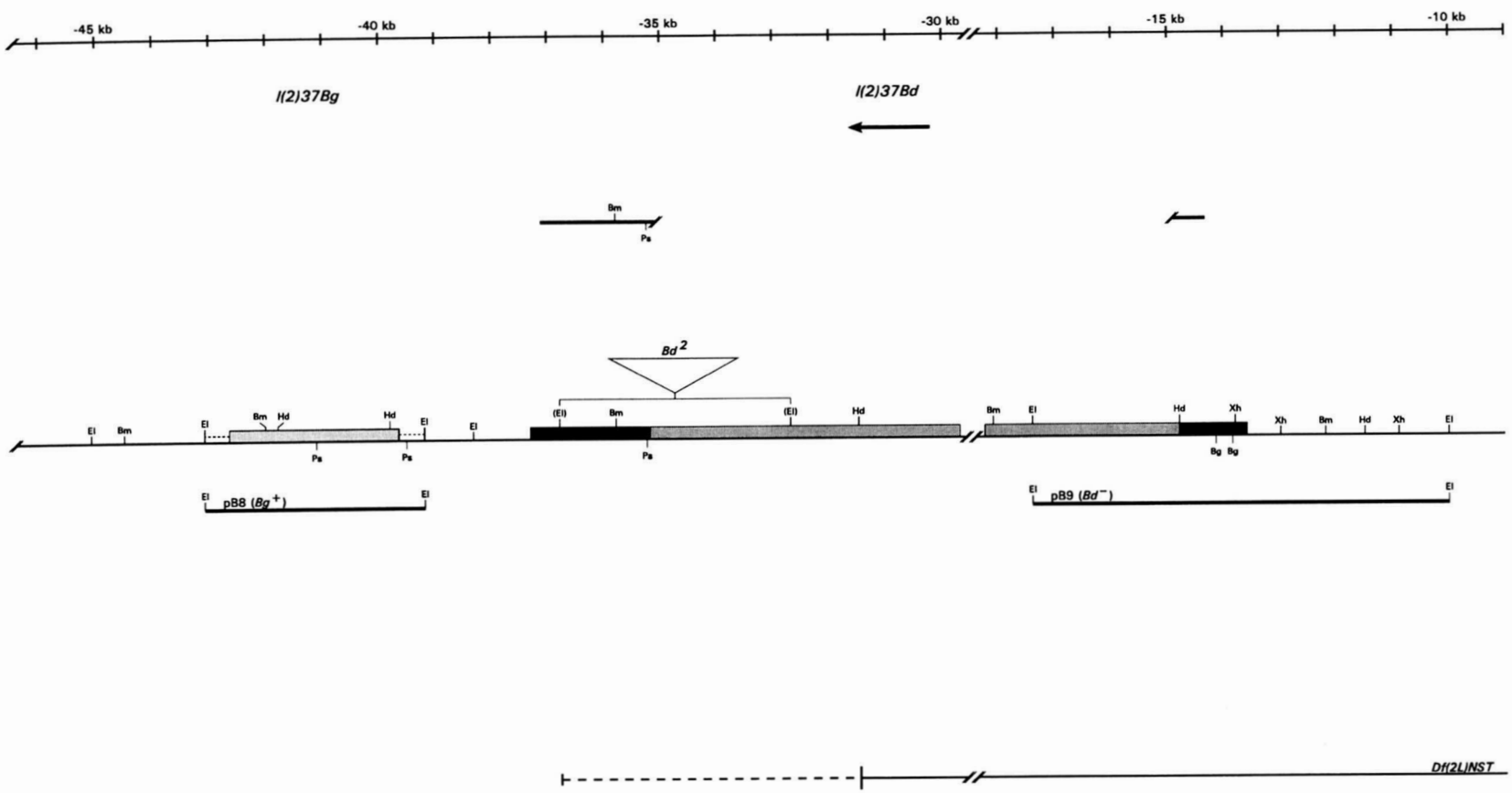

FIGURE 4.—Molecular organization of the *l(2)37Bg-l(2)37Bd* region from the *Ddc* gene cluster. See Figure 3 legend for description of common features. Note the break in numbering at −30 kb. The *Eco*RI sites in parentheses correspond to sites from the phage clones λ7 and λ11 and not from the genomic region. The genomic map portrays the location of the Bd^2 RFLP determined in this study (Table 7). The map also includes the deduced position and structure for *Bd*. *Bg* is represented by a light grey rectangle since its transcript size, but not its precise location, was determined.

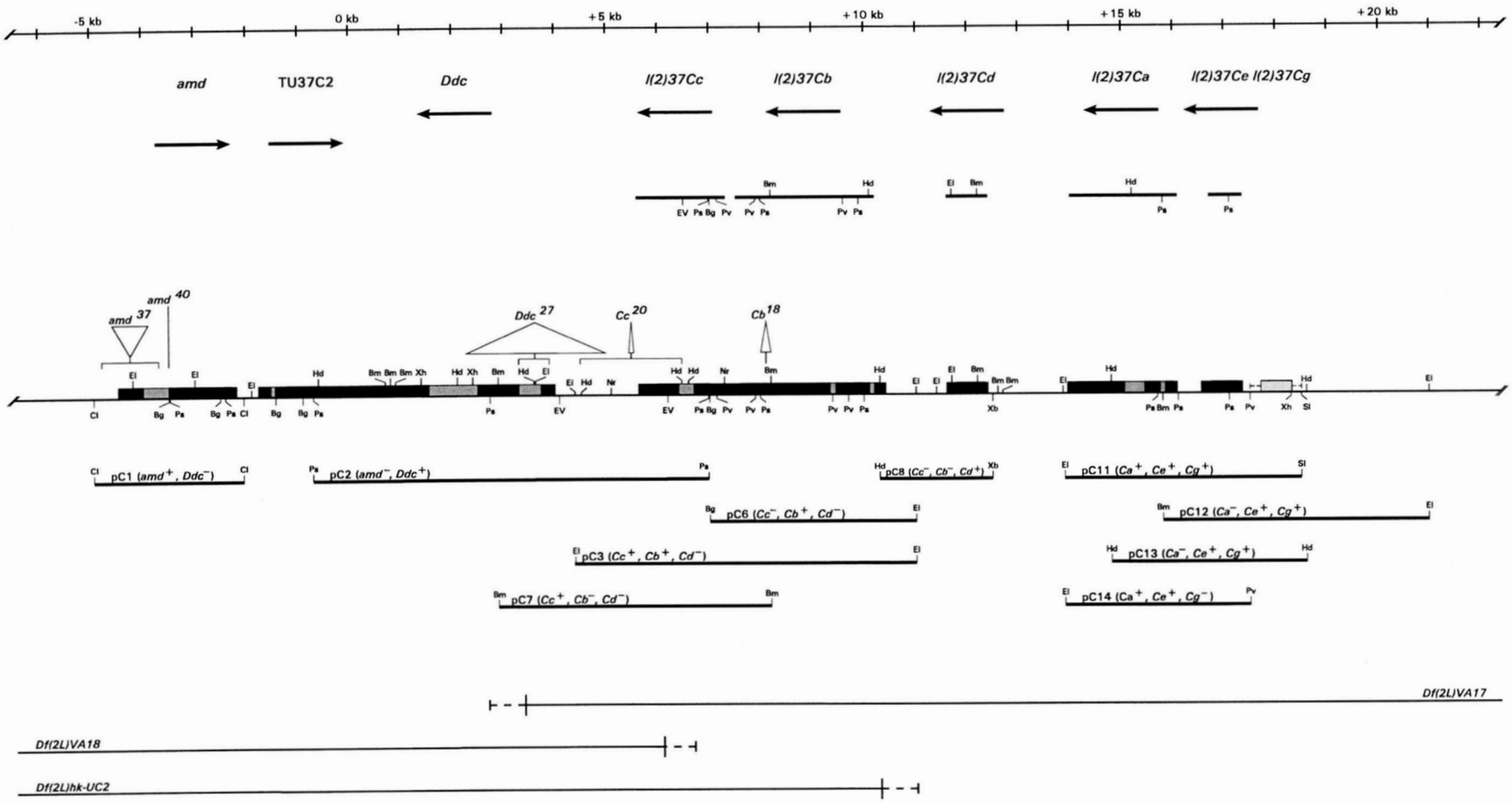

FIGURE 5.—Molecular organization of the proximal subcluster region from the *Ddc* gene cluster. See Figure 3 legend for description of common features. Restriction enzyme symbols are as follows: Bg, *Bgl*II; Bm, *Bam*HI; Cl, *CLa*I; EI, *Eco*RI; EV, *Eco*RV; Hd, *Hind*III; Nr, *Nru*I; Pv, *Pvu*II; Ps, *Pst*I; Sl, *sal*I; Xb, *XbaI; Xh, Xho*I. This genomic map portrays the location of all RFLPs determined in this study or reported previously (Table 7) [amd^{37} and amd^{40} (BLACK *et al.* 1987), Ddc^{27} (GILBERT *et al.* 1984)]. *Cg* is represented by a light grey rectangle since only its transcript size, but not its precise location, was determined.

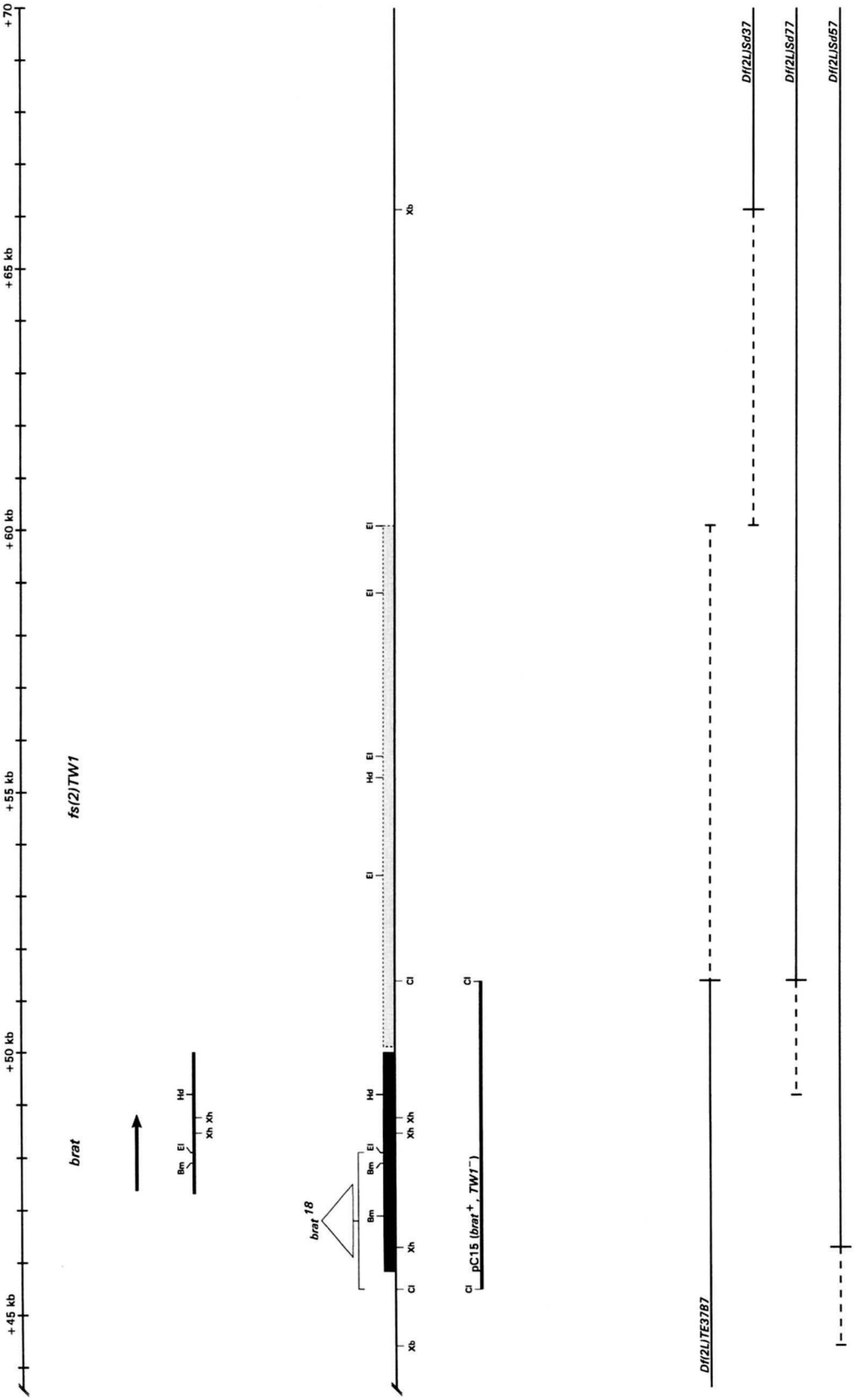

FIGURE 6.—Molecular organization of the *brat*-fs(*2*)*TW1* region from the *Ddc* gene cluster. See Figure 3 legend for description of common features. The genomic map portrays the location of the $brat^{18}$ RFLP. *fs(2)TW1* is represented by a dashed-outline, light gray rectangle since its transcript size and precise location were unknown.

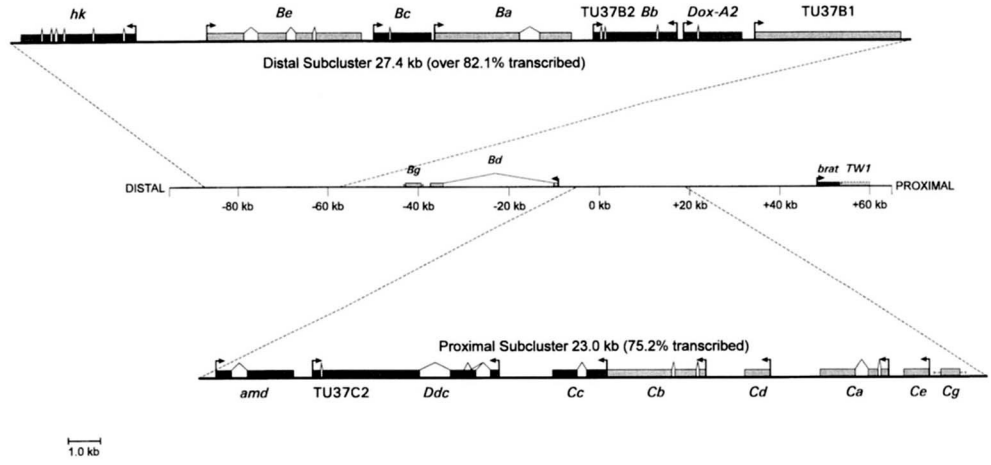

FIGURE 7.—Molecular organization of subclusters within the *Ddc* gene cluster. The deduced location, structure and transcriptional direction of genes within the distal and proximal subclusters are shown. Exons are represented by rectangles and introns by lines. Genes with darker-colored exons indicate structure known by DNA sequencing, genes with light-colored exons indicate position inferred by restriction enzyme mapping. The direction of transcription is indicated by the bent arrow. Table 9 contains additional information on each gene. See Figure 1 for a description of the DNA coordinate system. The diagram is drawn to scale, with the 1.0-kb scale corresponding to the enlarged subcluster regions.

sense and antisense RNA probes revealed the transcriptional direction of 15 genes and two unmutated transcription units (Table 9, Figures 2–6). The cDNA and transcript analysis of eight genes and two unmutated transcription units was confirmed by sequence determination (Table 9). This molecular genetic analysis indicates the distal subcluster contains six genes and two unmutated transcription units (see below) within ~27.4 kb (Table 9, Figures 2 and 3), while in the proximal subcluster there are eight genes and one unmutated transcription unit contained within a maximum of 22.7 kb (Table 9, Figure 5).

In the distal subcluster, cDNA clones for *Be, Bc, Ba* and *Bb* were isolated from a pNB40 12–24 hr embryonic cDNA library (Table 9). The deduced gene structure and location for each locus indicates that, with the ex-

TABLE 7

RFLP analysis

Locus[a]	Total alleles examined	Mutagen used[b]						RFLP		Figure[d]
		EMS	EMS/F	EMS/γ-ray	γ-ray	X-ray	DEB	Detected[c]	None detected[c]	
hook	4	—	—	2 (2)	—	2	—	492, C1	127, 131	3
l(2)37Be	5	2	—	3 (1)	—	—	—	5	1–4	4
l(2)37Bc	14	3	4 (1)	7	—	—	—	12	1, 11, 13, 14, 22–25, cs1, cs2, fs1	4
l(2)37Ba	3	—	—	2	1	—	—	—	8, 11, 12	4
l(2)37Bb	2	—	1	1 (1)	—	—	—	11	9	4
Dox-A2$^{\beta}$	4	—	—	4 (2)	—	—	—	1, 2	3, 4	4
l(2)37Bg	0	—	—	—	—	—	—	—	—	—
l(2)37Bd	4	—	3 (1)	1	—	—	—	2	3, 5, 7	5
amd$^{\gamma}$	7	—	—	6 (2)	—	1	—	37, 40	12, 36, 38, 39, 41	6
Ddc$^{\alpha}$	14	—	—	5	5 (1)	—	4	27	28–42, 44, 45	6
l(2)37Cc	25	14	3	7	—	—	1 (1)	20	1–19, 21, 22, ts1, ts2, ts1	6
l(2)37Cb	32	20	4	8 (1)	—	—	—	18	1–17, 19–30, ts1, ts2	6
l(2)37Cd	11	3	4	3	1	—	—	—	—	—
l(2)37Ca	44	24	12	7	1	—	—	—	—	—
l(2)37Ce	8	5	1	2	—	—	—	—	—	—
l(2)37Cg	4	1	1	2	—	—	—	—	—	—
brat	*30*	*18*	*5*	*6 (1)*	—	—	*1*	*18*	*1–17, 19–24, ts1–ts3, fs1–fs3*	*7*
fs(2)TW1	0	—	—	—	—	—	—	—	—	—
Total	211 (14)	90	38 (2)	66 (10)	8 (1)	3	6 (1)			

[a] Information previously reported: α, GILBERT *et al.* (1984); β, PENTZ and WRIGHT (1986); γ, BLACK *et al.* (1987).
[b] Figure in parentheses indicates the number of alleles with RFLPs.
[c] Numbers represent allelic designations for corresponding gene.
[d] Figure in which RFLP is depicted.

TABLE 8

***P*-element-mediated germline transformation analysis**

Construct[a]	Vector	DNA fragment	λ clone	Mutant[b] Rescued	Mutant[b] Not rescued	Insertion chromosome[c]	Figure[d]
pHOOK[γ]	pCaSpeR	6.5 kb *Bam*HI–*Bam*HI	λRS25	*hook*	—	3 (2)	3
pB1	pW8	9.1 kb *Bam*HI–*Sst*II	λRS24	*Be, Bc*	*Ba*	2 (1), 3 (1)	4
pB2	pW8	6.9 kb *Eco*RI–*Eco*RI	λRS24	*Bc*	*Be, Ba*	1 (1), 3 (1)	4
pB3	pCaSpeR	4.0 kb *Eco*VR–*Eco*RI	λRS24	None	*Be, Bc, Ba*	3 (1)	4
pB4	pW8	8.7 kb *Pst*I–*Bam*HI	λRS22	*Ba, Bb*	*Bc, Dox-A2*	1 (2), 3 (6)	4
pB5	pW8	6.2 kb *Pst*I–*Bam*HI	λRS22	*Ba*	*Bc, Bb, Dox-A2*	3 (1)	4
pB6	pCaSpeR	5.2 kb *Eco*RI–*Xho*I	λRS22	*Dox-A2*	*Ba, Bb*	1 (1), 3 (4)	4
pB7	pCaSpeR	4.3 kb *Eco*RI–*Eco*RI	λRS22	None	*Ba, Bb, Dox-A2*	3 (4)	4
pB8	pCaSpeR	3.9 kb *Eco*RI–*Eco*RI	λ11	*Bg*	—	1 (1), 2 (1)	5
pB9	pCaSpeR	7.4 kb *Eco*RI–*Eco*RI	λ5	None	*Bd*	1 (2), 3 (2)	5
pC1[β]	pCar20	2.9 kb *Cla*I–*Cla*I	λ1	*amd*	*Ddc*	3 (1)	6
pC2[α]	pry3, pHD10	7.8 kb *Pst*I–*Pst*I	λ2	*Ddc*	*amd*	1 (2), 2 (11), 3 (13)	6
pC3	pUChsNeo	6.6 kb *Eco*RI–*Eco*RI	λ6	*Cc, Cb*	*Cd*	1 (1), 3 (3)	6
pC4	pUChsNeo	3.7 kb *Eco*RI–*Nru*I	λ6	None	*Cc, Cb*	1 (1), 3 (3)	Not shown
pC5	pUChsNeo	3.3 kb *Nru*I–*Eco*RI	λ6	None	*Cc, Cb*	1 (1), 3 (1)	Not shown
pC6	pUChsNeo	4.0 kb *Bgl*II–*Eco*RI	λ6	*Cb*	*Cc, Cd*	3 (2)	6
pC7	pUChsNeo	5.3 kb *Bam*HI–*Bam*HI	λ6	*Cc*	*Cb, Cd*	3 (2)	6
pC8	pUChsNeo	2.2 kb *Hind*III–*Xho*I	λ6	*Cd*	*Cc, Cb*	3 (1)	6
pC9	pUChsNeo	2.2 kb *Eco*RI–*Eco*RI	λ6	None	*Cd*	1 (1), 3 (1)	Not shown
pC10	pUChsNeo	6.8 kb *Eco*RI–*Eco*RI	λ13	*Ca, Ce, Cg*	—	1 (2), 3 (1)	Not shown
pC11	pW8	4.6 kb *Eco*RI–*Sal*I	λ13	*Ca, Ce, Cg*	—	1 (2)	6
pC12	pUChsNeo	5.0 kb *Bam*HI–*Eco*RI	λ13	*Ce, Cg*	*Ca*	2 (1), 3 (2)	6
pC13	pW8	3.8 kb *Hind*III–*Hind*III	λ13	*Ce, Cg*	*Ca*	1 (3), 3 (1)	6
pC14	pW8	3.6 kb *Eco*RI–*Pvu*II	λ13	*Ca, Ce*	*Cg*	1 (1), 3 (6)	6
pC15	pW8	5.8 kb *Cla*I–*Cla*I	λ18	*brat*	*fs(2)TW1*	2 (1)	7

[a] Information previously reported: α = SCHOLNICK *et al.* (1983); MARSH *et al.* (1985); β = WANG and MARSH (1995); γ = M. PHISTRY and H. J. KRÄMER, personal communication.

[b] For simplicity, all lethal genes are abbreviated without the *l(2)37* designator, i.e., *l(2)37Be* is *Be*, *l(2)37 Cc* is *Cc*, *etc.*.

[c] Figures indicate the chromosome the *P*-element construct integrated into (number not in parenthesis) and the number of different insertion events isolated (number in parenthesis).

[d] Figure in which transformation fragment is depicted.

ception of *hk*, all loci within the distal subcluster are separated by no more than 600 bp from adjacent genes, with an average distance of only 260 bp (Figures 2 and 3). Sequence analysis of the *Bc* gene and flanking regions indicates that *Be* is 247 bp distal to *Bc* (D. G. STATHAKIS and T. R. F. WRIGHT, unpublished data), while sequencing regions distal to *Dox-A2* reveals *Bb* is 190 bp from *Dox-A2* (PENTZ *et al.* 1991) (Figure 3). All loci except *hk* and *Bb* are transcribed distal to proximal (Table 9). Two unmutated transcription units were also identified within the distal subcluster during screens to isolate cDNAs for known loci (Table 9, Figure 3). Two partial length cDNAs mapping proximal to *Dox-A2* identify a 4.6-kb transcript on Northern blots and represent the first unmutated transcription unit in the region. Since these cDNAs did not correspond to any known loci in the subcluster, they were designated Transcription Unit 37B1 (TU37B1). The TU37B1 transcript is detected strongly by the 4.0-kb *Pst*I fragment from −65.8 to −61.8 and detected weakly by the 1.2-kb *Pvu*II fragment from −63.0 to 61.8, placing TU37B1 only 400–600 bp proximal to *Dox-A2* (data not shown). Therefore, the location for TU37B1 lies somewhere between −62.4 and −57.8 (Figure 3). Two cDNAs corresponding to the second unmutated transcription unit, designated TU37B2, were isolated during the screen for *Ba* cDNAs. Genomic Southern blotting, restriction enzyme mapping and DNA sequencing revealed the exact position of TU37B2, proximal to *Ba*, in the region from −67.5 to −66.6 (Table 9). Northern blot analysis using single-stranded RNA probes detected a 0.9-kb transcript transcribed in a distal to proximal direction (Table 9). Thus, in addition to TU37C2, formerly designated *Cs* (EVELETH and MARSH 1987), the number of unmutated transcription units now identified within the *Ddc* gene cluster is three.

Two cDNAs for the scattered *B* gene *Bd* were isolated from a pNB40 12–24 hr embryonic cDNA library using a 5.8-kb *Eco*RI fragment from λ11 that hybridized to a 3.0-kb transcript in embryos and pupae (Table 9). Mapping of the cDNAs to the genomic DNA produced a unexpected result. The cDNAs hybridized to the expected region of the *Eco*RI probe fragment but also hybridized to the region of the 7.4-kb *Eco*RI fragment used in unsuccessful attempts to rescue *Bd* alleles by *P*-element transformation experiments (PEARLSON 1991). Southern blotting revealed that both cDNAs hybridized with probes derived from the distal 5.8-kb fragment and the proximal 7.4-kb region. The 5.8-kb probe hybrid-

TABLE 9

Molecular mapping analysis

Gene information			cDNA clone information				Transcript information		
Gene[a]	Position[b]	Size (kb)[c]	Number isolated[d]	Probe fragment and coordinates	Size (kb)[e]	Source[f]	Size (kb)[g]	Direction[h]	Figure[i]
hook[θ]	−85.2:−82.5	**2.7**	2	6.5 kb *Bam*HI–*Bam*HI (−85.9 to −79.4)	2.3	L3	ND	*D*	3
l(2)37Be	−79.4:−74.7	4.7	2	6.9 kb *Eco*RI–*Eco*RI (−78.2 to −71.3)	3.9	E3	3.9	***P***	4
l(2)37Bc	−74.5:−72.4	**1.9**	1	6.9 kb *Eco*RI–*Eco*RI (−78.2 to −71.3)	1.7	E3	1.9	***P***	4
l(2)37Ba	−72.3:−68.1	4.2	2	6.2 kb *Pst*I–*Bam*HI (−73.0 to −66.8)	2.1	E3	3.2	***P***	4
TU37B2	−67.5:−66.6	**0.9**	2	6.5 kb *Eco*RI–*Eco*RI (−66.4 to −59.9)	0.6	E3	0.9	***P***	4
l(2)37Bb	−66.6:−64.8	**1.8**	2	6.5 kb *Eco*RI–*Eco*RI (−66.4 to −59.9)	1.7*	E3	1.7	***D***	4
Dox-A2[ζ]	−64.5:−62.8	**1.7**	6	4.0 kb *Pst*I–*Pst*I (−65.6 to −61.6)	1.7*	E3, L1, P3	1.7	***P***	4
TU37B1	−62.4:−57.8	4.6	2	4.0 kb *Pst*I–*Pst*I (−65.6 to −61.6)	1.1*	E3	4.6	*P*	4
l(2)37Bg	(−43.2:−39.3)	(3.0)	NI	—	—	—	3.0	ND	5
l(2)37Bd	−37.4:−13.6	23.8	2	5.8 kb *Eco*RI–*Eco*RI (−32.7 to −38.5)	2.1	E3	3.0	**D**	5
amd[δ]	−4.5:−2.2	2.3	4	9.5 kb *Eco*RI–*Hind*III (−10.0 to −0.5)	1.8	E1, P1	2.0	**P**	6
TU37C2[η]	−1.7:+0.2	**1.9**	2	3.6 kb *Hpa*I–*Hpa*I (−3.6 to 0.0)	1.7	A	1.9	**P**	6
Ddc[α,β,ε]	+0.1:+4.1	**4.0**	13	3.5 kb *Hpa*I–*Hpa*I (0.0 to +3.5)	2.0, 2.3	E4, L2, P2, A	2.0, 2.3	D	6
l(2)37Cc[γ]	+5.8:+7.4	**1.6**	4	7.2 kb *Pst*I–*Pst*I (+2.7 to +9.9)	1.3	E1, P1	1.6	D	6
l(2)37Cb	+7.3:+10.5	3.2	3	2.1 kb *Bam*HI–*Hind*III (+7.2 to +9.3)	2.6	E2	3.2	D	6
l(2)37Cd	+11.6:+12.4	0.8	2	2.2 kb *Hind*III–*Xho*I (+10.4 to +12.6)	0.8	E1, L1	0.8	**D**	6
l(2)37Ca	+14.0:+16.1	2.1	1	6.8 kb *Eco*RI–*Eco*RI (+13.9 to +20.7)	2.1	E1, E3, L1	2.1	*D*	6
l(2)37Ce	+16.5:+17.3	0.8	1	6.8 kb *Eco*RI–*Eco*RI (+13.9 to +20.7)	0.6	E1, E3, L1	0.9	*D*	6
l(2)37Cg	(+17.5:+18.5)	(0.6)	NI	—	—	—	0.6	ND	6
brat	+45.7:+50.0	**4.3**	3	8.5 kb *Eco*RI–*Eco*RI (+38.5 to +47.1) 5.3 *Eco*Ri–*Eco*RI (+47.1 to +52.4)	2.8	E3, E4	4.2	*P*	7
fs(2)TW1	+50.1:+60.1	ND	NI	—	—	—	ND	ND	7

[a] Information previously reported: α, BEALL and HIRSH (1984); β, EVELETH *et al.* (1986); γ, EVELETH and MARSH (1986b); δ, MARSH *et al.* (1981); ε, MORGAN *et al.* (1986); ζ, PENTZ and WRIGHT (1986); η, EVELETH and MARSH (1987); θ, M. PHISTRY and H. J. KRAMER, personal communication.

[b] DNA coordinates give the gene position within the phage λ-cloned DNA and are relative to the *Hpa*I restriction site near the 3′ end of the *Ddc* gene. Coordinates in parenthesis are approximate locations based on *P*-element rescue data.

[c] Numbers in bold-face text are derived from DNA sequence data while numbers in normal text are deduced from transcript size, restriction enzyme mapping, RFLP analysis and *P*-element germline transformation data. Numbers in parenthesis are deduced only from transcript size and may represent an underestimation of gene size. ND = Not determined.

[d] NI = Not isolated.

[e] An asterisk (*) next to a size indicates a composite of several smaller, overlapping cDNAs.

[f] E1, λgt10 1.5–5 hr embryonic cDNA library (HOGNESS and GOLDSCHMIDT-CLERMONT, unpublished data); E2, λgt10 3–12 hr embryonic cDNA library (POOLE *et al.* 1985); E3, pNB40 12–24 hr embryonic cDNA library (BROWN and KAFATOS 1988); E4, λgt10 12–24 hr embryonic cDNA library (POOLE *et al.* 1985); L1, λgt10 crawling third instar larval cDNA library (WHARTON *et al.* 1985); L2, λgt10 crawling third instar larval cDNA library (POOLE *et al.* 1985); L3, pNB40 third instar larval imaginal disc cDNA library (BROWN and KAFATOS 1988); P1, λgt10 24 hr pupal cDNA library (HOGNESS and GOLDSCHMIDT-CLERMONT, unpublished data); P2, λgt10 24–48 hr pupal cDNA library (POOLE *et al.* 1985); P3, λgt10 24–48 hr pupal cDNA library (WHARTON *et al.* 1985); A, λgt10 0–2 hr adult cDNA library (EVELETH *et al.* 1986).

[g] ND = Not determined.

[h] Direction of transcription with respect to the centromere: D, proximal to distal; P, distal to proximal. Italicized letters indicate direction determined by restriction endonuclease mapping of directional-cloned inserts. Bold-face letters indicate direction confirmed by Northern blot analysis using sense and antisense probes.

[i] Figure in which gene location and structure is depicted.

ized to all fragments of the cDNA, but the 7.4 kb probe only hybridized to the proximal 0.67 kb of the cDNA. There is no cross hybridization between the 5.8- and 7.4-kb regions. Comparison of cDNA and genomic restriction maps shows there must be a nearly 20-kb intron interrupting the *Bd* cDNA (Figure 4).

The precise position of the scattered *B* gene *Bg* was not determined. The 3.9-kb fragment that rescues *Bg* mutants identifies a 3.0-kb mRNA on Northern blots and allows an approximate size for *Bg* to be inferred (Figure 4). However, since RFLPs were not found in the two *Bg* alleles, and cDNAs corresponding to the *Bg* gene were never isolated, no additional information on *Bg* was obtained.

In the proximal subcluster, cDNA clones for *Cb* were isolated from a λgt10 3–12 hr embryonic library, while clones for *Cd, Ca* and *Ce* were recovered from a λgt10 crawling third instar larval library (Table 9). The molecular analysis of loci within the proximal subcluster also indicate a dense organization, with an average of 700 bp separating adjacent loci (Figure 5). With the exception of *amd* and TU37C1, all are transcribed proximal to distal (Table 9). No cDNAs were recovered for the *Cg* gene. However, using the 4.6-kb *Eco*RI-*Sal*I λ13 fragment that rescued *Ca, Ce* and *Cg*, a 0.6-kb mRNA is detected in addition to the 0.9- and 2.1-kb transcripts (data not shown). Since the latter two transcripts are accounted for by the *Ca* and *Ce* genes, the *Cg* gene most likely encodes the 0.6 kb mRNA.

Three *brat* cDNAs were isolated using a 8.5-kb *Eco*RI fragment from λCS6.12 or a 5.3-kb *Eco*RI fragment from λLL18 to screen both pNB40 and λgt10 12–24 hr embryonic libraries (Table 9). Sequence analysis of *brat* shows the gene is transcribed distal to proximal and the 4.2-kb transcription unit does not contain any introns (Figure 6).

DISCUSSION

Mutagenesis screening within the *Ddc* gene cluster

Effectiveness of deficiency screening strategies: The efficiency with which deficiencies were recovered varied widely among screens (Table 1). The most productive, albeit labor intensive, screening strategy was first to screen individual chromosomes for lethal mutations in the *Ddc* region and then determine which lethal chromosomes were deficiencies. Of the 16,985 chromosomes screened in these five single chromosome F_2 screens (Table 1) (DF 4, 9, 14–16), 123 *Ddc* region lethal chromosomes were isolated of which six were deficiencies (0.035% recovery). The F_1 screens that involved uncovering *rdo, hk,* or *pr,* with or without the reversion of *Tft,* were effective at producing deficiencies and required significantly less effort (Table 1) (DF 1–3, 8 and 12). Nonetheless, while generating 30 deficiencies among the ~206,000 chromosomes screened, only nine of these deleted specifically into the *Ddc* region (0.004% recovery). The yield of deficiencies from the seven *Ddc* escaper screens was at least fivefold lower than the *rdo hk pr-Tft* screens (Table 1) (DF 10, 11, 13, 17–20) with only seven deficiencies isolated out of ~234,000 chromosomes screened (0.003% recovery). However, all were deletions within the *Ddc* region, a frequency similar to the 0.004% recovery from the *rdo hk pr-Tft* screens.

Our attempts to recover deficiencies by reverting the w^+ marker in TE37 were unsuccessful since only one reversion deficiency was recovered in three screens (Table 1) (DF 5–7) involving 132,000 chromosomes (0.0008% recovery). The deficiency recovered in screen 8 was a *Tft* revertant, not a w^+ revertant. The production of deficiencies by reverting the w^+ marker in TE-bearing chromosomes has, however, been carried out quite successfully by others (see ASHBURNER 1989a).

Effectiveness of using double mutagens in recessive lethal screens: The rationale for employing two different mutagens in the same screen is to recover small aberrations, which facilitates the molecular mapping of a gene, while still inducing a reasonable number of point mutations. While the focus of our work was not to document precisely the effectiveness of double mutagen screening, a few points can be made. The double mutagen combinations used did not significantly increase the mutation rate. In fact the EMS and formaldehyde rate was only half that of EMS alone (Table 2). However, the recovery of RFLPs in various loci is significantly higher, particularly with the EMS and γ-rays combination. Of the 211 mutations analyzed, none of the 90 EMS alleles had RFLPs, while 15.2% of the EMS and γ-ray-induced mutants and 5.3% of the EMS and formaldehyde-induced mutants had detectable aberrations (Table 7). The very high frequency of RFLPs in alleles induced by γ-rays (12.5%) or DEB (16.7%) alone may be comparable to the EMS/γ-ray double mutagen combination, however, the small sample size precludes any definitive statements. Therefore, it appears γ-ray exposure in combination with EMS or, possibly alone, is a good mutagen for inducing intragenic RFLP aberrations. While not used extensively by us, DEB also appears to be a mutagen that induces a high percentage of intragenic aberrations, although it may be gene specific (REARDON *et al.* 1987; WOHLWILL and BONNER 1991). A second unpredicted benefit of using the combination of EMS and γ-rays was the recovery of mutations in two previously unknown loci, *Dox-A2* and *Bg* (Table 2). Therefore our double mutagen strategy was successful in yielding both useful intragenic aberrations and identifying new loci.

Loci within the *Ddc* gene cluster are not equally mutable: The screening data from the *Ddc* region indicate genes from this area of the second chromosome are not equally mutable. First, a wide range of alleles was recovered from loci within this region. Thus while only one allele of *Bg* was isolated, 42 alleles of *Ca* were recov-

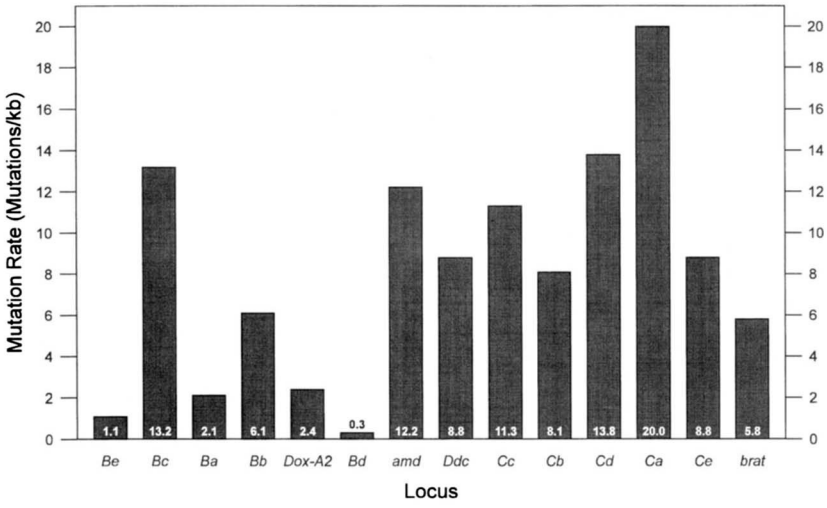

FIGURE 8.—Mutation rates of lethal genes within the *Ddc* gene cluster. Mutation rates were calculated by dividing the number of mutations recovered (Table 2) by the gene size (Table 9). Four genes were excluded: *Bg* and *Cg*, their gene size was unknown; *hk* and *fs(2)TW1*, not recessive lethal mutations.

ered, even though the same mutagenized chromosomes were screened for both loci (Table 2). If the mutation data for recessive lethal loci are normalized for target size, a 30-fold difference in gene mutability is seen (Figure 8). Even if *Bd* is excluded because its large intron may artificially reduce its mutation rate, a 20-fold difference is still observed. Second, recessive lethal mutations were recovered in previously unmutated genes only after we conducted screens using different mutagens. After completing the 600 series screen, over 22,000 chromosomes had been analyzed with 14 vital loci isolated (Table 2). Every complementation group had from 2 to 34 alleles with a mean of 13 alleles per gene. Thus, by convention the *Ddc* gene region was saturated for recessive lethal mutations. Nevertheless, changing the mutagen combination in the 7300/7400 series screens resulted in the recovery of mutations in two previously unidentified recessive lethal loci, *Dox-A2* and *Bg*. Third, mutation rates appear enhanced for genes located within more highly packed chromatin. The mutability of proximal subcluster loci are generally higher than loci contained within the distal subcluster (Figure 8). Using mutagenesis data for loci comprising approximately one-third of the first chromosome, LEFEVRE and WATKINS (1986) convincingly argued that genes from four different regions (1A1-3E8, 6D1-8A5, 9E1-11A7 and 19A1-20F4) are not equally mutable. Studies on the third chromosome also indicate that genes are not equally mutable. In the *rosy-Ace* region, three times as many transcripts were detected by reverse Northern blots as compared to complementation groups recovered by mutagenesis screens (43 transcripts to 12 loci) (HALL *et al.* 1983; BOSSY *et al.* 1984). In addition, WOHLWILL and BONNER (1991) also observed an unequal mutability of genes within chromomere 63B.

Taken together, these data extend the generality of the arguments presented by LEFEVRE and WATKINS (1986). First, it appears that the unequal mutability of genes is a general condition for the entire *D. melanogaster* genome. This assertion argues that the Poisson distribution is an invalid statistical approach to determine mutagenesis saturation, and the negative binomial distribution (JOHNSON and KOTZ 1969) is a far better statistical treatment of mutability distributions (see arguments of LEFEVRE and WATKINS 1986). Second, it is generally accepted that when multiple alleles of each locus are found, a region is saturated for mutations, even if only a single mutagen is used. However, the arguments presented by LEFEVRE and WATKINS (1986) and the results of our screens suggest this assumption is not valid. Therefore, it may be incorrect to state that a region is fully saturated for mutations if only a single mutagenesis strategy is used.

Organization of the *Ddc* gene cluster

Defining the cytogenetic region containing the *Ddc* gene cluster: In the past we arbitrarily defined the *Ddc* region as being encompassed by *Df(2L)TW130*. We have now established that except for the distal part of *hk*, the entire *Ddc* gene cluster is located between the proximal breakpoint of *Df(2L)TW203* (37B9-C1,2) and the distal breakpoint of *Df(2L)Sd37* (37C6-D1), a maximum of 11 bands. Of the 162.5 kb of cloned DNA in this region, a maximum of 152.5 kb extends between the breakpoints of these deficiencies (DNA coordinates −85.4 to +66.8) (Table 5). HEIRO *et al.* (1994) estimate these 11 bands (37B10-C7) represent 193.5 ± 19.4 kb of DNA, a figure 15–20% larger than the actual 152.5 kb. Considering that the values of HEIRO *et al.* (1994) represent averaged lengths based upon several assumptions and rough estimations using haploid DNA content (SORSA 1988) and that local variations undoubtedly exist, their estimations approximate our precise values remarkably well.

Genetic and molecular organization of genes within the *Ddc* gene cluster: Our extensive genetic study of the *Ddc* gene cluster resulted in a set of 377 mutations, including 38 chromosomal deletions that enabled us to define 18 complementation groups within the 37B10-

D1 interval. All 18 loci were assigned to transcription units by locating RFLPs in mutant alleles (Table 7) (14 in 11 different genes) or by the rescue of mutant alleles with known fragments of genomic DNA (Table 8, Figures 2–6) (26 constructs). Besides the 18 loci, three additional transcription units were discovered during screens for cDNAs using probes derived from this cloned region. The 21 genes are not randomly distributed, but rather 14 loci and three transcription units are located in two densely packed subclusters (Figure 7). The distal subcluster contains six loci (*hk, Be, Bc, Ba, Bb* and *Dox-A2*) and two unmutated transcription units (TU37B1 and TU37B2) within ~27.4 kb of DNA (Figures 3 and 4). The proximal subcluster spans ~23.0 kb of DNA and contains eight loci (*amd, Ddc, Cc, Cb, Cd, Ca, Ce* and *Cg*) and one unmutated transcription unit (TU37C2) (Figure 5). Approximately 53.3 kb of DNA separate the two subclusters. Four additional loci are also located within the *Ddc* gene cluster but are not within the two subclusters. These genes, *Bg, Bd, brat* and *fs(2)TW1* are referred to as the "scattered genes". Both *Bd* and *Bg* are located between the two subclusters (Figure 6). Interestingly, because of the large size deduced for *Bd* (~23.8 kb), over 44 % of this intervening region is occupied by this gene (Figure 4). The last two scattered genes, *brat* and *fs(2)TW1* are ~27.2 kb proximal to the proximal subcluster within an area of no more than 14.4 kb (Figure 6).

Indicative of the dense packing of genes, two pairs of genes found within the *Ddc* gene cluster overlap. The 3′ noncoding regions of two unmutated transcription units overlap the 3′ noncoding region of the adjacent lethal locus (TU37B2-*Bb* and TU37C2-*Ddc*). While the functional significance of these overlapping gene pairs was not determined, a few interesting observations can be made. First, the codon usage for TU37B2 and TU37C2 is unusual compared to most *D. melanogaster* transcripts, suggesting these mRNAs may not be translated. In addition, the individual partners of both overlapping gene pairs (TU37B2-*Bb*, TU37C2-*Ddc*) are expressed differentially in time and space (SPENCER *et al.* 1986; FREEMAN 1989), suggesting that the high expression of one gene could suppress the expression of its overlapping neighbor by transcriptional interference (see SCHULZ and BUTLER 1989 for discussion). Recent work in the *serendipity-janus* gene cluster provides evidence to support the existence of a transcriptional interference mechanism in *D. melanogaster* (YANICOSTAS and LEPESANT 1990). Other studies, however, have indicated the presence of two overlapping genes may be fortuitous and not relevant to regulating gene expression at all (BOGGS *et al.* 1987; SCHULZ *et al.* 1990). In any event, more detailed studies are required before the significance of the overlapping gene pairs in the *Ddc* gene cluster can be ascertained.

The *Ddc* gene cluster is a remarkably dense region of genes, with the 21 genes from *hook* to *fs(2)TW1*, the most distal and proximal genes, respectively, contained within ~145.3 kb (Table 9). An aspect of this organization is to consider the percentage of the region that contains a gene actively transcribed at some time during development. As one would predict, the transcriptional activity of both subclusters is extremely high. At one time or another, the 27.4-kb distal subcluster is 82% transcriptionally active, while >75% of the 23.0-kb proximal subcluster is transcribed. Over 50% of the 53.3 kb between the two subclusters may be transcribed due to the large predicted size of *Bd*. Finally, if one considers the 145.3 kb containing the entire *Ddc* gene cluster, over 49% of the region is transcriptionally active. This figure, however, is an underestimation. First, since the size of *fs(2)TW1* is not known, the value for this gene was not included in the calculations. Second, reverse Northern blot experiments indicate that several unidentified transcription units, none of which correspond to any of the 21 identified genes, are present in the region between *Dox-A2* and *Bg*, suggesting the existence additional genes (E. S. PENTZ and T. R. F. WRIGHT, unpublished data). Third, even though extensive screens for recessive lethal and female sterile mutations were conducted, we did not systematically screen for visible or male sterile mutations. Thus, in all likelihood, several more genes may be present in the *Ddc* gene cluster. Therefore, it is reasonable to estimate over 50% of the entire 145.3-kb *Ddc* gene cluster is actively transcribed at some point during development. Excluding reiterated gene clusters, the only other large regions exhibiting similar levels of transcriptional activity are the ANTC and BXC with ~60 and 45% transcriptional activity, respectively (LINDSLEY and ZIMM 1992). While by their very organization, dense gene clusters also exhibit extraordinarily high transcriptional levels, these regions are generally <15 kb (see Introduction). The *Ddc* gene cluster, therefore, represents one of the few large regions known in the *D. melanogaster* genome to possess such a high transcriptional activity.

Gene to band correlation of region 37B10-C7: The entire *Ddc* gene cluster spans chromomeres 37B10-C7, and the position of each gene within the *Ddc* gene cluster was mapped to one of these 11 bands (Table 10, Figure 9). HEIRO *et al.* (1994) estimates the lengths of these chromomeres as follows: 37B10, 4.00 kb; 37B11, 8.25 kb; 37B12, 8.25 kb; 37B13, 19.5 kb; 37C1,2, 57.5 kb; 37C3, 23.25 kb; 37C4, 8.25 kb; 37C5, 19.5 kb; 37C+, 4.00 kb; 37C6, 23.25 kb and 37C7, 8.25 kb, with values carrying a ±10% error. In addition, an average value of 1.5 kb was used as the interband length between each chromomere (HEIRO *et al.* 1994; KOZLOVA *et al.* 1994). The distribution of genes in each chromomere was determined (Table 10, Figure 9) using the above values for the estimated size of each chromomere in conjunction with our own region-specific mapping data including genetic complementation of genes/deficiencies (Table 6), cytological location/DNA coordi-

TABLE 10

Gene to chromomere distribution in region 37B10-C7

Chromomere region	Number of chromomeres	Number of loci	Number of transcription units	Genes	Gene to band average
37B10-12	3	5	1	*hk, Be, Bc, Ba,* TU37B2, *Bb*	2.0
37B13	1	4	1	*Dox-A2,* TU37B1, *Bg,* (*Bd*)[a]	5.0
37C1, 2	1	4	1	(*Bd*), *amd,* TU37C2, *Ddc, Cc*	5.0
37C3	1	5	0	*Cb, Cd, Ca, Ce, Cg*	5.0
37C4	1	0	0		0
37C5	1	2	0	*brat, fs(2)TW1*	2.0
37C+	1	0	0		0
37C6	1	0	0		0
37C7	1	0	0		0

[a] Since the *Bd* gene spans chromomeres 37B13 and 37C1, 2, it was considered present in each band and, therefore, counted twice.

nates of deficiency breakpoints (Table 5) and molecular location/size of genes/transcription units (Table 9) in the cluster. There is an overall 2:1 ratio of genes to chromomeres (21 genes *vs.* 11 bands) (Table 10). The distribution of the genes within this region is not random. Thus, while chromomeres 37B10-13, 37C1,2-3 and 37C5 each contain at least two genes, bands 37C4, 37C+ and 37C7 appear silent (Figure 9). In addition, there does not appear to be a correlation with gene location and packing ratio of chromatin. The genes of the distal subcluster are contained within bands of low packing ratio of chromatin, while genes of the proximal subcluster span both high and average density chromomeres (Figure 9).

The one gene-one band hypothesis is invalid: Regardless of the placement of specific genes within specific chromomeres, the majority of the chromomeres within this region contain multiple genes. For example, the 19 genes from *hk* to *Cg* lie within the six chromosomal bands 37B10–C3 representing a concentration of 3.2 genes per band. Therefore, our data clearly demonstrate the one gene-one band hypothesis of BRIDGES (1935) is invalid. But is the tight clustering of genes within chromomeres 37B10–C5 an anomaly? Are the majority of the genes within the *D. melanogaster* genome more widely distributed or spread out? Several lines of work clearly demonstrate that this region is not unique with respect to gene to band ratios. First, current estimates on the number of genes now identified in *D. melanogaster* is 6898 (GELBART *et al.* 1994) while the latest refinement on the number of chromomeres within the *D. melanogaster* genome is 5113 (HEIRO *et al.* 1994). Therefore, research has already identified many more genes than chromomeres, and it is quite apparent that many more genes are still undiscovered while chromomere estimates have not varied much for decades. Second, as evident from our introduction, dense gene clusters are not uncommon and more are reported each year. Characteristically, this type of gene cluster contains from three to five genes within 5–15 kb of DNA. Since the size of a fine chromosomal band may range from 3 to 36 kb (KOZLOVA *et al.* 1994), it is apparent that most dense gene clusters could lie within a single chromomere. Lastly, two other large regions of the *D. melanogaster* genome were also extensively mapped at the genetic and molecular level and studied with respect to gene to band ratios. KOZLOVA *et al.* (1994) did a thorough analysis of the gene to band arrangement of the seven bands 9F12–10A6 from the *vermilion-sevenless* region on the first chromosome. They presented evi-

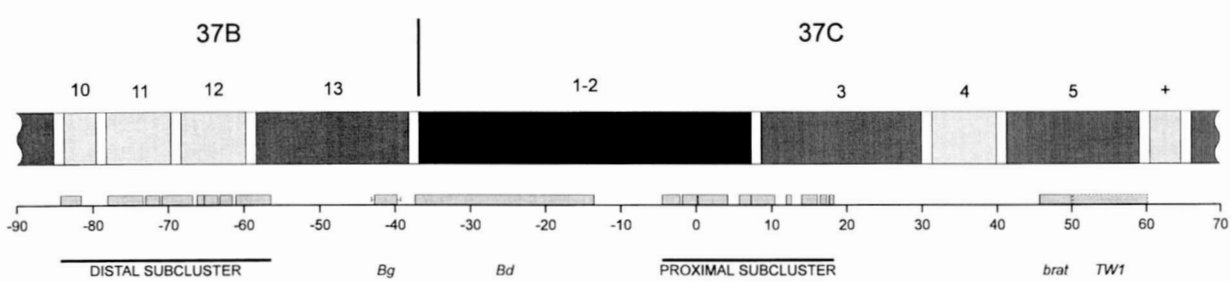

FIGURE 9.—Comparison of the linear DNA map of the *Ddc* gene cluster with 37B10-C+ chromomere structure. A diagram of chromosome region 37B10 to 37C+ is portrayed at the top. Individual chromomeres are numbered. Light grey bands represent a low packing ratio of chromatin; medium grey bands, an average packing ratio and dark gray bands, a high packing ratio (HEIRO *et al.* 1994). Interchromomeres are indicated by white bands. Below the chromosome region is a map with the locations of all 21 genes and coordinate scale. The diagram is drawn to scale.

dence indicating that ≥18 genes were located in this 285-kb region, representing a density of three genes per band. They also found that the DNA content and gene number varies dramatically and regions of small bands are genetically more dense than large bands. Using the criteria of KOZLOVA *et al.* (1994) for assessing band-size categories, all the bands in the *Df(2L)TW130* region correspond to small bands even chromomere 37C1–2. Thus, their findings and ours are in agreement. A second region extensively studied is the 315-kb *rosy-Ace* region from 87D5 to 87E5 (SPIERER *et al.* 1983). This 16-chromomere region contains 43 actively transcribed regions (HALL *et al.* 1983; BOSSY *et al.* 1984), representing a concentration of 2.7 genes per band. While a more detailed gene to chromomere association was not determined, it is quite apparent many of these bands are oligogenic. Therefore, our work, along with the work of LEFEVRE and WATKINS (1986) and KOZLOVA *et al.* (1994) clearly indicate the one gene-one band hypothesis is not valid and that multiple genes exist in a given chromomere.

Organization of the *Ddc* gene cluster is unique: Not only is the *Ddc* gene cluster one of the densest clustering of functionally related genes known in higher eukaryotic organisms, its structural organization is not strictly comparable to the three gene cluster types found in *D. melanogaster*. The presence of both structurally related and unrelated genes and the obvious lack of tandem organization indicate this gene cluster is not reiterated. In addition, the simple, straightforward intergenic complementation among loci suggests the *Ddc* gene cluster is not a gene complex. This is supported by the lack of overt global control of gene expression as evidenced by the observation of genes functioning individually in ectopic sites well enough to rescue lethality. Finally, while there are cases of more tightly clustered gene regions, such as the seven genes of the 15-kb heat shock protein gene cluster (AYME and TISSIÉRES 1985), the six genes of the 8-kb *serendipity-janus* gene cluster (VINCENT *et al.* 1985; YANICOSTAS *et al.* 1989) and the seven genes of the 10-kb 71E gene cluster (RESTIFO and GUILD 1986), these dense gene clusters encompass no more than 15 kb while the *Ddc* gene cluster extends over 145.3 kb with each subcluster occupying over 20 kb. Furthermore, since mutations in the majority of loci from the *Ddc* gene cluster appear to be involved in the same physiological process (WRIGHT 1995), it would seem inappropriate to split the two subclusters apart. Therefore, the *Ddc* gene cluster is too expansive to be considered a dense gene cluster. But is the organization of the *Ddc* gene cluster unique in the *D. melanogaster* genome? While genetic evidence suggests that the localization of loci apparently involved in similar functions from two other cytogenetic regions (UNDERWOOD *et al.* 1990; LITTLETON and BELLEN 1994), evidence indicates that the genes within these regions are not organized into gene clusters. Therefore, the *Ddc* gene cluster represents a unique arrangement of clustered, functionally related loci.

Why are the loci of the *Ddc* gene cluster so tightly clustered? Our first thought was that the *Ddc* gene cluster evolved due to the necessity of overt global control of gene expression. While enzymes as central to catecholamine biosynthesis such as tyrosine hydroxylase, N-acetyl dopamine transferase and N-β-alanyl dopamine transferase are not located within the *Ddc* gene cluster (WRIGHT 1987b), this cluster does contain genes both for structurally related (DDC and AMD) and structurally unrelated enzymes (DOX-A2) involved in catecholamine metabolism (EVELETH and MARSH 1986a; WRIGHT 1987a, 1995). Furthermore, of the 15 loci in the region affecting cuticle formation, cuticle sclerotization or cuticle melanization, eight affect catecholamine pool levels during prepupal and pupal development (WRIGHT 1987a, 1995), suggesting several of these genes may encode enzymes involved in catecholamine metabolism. Since the functions of a majority of these genes appear to be interrelated, one might predict global coordinate controls are operative. However, mitigating against this prediction is the fact that genomic DNA fragments encompassing only one or two genes relocated out of the subclusters to ectopic sites by *P*-element-mediated transformation can function well enough to rescue mutations in all the essential loci (Table 8). However, not all these transformed genes may be functioning optimally. We have observed significant variability in the efficiency of rescue by identical constructs inserted into different ectopic locations. Thus, transcription levels may be dependent on local enhancers. Furthermore, drastically reduced levels of gene activity may be adequate to effect rescue. For example, <10% wild-type DDC activity is sufficient to ensure the complete rescue of *Ddc* mutations (WRIGHT *et al.* 1982). Preliminary experiments to insulate the transformed fragments from local influences have been carried out using a DNA fragment known to rescue both lethal and female sterile *Dox-A2* mutations (E. S. PENTZ and T. R. F. WRIGHT, unpublished data). The *Dox-A2* fragment was flanked by the insulating SCS and SCS′ sequences (KELLUM and SCHEDL 1991) before transformation. These initial results are equivocal: one transformant still rescued both lethality and female sterility, but two different transformants rescued neither mutant phenotype. Note that all five of the transformed lines containing uninsulated fragments rescued *Dox-A2* mutations (Table 8). Thus, the possibility of global regulation of the genes in the subclusters cannot yet be completely ruled out.

The *Ddc* gene cluster appears evolutionarily conserved since subcluster integrity is maintained in related *Drosophila* species. In *D. virilis* and *D. psuedoobscura* while the proximal and distal subclusters are separated, the subclusters themselves are intact and are still located on the same chromosome (T. R. F. WRIGHT, unpublished

data). Therefore, the maintenance of subcluster integrity appears functionally important enough to conserve. Analyzing the temporal expression patterns of genes within the *Ddc* gene cluster does offer one insight. Eighteen of the 21 genes within the *Ddc* gene cluster are expressed during embryogenesis suggesting this common time of gene activity may be a reason for the clustering of these functionally related loci (Table 9) (T. R. F. WRIGHT, unpublished data).

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