GENETIC ANALYSIS OF THE DUMPY COMPLEX LOCUS IN *DROSOPHILA MELANOGASTER*: COMPLEMENTATION, FINE STRUCTURE AND FUNCTION

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ABSTRACT

An extensive genetic analysis of the *dumpy* locus is presented. This study includes complementation, fine structure mapping and allelic interaction. A number of complementing recessive lethals of the *dp* complex have been genetically mapped. Two alleles of the *ol(v)* type that complement *l* alleles map to the left portion of the locus. A number of *olv* alleles that complement both *l* and *lv* lethals map within the right portion of the locus.——Fine-structure analysis demonstrated that both *olv* and *o* alleles are distributed among various subloci. Evidence for spacer regions between subloci is presented.——An extensive discussion of the data considers whether the locus is unicistronic or multicistronic. The conclusion reached is that the locus is not a single functional cistron. The possibility of a single cistron encoding a multifunctional polypeptide is discussed.——The hypothesis is proposed that the left portion of the map and the *l* mutations function as regulatory sequences and that the right portion of the map encodes structural sequences.

GENETICALLY complex loci in Drosophila are interesting because they represent arrangements that may offer clues to the organization and intricacies of gene regulation in higher organisms. Most complex loci are characterized by pleiotropism and partial or complete allelic complementation. Based primarily on complementation patterns and the genetic map, many complex loci have been considered to be groups of closely linked genes (gene-clusters), *e.g.*, bithorax (LEWIS 1963, 1967, 1978), dumpy (GRACE 1970) and lozenge (GREEN and GREEN 1956). However, other complex loci may be composed of a single gene encoding a single polypeptide sequence with a single function, *e.g.*, vermilion (BAILLIE and CHOVNICK 1971). The complexity and relatively large size of many genetic loci have presented difficulties to workers attempting to understand their structure and function. In Drosophila, these difficulties have been, in part, related to the paucity of information about the biochemical products of these loci.

The isolation of single multifunctional polypeptides in eukaryotes other than Drosophila has led to an apparent resolution of this impasse. The data indicate that a single gene encodes a single polypeptide with discrete multiple functions (GAERTNER and COLE 1977; KIRSCHNER and BISSWANGER 1976). It appears that a single protein can be multifunctional; that is, discrete regions with unique

functions exist within a single polypeptide. Each region maintains its functional capabilities even when isolated (KIRSCHNER and BISSWANGER 1976).

Multifunctional proteins may also exist in Drosophila. Recent studies on the rudimentary locus, which encodes three enzyme activities in the pathway to the biosynthesis of pyrimidines (NORBY 1973; RAWLS and FRISTROM 1975), indicate that the three enzyme activities co-sediment as a single peak in sucrose gradients (BROTHERS *et al.* 1978). This suggests that the three discrete activities of the complex are contained on one polypeptide. The multifunctional protein concept provides a straightforward explanation for the rudimentary locus and possibly other genetically complex loci.

While the relative location of genes in Drosophila has been known for many years, the detailed features of each gene and its surrounding sequences are just beginning to be elucidated. A complete picture will become possible only with a thorough analysis of gene structure and gene function.

This paper describes extensive studies on the genetics of the dumpy complex locus. Mutants at this locus exhibit a variety of phenotypes (MULLER 1922; CARLSON 1959a,b). For some phenotypes, all alleles are restricted to a specific region within the locus; other phenotypes can result from a single mutation at any one of a number of sites.

In an earlier paper, I suggested that there may exist seven to ten genes within the dumpy complex (GRACE 1970). In light of the evidence related to multifunctional proteins, this hypothesis must be altered. This paper reports experiments on complementation between recessive dumpy lethals, on intralocus mapping and on fine structure.

MATERIALS AND METHODS

Different various *dp* mutants are designed by their phenotypic expressions, primarily affecting the wings and/or thorax: *o*, homozygous oblique (wing defect); *v*, homozygous vortex (thorax defect); *ov*, homozygous oblique-vortex; *olv*, recessive oblique-lethal-vortex (CARLSON 1959a,b). Allelic designations postscripted by "CS" were induced by ICR-170 (SOUTHIN and CARLSON 1962). Those postscripted by "HC" or "DG" were induced by n-nitrosomethylurea according to the method described by CORWIN (1968) or by ethyl methanesulfonate according to the method described by LEWIS and BACHER (1968). All other *dp* alleles referred to have been described elsewhere (CARLSON 1959a,b; LINDSLEY and GRELL 1967).

Early genetic studies led to the subdivision of the dumpy locus into *subloci* (CARLSON 1959a). These subloci corresponded to areas defined by map location, complementation tests and comparison of phenotypes. The inference was also made that each sublocus represents a functional unit. The term sublocus will be retained in preference to gene or cistron, because it can be used in a more general sense.

Stocks: Stocks were maintained in homozygous condition unless they were poorly viable or contained a secondary lethal. The lethal stocks were balanced over *In(2LR)Cy Stw^{DG} L^{4}* (*Stw^{DG}* is a chromosome *2* dominant, yellow-like mutant induced by X rays in the *In(2LR)Cy L^{4}* chromosome by OSTER and CARLSON, unpublished, and designated by them as straw dominant).

Phenotypic index: *dp* mutant phenotypes have been described previously; however, no attempt has been made to quantify the phenotypic effects. To measure the possible correspondence between the intensity of mutant phenotype and the position of the mutation within the locus, a scale was developed to express the severity of a mutant phenotype, the *phenotypic index*, which was determined by mating each mutant line to a selected *ov-1* stock. F_1 offspring were

classified according to a numerical scale ranging from 0 (wild type) to 5 (extreme dumpy) for both oblique wing and thoracic vortex (see Figure 1). Effects on the legs were noted. Since *dp* males are generally affected less than *dp* females, each phenotypic index was based on three- to six-day-old females at 22° or, in a few cases, at room temperature. The phenotypic index proved to be particularly useful in rating the recessive lethals.

Lethality: Some homozygous dumpy mutants express a very weak mutant effect, while others are semilethal. These extreme alleles (*e.g.*, *o-bm ov-h*) produce viable heterozygotes with most, if not all, *dp* lethals (LINDSLEY and GRELL 1967). In this study, alleles were classified as lethals and included in the lethal analysis only if they (1) were stable as balanced stocks, (2) showed a strong or extreme phenotype in combination with the tester allele *ov-1*, and/or (3) were lethal in combination with one or more of the *dp* lethal types *ol*, *lv*, *olv* or *l*.

Dumpy map: Based on the work of several investigators, a simplified general map of the *dp* complex has been constructed (CARLSON 1959a; SOUTHIN and CARLSON 1962; GRACE 1970; MONTGOMERIE 1974; and GRACE, unpublished).

o — *o* — *olv* — *l* — *lv* — *ov* — *ol* — *v*

The order *olv-l* and *l-lv* was determined by MONTGOMERIE, using a second lethal induced on the mutant *l* chromosome. Other *olv*'s exist throughout the locus and will be discussed in detail later. Alleles *ov* and *olv* are referred to as "polar" mutations because they show both *o* and *v* effects. Alleles *o*, *v*, *lv*, *ol* and *l* are referred to as nonpolar mutations. Each location shown on the map represents a possible sublocus rather than a particular allele. Each sublocus is represented by two or more phenotypically similar alleles and in some cases also by phenotypically different alleles, *i.e.*, both polar and nonpolar alleles. The problem of determining the boundaries of different subloci is addressed in detail in the RESULTS. For clarity, references to a mutant mapping within a particular sublocus will be designated by one of the allelic types as given above, even

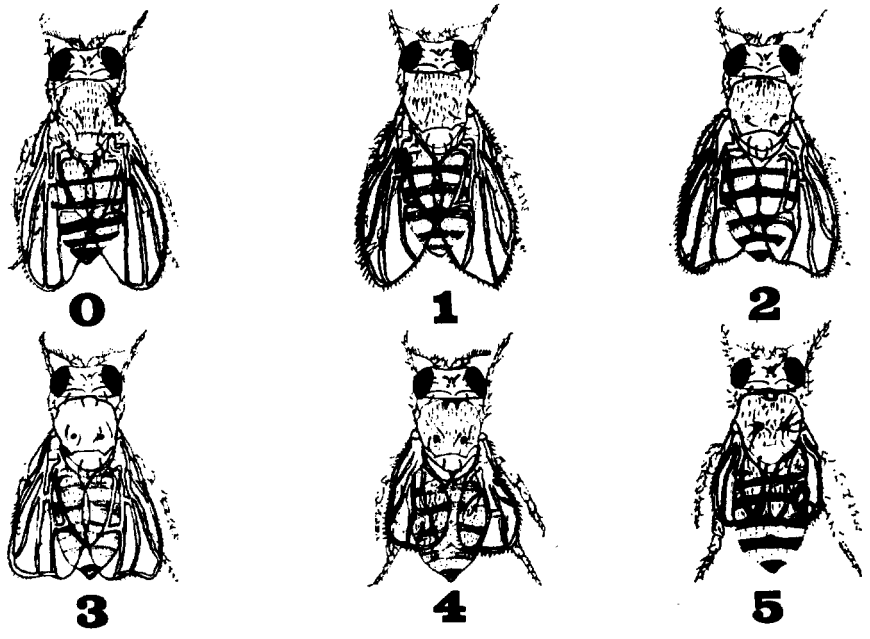

FIGURE 1.—Mutant index: A schematic drawing of the various *dp* mutant phenotypes representing the classifications of the mutant index. The wild type is rated 0, selected *ov-1* homozygote is rated 3 and the extreme *olv/ov* phenotype is rated 5. See text for details.

though the mutant under discussion represents a different phenotype if it maps within that sublocus, *e.g.*, an *olv* allele of the *ov* sublocus.

Lethal complementarity: A total of 45 lethal alleles, representing all known *dp* lethal types, were tested for complementarity by their ability to produce viable offspring when mated to other lethal alleles. The tests consisted of a series of crosses that involved mating each lethal line to the other 44 lines. Matings between different lines were made as mass cultures in shell vials containing six to ten virgin females of one line and five or more males of the other line. Each cross was kept at 16° and transferred to fresh medium twice at six-day intervals. The second transfer vial was maintained at room temperature. Complementation was determined by inspecting all culture vials for the presence of nonstraw progeny. Their presence was tentative evidence for complementation. The complementing individuals were verified as heteroallelic by mating them to the *ov-1* allele and demonstrating the presence of the two original lethals. If *l* was one of the two lethal alleles of the cross, both *ov-1* and a lethal, such as *olv*, were used to verify that complementation occurred between the two lethal alleles.

Mapping procedures: The four-point mapping procedure used for mapping *dp* alleles has been described by SOUTHIN and CARLSON (1962). In most cases, a stock containing a double *dp* lethal (*ol-olv-s* = *olv57*) *ed olv57 cl/InCy Stw*^{DG} *L*^{4} was used as the screening allele (CARLSON 1958). Otherwise, a marked balanced *lv* or *ol* stock was used.

RESULTS

Complementarity: Over 1,000 crosses were made using a random selection of various dumpy lethal types. Four *olv* alleles were identified, on the basis of these matings, that complemented all *lv* and *l* alleles (*olv*'s *DG27*, *61*, *HC39* and *59*). Two additional *olv* alleles that complemented only *l* (Table 1) map to the left

TABLE 1

Complementation between heterozygotes of mutants, l, ol, lv *and* olv

	olv	ol	lv	l
olv	—	—	±	±
ol		—	—	—
lv			—	—
l				—

The chart summarizes the results from more than a thousand crosses. (—) indicates that no complementation. (+) indicates that complementation was allele-specific. In each case, the specific allele was an *olv*. Any *olv* that complemented one *lv* or *l* complemented all *l* and *lv* mutations.

side of the locus. These two alleles, *ol(v)M51b* and *ol(v)DG20* are unusual in that they have less than 50% of the vortex defect when heterozygous with *ov-1*. Most *olv*'s show nearly complete penetrance for oblique and vortex (GRACE 1970). No complementation was found among combinations of *lv/ol*, *ol/olv* or *olv/olv*. A more exhaustive search might isolate complementing lethals for some of these combinations. In combinations that showed complementation in the first series of crosses, the number of nonstraw progeny was less than 5% of the total number of straw offspring. However, when some crosses were repeated several months later, the percentage of surviving nonstraw flies increased. Presumably, this change was due to an accumulation of *dp* modifiers that acted as dominants or were present in both lines (ALTENBURG and MULLER 1920). However, the complementation observed seems to depend on the nature of the mutations themselves in that these *olv* mutants have "less extreme" phenotypes than other typical *olv* mutants, rather than on modifiers. It seems highly unlikely that modifiers alone are involved because the complementing properties of the *olv* stocks are stable and the complementing lethals map to specific sites within the *dp* locus. In all cases, complementation tests occurred both at 16° and room temperature. Tests for temperature sensitivity for all lines were negative at 16°.

Phenotypes of complementing lethals: Viable combinations of *olv/l* that are phenotypically wild type can be maintained without difficulty as stable lines. The combination *olv/lv* is unable to fly, but generally has normal wings and legs. However, one combination, *lv/olvDG27*, produces a "balloon" or blistering on at least one wing in nearly 100% of the progeny. Usually, one or two pairs of vortices (one pair dorso-lateral, the other anterio-lateral) are present, as well as an anterior central "comma" effect. There is often a distortion of cell hair "pattern" on the thorax. Under conditions of stress, such as high temperature or nutritional restriction, a mild oblique wing can be found in these complementing lethal combinations. Among some *olv/lv* flies, an air bubble can occasionally be seen in the thorax under the cuticle. Rolling the fly back and forth maneuvers this bubble over the entire undersurface of the thorax, indicating that the flight muscles are absent. This absence may be due to factors such as muscle breakdown (METCALF 1970), abnormal tissue movement (KING 1964) or cell death, it may also be connected to the disturbance of bristle and cell hair pattern. A small percentage of the flies have their wings in a permanent vertical ("dead fly") position.

After the discovery of complementing *olv*'s, an attempt was made to recognize this particular type of *olv* by its phenotypic index (see MATERIALS AND METHODS). Virtually all noncomplementing *olv* alleles had been scored as #5 oblique (extreme) and had shortened legs (femurs). The complementing *olv* alleles were usually #4, or less, oblique and the leg effect was reduced. This pattern is consistent and has been successfully used to detect another *olv* (*olvDG62*), which also complements *lv* and *l* alleles. The vortex effect is inconsistent and apparently does not allow determination of a useful phenotypic index.

The recombination results have been divided into intersublocus mapping and intrasublocus mapping.

Intersublocus mapping: From a survey of the recombination data (Table 2,

TABLE 2

Summary of results from heterozygous crosses between various dumpy alleles

F_1 female	Verified single crossovers dp^+ (unverified)	Curly progeny $\times 10^3$	Frequency	Map order	"Conversions" (unverified)
1. *oDG33/ed o-2 cl*	*ed* + +	10.3	0.019	*o33–o-2*	——
2. *oDG53I/ed o-2 cl*	6 + + *cl*	74.2	0.016	*o-2–o53I*	——
3. *o-2/ed lv-1 cl*	*ed* + +	16.	0.012	*o-2–lv-1*	+ + +
4. *oDG56/ed o-2 cl*	6 + + *cl*	24.8	0.048	*o-2–o56*	——
5. *lDG82/ed o-2 cl*	3 + + *cl*	37.8	0.016	*o-2–l82*	——
	(+ + *cl*)		(0.020)		
6. *lDG83/ed o-2 cl*	+ + *cl*	87.4	0.002	*o-2–l83*	——
7. *lDG91/ed o-2 cl*	2 + + *cl*	31.8	0.013	*o-2–l91*	——
8. *oDG53I/ed lM57f cl*	2 *ed* + +	29.0	0.014	*o53I–lM*	——
9. *oDG56/ed lM57f cl*	2 + + *cl*	65.9	0.006	*lM–o56*	——
10. *ol(v)M51b/ed lM57f cl*	5 *ed* + +	16.9	0.059	*ol(v)–lM*	(+ + *cl*)
11. *ovDG37/ed lM57f cl*	——	3.4	——	——	——
12. *oDG/ed lM57f cl*	+ + *cl*	13.0	0.015	*lM–o6*	——
13. *olvDG61/ed lM57f cl*	3 + + *cl*	6.5	0.092	*lM–olv61*	——
14. *olvDG27/ed lM57f cl*	+ + *cl*	7.1	0.028	*lM–olv27*	——
15. *olvHC39/ed lM57f cl*	3 + + *cl*	9.6	0.062	*lM–olv39*	——
16. *oDG53I/ed lv-1 cl*	3 *ed* + +	30.9	0.019	*o53I–lv*	+ + *cl*
17. *oDG56/ed lv-1 cl*	6 + + *cl*	84.8	0.014	*lv–o56*	(*ed* + +)
18. *o(v)DG2/ed lv-1 cl*	——	5.7	——	——	——
19. *o(v)DG30/ed lv-1 cl*	2 + + *cl*	15.9	0.025	*lv-1–o(v)30*	——
	(+ + *cl*)		(0.038)		
20. *o(v)DG35/ed lv-1 cl*	2 + + *cl*	12.4	0.032	*lv-1–o(v)35*	——
21. *(ov)DG37/ed lv-1 cl*	2 + + *cl*	14.6	0.027	*lv-1–o(v)37*	——
22. *oDG41/ed lv-1 cl*	(+ + *cl*)	18.9	——	——	——
23. *oDG56/ed cm-2 cl*	——	8.5	——	——	——
24. *oDG53I/ed ov-1 cl*	2 *ed* + +	27.2	0.015	*o53I–ov-1*	+ + +
25. *oDG56/ed ov-1 cl*	*ed* + +	39.6	0.005	*o56–ov-1*	——
	(*ed* + +)		(0.01)		
26. *lvDG1/ed ov-1 cl*	——	6.4	——	——	——
27. *lvDG7/ed ov-1 cl*	*ed* + +	6.8	0.029	*lv-7–ov-1*	——
28. *lvDG10/ed ov-1 cl*	*ed* + +	9.7	0.020	*lv-10–ov-1*	——
	(*ed* + +)		(0.040)		
29. *lvDG38/ed ov-1 cl*	——	6.8	——	——	——
30. *lvCS42/ed ov-1 cl*	*ed* + +	5.3	0.038	*lv42–ov-1*	——
31. *lvDG49/ed ov-1 cl*	——	8.6	——	——	——
32. *lvDG50/ed ov-1 cl*	(*ed* + + *M*)	6.8	——	——	——
33. *lvDG57/ed ov-1 cl*	2 *ed* + +	5.4	0.074	*lv57–ov-1*	——
34. *lvDG58/ed ov-1 cl*	2 *ed* + +	19.7	0.020	*lv58–ov-1*	——
	(*ed* + +)		(0.030)		
35. *oDG41/ed ov-1 cl*	5 + + *cl*	45.7	0.021	*ov-1–o41*	——
36. *oDG41/ed cm-2 cl*	2 + + *cl*	54.1	0.007	*cm–o41*	——
37. *olvDG27/ed ov-1 cl*	6 + + *cl*	23.0	0.052	*ov-1–olv27*	——
38. *olvDG27/ed cm-2 cl*	5 + + *cl*	34.2	0.029	*cm-2–olv27*	——
39. *olvHC59/ed cm-2 cl*	6 + + *cl*	80.5	0.015	*cm-2–olv59*	——
40. *oDG41/ed v-2 cl*	2 *ed* + +	18.4	0.021	*o41–v-2*	——
	(2 *ed* + +)		(0.043)		
41. *olvDG27/ed v-2 cl*	5 *ed* + +	32.0	0.031	*olv27–v-2*	——

TABLE 2—Continued

F_1 female	Verified single crossovers dp^+ (unverified)	Curly progeny × 10^3	Frequency	Map order	"Conversions" (unverified)
42. *olvHC39/ed v-2 cl*	11 *ed* + +	20.7	0.106	*olv39–v-2*	———
	(*ed* + +)		(0.116)		
43. *olvHC59/ed v-2 cl*	7 *ed* + +	38.5	0.036	*olv59–v-2*	———
44. *olvDG61/ed v-2 cl*	8 *ed* + +	22.9	0.070	*olv61–v-2*	———
45. *oDG6/ed ov-1 cl*	*ed* + +	215.3	0.0009	*o6–ov-1*	———
46. *ovDG30/ed ov-1 cl*	———	35.6	——	———	*ed* + *cl*
47. *o(v)DG35/ed ov-1 cl*	———	60.0	——	———	———
48. *olvDG61/ed ov-1 cl*	5 *ed* + +	463.0	0.002	*olv61–ov-1*	———
49. *oDG6/ed cm-2 cl*	2 *ed* + +	79.3	0.005	*o6–cm-2*	———
50. *ovDG30/ed cm-2 cl*	———	12.6	——	———	———
51. *ovDG35/ed cm-2 cl*	———	15.5	——	———	———
52. *ovDG37/ed cm-2 cl*	———	5.6	——	———	———
53. *olvHC39/ed cm-2 cl*	———	66.1	——	———	———
54. *olvDG61/ed cm-2 cl*	*ed* + +	52.5	0.004	*olv61–cm-2*	———
	(*ed* + +)		(0.008)		
55. *oDG41/ed ol-s cl*	6 *ed* + +	92.0	0.013	*o41–ol-s*	2 + + + (*ed* + *cl*)
56. *olvDG27/ed o41 cl*	*ed* + +	84.6	0.005	*o41–olv27*	———
57. *olvHC39/ed o41 cl*	*ed* + +	113.8	0.002	*olv39–o41*	———
58. *olvHC59/ed o41 cl*	6 + + *cl*	69.3	0.017	*o41–olv59*	———
59. *olvCS103/ed cm-2 cl*	———	15.7	——	———	———
60. *olvCS108/ed cm-2 cl*	———	36.5	——	———	———
61. *olvCS69/ed o-2 cl*	———	22.6	——	———	———

The map order given refers to the sequence of the two alleles being tested. The number of verified recombinants and possible convertants are also shown. Those crossovers that died before verification are shown in parentheses under the data of the cross. For a description of the allelic designations, see MATERIALS AND METHODS.

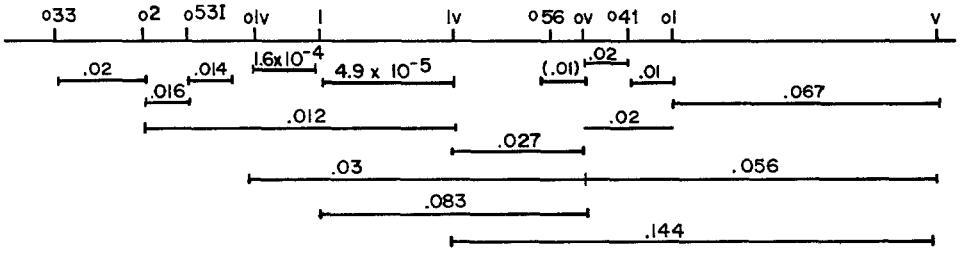

FIGURE 2.—A comprehensive map of the structural arrangement at the dumpy locus. Map distances shown are all based on a four-point test. The wide spatial arrangement of mutation sites is clearly evident. The *olv-l-lv* sequence has been determined by use of double mutants by MONTGOMERIE and involved a single allele. The alleles shown represent designations for the separate subloci. The left portion of the map is less precise than the right portion since mutant heterozygotes involving alleles from this region have a low viability. The distances between various subloci are averages taken from previous reports and the data in Table 2. Fine structure is not shown.

crosses 1–44), the locus appears to be composed of clusters of discontinuously distributed alleles. A map summarizing the recombination data (Figure 2) gives recombinational distances between various *dp* subloci. The distances are average recombination frequencies taken from published papers and the data of Table 2. Except for the *o* and *olv* alleles, each phenotypic type is limited to a unique sublocus. Virtually every sublocus that contains an *o* allele also includes an *olv* allele, suggesting that there is no "*olv* sublocus" *per se*. This result would be expected if *olv* results from a "polarity mutation" of the *o* sublocus. Since the *olv* alleles can be mapped by intersublocus and intrasublocus methods, they cannot be extensive deletions and are thought to be point mutations.

All *lv* and *ol* alleles appear to map to specific subloci. The order of *lv* and *ol* alleles within their respective areas cannot be determined by recombination frequencies because the necessary heterozygotes are lethal. The allele *lv-1* recombines with a number of different *ov* alleles at about the same frequency (Table 2, crosses 26–34). A comparable situation has been encountered with *ol* alleles, but these involve crosses where only one or a few recombinants have been recovered.

The intersublocus mapping data do not sharply delineate subloci boundaries. Figure 2 shows three possible subloci not found by other investigators. One is to the left of *o-2* at the site of the *oDG33* allele (Table 2, cross 1), a second may be between *l* and *ov* (Table 2, crosses 9 and 25), a third sublocus may be located between the *ov* and *ol* subloci (Table 2, crosses 36, 55 and 56). The existence of these subloci is tentative; each is represented by only one allele and additional recombination data will be needed to verify their existence.

Intrasublocus mapping data: Detailed intrasublocus mapping has been possible only at the *ov* sublocus. Previous investigators have mapped several *ov* alleles within this sublocus (SOUTHIN and CARLSON 1962; SEDEROFF 1967; GRACE 1970). Interlocus mapping provided evidence that alleles *oDG6* and *olvDG61* were within the *ov* sublocus. An attempt was made to decipher the intrasublocus positions of *olvDG61* and *oDG6* relative to *ov* alleles *ov-1* and *cm-2*. Crosses 46, 49, 50 and 55 (Table 2) show that *olvDG61* and *oDG6* are both to the left of *ov-1*. Figure 3 summarizes the fine-structure map of the *ov* sublocus, indicating the relative positions of six alleles. Except for the nonpolar *oDG6* allele, which has a mild oblique phenotype, the "strength" of the phenotypes of *ov* alleles

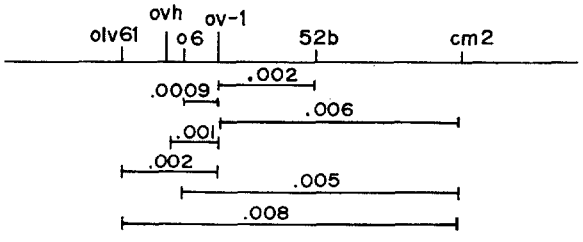

FIGURE 3.—The fine structure of the *ov* sublocus. The positions of the alleles are based on crosses with *ov-1* and *cm-2* and the frequency of recombination. Allelic phenotypes *olv*, *ov* and *o* are present within this sublocus.

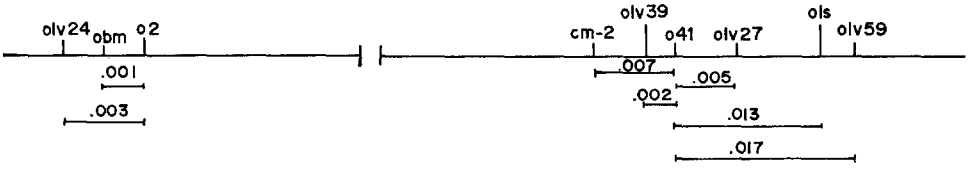

FIGURE 4.—A partial fine-structure map at portions of the map other than the *ov* sublocus. The sequence of alleles is based primarily on the frequency of recombination rather than direct mapping.

decreases progressively from left to right (see Figure 6c). This "gradient" may relate to the general function of the locus. Gradients within a single gene corresponding to the position of various polar (nonsense) mutations have been described thoroughly in prokaryotes (NEWTON *et al.* 1965; ZIPSER 1969). It would be meaningful to determine the map positions relative to *ov-1* and *cm-2* of the several unmapped mild phenotype *ov* alleles (*e.g.*, see Table 2, crosses 19, 20, 21, 50, 52, 46 and 47).

Tightly linked alleles were mapped in two other subloci (Figure 4), the *o-2* sublocus, which contains the alleles *o-2*, *o-bm* and *olvCS24* (SOUTHIN and CARLSON 1972; GRACE 1970) and the sublocus involving the *oDG41* allele to the right of *ov*. Crosses 56 and 57 of Table 2 show the comparative recombination frequencies between *oDG41* and two nearby *olv* alleles, *olvDG27* and *olvHC39*.

DISCUSSION

General nature of the dumpy locus: Based on recombination frequencies, dumpy is quite large, approximately 0.10 m.u. (SOUTHIN and CARLSON 1962). Other loci of comparable size are *lz*, 0.14 m.u. (GREEN and GREEN 1956), and *N*, 0.14 m.u. (WELSHONS and VON HALLE 1962). Two external dumpy alleles, *o-bm* and *v-2*, were shown by SOUTHIN and CARLSON (1962) to be separated by 0.11 m.u. Some *dp* alleles recombine with a higher frequency than do *o-bm* and *v-2* (SOUTHIN and CARLSON 1962; GRACE 1970). Most studies on recombination of complex loci demonstrate that internal distances are not additive (CARLSON 1959a); *lz*, however, does show additivity (GREEN and GREEN 1956). Although the exact length of the *dp* locus cannot be determined from recombination data, it seems unlikely, judging by the large number of recombinational events, that the true size could be less than 0.10 m.u. Thus, it is assumed in this discussion that *dp* is at least 0.10 m.u. long.

Unicistronic versus *multicistronic locus*: Much discussion concerning the structure of complex loci has concentrated on one central question: Do complex loci encode one or more than one structural enzyme? Evidence exists that apparently confirms both hypotheses. Support for the multicistronic hypotheses includes: (1) the uncommonly large genetic map, (2) the pleiotropic phenotypes observed, (3) the demonstration of complementation between certain alleles of the locus, and (4) the tendency for alleles of specific phenotypes to map to discrete areas of the locus. Data from other experiments bolster the single cistron

hypothesis: (1) mutations have been found that do not complement any other allele of the locus, and (2) recessive lethals from different subloci, having different phenotypes, are noncomplementary. This clearly demonstrates an interaction between products encoded by different regions of the locus. Thus, the question of a unicistronic or a multicistronic locus is unresolved.

In a review of complex loci, FRISTROM and YUND (1973) listed four useful criteria to determine whether or not a locus might consist of one or more cistrons: (1) distribution of mutant sites, (2) characteristics of complementation, (3) the ratio of classical recombinants to gene convertants, and (4) the molecular size of the intragenic recombinant unit. A genetic analysis incorporating criteria that evaluate both the structure and function of a locus can provide a strong argument for or against a single cistron locus, although such an analysis alone will not distinguish between a multicistronic and a multifunctional locus.

Distribution of mutant sites: If the distribution of mutant sites within a locus are continuous, either the genes themselves are continuous or only one cistron is involved. If the sites are discontinuous, then there is likely to be spacing between genes in a multigenic cluster. The conclusiveness of this test rests upon an extensive recombination analysis, not always an easy task. At the *dp* locus, there clearly appears to be "clustering" of allelic sites for different allelic phenotypes, *e.g.*, *lv*, *l*, *ol*, and *v* (Figure 2). In general, the regions of clusters are about 0.02 m.u. apart. In the rightmost region of the map, there is an unusually large distance, 0.067, between *ol* and *v*. A continuity of sites is suggested near the *ov* allele region, discussed in detail in the paragraph on fine structure. For most regions of the *dp* locus, however, discontinuities appear to exist (Figure 5). The genetic map, therefore, argues against a single cistron.

Characteristics of complementation: Theoretically, in a two-cistron locus, a fly heterozygous for mutations in separate cistrons would be wild type if there is no functional interaction between the gene products. A complicated pattern of complementation between *dp* alleles is observed, which suggests a functional interaction of gene products (CARLSON 1959). Different levels of complementation are found in crosses between different alleles. For example, complementing phenotypes can be (1) wild type, $l/v = +$; (2) viable individuals can be visibly mutant, $v/olv =$ viable v; or (3) a cross can produce fewer than normal (reduced function) offspring with a wild phenotype, *olv/l* or *lv-1/o-2* = wild type with

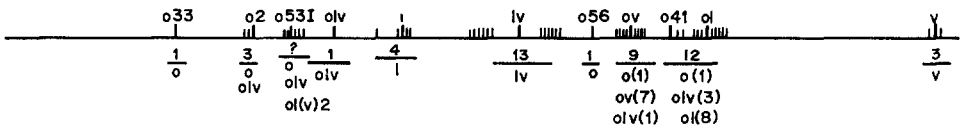

FIGURE 5.—The distribution of mutant sites at the *dp* locus. More than fifty alleles are represented. The lethal alleles are linearly ordered within each sublocus even though it is not possible to map these with respect to each other, *i.e.*, olv's within the same sublocus or the adjacent one cannot be mapped with respect to each other. The allelic positions are shown above the line and the various phenotypes represented are shown below. The left segment of the map is somewhat ambiguous since mapping data are limited by the inviability of heterozygotes. The actual sequence of alleles as determined by the four-point test are given in Figures 3 and 4.

low viability. More than one type of complementation occurs for different mutations within the same sublocus.

Since the *ov* allele is viable and noncomplementing with all known *dp* alleles (except *l*), it is possible to compare the type and severity of phenotypes of different *dp* alleles as a function of the genetic map position. In Figure 6a, the intensities (phenotypic index) of phenotypes of *olv* alleles as a function of map position are shown. Extreme *olv*'s are found on both sides of the map. One allele (*olvHC39*) has a low index, that is, it complements *ov* well, but is nonetheless lethal with *olv* and *ol* alleles. Of the *olv*'s mapped thus far, the most extreme allele is at the leftmost end of the map. Preliminary results indicate that *olv*'s (Table 2, crosses 58 and 59) may be close to or within the *ov* sublocus, making it apparent that mutations of the intense *olv* types exist at different sites.

The *o* alleles also map at a number of sites within the locus (Figure 6b). Most

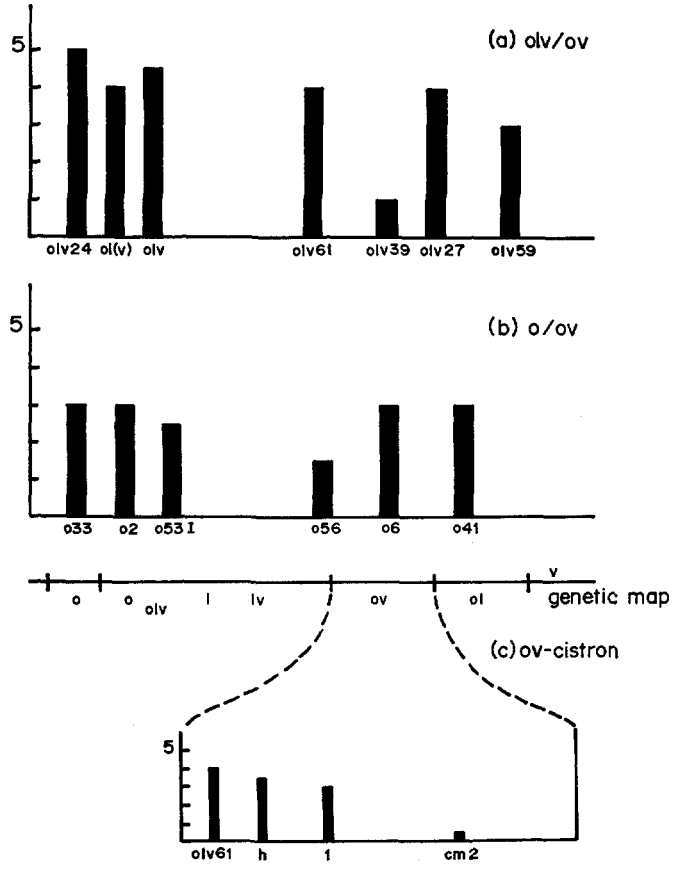

FIGURE 6.—A comparison of allelic complementation. The phenotypic index is used to plot the mutant phenotypes of *olv* (a) and o (b) alleles distributed throughout the map. The mutant index is based on the phenotype of the various mutants crossed to *ov-1* (see Figure 1). (c) Plot of the mutant index for alleles within the *ov* sublocus. There is a left-to-right decrease in the phenotypic expression of the alleles.

of those alleles available for testing do not show a variety of different oblique intensities with *ov-1*. An exceptional allele, *oDG56*, has a rather mild phenotype with an index of 1, and maps to the left of the allele *olvDG61* in the center of the map, while *olvHC39* maps to the right of *olvDG61*. The map distance between *oDG56* and *olvDG61* is estimated to be about 0.03 m.u., indicating that these alleles may be in separate subloci. The sublocus is noticeably complicated (Figure 6c). Alleles *olv*, *ov* and *o* map within 0.008 m.u. of one another. When the intensity of phenotypes of "polar" mutations is plotted according to map position, a left-to-right descending gradient is suggested. These "polar" mutations are not known to be terminators. It is assumed that these phenotypes reflect levels of gene activity. Gradients of gene activity are reminiscent of mutations in prokaryotes where nonsense (polar) mutants within a cistron exhibit a decrease in enzyme activity the closer the lesions are to the promotor. Clearly, these observations do not support a single cistron hypothesis.

The ratio of classical recombinants to gene convertants: Gene conversion usually occurs where there is tight linkage between a pair of heteroalleles. As the distance between alleles decreases, conversion increases (CHOVNICK, BALLANTYNE and HOLM 1971). When the distance is greater than a cistron (1000 bp), conversion is rare and can be confused with other rare events, such as reversion or multiple recombination. Conversion has been extensively studied in fungi (FOGEL and MORTIMER 1971), but only a few detailed analyses have been carried out in Drosophila (CARLSON 1971; CHOVNICK, BALLANTYNE and HOLM 1971; FINNERTY, DUCK and CHOVNICK 1970).

Virtually all recombination observed at the *dp* locus is coupled with the exchange of outside markers. Although over 500 dp^+ exceptionals have been recovered from crosses involving various alleles, only 16 recombinant events were not consistent with classical crossing over (SOUTHIN and CARLSON 1962; CARLSON 1959a; Table 1). It may be significant that seven of the 16 crosses involved *ov-1* as one of the alleles in the cross; one is tempted to consider them as convertants or revertants. However, except for *ovDG30* and *ov-n*, none of these alleles maps "close" to *ov-1*. Of the 16 events, two were associated with the exchange of outside markers, which suggests both a conversion event and recombination of outside markers. Since the *ov-1* allele was discovered by MORGAN in 1916, there has been only one reported reversion event, even though it has been maintained in a number of laboratories for over fifty years (MEYER, personal communication). In one cross, *oDG41* × *ol-s*, three possible convertants were recovered (Table 1, cross 55). Two of these cases were verified by progeny testing; the third was sterile. These data could be considered to be the upper limit for the actual rate of conversion at the *dp* locus. No experiment has been designed to screen specifically for conversion or reversion. It is concluded that crosses between most of the *dp* alleles involve distances that favor classical recombination, and the rarity of gene conversion suggests that the distances encountered in *dp* crosses are beyond those short intervals expected to favor gene conversion.

Molecular size of the intragenic recombination unit: *ov* is the only sublocus for which adequate fine-structure data are available, and the only sublocus dis-

cussed in detail. Fine-structure mapping at the *dp* locus between phenotypically identical alleles was first achieved with *ov-1* and *ov-52b* (SEDEROFF 1967). Since 1967, six alleles have been mapped to the *ov* sublocus. Their relationships are shown in Figure 3. The smallest distance between two alleles is 0.0009 m.u. (*oDG6* to *ov-1*), the largest is 0.008 m.u. (*olvDG61* to *cm-2*). Although only one of two crossovers between the latter alleles survived to be verified, 0.008 is also the sum of the map distances from *olvDG61* to *ov-1* plus *ov-1* to *cm-2*. Ten crossovers were verified from these crosses. Assuming 2.94×10^{-6} map units per base pair, one can estimate that the *ov* sublocus contains 2720 base pairs, enough to code for a polypeptide 900 amino acids long. The entire dumpy locus would be roughly equivalent to 34,000 nucleotide pairs.

Three enzymes in the pathway of pyrimidine synthesis are coded by the rudimentary locus (NORBY 1973; RAWLS and FRISTROM 1975; FALK 1976). The genetic size of *r* is 0.07 m.u., a distance sufficient to encode 24 genes of average size (FRISTROM and YUND 1973). There are several possibilities that might account for this DNA excess: (1) The enzymes are multimeric and many different polypeptides must be assembled for activity. (2) Genes are separated by large neutral (spacer) DNA regions. (3) The extra DNA sequence codes for regions that are later eliminated or are necessary for the processing or function of the enzymes. Transcription of the entire locus would produce a very large RNA molecule. (4) Each gene contains a specific region for autoregulation and control even though the locus may be coordinately transcribed as a single polycistronic message. (5) The calculations are misleading.

It is reasonable to speculate that the *dp* products function as a single enzyme unit. Support for such an enzyme unit may be suggested by the large number of noncomplementing mutations in different subloci. These points have been discussed by JUDD and YOUNG (1973 and JUDD (1976). At both the *r* and *dp* loci, 50% of the known alleles are noncomplementing. Of course, other events may account for the high rate of noncomplementation, including nonsense mutations. Clearly all of the genetic criteria discussed indicate that dumpy is too complex genetically to be classified as a single functional cistron. This distinction is not a trivial one even though the locus may be a single multifunctional cistron.

In summary, *dp* alleles appear to fall into more-or-less discrete clusters. The estimated number of these clusters, inferred from mapping analysis, is between four and ten. It is estimated that *dp* has sufficient DNA to encode 24–25 average sized genes. The locus could accommodate ten exceptionally large proteins, with 1000 amino acids each. It is not unreasonable, however, that a significant amount of the DNA does not directly code for a polypeptide. The concluding hypotheses are: (1) The *dp* complex consists of more than one functional unit. (2) Fine-structure analysis of the *ov* sublocus indicates that allele sites within this particular sublocus are continuously distributed and that gene conversion, of it occurs, is rare. (3) Regions of discontinuity (spacing) appear to exist between subloci. (4) Noncomplementing mutations are spaced throughout the locus.

Biochemical studies on complex loci in Neurospora offer a useful model for understanding the functions of complex loci. For example, the Neurospora *arom* gene cluster encodes five discrete enzyme functions (GILES *et al.* 1967). All the enzyme activities appear to be specified by a single locus that encodes a single multifunctional polypeptide (GAERTNER and COLE 1977).

BROTHERS *et al.* (1978) reported that the three enzyme activities specified by the r^+ locus in Drosophila co-sediment as a single peak in sucrose gradients. This is interpreted as indicating that all three enzyme activities reside in a single polypeptide.

If this hypothesis is substantiated, the apparent ambiguities observed at many complex loci in Drosophila could be resolved. The exciting results reported for the *r* locus are significant to this report because a close genetic similarity exists between *r* and *dp*. Many complex loci formerly considered to be multicistronic may be, in fact, single cistrons encoding a multifunctional protein.

The length of the dumpy locus, as determined by genetic recombination, stimulates speculation about possible intralocus regulatory regions. Some observations support the hypothesis that regulatory and structural elements are contiguous. Four mutations map as follows: *l* is approximately at the center of the locus; *o-2* is near the left end of the locus; *ol* and *v-2* are on the right half of the locus, *v-2* at the right end (see Figure 2). The recessive lethal *l* mutations are unique among dumpy alleles. These are the only alleles that have no mutant phenotype when heterozygous with nonlethal alleles or with the few recessive lethals that they complement (see RESULTS). Mutants from a cross between the double mutant *o-2–v-2* and *ov-1* express a more enhanced phenotype than either *o-2* or *v-2* separately with *ov-1*. However, offspring from the double mutant *ol–v-2* crossed to *ov-1* do not have an enhanced phenotype (CARLSON 1959a). The *o-2* allele might be a mutation in a regulatory sequence, with a *trans* effect. A working hypothesis is that the left half of the locus, including *l*, contains regulatory sequencies and the right half contains structural sequences.

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