RESTRICTION IN MATINGS OF *ESCHERICHIA COLI* STRAIN K-12 WITH STRAIN B¹

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RESTRICTION in *Escherichia coli* limits the acceptance of genetic elements such as bacteriophage, sex-factor, and donated bacterial chromosome. These extrinsic genetic elements carry a specificity determined by their last host. When the specificity differs from that of the new potential host, the genetic element is restricted by the new host cell. Restriction is manifest by low efficiency of plating for phage, diminished acceptance of sex-factors or chromosomal markers, or by reduction in linkage of markers known to be closely associated. Modification is a host-induced superimposition of a new specificity on these genetic elements and a conferring of this new specificity on their replicas. Once modified, the formerly restricted genetic elements can be freely accepted by other cells that have specificities either identical with, or similar to, the modifying host.

Restriction of the bacterial chromosome has been shown in matings between an *E. coli* K-12 nonlysogenic donor and an *E. coli* K-12 recipient lysogenic for prophage P1 (ARBER 1962). In such a cross, the frequency of recombinants is reduced compared to frequencies in crosses where neither parent is lysogenic. These results have been confirmed and extended by PITTARD (1964) who demonstrated that in a cross between a P1-sensitive donor and a P1-lysogenic recipient the frequency of selected recombinants and the linkage between markers is diminished.

A more complete analysis of modification and restriction in the bacterial conjugation system has been presented by BOYER (1964). Crosses between *E. coli* K-12 *Hfr* and B/r F⁻, give a frequency of recombination about 10 to 15 times lower for the selected marker, compared to a cross in which K-12 F⁻ is used as a recipient. BOYER shows that a region on the *E. coli* map between *pil* and *thr* controls the mating response between K-12 and B/r. Interrupted matings of the restricted K-12 × B/r cross gives surprisingly different results from the unrestricted K-12 × K-12 cross. Proximal markers, known to enter about 8 min after mating, do not appear in the recombinants until after 30 min in the restricted cross. The frequency of unselected markers is reduced about one half in the restricted cross compared to the unrestricted cross. Restriction and modification of other genetic elements, such as bacteriophage lambda, F-*lac*, F-*gal* and sex-factor F1, are found to be controlled by the same genetic region that controls the mating response

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for these two strains. BOYER concludes that the anomalous results obtained when K-12 and B/r strains are mated can be explained on the basis of modification and restriction.

Other publications and reviews relating to restriction and modification associated with conjugation are by: ARBER and MORSE (1965), GLOVER and COLSON (1965), PTASHNE (1965), COLSON *et al.* (1965), WOOD (1966), ARBER (1965) and KLEIN (1965).

As reported in this paper, restriction of K-12 genetic material by strain B is greatest early in the mating and later becomes constant. Unlike restriction in B/r, no delay in the appearance of a proximal marker is found. Also, the linkage between markers is not reduced by a constant amount, but is dependent upon the distance between the markers. Restriction in strain B is responsible for only part of the reduction in the inheritance of genetic markers from K-12, whereas in strain B/r restriction accounts for the entire effect.

MATERIALS AND METHODS

Table 1 lists the bacterial strains used and their characteristics. Strains AB312, AB259, and

TABLE 1

Bacterial strains employed

Strain designation	Characteristics
Derivatives of <i>E. coli</i> K-12:	
AB312	Hfr: Thr ⁻ Leu ⁻ Thi ⁻ . Order of genes transferred: O <i>arg mtl ile met</i>
AB259	Hfr: Thi ⁻ Lambda-s Str-s. Order of genes transferred: O <i>pil thr leu pro lac</i>
AB531	F ⁻ : Met ⁻ Pro ⁻ His ⁻ Thi ⁻ Gal ⁻ Mal ⁻ Xyl ⁻ Mtl ⁻ Lac ⁻ T6-r T1-r Lambda-r Str-r
KIM25	F ⁻ : derived from AB531: Ile ⁻ Arg-7 ⁻
W1177	F ⁻ : Thr ⁻ Leu ⁻ Thi ⁻ Mtl ⁻ Ara ⁻ Mal ⁻ Lac ⁻ Gal ⁻ Xyl ⁻ Str-r T6-r (lambda)
Derivatives of <i>E. coli</i> B:	
B	F ⁻ : Mal ⁻ Val-r
BIM36	F ⁻ : derived from B. Met ⁻ His ⁻ Pro ⁻ Ilv ⁻ Arg-7 ⁻ Val-r Mal ⁻ Mtl ⁻ T6-r T1-r
B(K)IM37	F ⁻ : Thr ⁺ recombinant AB259 × BIM36 Met ⁻ His ⁻ Pro ⁻ Ilv ⁻ Val-r Mal ⁻ Mtl ⁻ T6-r T1-r Arg-7 ⁻

Symbols: +, ability to synthesize or utilize; -, inability to synthesize or utilize; r, resistance; s, sensitive; F, fertility factor; Hfr, high-frequency donor; lambda phage; (lambda), lysogenic for lambda phage.
Auxotrophic markers: arginine-blocked at the condensing enzyme (*arg-7*); histidine (*his*); isoleucine (*ile*); isoleucine-valine (*ilv*); leucine (*leu*); methionine (*met*); proline (*pro*); thiamine (*thi*); threonine (*thr*); valine (*val*). Carbohydrate utilizations: arabinose (*ara*). Resistance markers: streptomycin (*str*).

AB531 were supplied by ADELBERG, strain W1177 by BACON, and strain B by WITKIN. In addition, T6 from laboratory stock was used.

Repeated attempts with ultraviolet light to induce *E. coli* B, under conditions which bring about the induction of known lysogenic K-12 (λ) strains, were unsuccessful in this study. Readily detectable inducible or uninducible prophage or colicinogenic factors for which strain K-12 is sensitive were not found in strain B.

Media: The following were used. (A) Nutrient-Yeast Extract (NByex/NAyex): 0.8% Difco nutrient broth, 0.1% Difco yeast extract, and 2.0% Difco agar for solid medium. (B) M-9 Minimal Medium: 0.4% dextrose, 0.2% MgSO_4 , 0.3% KH_2PO_4 , 0.05% NaCl , 0.1% N_4HCl , 0.6% Na_2HPO_4 , and 2.0% Difco agar for solid medium. (C) Brom Thymol Blue Sugar Agar (BTB): 0.8% Difco nutrient broth, 1.0% sugar, 0.012% Difco brom thymol blue, and 2.0% Difco agar. (D) Eosin Methylene Blue Sugar Agar (EMB): 2.74% Levine EMB agar (Baltimore Biological Laboratories, Baltimore, Maryland) and 1.0% sugar. (E) Phosphate Buffer Diluent (pH 6.8): 0.45% KH_2PO_4 and 0.58% K_2HPO_4 . Amino acids were added to obtain a final concentration of 50 $\mu\text{g}/\text{ml}$ of medium. Thiamine HCl was added at a final concentration of 0.4 $\mu\text{g}/\text{ml}$ of medium. All percentages were given as w/v.

Culture conditions: All incubator, water bath or shaker temperatures were 37°C. Cultures were grown in culture tubes or 125 ml Erlenmeyer flasks on a rotary shaker. Bacterial cultures used for obtaining mutants, or for matings, were inoculated from starter cultures that had been grown in 10 ml of medium inoculated from a stock slant and incubated for 17 to 18 hours. Stationary-phase cultures were made by inoculating 0.1 ml of 1:10 dilution of the starter culture into 10 ml of medium to be incubated 17 to 18 hours. Viable counts of stationary-phase cultures ranged from 2 to 4×10^9 cells/ml. Log-phase cultures were produced by inoculating 1.0 ml of starter culture into 10 ml medium and incubating the culture for 2 hours. When viable counts reached 3 to 8×10^8 cells/ml, the log-phase cultures were used. Generally, cells plated on complex agar media were incubated 18 to 24 hours and those plated on synthetic agar media for 40 to 48 hours.

Mutant production: Auxotrophic mutants were obtained by treating stationary-phase cells with ultraviolet light (UV, 2537 Å), or log-phase cells with ethyl methanesulfonate (EMS), and then subjecting the cells to the penicillin treatment (ADELBERG and MYERS 1953; GORINI and KAUFMAN 1960). For additional enrichment of mutants, the penicillin-selected cells were sometimes recycled a second time through the penicillin treatment. Mutants to phage resistance were selected by plating large numbers of cells with an excess of phage particles. Fermentation mutants were produced by treating the cells with UV or EMS and then plating onto EMB or BTB sugar agar containing a particular sugar.

Matings: Log-phase cultures grown in NByex were used for matings. Interrupted matings were performed according to the method of DEHAAN and GROSS (1962). The mating mixture was made by adding 0.5 ml of the donor culture to 4.5 ml of the recipient culture contained in a 125 ml Erlenmeyer flask. The mixture was then gently agitated in a rotary water bath shaker for 5.0 minutes, a period of time that allowed effective pairing to take place. The mating mixture was diluted into 100 ml of NByex in a water bath. The amount of dilution depended upon the particular cross. At appropriate time intervals, 1.0 ml samples were removed, put into screw-cap tubes, and blended with a Vortex-Junior Mixer for 1.0 minute to interrupt the mating pairs. To the blended mixture was added 0.1 ml of a high titer stock of phage T6 ($10^{11}/\text{ml}$) to kill the sensitive donor cells. The phage-treated mixture was incubated for an additional 15 minutes to permit efficient phage absorption. After this time, 0.1 ml of the mating mixture, or of a 1:10 dilution in NByex, was plated directly onto selective M9 minimal agar. Contraselection of the donor was made by T6 treatment in all crosses reported in this paper.

Unselected marker analysis: After the recombinant colonies were fully grown, they were picked and streaked for purification on the same kind of medium from which they had been isolated. A single colony isolate was spotted onto a master M9 agar plate of the same composition. This master plate was then replicated onto appropriate selective M9 agar to determine the genotype of each recombinant.

FIG. 1.—Interrupted mating AB312×KIM25. Mating mixture diluted 1:1000 into broth 5.0 min after mating.

FIG. 2.—Interrupted mating AB259×W1177. Mating mixture diluted 1:500 into broth 5.0 min after mating.

RESULTS

Unrestricted mating K-12 × K-12: In Figures 1 and 2, a typical unrestricted cross between a K-12 donor and a K-12 recipient is shown. Figure 1 represents the result of an interrupted mating experiment between donor Hfr AB312 and recipient KIM25. The time of entry for each of the three selected markers is distinct. The proximal marker *arg*⁺ entered about 8 minutes after the onset of mating. The rate of transfer and incorporation for this marker was linear from its time of entry. The kinetics of appearance for *ile*⁺ and *met*⁺ are like those for

FIG. 3.—Interrupted mating AB312×BIM36. Mating mixture diluted 1:100 into broth 5.0 min after mating.

FIG. 4.—Interrupted mating AB259×BIM36. Mating mixture diluted 1:100 into broth 5.0 min after mating.

arg⁺. The order of appearance of the selected markers is in agreement with their positions on the genetic map of *E. coli* (TAYLOR and THOMAN 1964).

Similar results are shown in Figure 2 for donor Hfr AB259 mated to K-12 recipient W1177. The kinetics of appearance of *thr*⁺ are similar to those for *arg*⁺ in the previous cross.

Restricted mating K-12 × B: The effect of restriction by strain B on the kinetics of appearance of K-12 genetic markers is shown in Figure 3. The same donor, AB312, was mated to the recipient BIM36. The rate of transfer and incorporation of *arg*⁺ is not linear, but curvilinear. The rate is low at the onset of the cross and increases until it becomes linear after about 30 min of mating. At best, the time of entry for the *arg*⁺ marker can be estimated since the early part of the curve is not linear; it seems to be in general agreement with the time of entry for the same marker in the K-12 × K-12 cross.

Kinetics for *ilv*⁺ and *met*⁺ are not changed in any noticeable way from those for K-12 × K-12 crosses. Unlike BOYER's (1964) results for K-12 × B/r matings, each marker in the K-12 × B cross has a distinct time of entry and no significant delay in entry time is found for the most proximal marker. The frequencies of appearance for all three markers are reduced by about a factor of 100 compared to frequencies obtained for these markers in the K-12 × K-12 matings.

To demonstrate that restriction is not dependent on the donor strain, or on the genetic region transferred, Hfr AB259 was mated to the recipient BIM36 as shown in Figure 4. The rate of transfer and incorporation for *thr*⁺ is low and increases with time or amount of donor chromosome transferred until the rate becomes linear. The frequency of appearance for this marker is reduced compared to the similar K-12 × K-12 cross.

The points obtained in the early portions of Figures 3 and 4 are significantly higher than background at the 5% level based on repeatable and numerous platings and on comparisons made to appropriate control plates.

From the mating of AB259 with BIM36, a modified B strain, B(K)IM37, was selected. This hybrid recombinant remained *pro*⁻, and thus could maximally contain a K-12 derived genetic segment extending from the origin of AB259 to *pro*. This segment is exclusive of the region studied with the donor AB312. Of 48 *thr*⁺ recombinants, 79% were found to give a frequency of recombination for *arg*⁺, upon backcrossing with AB312, higher than that for a B recipient.

Unrestricted mating K-12 × B(K): Matings of AB312 to the modified B(K)-IM37 are not restricted as shown by the linear appearance of *arg*⁺ in Figure 5. However, the frequency of the *arg*⁺ recombinants in this cross was lower than in K-12 × K-12 crosses indicating another independent effect acting to reduce the acceptance of transferred genetic material. Further evidence for the absence of restriction in the K-12 × B(K) cross will be given later from the unselected marker analysis.

The appearance of *ilv*⁺ and *met*⁺ differs considerably from either of the two matings described before. The rate of appearance for these markers is linear, and their times of entry are in agreement with those found in the K-12 × B crosses. However, the frequency of their appearance, relative to each other, is now

FIG. 5.—Interrupted mating AB312 $\times$ B(K)-IM37. Mating mixture diluted 1:100 into broth 5.0 min after mating.

FIG. 6.—Recovery of selected recombinants from interrupted matings of donor AB312 with recipients K12, B(K), B, and B(K)-IM37, B(K).

reversed. Unlike the results obtained in the K-12 $\times$ B cross, *met*⁺ appears more frequently throughout the cross than does *ilv*⁺.

Two other hybrids were mated to AB312 and the kinetics for the appearance of *arg*⁺ were followed. The results obtained were in agreement with those for K-12 $\times$ B(K)IM37 matings.

Comparative amounts of recovery of selected markers: To facilitate comparison of the number of recombinants per ml recovered for each selected marker from K-12 $\times$ B and K-12 $\times$ B(K) crosses to the K-12 $\times$ K-12 cross, the data are plotted semi-logarithmically in Figure 6. The numbers of donor and recipient cells for these crosses are essentially the same. By averaging the individual values for points on each curve, the following comparisons can be made. The amount of recovery for selected markers in the K-12 $\times$ B(K) cross compared to the K-12 $\times$ K-12 cross is 12% for *arg*⁺, 11% for *met*⁺ and only 2.3% for *ilv*⁺, about five times less than for the other two markers. Compared in the same way, the amount of recovery for these markers in K-12 $\times$ B crosses is 0.4% for *arg*⁺, 1.3% for *ilv*⁺, and 1.9% for *met*⁺.

Restriction accounts for a reduction of about ten times in the recovery of a selected marker compared to the unrestricted cross. However, the recovery for markers in the unrestricted K-12 $\times$ B(K) cross is about ten times lower than for these markers in the K-12 $\times$ K-12 cross, showing the magnitude of the remaining effect to be comparable to that of restriction. Suggestive evidence to explain the anomaly of the *ilv*⁺ marker in the B(K) cross will be given later in the unselected marker analysis. The low recovery for *arg*⁺ in the K-12 $\times$ B cross represents the increased effect of restriction on this proximal marker early in the cross.

Unselected marker analysis: Unselected marker analysis of matings provides a means of assessing the effect of restriction that is independent of selective

marker kinetics; restriction has been shown to unlink markers known to be closely associated (PITTARD 1964; BOYER 1964). Recombinants inheriting the last selected marker, *met*⁺ were used for the unselected marker analysis. These recombinants had received all of the unselected markers scored, since they had entered the recombinant before the selected marker *met*⁺. Table 2 gives the result. Hfr AB312 is the donor and K-12, B and B(K) strains are recipients. The *met*⁺ recombinants are grouped according to the time during the interrupted mating when they were selected.

All data have been examined statistically for differences or similarities by using chi-square. If values are not significantly different at the 5% level with respect to time of sampling for a given marker within a particular cross, then they were averaged. This average was used for further significance testing, between crosses, for that marker.

For the *met*⁺ recombinants, all samples within each group of unselected markers are homogeneous without reference to time except for the *arg*⁺ group from the K-12 × B cross. In this group, samples are significantly different from each other and show a trend of linkage that increases with time of mating. No significant variations are found for any of the other groups represented. These data show that sampling at different times for the unrestricted crosses, K-12 × K-12 and K-12 × B(K), has no effect on the linkage between unselected and selected markers. By comparison, sampling at different times during the restricted K-12 × B cross shows an effect on linkage, but only on the proximal marker. Thus, the effect of restriction is greatest early in the cross and becomes constant later.

Independent evidence for absence of restriction in the K-12 × B(K) cross is shown by the lack of any significant difference between the values obtained for unselected markers in this cross compared with the K-12 × K-12 cross.

Linkage of *ilv*⁺ to *met*⁺ in the K-12 × B cross is not significantly different from that of *ile*⁺ to *met*⁺ in the K-12 × K-12 cross. However the linkage of *mtl*⁺ and *arg*⁺ to *met*⁺ in the K-12 × B mating does differ significantly from the K-12 × K-12 cross. The decreased linkage for *arg*⁺ is much larger than for *mtl*⁺.

TABLE 2
Unselected marker analysis for met⁺ recombinants

Recipient	Time sampled (min)	Total number	Percent of total		
			<i>ile</i> ⁺ or <i>ilv</i> ⁺	<i>mtl</i> ⁺	<i>arg</i> ⁺
KIM25	30-35	58	85	83	41
	60	58	88	78	42
	90	58	85	71	38
B(K)IM37	30-35	60	93	80	39
	60	60	98	73	38
	90	60	93	70	40
BIM36	30-35	54	89	35	2
	60	56	93	45	5
	90	56	98	45	16

However, the polarity of the conjugation process must be taken into account. Low recovery of the *arg*⁺ unselected marker is dependent upon its distance from the selected *met*⁺ marker and on its being more restricted because of its early introduction into the recipient. The *mtl*⁺ and *ilv*⁺ unselected markers enter the recipient at a time when the amount of restriction is nearly constant. These results show that the unlinking of closely associated markers by restriction is dependent upon the distance between these markers. Markers closely associated, i.e., *ilv* and *met* show little to no reduction in linkage; markers further apart, i.e., *mtl* and *met*, show reduction in linkage; and markers still further apart, i.e., *arg* and *met*, show further reduction in linkage. These results differ from the data of BOYER (1964); he found that the unselected markers are uniformly reduced by about one half in the K-12 × B/r mating compared to the K-12 × K-12 cross. The distance between the markers examined in his crosses are very close to the distance between markers used in the present experiments. In Table 2, where linkage to *met*⁺ is being measured, the averaged values for *ilv*⁺ in the K-12 × B(K) cross approach a significant difference from comparable values for *ile*⁺ in the K-12 × K-12 cross. This disparity suggests greater linkage in the B(K) mating than in the K-12 mating. Linkage between *ilv*⁺ and *met*⁺ in the K-12 × B cross shows the same tendency. To examine this point further, an unselected marker analysis is made for *ile*⁺ or *ilv*⁺ selected recombinants in Table 3. It should be remembered that the *met*⁺ unselected marker enters after the *ile*⁺ or *ilv*⁺ marker in all of the crosses analyzed. For early samples, 30 to 35 min, the *met*⁺ marker would enter the recipient at about the time this sample was taken. Presence or absence of the unselected *met*⁺ marker would be dependent upon its transfer and not only on its linkage to the selected marker. For this particular sampling time, data for the *met*⁺ unselected marker are not included in the analysis.

Linkage of the unselected *met*⁺ to selected *ile*⁺ or *ilv*⁺ does not differ significantly when the K-12 × B and K-12 × K-12 crosses are compared, but both of these differ significantly from the K-12 × B(K) cross. In the latter, linkage is greater. These data are consistent with there being a possible homology difference

TABLE 3

Unselected marker analysis for ile⁺ or ilv⁺ recombinants

Recipient	Time sampled (min)	Total number	Percent of total		
			<i>met</i> ⁺	<i>mtl</i> ⁺	<i>arg</i> ⁺
KIM25	30-35	20	..	100	90
	60	20	50	85	80
	90	20	45	70	70
B(K)IM37	30-35	20	..	95	70
	60	20	80	75	80
	90	20	75	65	80
BIM36	30-35	20	..	25	5
	60	20	35	40	15
	90	20	55	40	25

at the *ilv* locus between B and K-12. Lack of a significant difference in the K-12 $\times$ B cross is probably the result of the restriction effect.

Other relationships from this analysis are consistent with those of the earlier *met*⁺ unselected marker analysis. The values for the *arg*⁺ group in the K-12 $\times$ B cross are not significantly different, probably because of small sample size, but they show a trend toward significant dichotomy dependent upon time of sampling. Also, *mtl*⁺ and *arg*⁺ from the K-12 $\times$ B cross differ significantly in recoverability from those markers in the K-12 $\times$ K-12 and K-12 $\times$ B(K) crosses. Linkage between *ilv*⁺ and *arg*⁺ is reduced more than that between *ilv*⁺ and *mtl*⁺.

DISCUSSION

During mating between *E. coli* strains K-12 and B, the effect of restriction is greatest early in the cross and become constant after about 30 minutes. This agrees with and supports the hypothesis made by BOYER (1964) for restriction in B/r, that early in mating the DNA being transferred into the recipient is rapidly degraded. As more DNA is transferred into the recipient cell, the "restricting enzyme" becomes saturated by an increased amount of substrate. Integration and modification reactions probably compete with the restriction reaction within the bacterial cell. All of these processes taken together presumably determine the final reaction rate for the appearance of a marker among recombinants.

The reduction in linkage of closely linked markers by the restriction reaction is dependent upon the distance between them. If two markers are very closely linked, the probability that they will be separated is less than that for two markers not as closely linked. In the restricted K-12 $\times$ B cross, the linkage between *ilv*⁺ and *met*⁺ is unaffected. The distance between these two markers corresponds to about one-hundredth of the entire *E. coli* chromosome. This observation may indicate that chromosome breakage by the restriction reaction occurs at points separated by rather large nucleotide distances.

When the genetic region controlling restriction and modification of K-12 is introduced into a B recipient, then the effect of restriction on the K-12 chromosome is eliminated in crosses of K-12 donor with the resulting B(K) hybrid.

The frequency of recovery for the *ilv*⁺ selected marker in the K-12 $\times$ B(K) cross is not appreciably different from that found in the K-12 $\times$ B cross. As noted before, linkage between the unselected *met*⁺ marker and the selected *ilv*⁺ marker in this cross is significantly higher than expected. These data are consistent with the hypothesis that the *ilv* region on the B chromosome may lack homology with the same region on the K-12 chromosome. Because of this difference, the K-12 *ilv*⁺ marker would be incorporated into a recombinant chromosome only when pairing and recombination take place in regions adjacent on the chromosome where homology exists. This would lead to an increased association between two adjacent or closely linked markers and could account for the result observed in the unselected marker analysis.

Lack of homology at any given region of the chromosome would interfere with pairing at that region and, as a result, with the recombination reaction for that

region. Thus differences in homology would affect recovery of the region whether or not the restriction effect were present. The observation that even after removal of restriction the selected *ilv*⁺ marker was recovered at only a slightly higher frequency than when restriction was acting agrees with this proposal.

A lack of homology between the B and K-12 strains at the *ilv* region of the chromosome may or may not occur in nature and could be caused by differences in nucleotide sequences in the *ilv* region of these two strains. Such differences might have arisen in either strain by deletion, rather than point mutations. The experiments reported do not allow a choice between these alternatives.

In the unrestricted K-12 $\times$ B(K) cross there remains approximately a tenfold difference in recovery of markers other than *ilv*⁺, when compared to the control K-12 $\times$ K-12 cross. A different result is obtained when B/r is used as a recipient. The modified B/r recipient shows complete recovery of markers as shown in K-12 $\times$ B/r(K) matings (BOYER 1964). Lower recovery of markers in the K-12 $\times$ B/r matings can be accounted for completely by the restriction process. The amount of restriction observed in the K-12 $\times$ B/r matings, as estimated from the published data, is about the same as found in the K-12 $\times$ B matings. This difference in recovery, after restriction has been removed, suggests a lack of similarity in the B and B/r strains used. The B/r strain was obtained by BOYER from WITKIN (BOYER personal communication) as was the parent-type B strain used in our own study.

A difference between B and B/r that could account for the reduced recovery of K-12 genetic material in modified B, but complete recovery in modified B/r, may involve cell wall factors that lower the efficiency of mating of B with K-12 donors. Recently, LANCASTER, GOLDSCHMIDT, and WYSS (1965) reported that cell wall fractions of strain B did not completely inhibit a K-12 $\times$ K-12 mating assay as did cell wall fractions from K-12 donors and recipients.

An alternative hypothesis can account for this difference in recovery and for some other observations reported in the literature. Strains B and B/r may differ in recombinability analogous to the radiation sensitive, recombinationless mutant of K-12 and its radiation resistant, wild-type parent strain (CLARK and MARGULIES 1965). The difference in radiation sensitivity between B and B/r may be associated with a difference in recombinability. The "recombinase(s)" in the B strain may not be totally lacking in ability to bring about recombination, but may be less efficient in carrying out the necessary steps. Such a difference in recombinase activity could then account for the observed incomplete recovery, even after removal of the restriction effect in the B(K) matings.

It is interesting to note that if recombinase activity is responsible for this difference between B and B/r in matings with K-12, then one would expect to see no discrepancy for factors that do not undergo recombination, i.e., formation of recombinant DNA molecules from two parental molecules. Only the effect of restriction would be observed on phage and other genetic elements such as episomes and plasmids. The levels of restriction observed by BOYER (1964) on extra-chromosomal elements in B/r is in agreement with those presented (ARBER and LATASTE-DOROLLE 1961; ARBER, HATTMAN and DUSOIX 1963; ARBER and MORSE

1965) for similar elements in strain B. Thus only the restrictive effect is observed.

In summary, at least three different effects may act to limit the exchange of genetic information between strains of *E. coli*. (1) Restriction is a well-documented effect that leads to a lower probability of acceptance, or additionally of incorporation of a genetic element donated from one cell to another each having a particular host specificity. (2) Lack of genetic homology between regions of two chromosomes having similar functions is yet another way in which an exchange of genetic information may be limited. This effect would act only to diminish the incorporation of genetic information into a recombinant chromosome; it would not reduce the acceptance of genetic elements that can remain in an autonomous state. (3) Also, deficiencies in recombinases would act to reduce exchanges of genetic information and, as when homology differences exist, would not limit the acceptance of factors that can reside autonomously in the host cell.

Restriction thus stands distinct as resulting in the reduced incorporation of genetic information on the basis of recognition patterns that need not coincide exactly with conventional pairing homology of DNA molecules. Restriction effects, lack of nucleotide homology, and deficient recombinases need to be identified as factors in reduced recovery of genetic markers following interstrain crosses of bacteria.

SUMMARY

The amount of restriction is not uniform but decreases with increasing amount of donor chromosome transferred and then becomes constant. Because of the polarity of the conjugal process, early proximal markers are restricted to a greater extent than later markers. The unlinking of linked markers is dependent upon the distance between them; closely linked markers will be unlinked less often than markers further apart.—A probable lack of homology between a region of the K-12 and B chromosome complicates the observed effect of restriction. Homology differences may limit pairing of the chromosome regions involved, and thus recombination. This interference would limit the amount of recovery for the nonhomologous region, whether or not restriction is acting.—An apparent difference exists between strain B and B/r of *E. coli*. Strain B shows a lack of recovery for genetic markers even after restriction has been removed. Strain B/r shows complete recovery of genetic markers after removal of restriction (BOYER 1964). This difference may be attributed to a possible deficiency in the recombination reactions in B, analogous to the recently discovered recombination deficient and radiation-sensitive mutants of K-12 (CLARK and MARGULIES 1965).

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