COMPARISON OF MICROMAPS OBTAINED BY DIRECT AND INDIRECT METHODS OF RECOMBINATION IN THE DUMPY REGION OF *DROSOPHILA MELANOGASTER*

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TWO mapping procedures have been used for the analysis of multiple allelic series. Both of these methods are based on techniques successfully used for genes which are relatively far apart on the chromosome map. In one procedure, a particular set of genes is singled out to establish some standard points of reference. Other genes, known to segregate with the same linkage group, may be tested individually with one or more of these reference loci. The series of recombination frequencies which are obtained can then be arranged from the smallest to the largest frequency of recombination obtained with each reference point. Assume, for example, that a gene close to the telomere of a chromosome is chosen as a reference point. If two genes tested with it give different frequencies of recombination, the smaller frequency would permit the placement of its gene between the reference point and the locus of the gene used for obtaining the larger frequency of recombination. If the recombination frequencies are based on large counts, the approximate map distances of all the genes used in the investigation can be calculated. From such a tentative ordering of the map the predicted localizations can be more accurately determined. This method is particularly useful for making a rapid survey of the chromosome map if several genes are available. Since no other genetic tools are employed in this procedure, it is necessarily an indirect approach, employing as its basic assumption the well-established view that map distance can be measured by the frequency of recombination. When employed for very small distances, especially pseudoallelic systems, this method must also assume that there is no special feature of recombination that would disturb the relation between distance and recombination frequency. This method, as used here, shall be referred to as the *indirect method of linkage mapping by overlapping recombination frequencies*.

For determining the sequential order, but not the map distance of two genes, another approach may be used. Two genes (or alleles) may be mapped with respect to one another by using one or more additional genes ("markers") readily distinguishable from the ones being investigated. In practice, two marker genes are used to identify, respectively, a locus to the left of the two genes being tested and a locus to their right. If a recombination does occur between the two middle genes, the distribution of the marker genes in the recovered recombinant indicates the proper linear sequence of all four of the genes involved. This method

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directly determines the sequence with a single appropriate crossover, but it does not provide sufficient information on the map distances separating the various members (unless very large numbers of progeny are counted to accumulate sufficient recombinants in the appropriate regions to make the recombination frequencies meaningful). The use of one marker identifying the left part of the map and the other marker identifying the right part of the map, uniquely distinguishes single crossover events from reverse mutations. Since only one isolated recombinant between the middle two members is sufficient to place all four loci in a linear sequence, it is obvious that this method becomes very useful for exploring small regions of the chromosome, particularly in the regions of multiple allelic series. In contrast, the indirect method of localization would require far larger numbers of progeny for counting than could be managed efficiently in *Drosophila*. The recombinational technique employing identifying marker genes shall be referred to in this paper as the *direct method of linkage mapping by a four point test*.

Both methods of linkage mapping have been employed for the dumpy series of alleles in *D. melanogaster*. In this paper, the differences between these techniques and their limitations are explored in detail. A revised, extended map of the dumpy region, as well as the problems involved in its construction, is also presented.

MATERIALS AND METHODS

The dumpy gene, *dp*, located at 13.0 on the second chromosome, has been studied intensively for its genetic features. Three major pleiotropic effects are manifested, alone or in combination, by the various alleles of this system: a reduction in wing size resulting in oblique wings (o); a disturbance in bristle distribution with pigmented pits or mounds on the thorax, resulting in a vortex (v) appearance; and an embryonic or larval lethality (l). All of these effects are recessive and only when two alleles have traits in common are they expressed. Thus *o/ov* is (o); *o/lv* is (+); and *ov/lv* is (v). An additional effect, resulting in a pair of pigmented indentations at the anterior end of the thorax is called a comma effect (cm). This comma effect is usually associated with the vortex effect, but in some alleles it is not expressed. Because it is a less obvious pleiotropic manifestation, the comma effect is not represented in the allelic symbols for any of the alleles except the mutant *cm* itself, which manifests this phenotype as a homozygote without noticeable (o) or (v) effects. For a more thorough illustrated description of these phenotypes see CARLSON 1959.

In the studies reported by CARLSON 1959, all the mapped members were tested by using echinoid eye (*ed*—2, 11.0) and clot eye color (*cl*—2, 16.5) as the outside marker genes. Females bearing two alleles to be tested for recombination with each other were mated to *ed ov¹ cl* males. In such cases an immediate four point test could be completed if the wild-type or doubly mutant recombinant appeared. In all cases, however, the recovered recombinant was mated to the tester *ed ov¹ cl* stock to verify the genotypes of the paternal and maternal chromosomes. Because of the lethality or inviability of certain allelic heterozygotes (e.g. *l^m/ol^s*; *o²/olv¹*)

and because of the complementation which would exist in the case of $l^m/ed\ ov^1\ cl$ progeny derived from a cross involving the l^m allele, several partially selective tester stocks were designed. For example, heterozygous females, $l^m/ed\ ov^1\ cl$, were mated to $ed\ ol^s\ cl/Cy$ males because the ol^s allele is lethal in heterozygous compound with l^m and the ol^s allele expresses an (o) phenotype in heterozygous compound with ov^1 . This scheme poses problems for calculating the frequency of recombination obtained, but it does not affect the validity of the four point test because the placement of the markers in the wild-type recovered chromosomes in the presence of the paternal tester chromosome unambiguously defines the correct linear order of the alleles. The Curly chromosome in the male tester contains inversions which prevent the loss or breakdown of the $ed\ ol^s\ cl$ tester chromosome and it also carries the dominant visible marker Curly which identifies this chromosome balancer. Note that the balancer plays no direct part in detecting the crossover chromosomes produced by the females. The most satisfactory male tester stock used was $olv^{57}\ cl/Cy$ which greatly reduced the number of non-Curly progeny because most of the dumpy alleles are lethal or inviable with this allele. The wild-type crossovers picked up by the $olv^{57}\ cl$ chromosome were mated to individuals of $ed\ ov^1\ cl$ composition to verify the presence of the $olv^{57}\ cl$ paternal chromosome and the presence of the ed or cl markers in the maternal nondumpy recombinant chromosome.

Excluding, for the moment, the calculation of the fiducial limits for the recombination frequencies, let us examine how map distances can be determined for either tester scheme. In the case of the $ed\ ov^1\ cl$ tester method, the number of verified wild-type recombinants is doubled and divided by the total progeny obtained. There are some sources of heterogeneity in this approach. For example, some alleles (e.g., olv^1) are semilethal in heterozygous compound with ov^1 . Thus crosses involving this allele (as in $olv^1/ed\ v^2\ cl \times ed\ ov^1\ cl$) will reflect a *higher* recombination frequency than would a cross involving "neutral" alleles (as in $o^2/ed\ v^2\ cl \times ed\ ov^1\ cl$). To correct for this difference one could increase the total number of progeny by equalizing the two classes of $ed\ ov^1\ cl$ heterozygotes obtained (or more simply, by doubling the larger class and omitting the smaller class). At best this calculation rests on an additional assumption that there is no *supravital* effect of any of the allelic heterozygotes (e.g., v^2/ov^1 in the example cited). For this reason the frequencies in Table 2 using this method are calculated simply on the total progeny obtained.

In the partially selective scheme, the interpretation of a cross becomes more complicated. For example, the cross $l^m/ed\ o^2\ cl \times olv^{57}\ cl/Cy$ produced 2 (+cl) crossovers among 22,532 progeny, of which 19,554 were (Cy) in phenotype. If the (Cy) progeny were ignored, the recombination frequency based on the surviving non-Curly class would be $2 \times 2 (+cl) / 2,978$ or 0.133 map units (or 0.067, if the non-Cy class is doubled to account for the lethal heterozygous class). If, however, one assumes that the number of Curly progeny represents *half* of all zygotes formed, then the number of recombinants can be multiplied by four (since half the recombinants go undetected with the Curly balancer and half the recombinants are presumed to be the double mutant class) and the number of

Curly progeny can be doubled (to account for the remaining zygotes, most of which are lethal or semiviable with $olv^{57} cl$). This gives $\frac{4 \times 2 (+cl)}{2 \times 19,554 (Cy)}$ or more simply, doubling the number of wild-type recombinants and dividing by the number of Curly progeny obtained. In this example, the frequency becomes $4/19,554$ or 0.02 map units, which is considerably less than the 0.133 or 0.067 map units which would be calculated from the non-Curly class. It is assumed, also, that the Curly heterozygotes have approximately the same viability with any dumpy allele. If this does not prove to be valid, then a hidden heterogeneity exists in the calculated map distances based on this scheme.

RESULTS

In 1959 CARLSON used both the direct and the indirect methods of recombination to map seven of the alleles in the dumpy series. The right-hand portion of the map, consisting of the sequence $o^2 lv^1 olv^1 v^2$ was determined by the four point test for each consecutive pair of alleles. The left-hand portion, consisting of the alleles $l^m ol^s olv^1 o^2$, was more provisionally localized by the method of overlapping recombination frequencies using these four alleles in individual tests with the standard reference locus, olv^1 . Direct mapping of this left-hand region was not possible at that time because of the lethality of the ol^s/l^m , olv^1/ol^s and l^m/olv^1 compounds. Similarly, the compound o^2/olv^1 was inviable and the few surviving individuals of this genotype were too deformed to survive long enough after mating to yield the progeny required for a recombination test. The possible viable compounds, ol^s/o^2 and l^m/o^2 , were not tested at that time.

The discovery of the allele, comma, cm^2 , by MEYER (1955), at first suggested that this mutant was a quantitatively weaker variant of the ov region. This was particularly likely because a quantitatively stronger variant (humpylike, h) of the ov phenotype was in fact localized between lv and v . When tested by SOUTHIN in a four point test using females of the composition $cm^2/ed lv^1 cl$, the cm^2 site was found to be to the left of lv^1 . This made it a very promising allele for studying the left end of the dumpy region because l^m , ol^s , and olv^1 were all viable in heterozygous compound with the cm^2 allele. An intensive analysis of the left end of the dumpy region employing these lethal bearing alleles with cm^2 and other crosses, established the new micromap represented in Figure 1. Altogether 52 allelic four point tests have been studied in the dumpy series (see Table 1). Notice that none of these series contradicts the linearity of the sequence established for this series.

DISCUSSION

There are many features of the map of the dumpy region which need to be explored in detail. One of these is the calculation of the distance between the various allelic sites. The four point test presents a major difficulty in determining the distances between the sites in this region and in other pseudoallelic regions of *Drosophila*. Because of the reliability of the sequence determined from this test, very few crossover events are recorded before a series is terminated. This makes the fiducial limits very large for the recovered frequency of verified cross-

FIGURE 1.—Map of the dumpy region. The above map of the dumpy region is based on 48 recombination series, each of which employed the unrelated marker genes echinoid and clot. Their position with respect to one another orders the series in a linear array of sites. The alleles olv^w and l^m cannot be separated from one another at present because their heterozygous compound is lethal. Similarly the alleles ol^s and lv^1 form a lethal compound when mutually heterozygous. Designations for the symbols are: o = oblique wings; l = lethality; v = thoracic vortices; cm = comma, a weak (ov) mutant with prominent thoracic commas; h = humpy-like, an extreme (ov) mutant without an obligate lethal effect. See text for further details.

overs (STEVENS 1942). If a larger number of crossovers is obtained, however, then the reliability of the map distances may become more meaningful. Thus, in the case of the lozenge region, the three sites reported by GREEN and GREEN (1956) appear to agree with the hypothesis that the principles of direct and indirect mapping extend to the smallest levels of recombination analysis. Various alleles of lz^s and lz^1 yielded 56 crossovers in 134,865 progeny, for a map distance of 0.83 map units. In the adjacent region, alleles of lz^1 and lz^{91u} yielded 20 cross-

TABLE 1

Series 1 through 48 in this table are presented in Figure 1. The map order in each case has been determined by the four point test. So called conversions may represent reverse mutations, double crossovers, or other unexpected marker associations with the wild-type exceptions. No attempt to distinguish these alternatives has been made

Series	P ₁ females	P ₁ males	Verified single recombinants	Total progeny	Curly progeny	Frequency	Map order	Conversions
1	<i>o^{bm}/ed o² cl</i>	<i>olv⁵⁷cl/Cy</i>	1 ed +	124,913	123,105	0.0016	<i>o^{bm}_o²</i>	0
2	<i>l^m/ed cm² cl</i>	<i>olv⁵⁷cl/Cy</i>	2 ed +	11,892	9,631	0.0415	<i>l^m_cm²</i>	0
3	<i>ed lv¹ cl/h</i>	<i>ed ov¹ cl</i>	1 + cl	3,548		0.0564	<i>lv¹_h</i>	0
4	<i>v²/ed ov¹ cl</i>	<i>ed ov¹ cl</i>	7 + cl	35,200		0.0397	<i>ov¹_v²</i>	0
5	<i>ed o² cl/l^m</i>	<i>ed ol^s cl/Cy</i>	2 + cl	22,531	19,554	0.0205	<i>o²_l^m</i>	0
6	<i>cm² cl/ed lv¹</i>	<i>olv⁵⁷cl/Cy</i>	1 ed + cl	7,890	6,392	0.0313	<i>cm²_lv¹</i>	0
7	<i>h/ed ov¹ cl</i>	<i>olv⁵⁷cl/Cy</i>	1 ed +	145,752	132,183	0.0015	<i>h_ov¹</i>	0
8	<i>olv¹⁰/ed o² cl</i>	<i>olv⁵⁷cl/Cy</i>	1 + cl	3,472	3,187	0.063	<i>o²_olv¹⁰</i>	0
9	<i>ed v²/ol^s cl</i>	<i>ed ov¹ cl</i>	6 ed + cl 2 ol ^s v ²	16,118		0.074	<i>ol^s_v²</i>	0
10	<i>olv¹⁰/ed cm² cl</i>	<i>olv⁵⁷cl/Cy</i>	1 ed +	71,269	53,918	0.0037	<i>olv¹⁰_cm²</i>	0
11	<i>ol^m/ed ov¹ cl</i>	<i>ed ov¹ cl</i>	9 ed +	32,124		0.056	<i>ol^m_ov¹</i>	1 +
12	<i>olv^{54e}/ed o² cl</i>	<i>olv⁵⁷cl/Cy</i>	2 + cl	22,015	21,195	0.019	<i>o²_olv^{54e}</i>	0
13	<i>cm²/ed ol^s cl</i>	<i>olv⁵⁷cl/Cy</i>	3 ed +	65,818	49,811	0.012	<i>cm²_ol^s</i>	0
14	<i>h/ed v² cl</i>	<i>ed ov¹ cl</i>	1 ed +	2,955		0.0677	<i>h_v²</i>	0
15	<i>o^{bm}/ed cm² cl</i>	<i>olv⁵⁷cl/Cy</i>	7 ed +	48,971	41,741	0.034	<i>o^{bm}_cm²</i>	1 + cl
16	<i>ed lv¹/v^w cl</i>	<i>ed ov¹ cl</i>	14 +	26,280		0.107	<i>lv¹_v^w</i>	0
17	<i>o²/ed ov¹ cl</i>	<i>ed ov¹ cl</i>	6 ed +	57,207		0.021	<i>o²_ov¹</i>	0
18	<i>ed v²/o² cl</i>	<i>ed ov¹ cl</i>	7 ed + cl 10 o ² v ²	15,505		0.090	<i>o²_v²</i>	0
19	<i>o^{bm}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	6 ed +	13,315	10,789	0.111	<i>o^{bm}_v²</i>	0
20	<i>o²/ed ol^s cl</i>	<i>ed ol^s cl/Cy</i>	1 ed +	1,147	977	0.205	<i>o²_ol^s</i>	0
21	<i>ovⁿ/ed v² cl</i>	<i>olv¹/Cy</i>	2 ed +	4,539	3,819	0.105	<i>ovⁿ_v²</i>	0
22	<i>olv¹/ed cm² cl</i>	<i>olv⁵⁷cl/Cy</i>	1 ed +	48,077	39,610	0.005	<i>olv¹_cm²</i>	0
23	<i>ovⁿ/ed lv¹ cl</i>	<i>olv¹/Cy</i>	2 + cl	22,766	19,800	0.02	<i>lv¹_ovⁿ</i>	0
24	<i>olv^{bm}/ed cm² cl</i>	<i>olv⁵⁷cl/Cy</i>	1 ed +	1,151	943	0.21	<i>olv^{bm}_cm²</i>	0
25	<i>ov^{52b}/ed v² cl</i>	<i>olv¹/Cy</i>	1 ed +	20,820	17,950	0.011	<i>ov^{52b}_v²</i>	0
26	<i>ov^{52b}/ed lv¹ cl</i>	<i>olv⁵⁷cl/Cy</i>	1 + cl	13,607	11,220	0.018	<i>lv¹_ov^{52b}</i>	0
27	<i>ed lv¹/o² cl</i>	<i>ed ov¹ cl</i>	5 ed + cl 1 o ² lv ¹	49,692		0.012	<i>o²_lv¹</i>	0
28	<i>ov^{51f}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	1 ed +	7,398	7,117	0.028	<i>ov^{51f}_v²</i>	0
29	<i>olv^{x5}/ed o² cl</i>	<i>olv⁵⁷cl/Cy</i>	2 + cl	7,509	7,307	0.055	<i>o²_olv^{x5}</i>	0
30	<i>ov^{51f}/ed lv¹ cl</i>	<i>olv¹/Cy</i>	1 + cl	34,544	29,504	0.007	<i>lv¹_ov^{51f}</i>	0
31	<i>cm²/ed o² cl</i>	<i>olv⁵⁷cl/Cy</i>	3 + cl	22,698	17,398	0.034	<i>o²_cm²</i>	0
32	<i>ov^x/ed v² cl</i>	<i>ed lv¹ cl/Cy</i>	2 ed +	7,894	6,516	0.061	<i>ov^x_v²</i>	0
33	<i>ov^x/ed lv¹ cl</i>	<i>olv¹/Cy</i>	3 + cl	28,059	22,071	0.027	<i>lv¹_ov^x</i>	0
34	<i>olv^{bm}/ed ov¹ cl</i>	<i>ed ol^s cl/Cy</i>	4 ed +	9,907	7,565	0.106	<i>olv^{bm}_ov¹</i>	0
35	<i>lv¹/ed ov¹ cl</i>	<i>ed ov¹ cl</i>	5 ed + 1 lv ¹ ov ¹ cl	66,009		0.015	<i>lv¹_ov¹</i>	1 ed + cl
36	<i>l^m/ed ov¹ cl</i>	<i>ed ol^s cl/Cy</i>	4 ed +	25,116	19,693	0.041	<i>l^m_ov¹</i>	0
37	<i>olv¹/ed v^w cl</i>	<i>ed ov¹ cl</i>	6 ed +	7,785		0.152	<i>olv¹_v^w</i>	0
38	<i>lv^{x1}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	1 ed +	1,795	1,470	0.136	<i>lv^{x1}_v²</i>	0
39	<i>olv^{bm}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	7 ed +	18,534	14,130	0.099	<i>olv^{bm}_v²</i>	0
40	<i>lv^{x3}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	2 ed +	3,835	2,876	0.239	<i>lv^{x3}_v²</i>	0

TABLE 1—Continued

Series 1 through 48 in this table are presented in Figure 1. The map order in each case has been determined by the four point test. So called conversions may represent reverse mutations, double crossovers, or other unexpected marker associations with the wild-type exceptions. No attempt to distinguish these alternatives has been made

Series	P ₁ females	P ₁ males	Verified single recombinants	Total progeny	Curly progeny	Frequency	Map order	Conversions
41	<i>olv^{x1}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	2 ed +	16,947	12,825	0.031	<i>olv^{x1}_v²</i>	0
42	<i>lv^{x4}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	3 ed +	11,541	9,316	0.064	<i>lv^{x4}_v²</i>	0
43	<i>olv^{x2}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	4 ed +	7,681	5,885	0.136	<i>olv^{x2}_v²</i>	0
44	<i>lv^{x5}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	3 ed +	4,597	3,277	0.183	<i>lv^{x5}_v²</i>	0
45	<i>olv^{x3}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	1 ed +	13,569	10,264	0.019	<i>olv^{x3}_v²</i>	0
46	<i>lv^{x18}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	6 ed +	12,428	8,995	0.133	<i>lv^{x18}_v²</i>	0
47	<i>olv^{x5}/ed v² cl</i>	<i>olv⁵⁷cl/Cy</i>	5 ed +	28,270	22,031	0.045	<i>olv^{x5}_v²</i>	0
48	<i>olv^{x4}/ed ov¹ cl</i>	<i>ed ov¹ cl</i>	1 ed +	5,860		0.034	<i>olv^{x4}_ov¹</i>	0
49	<i>ov^{51f}/ed ov¹ cl</i>	<i>ed ov¹ cl</i>	0	51,477				.
50	<i>ov^x/ed ov¹ cl</i>	<i>ed ov¹cl/lv¹Cy</i>	0	50,457	27,342			.
51	<i>ov^{52b}/ed ov¹cl</i>	<i>ed ov¹cl/lv¹Cy</i>	0	47,581	26,565			.
52	<i>ovⁿ/ed ov¹ cl</i>	<i>ed ov¹ cl</i>	0	66,313				1ed+cl

overs among 69,971 progeny, for a calculated frequency of 0.057 map units. The sum of these two distances agrees almost perfectly with the 0.142 map units found for alleles of *lz^s* and *lz^{g1y}* which yielded 70 crossovers in 98,399 progeny. It was on this basis that the left end of the dumpy region was determined in 1959 by the indirect method of overlapping recombination frequencies. Inspection of the revised map in Figure 1 clearly shows that this sequence was incorrect. The four point test may safely be assumed to be more reliable because all of the tests involving alleles separated by two or more sites agree with the linear sequence established by tests with alleles at adjacent sites. There are two possible explanations that might resolve the differences in agreement between those reported for lozenge and those obtained in dumpy. If the numbers of crossovers involved in the calculation for mapping by overlapping frequencies is very small, then the fiducial limits might be so large that no meaningful comparisons can be made. Alternatively, we might find that even when we do use large enough numbers, with fiducial limits comparable to those which can be calculated for the lozenge region, the map distances obtained are not additive. This would imply that other events are imposed in pseudoallelic regions which obscure the significance of the map distances.

In the dumpy series the map distances for the regions *lv* to *ov* and *ov* to *v* are, at first glance, not additive when compared to the calculated map distances for the *lv* to *v* region. Note, in Table 2, that virtually the same frequency of recombination is obtained whether the paternal tester chromosome is *ed ov¹ cl* or *olv⁵⁷ cl/Cy*. The fiducial limits at the five percent level of significance for these map distances are not significant, but they are barely significant at this level if the calculations are based on the additivity of the frequencies of verified wild-type crossovers in this series or by doubling these limits to obtain map distances. This

TABLE 2

The recombination frequency is calculated by doubling the number of nondumpy recombinants and dividing by the listed progeny. Fiducial limits are calculated from the uncorrected frequency, then doubled

I. Apparent nonadditivity of map distances in the dumpy region					
A = Series of alleles using <i>olv</i> ⁵⁷ <i>cl</i> /Cy tester males					
Series	<i>lv—ov</i>	Series	<i>ov—v</i>	Series	<i>lv—v</i>
23	2/19,800	21	2/3,819	42	3/9,316
26	1/11,220	25	1/17,950	38	1/1,470
30	1/29,504	28	1/7,117	40	2/2,876
33	3/22,071	32	2/6,516	44	3/3,277
				46	6/8,995
	7/82,595		6/35,402		15/26,034
	= 0.017		= 0.034		= 0.115
B = Series of alleles using <i>ed ov</i> ¹ <i>cl</i> tester males					
Series	<i>lv—ov</i>	Series	<i>ov—v</i>	Series	<i>lv—v</i>
35	5/66,009	4	7/35,200	16	14/26,280
	= 0.015		= 0.04		= 0.107
C = Total frequencies expressed in map units					
	<i>lv—ov</i>		<i>ov—v</i>		<i>lv—v</i>
	12/148,614		13/70,602		29/52,314
	= 0.016 m.u.		= 0.037 m.u.		= 0.111 m.u.
D = Fiducial limits at five percent confidence level					
	= 0.0083 m.u.		= 0.0196 m.u.		= 0.074 m.u.
	= 0.0282 m.u.		= 0.0635 m.u.		= 0.159 m.u.
II. Additivity of map distances in the lozenge region (from GREEN and GREEN 1956)					
A = Map distances in the lozenge region					
	<i>lz</i> ⁸ — <i>lz</i> ¹		<i>lz</i> ¹ — <i>lz</i> ^{91y}		<i>lz</i> ⁸ — <i>lz</i> ^{91y}
	56/134,865		20/69,971		70/98,399
	= 0.083 m.u.		= 0.057 m.u.		= 0.142 m.u.
B = Fiducial limits of map distance at five percent confidence level					
	= 0.054		= 0.0292		= 0.101
	= 0.116		= 0.098		= 0.192

means that care must be exercised in interpreting how the figures should be compared. To be conservative the frequency of recovered verified wild-type cross-overs and not the frequency of recombination should be the basis for calculating fiducial limits. If this had not been done for this series the apparent significance would have required the formation of a new hypothesis to account for the discrepancy in additivity. Note also that there is a considerable spread in the limits for the frequencies of wild-type crossovers recovered in each of the three series cited for the lozenge alleles. Even the more generous use of recombination frequencies (doubling the wild-type exceptions in each series before consulting statistical tables) gives fiducial limits to the map distances which would make the values listed subject to a 30 to 50 percent error. It does not seem likely therefore,

that meaningful map distances can be placed between the intervals of the pseudoallelic sites in the dumpy region or in any pseudoallelic region unless the recovered crossovers in each series numbered about 100, which would reduce the error in mapping to about ten percent.

Another problem of interest in the analysis of the micromap for dumpy is the degree of "fine structure" which can be estimated. The approach used by PONTECORVO and ROPER (1956) divides the map distance for the end points of the region by the map distance separating the closest pair of sites. In the dumpy region this would be 0.111 for o^{bm} to v^2 and 0.0015 for h to ov^1 . This gives an estimated 74 sites. However, at the ten percent confidence level the maximal fiducial limit for the distance from h to ov^1 is 0.0059 and the minimal distance based on the fiducial limit for o^{bm} to v^2 is 0.0584. This estimate (which now provides a four percent confidence level for the quotient) would indicate only 9.9 sites in the dumpy region. If the maximal fiducial limit for o^{bm} to v^2 (0.1952) is divided by the minimal fiducial limit for h to ov^1 (.000158) the estimate is 1228 sites. This method is, therefore, not very useful for frequencies usually obtained by the four point test, although it is valid in tests employing the indirect method of overlapping recombination frequencies where the number of recombinants is large (as in several selective assay schemes employed in microbial organisms).

In the dumpy region, however, it is possible to detect fine structure by obtaining recombinants between members of similar phenotype which have been localized to the same subregion. This has been done for two cases: o^{bm} with o^2 and h with ov^1 . However, these cases are only suggestive and not critical proof of fine structure because there are quantitative phenotypic differences between each of these alleles.

In conclusion, this analysis indicates that there are few reliable map distances for pseudoallelic micromaps in *Drosophila*. Additionally, the most reliable basis for mapping alleles in the absence of selective techniques is the four point test which establishes the linearity of the sites in the map. Calculation of the fiducial limits for these series very clearly limits the usefulness of recombination frequencies or estimates of fine structure in the dumpy series or in any pseudoallelic system presently reported in *Drosophila*. It is only with very large numbers (about 100) of crossovers for any given pair of alleles that either the mapping or fine structure calculations can be determined arithmetically. In the absence of such efforts, alternative techniques must be employed to obtain evidence for fine structure or for estimating the relative distances between sites.

SUMMARY

1. Direct and indirect methods of mapping pseudoalleles at the dumpy locus are compared and their limitations are discussed.
2. The indirect method of mapping does not necessarily agree with that directly determined with the use of outside marker genes.
3. Tests of significance employing fiducial limits rule out the possibility of constructing accurate map distances for *Drosophila* pseudoalleles from published data presently available.
4. Determinations by calculations from map distances of the number of sites in a pseudoallelic region are inconclusive for all reported cases in *Drosophila*. However, current data indicate the existence of at least ten sites or as many as 1200 sites in the dumpy locus.
5. A revised and enlarged map of the dumpy region based on direct mapping techniques is presented.

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