Genet. Res., Camb. (1963), **4**, *pp.* 427–440
With 5 text-figures
Printed in Great Britain

Transduction analysis using leucine requiring mutants of *Salmonella typhimurium*

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(*Received* 4 *May* 1963)

1. INTRODUCTION

In a recent paper, Dawson (1963) has proposed a method whereby the data from transduction experiments using *Salmonella typhimurium* can be quantitatively analysed. The method of analysis is based on the argument of Ozeki (1959) that all the transduced fragments carrying a specific genetic marker arise by breakages at constant positions in the genome of the donor bacteria. In this paper we shall present firstly, the results of some transduction experiments in support of this hypothesis, and secondly, an analysis of results demonstrating how the data from a series of two-factor transductions can be used to construct a linkage map.

2. MATERIALS AND METHODS

The wild type and all the mutants used were of the LT-2 strain of *Salmonella typhimurium*, and genetic material was transduced with temperate phage PLT-22. The mutant *leu-39 araB-9* was obtained from the Carnegie Department of Genetics; the mutants *leu-121 araB-9*, *leu-126*, *araB-9*, *leu-128 araB-9* and *leu-129 araB-9* were isolated by and obtained from Dr P. Margolin, the Biological Laboratory, Cold Spring Harbor, Long Island, New York.

The preparation of the phage, the method of performing the transductions and the media used have been previously described (Smith-Keary, 1960; Dawson & Smith-Keary, 1960).

3. THE LENGTHS OF THE FRAGMENTS WHICH INCLUDE *leu* AND *araB*

The genetic fragments participating in the transduction of a specific marker may be identical as proposed by Ozeki (hypothesis A), or they may be of identical lengths but not have identical ends (hypothesis B), or they may be of random lengths and so can not have identical ends (hypothesis C), or the fragments may have a fixed breakage point at one end while the second break occurs at random (hypothesis D).

Consider the transductions *leu-x araB-9* (x) $^{+}$ $^{+}$ and *leu-y araB-9* (x) $^{+}$ $^{+}$ which

involve the closely linked *leu* and *araB* loci, and where x and y are different sites within *leu*, y being the nearer to *araB-9* (Fig. 1).

If hypothesis A is correct the frequency of transduction of *leu-x* to *leu-x*$^+$ (t^x) is $k_1 a^x(b^x + c)$, where k estimates the efficiency of transduction and a^x, b^x and c are the magnitudes of regions I, II and III in terms of recombination frequency.

Similarly, $$t^y = k_2 a^y(b^y + c)$$

and $$t^{ara} = k_3(a + b)\,c$$

Thus if the efficiency of transduction is the same for each marker (i.e. $k_1 = k_2 = k_3$), the frequency of transduction of a marker will be directly related to the position of

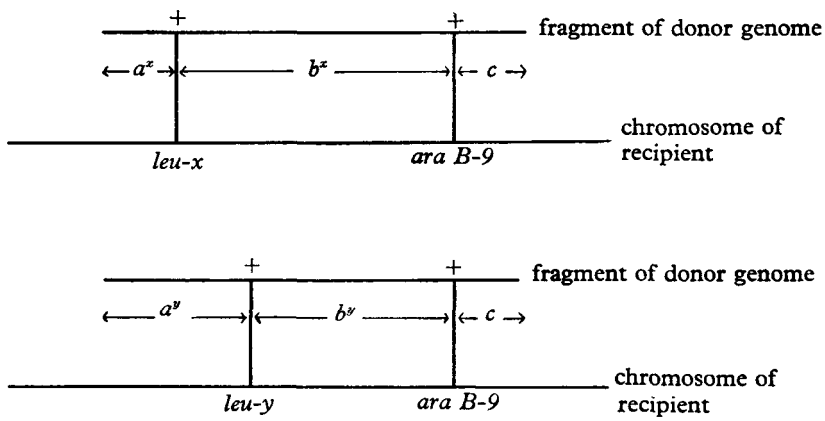

Fig. 1. The transductions *leu-x araB-9* (x) + + and *leu-y araB-9* (x) + + (for explanation see text).

that marker in relation to the ends of the fragment. On the other hand, if either hypothesis B or C is correct

$$t^x = k_1$$

$$t^y = k_2$$

$$t^{ara} = k_3$$

and if the efficiency of transduction (i.e. of pick up, transfer, pairing, integration and expression) is the same for each marker all markers should be transduced at the same frequency.

It should be possible to distinguish hypothesis A from hypotheses B and C by comparing the estimates of t (the frequency of transduction) for a series of markers. The most reliable method for estimating t is by expressing it in terms of a given number of survivors (Smith-Keary, 1960; Dawson & Smith-Keary, 1960), but even this method is insufficiently precise to detect the small differences in t which are expected if hypothesis A is correct; variation between repeat experiments using the same phage preparation being as great as the variation between experiments

involving different markers. This variation in repeat experiments is probably attributable to differences in the efficiency of transduction due, for example, to the recipient cultures containing different proportions of recipient cells in the right phase of growth, and to inaccuracies in estimating the numbers of survivors.

This difficulty can be overcome by using as recipient a double mutant *leu-x araB-9* (as in Fig. 1) and plating samples of the same transduction mixture (using wild-type phage) on different media to select for *leu*$^+$ transductants (EMM) and for *araB*$^+$ transductants (EMM(arab) + LL).

With hypothesis A the ratio of the frequencies of transduction is

$$\frac{\text{number } leu^+ \text{ transductants}}{\text{number } araB^+ \text{ transductants}} = \frac{a^x(b^x+c)}{(a+b)c}$$

there being no reason to suppose that k is different for the two series of platings. This ratio will only equal unity when $a = c$, and may have any value between 0 (when $a^x = 0$) and

$$\frac{[\frac{1}{2}(a+b+c)]^2}{(a+b)c}$$

(when $a^x = b^x + c$).

On the other hand, with hypotheses B and C this ratio should not differ significantly from unity.

Table 1 sets out the results of 30 transductions using four different *leu* mutants each in combination with the *araB-9* marker. The transduction mixtures were simultaneously plated on EMM (selecting for *leu*$^+$) and on EMM(arab) + LL (selecting for *araB*$^+$) and the transductions scored after 5 days' incubation. In each group of experiments involving a particular *leu* site there is a significant departure from a 1:1 ratio of *leu*$^+$:*araB*$^+$ transductants (P always $\ll 0{\cdot}001$).

The only objection implicit in this method is that the ratio observed may be biased because of differences in the numbers of residual divisions necessary for the complete expression of *leu*$^+$ and *araB*$^+$ transductants. This is unlikely, however, for the following reason: plate-washing experiments have shown that when about 10^8 *leu-x araB-9* bacteria are plated (and in our experiments the number of survivors per plate was never in excess of this), about four residual division cycles occur on EMM and about six on EMM(arab) + LL. Since Witkin (1956) has shown that for the *try-3* mutant the maximum number of transductants is expressed after only two residual division cycles, it is most improbable that full expression of the transductants does not occur on the media we used. Furthermore, the additional residual division cycles that occur on EMM(arab) + LL would, if anything, be expected to increase the proportion of *araB*$^+$ transductants, whereas we have observed an excess of *leu*$^+$ transductants. We conclude that it is unlikely that the observed deviations from a 1:1 ratio can be accounted for by differences in the expression of the two classes of transductant. This conclusion can be confirmed by comparing the t^{leu}/t^{ara} ratios using a series of different leucine mutants of known linkage order, each in combination with *araB-9*.

Consider the transductions represented in Fig. 1 in relation to hypothesis A. We have shown that

$$\frac{\text{number } leu\text{-}x^+ \text{ transductants}}{\text{number } araB^+ \text{ transductants}} = \frac{t^x}{t^{ara}} = \frac{a^x(b^x+c)}{(a^x+b^x)c}$$

and

$$\frac{\text{number } leu\text{-}y^+ \text{ transductants}}{\text{number } araB^+ \text{ transductants}} = \frac{t^y}{t^{ara}} = \frac{a^y(b^y+c)}{(a^y+b^y)c}$$

and, since

$$a^x+b^x = a^y+b^y$$

$$\frac{t^x}{t^{ara}} : \frac{t^y}{t^{ara}} = t^x : t^y$$

Similarly, for four leucine mutants *leu-w*, *leu-x*, *leu-y* and *leu-z* we can measure

$$t^w : t^x : t^y : t^z$$

and if the order of these sites in relation to *araB-9* is

distal end — *w x y z* (*leu*) — *araB-9* — proximal end

the ratio should be

(1) an ascending series when $a^z < b^z+c$; i.e. when all the *leu* sites are located on the distal half of the fragment,

or

(2) a descending series when $a^w > b^w+c$; i.e. when all the *leu* sites are located on the proximal half of the fragment,

or

(3) an ascending-descending series when $a^w < b^w+c$ and $a^z > b^z+c$; i.e. the *leu* sites span the centre of the fragment.

With either hypothesis B or C

$$t^w : t^x : t^y : t^z = 1:1:1:1$$

With hypothesis D, there are two alternatives which must be considered

(a) when the fixed breakage point is to the left of the *leu* locus, and
(b) when the fixed breakage point is to the right of the *araB* locus.

If the fixed breakage point is to the left of *leu*, and if the second break occurs at random then the nearer the *leu* site is to *araB-9* the lower will be the number of fragments carrying that site and the lower will be its frequency of transduction, approaching the frequency of transduction of *araB-9* which will always be lower than that of *leu*, thus $t^w : t^x : t^y : t^z$ will be a descending series approaching unity. If the fixed breakage point is to the right of *ara* the reverse order of frequencies of transduction will be found and t^{leu}/t^{ara} will always be less than unity.

To test between these we used the four double mutants *leu-121 araB-9*, *leu-129*

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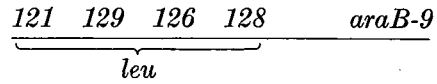
araB-9, *leu-126 araB-9* and *leu-128 araB-9*, whose linkage order is as follows (Margolin *et al.*, 1960)



Each mutant was infected with phage grown on a wild-type donor and the transduction mixture plated on EEM (selecting for *leu*$^+$ transductants) and on EMM(arab) + LL (selecting for *araB*$^+$ transductants). After five days' incubation the *leu*$^+$ transductants (L) and *araB*$^+$ transductants (A) were scored. The results and analyses of these transductions are set out in Table 1.

These results show that

1. $t^w : t^x : t^y : t^z \neq 1:1:1:1$ making improbable either hypothesis B or C.
2. $t^w : t^x : t^y : t^z$ is not a descending series as predicted with hypothesis D (with a fixed breakage point to the left of *leu*).
3. t^{leu}/t^{ara} is never less than unity as predicted with hypothesis D (with a fixed breakage point to the right of *araB*).

In section 4 evidence will be presented which shows that if hypothesis A is correct then *leu-121*, *leu-129* and *leu-126* must be located distally to the centre of the fragment, and that *leu-128* can not be located farther from the centre than *leu-126*. If therefore hypothesis A is correct we predict that $t^{121} : t^{129} : t^{126} : t^{128}$ will be an ascending series. It is conspicuous that the observed ratios form an ascending series (1·361 : 1·432 : 1·493 : 1·605, means of ratios, or 1·220 : 1·286 : 1·439 : 1·457, ratio of totals) in precise agreement with this hypothesis. Since the probability of obtaining an ascending series of four values by chance is 1 in 24, we conclude that these results are very strong evidence for Ozeki's hypothesis that all the fragments participating in the transduction of a particular linkage group are identical (hypothesis A).

4. ANALYSIS OF THE *leu* LOCUS

In this analysis we used the four *leu araB-9* mutants used in the experiments described in section 3, *leu-121*, *leu-129*, *leu-126* and *leu-128*, which are representatives of complementation groups I, II, III and IV respectively, and *leu-39 araB-9*. *leu-39* does not (except very rarely) yield prototroph transductants in transductions with group III and group IV mutants, but does yield prototrophs in transductions with *leu-129*; abortive transductions are only observed when *leu-39* is infected with phage grown on a wild-type donor or when it is used in transductions with a group I mutant. Thus *leu-39* is probably a multisite mutant covering the whole of the group III and group IV regions and part of the group II region. Since the *leu-39* strain has no requirement other than for leucine, it is unlikely that the proximal end of the mutant region lies outside the *leu* locus; probably, since multisite mutants have a propensity for ending at the end of a locus (Demerec, 1960) the proximal ends of the *leu* locus and the *leu-39* mutation coincide.

Table 1. *Each recipient strain was infected with phage grown on a wild-type donor strain and plated on EMM (selecting for* leu$^+$ *transductants) and on EMM(arab)+LL (selecting for* araB$^+$ *transductants).* leu$^+$ (L) *and* araB$^+$ (A) *transductants were scored after 5 days' incubation*

Recipient	*leu-121 araB-9*			*leu-129 araB-9*			*leu-126 araB-9*			*leu-128 araB-9*		
	L	*A*	*L/A*	*L*	*A*	*L/A*	*L*	*A*	*L/A*	*L*	*A*	*L/A*
Transductants observed	289·3*	225·0	1·286	206·0	131·0	1·573	92·0	113·3*	0·812	187·0	72·0	2·598
	290·0	206·6*	1·403	295·0	240·0	1·229	270·0	120·0	2·250	300·0	169·3*	1·772
	241·0	176·6*	1·365	248·0	212·0	1·170	73·0	58·0	1·259	593·3*	415·0	1·430
	227·0	307·5*	0·738	410·0	395·0	1·038	273·0	206·0	1·325	144·0	118·0	1·220
	480·0	222·0	2·162	475·0	208·5*	2·278	350·0	226·0	1·549	134·0	106·0	1·264
	146·0	90·0*	1·622	218·6*	113·0	1·935	646·0	580·0	1·114	268·0	306·0	0·876
	393·0	492·0	0·799	300·0	375·0	0·800	524·0*	245·0	2·139	317·0	168·0	1·887
	168·0	111·0	1·513							158·0	88·0	1·795
Totals	2234·3	1830·7		2152·6	1674·5		2228·0	1548·3		2101·3	1442·3	
Mean *L/A* ratios			1·361			1·432			1·493			1·605
L/A ratios of totals			1·220			1·286			1·439			1·457

Totals marked * are scaled to compensate for differing numbers of EMM and EMM(arab) + LL plates scored.

Each double mutant (*leu-z araB-9*) was transduced with phage grown on a wild-type donor as described in section 3. The *leu*$^+$ transductants were then characterized for their ability to ferment arabinose by subculturing each transductant onto EMB, and the *araB*$^+$ transductants were characterized for their leucine requirement by subculturing onto MM. The results, presented in Table 2, are the sum of at least eight independent experiments with each mutant.

Table 2. *Results of the transductions* leu-z ara-B9 (x) + +

(*a*) *selecting on EMM for* leu$^+$ *transductants; these characterized for their ability to ferment arabinose*

		leu-z				
		-121	*-129*	*-126*	*-128*	*-39*
Transductants observed	*leu*$^+$ *araB* (*x*)	1607	1565	1615	1315	980
	leu$^+$ *araB*$^+$ (*y*)	1283	1459	1590	1636	1883
	Total	2890	3024	3205	2951	2863
	d	26·80	27·50	28·29	26·87	25·60
	d′	26·58	27·43	28·28	27·09	25·69

(*b*) *Selecting on EMM(arab)+LL for* araB$^+$ *transductants; these characterized for ability to grow in the absence of leucine*

		leu-z				
		-121	*-129*	*-126*	*-128*	*-39*
Transductants observed	*leu*$^+$ *araB*$^+$ (*x′*)	1526	1546	1500	1605	1813
	leu$^-$ *araB*$^+$ (*y′*)	1498	1395	1294	1295	888
	Total	3024	2941	2794	2900	2701
	d	27·52	27·09	26·41	26·86	24·74
	d′	27·50	26·99	26·26	26·65	24·06

Consider the transduction *leu-z araB-9* (x) + + (Fig. 2), where *a*, *b* and *c* are the relative frequencies of recombination in regions I, II and III. Let *x* and *y* be the observed numbers of *leu*$^+$ *araB-9* and *leu*$^+$ *araB*$^+$ transductants respectively when

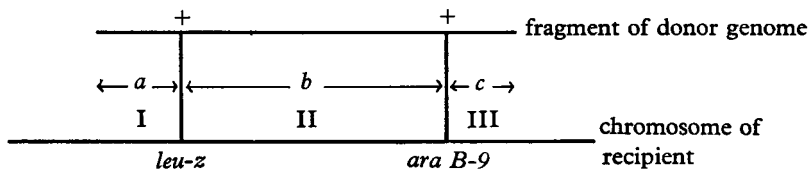


Fig. 2. The transduction *leu-z araB-9* (x) + + (for explanation see test).

selection is for *leu*$^+$, and let *x′* and *y′* be the observed numbers of *araB*$^+$ *leu*$^+$ and *araB*$^+$ *leu*$^-$ transductants respectively when selection is for *araB*$^+$.

From *x* and *y* we can derive estimates of the relative frequencies of recombination in regions II and III (*b*/*c*), and from *x′* and *y′* we can obtain similar estimates of the relative frequencies of recombination in regions I and II (*a*/*b*). Knowing the

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maximum and minimum values of b/c and a/b a range of values for a/c is obtained by multiplication. Since region III is identical in all the transductions, c can be given the arbitrary value of unity, and then a/c and b/c provide estimates of the sizes of regions I and II respectively, in terms of the frequency of recombination in region III (c).

The calculations, following the method described by Dawson (1963), proceed as follows:

The maximum and minimum values of X/Y (the true value of x/y, i.e. x/y when an infinite number of transductants is scored) with which x/y is consistent, are ($P = 0{\cdot}05$)

$$\frac{x+2d}{y-2d} \quad \text{(maximum)}$$

where $$d = \frac{(x-y)+\sqrt{[(x-y)^2+(x+y+4)\,xy]}}{x+y+4}$$

and

$$\frac{x-2d'}{y+2d'} \quad \text{(minimum)}$$

where $$d' = \frac{(y-x)+\sqrt{[(y-x)^2+(x+y+4)\,xy]}}{x+y+4}$$

The values of d and d' for each set of data are shown in Table 2. Then, when $x > y$, the value of b/c will fall within the limits ($P = 0{\cdot}05$)

$$\frac{X}{Y} \quad \text{(maximum)} = \frac{x+2d}{y-2d}$$

and $$\frac{X+Y}{2Y} \quad \text{(minimum)} = \frac{x+y}{2(y+2d')}$$

and, when $x < y$, the corresponding limits are

$$\frac{X}{Y} \quad \text{(minimum)} = \frac{x-2d'}{y+2d'}$$

and $$\frac{2X}{X+Y} \quad \text{(maximum)} = \frac{2(x+2d)}{x+y}$$

A similar range of values for a/b is calculated from x' and y'. These limits for b/c and a/b (Table 3) are such that there is only 1 chance in 20 that the true values be outside these limits ($P = 0{\cdot}05$). The 0·05 probability limits of a/c were calculated by multiplying together the maximum limits of a/b and b/c and the minimum limits of a/b and b/c using $\sqrt{(0{\cdot}05)}$ probability limits.*

* The limits of b/c and a/b were also calculated for $P = \sqrt{(0{\cdot}05)}$ and for $P = \sqrt[4]{(0{\cdot}05)}$ by substituting $1{\cdot}217d$ and $1{\cdot}217d'$ [for $P = \sqrt{(0{\cdot}05)}$], and $0{\cdot}720d$ and $0{\cdot}720d'$ [for $P = \sqrt[4]{(0{\cdot}05)}$] for $2d$ and $2d'$ in the given formulae. The $\sqrt{(0{\cdot}05)}$ probability limits of a/c were obtained by multiplying together the maximum limits of a/b and b/c and the minimum limits of a/b and b/c, using the $\sqrt[4]{(0{\cdot}05)}$ probability limits of a/b and b/c.

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Maximum and minimum values for the total length of the fragment were obtained from the data of each pair of transduction experiments by adding the maximum values (to give a maximum limit) or minimum values (to give a minimum limit) of a and b [$P = \sqrt{(0{\cdot}05)}$] to unity (i.e. c/c). These limits will correspond to a probability of approximately 0·05.

Table 3. *Analysis of the data presented in Table* 2. *All values have a probability of* 0·05

		leu-121	*leu-129*	*leu-126*	*leu-128*	*leu-39*
1. $\frac{b}{c}$	max.	1·351	1·154	1·090	0·928	0·720
	min.	1·081	0·999	0·973	0·746	0·480
2. $\frac{a}{b}$	max.	1·096	1·193	1·251	1·336	2·221
	min.	0·974	1·015	1·038	1·075	1·443
3. $\frac{a}{c}$	max.	1·396	1·300	1·287	1·185	1·517
	min.	1·085	1·043	1·039	0·839	0·730
4. Location of centre of fragment	max.	1·828				
	min.	1·602				
5. Length of fragment	max.	3·656	3·375	3·264	3·065	3·173
	min.	3·204	3·076	3·045	2·632	2·250

The linkage map (Fig. 3) was constructed as follows:

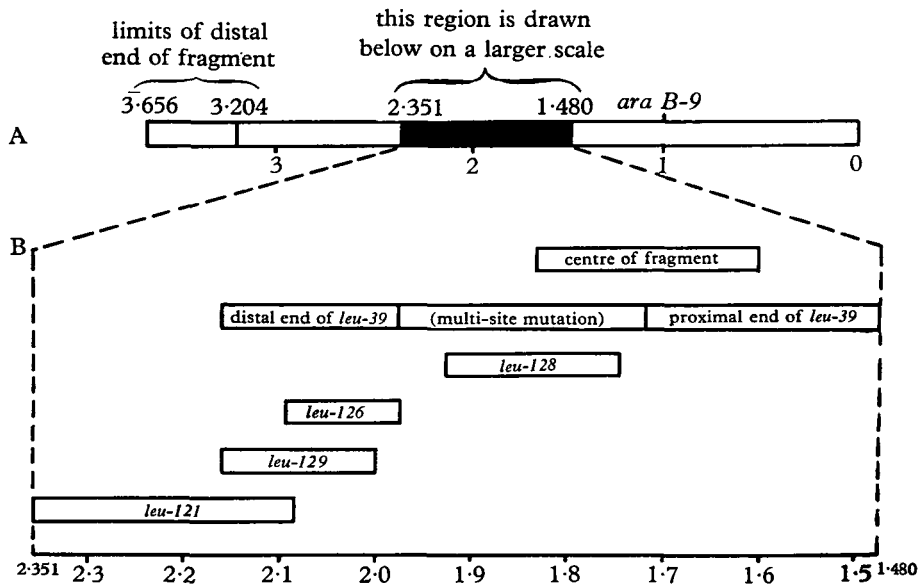

Fig. 3. Linkage map of the fragment participating in the transduction of the *leu* and *araB* markers in *Salmonella typhimurium* (for explanation see text). A, map of the entire fragment; B, the *leu* region enlarged.

1. The proximal end of the fragment is taken as zero, and all map distances are measured from this point.
2. Since c is taken as unity, the *araB-9* site will be located at unity.

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3. The limits of the positions of the *leu-121*, *-129*, *-126* and *-128* sites, and of the proximal end of the *leu-39* multisite mutation are given by the corresponding limits of b/c ($P = 0{\cdot}05$, Table 3, row 1).
4. The distal end of the *leu-39* mutation must, since *leu-39* recombines with *leu-129* but not with *leu-126* (or *leu-128*), be positioned within the region covered by the maximum limit of *leu-129* and the minimum limit of *leu-126*.
5. The limits of the distal end of the fragment were plotted from the data using *leu-121* as this data is probably the least biased by negative interference (see discussion).
6. The limits of the centre of the fragment are one-half of the limits of the total length of the fragment.

All limits on the map have a probability of approximately 1 in 20.

5. DISCUSSION

In section 3 we presented the results of a series of transduction experiments which confirm the hypothesis of Ozeki (1959) that the genetic fragments participating in the transduction of a particular linkage group are of identical length and of homologous genetic constitution. These results have already been discussed in relation to this and to alternative predicates and will not be further discussed here. Section 4 presented an analysis of some results which has enabled us to construct a linkage map of the genetic fragment participating in the transduction of the *leu–araB* linkage group in terms of relative recombination frequencies. The method of analysis used assumes the validity of the predicate confirmed in section 3, namely that all the fragments carrying a particular genetic marker are of identical length arising by breakages occurring at constant positions in the genome of the donor bacteria.

Before discussing these results further, we must account for a discrepancy in the analysis set out in Table 3; whilst, as expected, the values of b/c decrease and the values of a/b increase as the site of the *leu* mutation approaches *araB-9*, the corresponding values of a/c decrease, although region I (and hence a/c) should increase in magnitude the nearer the *leu* site is to *araB-9*. This can be accounted for by localized negative interference, a phenomenon which Pritchard (1959, 1960) has shown in *Aspergillus* to increase in magnitude with decrease in the size of the region in which the obligatory recombination event occurs.

Consider the transductions *leu-1 araB-9* (x) + + and *leu-2 araB-9* (x) + + (where *leu-2* is the nearer to *araB-9*) when selection is in region III for $araB^+$ transductants (Fig. 4). If there is negative interference there will be a cluster of crossovers in that part of region II adjacent to *araB-9* (region *d*) in addition to less frequent random crossovers in the remainder of region II and in region I. Thus changing the position of the *leu* site from *leu-1* to *leu-2* will, provided that *leu-2* does not fall within *d*, result in only a relatively small decrease in b and the difference between a_1/b_1 and a_2/b_2 will be less than if negative interference were absent, and

relative to a_1/b_1, a_2/b_2 will be underestimated. The degree by which a/b is underestimated will increase as the *leu* site approaches *araB-9* so that the most reliable estimate of an a/b value is that provided by the data from the transductions using *leu-121*.

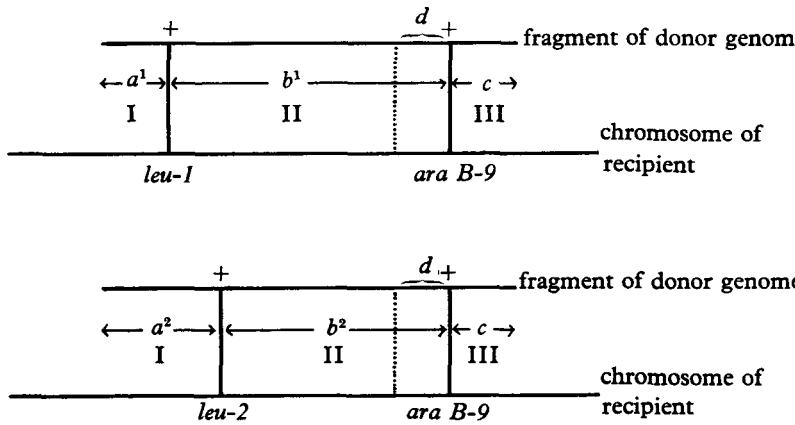

Fig. 4. The regions of high negative interference (*d*) in the transductions *leu-1 araB-9* (x) + + and *leu-2 araB-9* (x) + + when selection is for *araB*$^+$.

Consider now when selection is in region I for *leu*$^+$ (Fig. 5). Since a_1 is less than a_2 the cluster of crossovers in region II (*d*) will be greater with *leu-1* than with *leu-2*. We cannot however predict which of the b/c values is the most reliable, for although

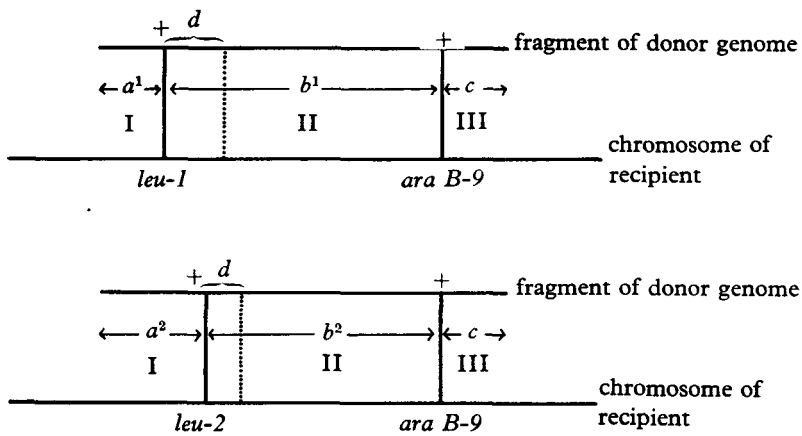

Fig. 5. The regions of high negative interference (*d*) in the transductions *leu-1 araB-9* (x) + + and *leu-2 araB-9* (x) + + when selection is for *leu*$^+$.

we believe that the size of the cluster decreases as *a* increases, we do not know the relationship between the size of the cluster and the magnitude of region II; that is to say, we do not know if the number of crossovers due to interference per unit length of region II is greater or less for transductions using *leu-1* than for transductions using *leu-2*. Since our most reliable estimate of a/b is from the data of

transductions using *leu-121* (i.e. the least biased by negative interference) we consider that the most reliable estimate of a/c is also obtained from this data, and we have accordingly used these a/c and b/c values to estimate the maximum and minimum lengths of the fragment. Furthermore, it is believed that the degree of negative interference in adjacent regions varies as the size of the region in which selection is made and hence b will have the same value in a/b and b/c when $a = c$. As this condition is approached with *leu-121* there is no reason to suppose that our estimate of a/c using this marker is significantly biased by negative interference. Similarly, our estimate for the total length of the fragment $(a/c + b/c + c/c)$ obtained from the *leu-121* data will approximate to the true value for if a/c is underestimated, b/c will be correspondingly overestimated.

The number of functional units on the fragment

The maximum and minimum magnitudes of the *leu* locus are given by the differences between

the most distal limit of *leu-121* and the most proximal limit of *leu-39* (maximum)
and
the most proximal limit of *leu-121* and the most distal limit of *leu-39* (minimum)

using the $\sqrt{(0{\cdot}05)}$ probability limits.

Thus the *leu* region has a maximum extent of 0·815 and a minimum extent of 0·393 (P about 0·05). The entire *leu* region consists of four complementation groups or functional units, each of which appears to control a different step in the biosynthesis of leucine (Freundlich *et al.*, 1962; Gross *et al.*, 1962). The region between *leu-121* and *leu-39* covers the whole of complementation groups II and III, the whole or the greater part of group IV (no known *leu* site lying proximal to the proximal end of *leu-39*), and the greater part of group I (of eight other group I mutants whose sites have been plotted, only two are distal to *leu-121*). We conclude that the region between *leu-121* and *leu-39* covers about $3\frac{1}{2}$ functional units. Each functional unit has therefore an average length of between 0·112 (minimum) and 0·233 (maximum). Since the fragment has a maximum length of 3·656, the maximum number of functional units included on the fragment is $3{\cdot}656/0{\cdot}112 = 32{\cdot}643$ and the minimum number is $3{\cdot}204/0{\cdot}233 = 13{\cdot}751$. We conclude that the fragment is between 13 and 33 times the average length of a *leu* functional unit. This is to be compared with the minimum estimate of 19, for the number of loci on the fragments participating in the transduction of the *try–cys* linkage group (Dawson, 1963).

In this analysis we have considered only the *leu* and *araB* loci but would point out that two other loci have also been located on this fragment, *su-leuA* located distally to *leu*, and *araA* located between *leu* and *araB*. Thus seven distinct functional units have been located on the fragment, although it cannot be ruled out that separate functional units also exist within the *araA*, *araB* and *su-leuA* loci.

The usefulness of this method of analysis for two-factor transduction data is two-fold. First, it permits estimates to be made of the relative frequencies of

recombination in the different regions of the transduced fragment involved—thus permitting the construction of a linkage map, whereas other methods of analysis only permit the deduction of linkage order. Second, it demonstrates the reliability of the deduced linkage order. For example, in Table 2 (a) the proportion of joint: single transductants for *leu-121* is 1283:1607 and for *leu-129* is 1459:1565. These ratios being significantly different ($P < 0{\cdot}001$) it might be concluded that *leu-129* is the site nearer to *araB-9*; yet our analysis of these data shows that the probability of *leu-121* being the nearer site to *araB-9* is just on the borderline of significance. This analysis further demonstrates the very large numbers of transductants that must be characterized for the unselected marker for reliable conclusions about the order of the sites to be drawn.

Lastly we would draw attention to the fact that it is possible to construct accurate linkage maps from the data of transduction experiments, provided that sufficiently large totals of transductants are analysed. The linkage map we have constructed is in complete agreement with the linkage order established by three point test crosses, and the only discrepancy can be fully accounted for by negative interference—a phenomenon which is not confined to transduction experiments.

6. SUMMARY

1. Data is presented in support of the hypothesis that in *Salmonella typhimurium* the fragments participating in the transduction of a particular linkage group arise by breakages at constant positions in the genome of the donor bacteria.

2. Data from three factor transduction experiments are used to construct a linkage map of the *leu–araB* region which defines not only the relative order of the markers but also the relative distances between them.

The research reported herein has been sponsored by the Office, Chief of Research and Development, U.S. Department of the Army, through its European Research Office.

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