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Gross Map Distances and Hfr Transfer Times in *Escherichia coli* K-12

PAUL BRODA AND J. F. COLLINS

MRC Molecular Genetics Unit and Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland

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Hfr strains B4 and B8 transfer the *Escherichia coli* chromosome in opposite directions, each transferring *lac*$^+$ as the last known marker. They were mated in concurrent crosses with the *proA leu metE lys trp purE lac* strain χ462. Analysis of the time of entry values for these markers showed that Hfr strain B8 transfers the whole chromosome more rapidly than does Hfr strain B4. In both crosses, the rate of transfer observed decelerates. If deceleration occurs as a function of the amount of chromosome transferred, the data are consistent with the markers examined being very accurately placed on the Taylor-Trotter map of the *E. coli* K-12 genome.

The basis of the well-filled linkage map of the *Escherichia coli* K-12 chromosome (10) was the location of a number of markers by interrupted mating experiments (9). Other markers have been fitted into this map by using either conjugation or transduction as the tool. There is little doubt that this ordering of markers is generally correct, and this map has proved of the greatest value to all workers using *E. coli*. However, there are a priori grounds for questioning the overall relative dimensions, since positioning the original "landmarks" involved using several assumptions that had not, at that time or indeed subsequently, been tested adequately.

These assumptions include the following: (i) that different Hfr strains and, in particular, sibling Hfr strains transfer the chromosome at similar rates; (ii) that a given Hfr strain transfers its chromosome so that the intervals between the times of entry (t_E) of successive markers are proportional to the distances between them; (iii) that different recipient strains can accept chromosomal deoxyribonucleic acid (DNA) at the same rate; (iv) that the mating conditions and methods of deriving entry times from the data thus obtained in different laboratories yield comparable entry times. Wood (11), studying the physical conditions affecting mating parameters, concluded that in the *thr-his* region (38.5 min on the current map) chromosome transfer did not vary in rate by more than 10%.

The sibling Hfr strains B4 and B8 transfer the chromosome in opposite directions (see Fig. 1), and each transfers *lac*$^+$ as the last known marker (2, 3). In the main experiments to be described, the t_E values for a number of different markers, including *lac*$^+$, were determined by using a single recipient strain in concurrent matings with these Hfr strains. By relating the t_E values obtained in these crosses to the published map, it was possible to test whether the dimensions of this linkage map are compatible with the kinetics of chromosome transfer.

MATERIALS AND METHODS

Particulars of the strains used are given in Table 1. Hfr strains B4 and B8 have origins of transfer that are represented in Fig. 1.

Matings were performed by the procedures described in the preceding paper (3), with the following additional detail. About 15 min after beginning the matings, a 1-ml sample was diluted into 24 ml of prewarmed broth, using a 10-ml pipette to minimize shear. This diluted culture was used for determining t_E values of later markers; samples of either 0.1 or 0.2 ml were withdrawn and pipetted directly into soft agar for interruption.

Except for earliest markers, selection was always for more than one marker. For instance, selection for the transfer of the late marker *purE*$^+$ by strain Hfr B4 was accompanied by simultaneous selection for transfer of *leu*$^+$ (an early marker) and *lys*$^+$ (a middle marker). The purpose of the procedure was (i) to minimize the growth of revertants in the recipient population, and (ii) to ensure that the marker had indeed been transferred through transfer of the whole chromosome, rather than by an inverted or otherwise altered mode of transfer (7); this is very important for the late markers since such progeny could also arise through the formation of F′ factors in the donor population.

TABLE 1. *Strains employed*[a]

Strain	Genotype	Origin or reference
Hfr B4	*metB*λ⁻λ^R	2
Hfr B8	*metB*λ⁻λ^R	2
χ462	*proA leu metE lys trp purE lac str*	1
JC411	*leu met his argG str*	4
ED1195	*tyr argE his str*	Str^R (spontaneous) ex AT2273 (8)

[a] The nomenclature is that recommended by Taylor and Trotter (10).

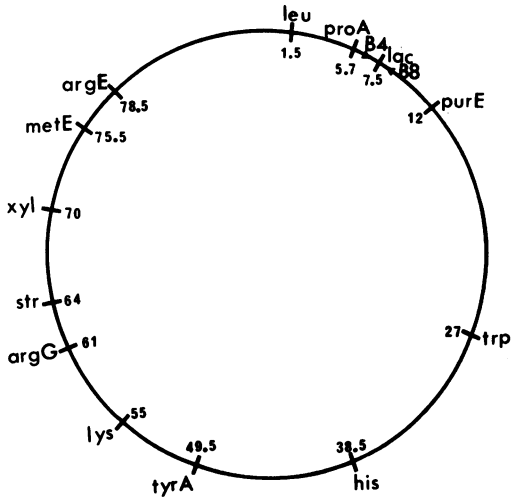

FIG. 1. *E. coli linkage map, with the origins of Hfr strains B4 and B8, and the positions of the markers used in this study. These are placed according to Taylor and Trotter (10) except that proA is moved from 6.5 to 5.7 min, and lac is moved from 9 to 7.5 min (3).*

Streptomycin was included in all plates. It did not appear that its presence altered the time of entry for markers transferred after the *str*⁺ allele.

Plates were incubated for at least 60 h; it was noticed that colonies arising from the transfer of later markers took longer to appear than those arising only from the transfer of earlier markers.

RESULTS

Interrupted matings were performed simultaneously between strain χ462 and Hfr strains B4 and B8, in flasks in the same shaking water bath. There was sufficient broth present to allow normal growth and conjugation throughout the duration of the mating experiments. With later markers, there are much lower yields of progeny. It was nevertheless possible to define adequate entry curves by plating as many samples as possible (generally every 1 or 2 min for any marker). Since in these crosses Lac⁺ progeny colonies were small and the yield of such colonies was variable, the data for *lac*⁺ were obtained by scoring PurE⁺ progeny of strain Hfr B4 and Pro⁺ or Leu⁺ progeny of strain Hfr B8, for the Lac⁺ phenotype. These gave t_E values for *lac*⁺ which were greater than those for *purE*⁺ and *pro*⁺; moreover, there were no Lac⁺ clones among earlier recombinant classes (e.g., Trp⁺ for strain B4 and MetE⁺ for strain B8). This is therefore a valid method for obtaining t_E values for *lac*⁺. The data from a pair of crosses (experiment number 4 in Table 2) are given in Fig. 2.

The t_E values inferred from the five pairs of crosses performed are presented in Table 2. Three minutes are deducted from all t_E values, since it has been shown (3) that this was the approximate "origin entry time" of these two strains. It appears that strain Hfr B4 transfers the chromosome in about 1.07 times the time taken by strain Hfr B8. We conclude that even sibling Hfr strains may transfer the chromosome at different rates. However, it should be noted that these strains arose from a common ancestor over ten years ago.

Perhaps the most important marker for this analysis other than *lac* is *lys*, which is situated about halfway round the chromosome from *lac*, at 55 min. Checks were made to show that the *lys*⁺ allele on the donor strains was not anomalous in being transposed or specified by two loci, for instance. Strains Hfr B4 and Hfr B8 were mated with strain JC411, which is ArgG⁻ His⁻, and strain ED1195, which is Tyr⁻ His⁻. *argG* and *tyr* lie on either side of *lys*, at 61 and 49.5 min, respectively. The t_E values obtained (Table 3) are consistent with these markers and *lys* being in their expected positions with respect to each other in strains B4 and B8.

The sum of the t_E values for any given marker from strains Hfr B4 and Hfr B8 represent the time taken to transfer DNA equal to one complete chromosome, since the two Hfr strains have closely located origins but opposite directions of transfer. This sum falls to a minimum for *lys* (Table 2); in other words, the first half of the chromosome is transferred by each Hfr strain faster than the second half. The speed of transfer does not appear to be determined by the specific segment of DNA transferred, but by the proximity of the DNA to the origin of transfer in each case. Deceleration in the rate of transfer of the chromosome in a mating pair has been proposed previously (5, 6; see 11). However, an alternative explanation is that not all donor cells transfer DNA at the same rate

TABLE 2. *t_E values (minutes) for markers in crosses between Hfr strains B4 and B8 and the F⁻ strain χ462*[a]

Donor	Expt no.	*proA*⁺	*leu*⁺	*metE*⁺	*lys*⁺	*trp*⁺	*purE*⁺	*lac*⁺
B4	1	1	6		51	107	134	139
	2	1	6.5		49	98	131	133
	3	1.5	6	28	58		129	141
	4	1	7.5	28	59	111	136	143
	5	1.5	7	28.5	55	98	128	133
	Avg	1.2	6.5	28	54.5	103.5	131.5	138
	% of t_E for *lac*	0.8	4.7	20	39.5	75	96	100
		purE⁺	*trp*⁺	*lys*⁺	*metE*⁺	*leu*⁺	*proA*⁺	*lac*⁺
B8	1	5.5		60		113	121	125
	2	5		60		120	127	131
	3	5.5	23	59	92	126	129	131
	4	5.5	23	58	94	125		132
	5	4.5	24	58	86	113		125
	Avg	5.2	23.5	59	91	119.5	126	129
	% of t_E for *lac*	4	18.2	46	70.5	93	97.5	100
Sum of t_E values for B4 and B8		136.7	127	113.5	119	126	127.2	

[a] The experiments are numbered to indicate concurrent matings. All the t_E values have had 3 min subtracted, to allow for the origin entry times determined by Broda (3). The ratios of the t_E values for *lac*⁺ transferred by Hfr strains B4 and B8 in the five experiments are 1.11, 1.02, 1.08, 1.08, 1.06, giving an average of 1.07.

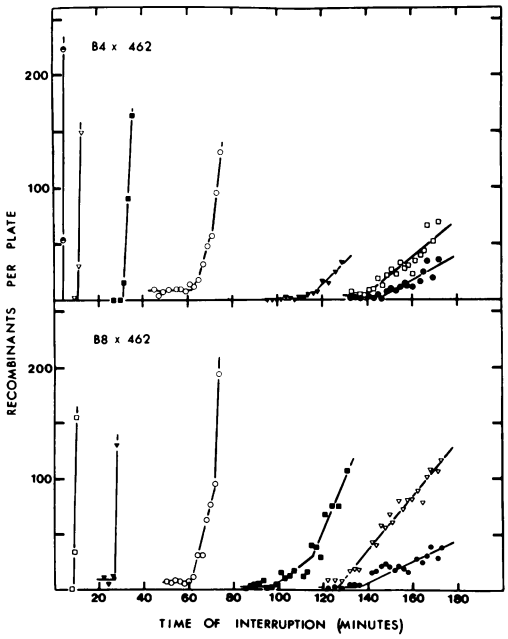

FIG. 2. *Plots of recombinant formation in concurrent crosses between strain χ462 and Hfr strains B4 and B8. The B4 × χ462 cross was started 1 min before the B8 × χ462 cross to obtain the t_E values given in Table 2, experiment 4; 3 minutes were subtracted from all values to allow for the origin entry times.*

during conjugation, and that the faster the rate of transfer, the greater the chance of the DNA breaking and transfer terminating. The recombinants for late markers would, therefore, arise from donors that transferred DNA more slowly than average, thus delaying the t_E from that expected from the chromosomal position of the late marker.

Whether the deceleration arises from the changing rate of transfer in each mating pair or from more complex properties of a heterogeneous population, it seemed worthwhile to characterize the process algebraically so that the behavior of the different Hfr strains could be compared.

For the ideal transfer process proceeding at a constant rate, the rate of transfer of DNA (x) during conjugation (dx/dt) equals a constant, k. If the process is decelerating, then this expression changes to include a negative term dependent in the simplest case on either x (model A) or t (model B).

Model A. If the deceleration were a function of the amount of DNA transferred (e.g., the donor cell is running out of energy or the recipient cell offers increasing resistance to the entry of the DNA) then the rate equation is $dx/dt = k - ax$, where a is a constant. This has an integral in the form: $k - ax = C\,e^{-at}$, where C is a constant. Subject to the boundary condition, $t = 0$

when $x = 0$, C must equal k, and the expression can be rewritten $x = k(1 - e^{-at})/a$. The second boundary condition used is that transfer of the *lac*$^+$ marker indicates complete transfer of the chromosome (i.e., $x = 90$ when $t = t_E$ for *lac*). This value of 90 min for total chromosome length is assumed in both models so as to fit with current usage, e.g., in the Taylor-Trotter map, but does not imply that this figure has any specific physical significance. The expression for the transfer of amount x DNA is now given as: $x = 90(1 - e^{-at})/(1 - e^{-at_E\text{-}lac})$. The value of a was obtained by computing the least-squares fit for the experimental data, and a was calculated to be 0.00412 for strain Hfr B4, and 0.00433 for strain Hfr B8.

Model B. If the deceleration is dependent on time (e.g., the cells change their condition steadily to become less able to transfer DNA) then the rate equation is given by $dx/dt = k - at$. This has the integral form $x = C + kt - at^2/2$. If $x = 0$ when $t = 0$, then C, the constant of integration, is 0. If we take as the second condition that undecelerated transfer would have taken 90 min, then $k = 1$. The value of a is now fixed by the observed time to transfer the total chromosome, which give values of a equal to 0.00252 for strain Hfr B4, and 0.002344 for strain Hfr B8.

In Table 4, the calculated t_M (map position)

TABLE 3. *t_E values for markers in crosses between Hfr strains B4 and B8 and F$^-$ strains JC411 and ED1195*[a]

Donor	Recipient	t_E values (min)					
		leu$^+$	*argE*$^+$	*xyl*$^+$	*argG*$^+$	*tyr*$^+$	*his*$^+$
B4	JC411	6.5			53		89
	ED1195		23	37		69	93
B8	JC411				69		38
	ED1195					54	37

[a] All t_E values have had 3 min subtracted.

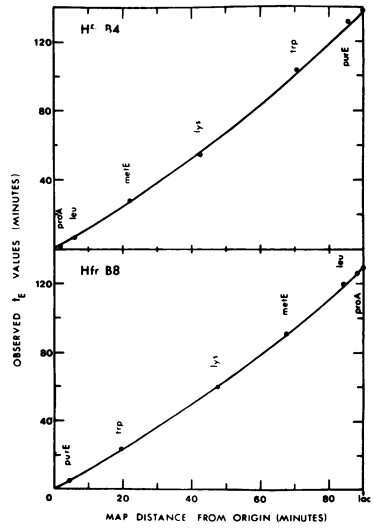

FIG. 3. *Mean observed t_E values in crosses between Hfr strains B4 and B8 and strain χ462, plotted against distance of markers from origin of chromosome transfer (see text). The curves are the calculated best-fit curves for these data, assuming deceleration according to model A (see text).*

TABLE 4. *Calculated mean distance of markers from origins of chromosome transfer of Hfr strains B4 and B8, based on the calculated best fit of all the observed t_E values given in Table 2 to model A*[a]

Donor (1)	Marker (2)	Map position[a] (t_M) (3)	Avg observed t_E (4)	Calculated t_M on model A (5)	3–5 (6)	Calculated t_M on model B (7)	3–7 (8)
Hfr B4	*proA*$^+$	1.8	1.2	1.0	+0.8	1.2	+0.6
	leu$^+$	6.0	6.5	5.6	+0.4	6.4	−0.4
	metE$^+$	22.0	28.0	22.8	−0.8	26.0	−4.0
	lys$^+$	42.5	54.5	41.7	+0.8	47.0	−4.5
	trp$^+$	70.5	103.5	72.1	−1.6	76.5	−6.0
	purE$^+$	85.5	131.5	86.9	−1.4	87.9	−2.4
	lac$^+$	90.0	138.0	90.0	0	90.0	0
Hfr B8	*purE*$^+$	4.5	5.2	4.7	−0.2	5.1	−0.6
	trp$^+$	19.5	23.5	20.2	−0.7	22.2	−2.7
	lys$^+$	47.5	59.0	47.5	0	50.8	−3.3
	metE$^+$	68.0	91.0	68.3	−0.3	71.6	−3.6
	leu$^+$	84.0	119.5	84.9	−0.9	86.0	−2.0
	proA$^+$	88.2	126.0	88.3	−0.1	88.8	−0.6
	lac$^+$	90.0	129.0	90.0	0	90.0	0

[a] Distance from origin of chromosome transfer, based on the Taylor-Trotter map (10), revised for *lac* and *proA* (3).

values for both models are presented, and those for model A are plotted in Fig. 3. *proA* and *lac* are placed at 5.7 and 7.5 min, respectively (3). In crosses with each Hfr strain, model A gives an excellent fit. The χ^2 value for the strain B4 data (which have 31 degrees of freedom) was 6.2. For the strain B8 data (29 degrees of freedom) it was 1.4. Both these values are highly significant. Model B generates less well-fitting curves, and for both Hfr strains the observed t_E values correspond to calculated t_M values which are consistently less that those expected from the Taylor-Trotter map. The systematic nature of these discrepancies shows that model B cannot account adequately for the physical characteristics of the transfer process.

DISCUSSION

Since the physical basis of the Taylor-Trotter genetic map is a single DNA molecule in which the genes have fixed relationships to each other, the observation of nonlinear patterns of transfer by both the Hfr strains cannot have its origin in any inaccuracy in the map, but must arise as a consequence of the transfer process itself having nonlinear properties. The data presented in Table 2 also show that two sibling Hfr strains transfer the whole chromosome at different and decelerating rates. Therefore, one cannot, without qualification, directly compare transfer times for markers at different distances from the origin of a given Hfr or times obtained with different Hfr strains.

The problem of obtaining t_E values from plots of recombinant formation against time has been mentioned in the preceding paper (3). The method used here, which uses the very early recombinants, probably overestimates t_E values for late markers since with these markers the first recombinants to be observed represent a larger proportion of the total recombinant population. This would make the true deceleration in transfer somewhat less than the apparent deceleration.

The computed best-fit curves with model A are surprisingly good. If, therefore, deceleration does occur as a linear function of the amount of chromosome already transferred, the markers examined here are very accurately placed relative to each other on the Taylor-Trotter map using the revised positions of *proA* (5.7 min) and *lac* (7.5 min) given in the previous paper (3). Since the scatter of points about the curves is small and, on comparison of the data for Hfr strains B4 and B8, it is random, there is no case for moving any of the other markers to new positions. It is of interest to note that on model A the segments 0 to 10, 10 to 20, 20 to 30, and 30 to 40 min will be transferred by Hfr strain B8 in 11.25, 11.83, 12.47, and 13.18 min, respectively. However, we can only equate corrected transfer distance with physical distance (i.e., the number of bases transferred) if we assume that there are no chromosomal regions which have the intrinsic property of being transferred faster or slower than other regions. Discrete regions behaving anomalously should, however, be detected in experiments with paired Hfr strains such as B4 and B8, as localized regions of poor fit, with one or both Hfr crosses.

These experiments underline the need for caution when comparing and compiling segments of the genetic map. The first two assumptions listed above—that different Hfr strains transfer the chromosome at similar rates, and that a given Hfr strain transfers markers at time intervals proportional to the distance apart—are clearly incorrect. Although the difference in transfer rate, about 7%, between the two Hfr strains, is small, it is believed to be real from the consistency of the trend observed in the five pairs of crosses.

The non-linearity of the entry times for different markers when plotted against map distances based on the Taylor-Trotter map is quite pronounced with both Hfr strains. The form of the curve fits in each case a similar decelerating function (model A), as though the origin of this behavior is some property of the transfer process itself or of the recipient used. Possible variations in behavior between different recipient strains—a test of the third assumption given above—have not been examined here.

With regard to the fourth assumption, that data from different laboratories should give comparable entry times, it is likely that the improvement and standardization of techniques may increase agreement, although the experimental results shown in Table 2 contain more variation than one would ideally like to see. Since in each experiment there is a tendency for all the t_E values to be earlier or later than the average, slight variations in the physiological state of the mating cells or of the conditions of mating influence the gross transfer rates and limit the absolute accuracy which can be reached at the present.

The fact that model A, showing deceleration with amount of DNA transferred, clearly gives the better fit to the experimental data cannot be taken to define the physical basis of the deceleration, though two possibilities—limitation of energy sources or packing problems in the recipient—have been mentioned. In particular, it has not been shown whether the deceleration process is a property of each individual mating

or whether it arises from heterogeneity in the transfer behavior of different donor cells. Further experiments are needed to clarify this aspect. However, model A does have good predictive value for transfer times and chromosome distances even though at present it represents only an empirical relationship.

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