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Pairing interaction as a basis for negative interference

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The recurring problem of negative interference in conjugal crosses of *Escherichia coli* K-12 (Rothfels, 1952; Cavalli-Sforza & Jinks, 1956; Wollman, Jacob & Hayes, 1956) has been periodically overshadowed by new discoveries concerning the mechanism of genetic transfer in this organism, such as unidirectional and partial transfer (Hayes, 1953; Wollman *et al.*, 1956) and the genetic heterogeneity of zygotes formed in $F^+ \times F^-$ crosses (Jacob & Wollman, 1957), which have led to reappraisal of the interpretation of the genetic data. While some of those data (e.g. those of Rothfels, 1952) can be interpreted entirely on the basis of prezygotic elimination of the male genetic contribution, others (e.g. those of Cavalli-Sforza & Jinks, 1956) could not be, and were not, explained entirely on such a basis but demanded the introduction of the notion of incomplete pairing. Meanwhile the problem of negative interference became an important issue in other microorganisms such as bacteriophage, *Aspergillus*, *Neurospora* and yeast (review, Pritchard, 1960). The most plausible current model of localized negative interference in these organisms postulates that chromosome pairing is discontinuous and random, and that recombination only occurs within those small regions where pairing is effective (Pritchard, 1955; Chase & Doermann, 1958); thus the 'coincidence of recombination in two intervals would occur with greater than random frequency if these intervals were short enough and close enough to be frequently included within one effectively paired segment' (Pritchard, 1960). The mean distance over which such negative interference operates is very small, being of the order of only a few cistrons in both bacteriophage and *Aspergillus* (Pritchard, 1960).

In crosses mediated by phage P1 transduction in *E. coli*, it has been found that when inheritance of the closely linked donor loci, *thr*$^+$ and *leu*$^+$ (ability to synthesize threonine and leucine), is selected, intermediate donor loci such as ara_2 (fermentation of arabinose) are excluded from the *thr*$^+$ *leu*$^+$ recombinants with significant frequency (Lennox, 1955; Gross & Englesberg, 1959) (see Fig. 1). On the other hand, when the same two loci are selected in crosses mediated by conjugation between the same two strains, the intermediate ara_2 locus is virtually never excluded from *thr*$^+$ *leu*$^+$ recombinants (our unpublished data; see also Cavalli, Lederberg & Lederberg, 1953). This anomaly is readily explicable on the above model of negative interference. In transduction, the distance between the selected loci *thr*$^+$ and *leu*$^+$ approaches the maximum length of a transducible fragment (Lennox, 1955), so that the inheritance of these loci must be associated

with recombinational events occurring very close to them with the result that negative interference becomes operative. In conjugation, however, because the segment *thr–leu* is only a small fraction of the total length of the donor chromosome generally transferred to the zygote, there is a high probability that inheritance of this segment as a whole will result from recombination events occurring far from its extremities, i.e. outside those regions where the loci will be affected by negative interference.

On the other hand, some previous studies of conjugal crosses in *E. coli* K-12 (mentioned above) have suggested that negative interference may operate over regions much greater than those of effective pairing as postulated by the current model (Pritchard, 1960). Our attention was directed to this problem by a peculiarity in the inheritance of fimbriation (i.e. possession of non-flagellar appendages called fimbriae) in *E. coli* K-12. Before presenting the data, however, it is necessary to summarize those unique features of chromosome transfer in ♂ Hfr × ♀ F^- crosses which are relevant to the present problem.

(1) The cells of any given ♂ Hfr strain conjugate with ♀ F^- cells with an efficiency of the order of 100% under optimal conditions and then transfer their single chromosomes in a homogeneous, oriented way, such that one particular chromosomal extremity (*O*) is always the first to penetrate the ♀ cells which thus become zygotes. Thereafter the various ♂ loci enter the zygotes in the order of their arrangement on the chromosome (Wollman *et al.*, 1956). During chromosomal transfer, however, there is a tendency for the chromosome to break, with the result that loci distal to the break do not enter the zygote and cannot appear in recombinants. The probability of any locus being excluded in this way is an exponential function of its distance from the extremity *O* (Wollman & Jacob, 1959).

(2) Under standard conditions, each ♂ Hfr locus begins to enter ♀ F^- cells at a very precise time after the commencement of mating. There is good evidence that the speed of chromosomal transfer is constant over the region we have studied (Fuerst, Jacob & Wollman, 1956), so that the distance between loci on the ♂ Hfr chromosome can be precisely mapped as a function of time. Since the time intervals relate to chromosomal transfer, they are independent of subsequent recombinational events, and so can serve as an absolute standard for measuring the frequency of such events in terms of chromosome lengths.

(3) By means of ^{32}P decay experiments the length of chromosome transferred in a given interval of time can be translated into molecular terms (Fuerst, Jacob & Wollman, 1956). It is found that the decay of ^{32}P atoms (incorporated at high concentration) in ♂ Hfr bacteria *prior to mating* prevents them transferring their loci to zygotes at a rate proportional to the distances of these loci from the chromosomal extremity, *O*. Moreover, the relative rates at which transfer of the various loci decline as a function of ^{32}P decay are found to be closely similar to the relative distances, in time units, of these loci from *O*, so that a correlation can be established between genetic distance and ^{32}P decay. From the amount of DNA per nucleus and specific activity of the radio-phosphorus, genetic distance can be the equated with

the number of phosphorus atoms in the chromosomal DNA, provided that two assumptions are made. The first is that the ^{32}P atoms are uniformly distributed among the phospho-diester bonds which constitute the 'backbone' of the DNA double helix. The second is that the probability that a ^{32}P disintegration will prevent further chromosome transfer is the same as that producing death of a bacterium or a phage particle, which is known. Making these assumptions, the data give the result that the whole ♂ Hfr chromosome, whose transfer takes about 100 minutes at uniform speed, contains about 10^7 nucleotide pairs, a figure that agrees well with the amount of DNA per nucleus as estimated by chemical means. Thus approximately 10^5 nucleotide pairs along the double helix of DNA are transferred in one minute at 37° C. (Jacob & Wollman, 1958). This number of nucleotide pairs is of the same order of magnitude as that in the DNA of a phage particle, while the equivalent length of *E. coli* chromosome is also the maximal segment transducible by phage P1.

The genetic data to be presented emerged from crosses between the fimbriated (Fim$^+$) ♂ strain Hfr*H* and the non-fimbriated (Fim$^-$) ♀ strain W945.F$^-$, or derivatives of these strains (see Maccacaro & Hayes, 1961). The relevant loci of ♂ Hfr*H*, the order of their arrangement on the chromosome and the approximate distances between them in terms of times of transfer in broth at 37° are given in Figure 1. The ♀ F$^-$ strain was complementary to the ♂ strain in all these characters.

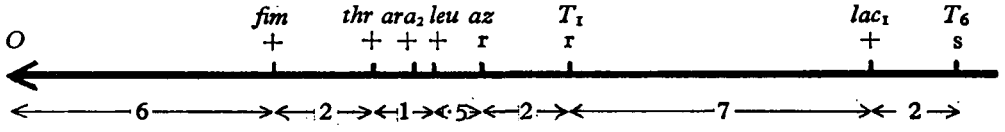


Fig. 1. The relevant segment of chromosome of the ♂ strain Hfr*H*, showing the arrangement of loci and the approximate distances between them (to scale) in terms of times of transfer in broth at 37°. The figures refer to times of transfer in minutes.

O = leading locus; *fim* = fimbriation; *az* = sodium azide resistance (r) /sensitivity(s).

thr = threonine, *leu* = leucine } synthesis

ara$_2$ = arabinose, *lac*$_1$ = lactose } fermentation

T$_1$ = phage T$_1$, *T*$_6$ = phage T$_6$ } resistance (r)/sensitivity (s)

When recombinants inheriting *thr*$^+$ and *leu*$^+$ from the ♂ Hfr parent were selected, 25% were found to be Fim$^-$ and 75% Fim$^+$. This could be interpreted formally in terms of the ratio of the probability of recombination excluding *fim*$^+$ in the short region *fim–thr* (2 minutes) to that of recombination in the much longer region *O–fim* (6 minutes). However, when analysis was made of the distribution of the Fim$^+$ and Fim$^-$ character among the various recombinant classes determined by inheritance of the unselected loci *az*^{r} and *lac*$^+$ on the opposite side of *thr*$^+$ *leu*$^+$, a striking correlation was discovered. The greater the length of ♂ chromosome to the right of *thr*$^+$ *leu*$^+$ that was inherited by recombinants, the higher was the proportion of *fim*$^+$ among them, as Table 1 shows.

Table 1. *Percentage inheritance of the ♂ locus* fim+ *among recombinant classes inheriting various lengths of ♂ Hfr chromosome to the right of the selective loci* thr+leu+. *Mean chromosome lengths inherited are indicated by the uninterrupted lines. Distances are not drawn to scale.*

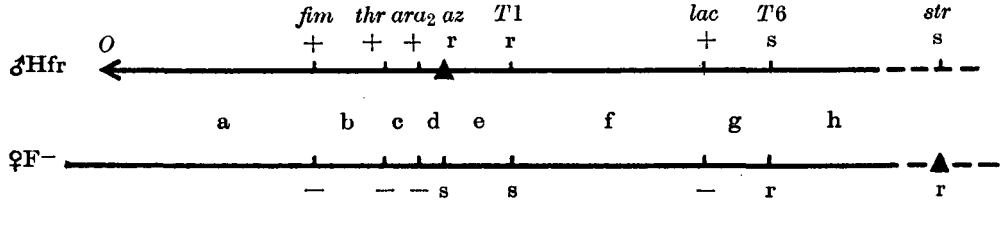
Lengths of ♂ Hfr chromosome inherited by various recombinant classes	*fim*+ inheritance (per cent)	
fim+ *thr*+ *leu*+ *az*r *lac*+	50	Significance in relation to total no. recombinants tested:
	68	$\chi^2 = 7{\cdot}55$ $P = < 0{\cdot}05$
	89	

Similar results were obtained in crosses reversed with respect to the *fim* locus, i.e. when the ♂ strain was Fim− and the ♀ strain Fim+, the inheritance of the *fim*− locus was positively correlated ($P < 0{\cdot}05$) with that of increasing lengths of chromosome to the right of *thr*+ *leu*+, showing that the effect was a consequence of the *position* of the locus rather than of the nature of its function. These results seemed to indicate an interaction between recombinational events on either side of the selected locus, such that the closer a crossover occurs to the right of *thr*.*leu*, the greater is the probability of a second crossover between *fim* and *thr*.*leu*, excluding the ♂ *fim* locus. The phenomenon can thus be interpreted as a type of negative interference, but the distances involved are much greater than those over which high, localized negative interference is displayed in *Aspergillus* or bacteriophage. For example, using the data of various authors (Pontecorvo, 1958; Pritchard, 1960; Barricelli & Doermann, 1960), one can estimate that the mean lengths of regions of 'effective pairing' in *Aspergillus* and of 'switch areas' in bacteriophage T4, over which high negative interference occurs, are approximately equivalent to 2×10^4 and 4×10^3 nucleotide pairs of DNA respectively. In comparison, the effect described above extends at least from *fim* to *lac*, a region of the order of 10^6 nucleotide pairs (12 minutes).

Another experiment was therefore performed using a similar cross but embracing a larger number of loci on either side of the selected locus. The genotype of the parental strains with respect to the region of chromosome studied is shown on the map surmounting Table 2, in which the loci are represented at their approximate distances on a time scale. Recombinants were selected for inheritance of the ♂ Hfr locus *az*r (sodium azide resistance) and of the ♀ F− locus *str*r (streptomycin resistance) by plating zygotes, formed in nutrient broth, on nutrient agar + sodium azide + streptomycin. The zygotes were plated before segregation, but after dilution and aeration in nutrient broth + streptomycin for sufficient time to allow full expression of the character *az*r (Hayes, 1957). Since, in this cross, the *str*s locus of the ♂ parent is not transferred to zygotes with significant frequency,

the incorporation of streptomycin in the selective medium merely eliminates ♂ parental growth and has no effect on the constitution of recombinants. The $az^r.str^r$ recombinants were initially classified into two groups determined by recombination (1) in the small regions *c* or *d* (i.e. $az^r.thr^-$), or (2) in the longer regions *a* or *b* (i.e. $az^r.thr^+$). One hundred and thirty-one recombinants from the first group, and one hundred and sixty from the second group were then scored for inheritance of unselected loci from the ♂ parent. An analysis of the results, showing the correlation between the occurrence of recombination in different regions on either side of the selective marker, is given in Table 2. Quadruple recombinants, of which fourteen were among the first group (9 %) and seven among the second group (4 %), are excluded from the analysis. It should be realized that in systems such as this, where the genetic contribution of one parent is partial, an even number of crossovers is required for the formation of viable recombinants.

Table 2. *Correlation between the occurrence of recombination in different regions on either side of the selective locus* az^r



Analysis of recombinants

A

No. of recombinants with respect to region *a*, *b*, *c* or *d*, and region *e* or $f+g+h$

	e	$f+g+h$	Total	Ratio $e/(e+f+g+h)$
a	10	86	96	0·10
b	12	45	57	0·21
c	17	32	49	0·34
d	30	38	68	0·44
Total	69	201	270	

$\chi^2_3 = 26{\cdot}75$ $P < 0{\cdot}01$

B

No. of recombinants with respect to region *e*, *f* or $g+h$, and region *a* or *b*

	a	*b*	Total	Ratio $a/(a+b)$
e	10	12	22	0·45
f	22	16	38	0·57
$g+h$	64	29	93	0·70
Total	96	57	153	

*$\chi^2_2 = 4{\cdot}73$ $P < 0{\cdot}10$

* The relatively low significance of the data in B is compensated by the concordant results of Table 1, and of many similar (though not identical) crosses.

It will be seen from Table 2A, for example, that when recombination occurs in the interval *d*, close to the left of the selected az^r locus, the ratio of recombination in interval *e* to that in the interval $e+f+g+h$ (0·44) is more than four times higher than that associated with recombination in interval *a* (0·10). A gradient of interaction exists between these two extremes.

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In Table 3, the same data are analysed in a different way. In the left-hand column are listed increasing numbers of ♂ Hfr loci located progressively to the right of the selected locus az^r; the other columns give the percentage of recombinants inheriting these ♂ loci, as a function of recombination in intervals *a*, *b* and *c* to the left of the selected locus (see map surmounting Table 2). It is evident that the gradient of inheritance of the loci located to the right of the selected locus is determined by the position of recombinational events to the left, and that this effect extends at least as far as the T_6 locus at a distance of 11 minutes, or approximately one-tenth the length of the whole chromosome, from the selected locus.

Table 3. *The gradient of inheritance by recombinants of loci on the ♂ chromosome distal to the selected locus* az^r, *as a function of proximal recombination regions. The loci and regions are specified in Table* 2

	Percentage recombinants of various classes among recombinants for regions		
Inheritance of ♂ loci	*a*	*b*	*c*+*d*
az^r	100	100	100
$az^r.T1^r$	90	80	59
$az^r.T1^r.lac^+$	67	50	41
$az^rT1^r.lac^+.T6^s$	46	35	26
Total number recombinants scored	(96)	(57)	(117)

This phenomenon, which could not plausibly be interpreted in terms of recombination within small paired regions, suggested a new model of negative interference to account for the extended effect. Like the previous model, it supposes that, before pairing, there is an equal probability that any region of the chromosomes will pair and that this region is small. It further supposes, however, that as soon as pairing has occurred at any region, the probability of pairing at any other region is no longer random but is increased in inverse proportion to its distance from the first region of pairing. It is apparent that this theory, which involves an interaction between *different regions of pairing*, in no way conflicts with the earlier model, but provides a more general framework within which the earlier model can be included. Since the probability of a single recombination event in an effectively paired region has been estimated as about unity in both *Aspergillus* and phage (Pritchard, 1960; Chase & Doermann, 1958), there is no need to postulate that the occurrence of a second, adjacent region of pairing reduces this probability (i.e. that there is a superimposed positive interference) in order to account for the observed results. The alternative possibility that negative interference over large distances might be due to greatly extended regions of effective pairing in a proportion of zygotes, was considered less likely if it is assumed (*a*) that the mechanism of recombination in transduction and conjugation is the same, and (*b*) that, given effective pairing, the probability of a recombinational event per unit dist-

ance remains the same irrespective of the length of the effectively paired region. The probability of such an event excluding the ♂ locus *ara*$^+$ from recombinants selected by inheritance of the two outside loci *thr*$^+$ and *leu*$^+$ (see above) would then remain the same irrespective of whether the region of pairing was small (as in transduction) or large. As has been mentioned, however, the locus *ara*$^+$ is virtually never excluded from such recombinants derived from conjugal matings, so that, if the assumptions are correct, the region of effective pairing is smaller than the region *thr–leu*.

In presenting these results, we have refrained from considering the many factors, such as the possible effect of the ♂ Hfr leading locus (*O*) in promoting pairing, the polarity of chromosomal replication or the occurrence of chromosomal breakage during genetic transfer, which may complicate the recombination process in *E. coli* in ways not yet understood. Whether or not the tentative theory of widespread negative interference which we have suggested is a correct one, we feel that the data themselves confirm the existence of the phenomenon in *E. coli* crosses and that this must be taken into account in recombinational analysis. An answer to the question whether two distinct mechanisms of negative interference operate, or only one, awaits the demonstration of either a continuous or a discontinuous gradient of effect when the interactions of a continuous series of loci, separated by distances ranging from intra-cistronic to those of the order we have examined, are analysed in a single system. Conjugation in *E. coli* is well suited to such a study since it is the only system where recombinational events can be equated with absolute chromosomal lengths.

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