Molecular Evolution of the *Escherichia coli* Chromosome. V. Recombination Patterns Among Strains of Diverse Origin

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ABSTRACT

Incorporation patterns of donor DNA into recipient chromosomes following transduction or conjugation have been studied in the progeny of a variety of *Escherichia coli* crosses in which donor and recipient nucleotide sequences differ by 1–3%. Series of contiguous or variously spaced PCR fragments have been amplified from each recombinant chromosome and digested with a commercial restriction endonuclease previously shown to distinguish the respective parents in a given fragment. We conclude that entering donor DNA fragments are frequently abridged (cut and shortened) before incorporation, the cutting being due to restriction systems, and the shortening presumably due to exonuclease activity. Analysis of several backcrosses confirms, and extends to conjugation, the importance of restriction in *E. coli* recombination in nature. The transmission patterns in conjugation are similar to those of transduction, but (as expected) on a much larger scale. Asymmetric results of reciprocal crosses imply that mismatch frequency is not a major factor. Marked differences among the results of simple crosses according to parental strain combinations are consistent with observations that *E. coli* strains in nature vary dramatically in their restriction-modification systems.

THE present study continues an effort to define the patterns of genetic exchange within the species *Escherichia coli* in nature, and the forces responsible for these patterns. The species is generally understood to display a largely clonal population structure (Whittam 1996): that is, its lineages have a tree-like, as opposed to a net-like (Maynard Smith *et al.* 1993), relationship. Intraspecific genetic exchange is thus too rare to obscure the general pattern of linkage disequilibrium observed among polymorphic alleles.

Of interest in this context, comparative DNA sequencing (Milkman and McKane Bridges 1993; Milkman and Mckane 1995; Milkman 1996) in and near the *trp* operon of 41 *E. coli* strains [K12, as well as 40 *Escherichia coli* reference (ECOR) strains (Ochman and Selander 1984; Herzer *et al.* 1990)] revealed abundant mosaic polymorphism consisting of *clonal segments* embedded in *clonal frames.* Each clonal frame is taken to be a remnant of the chromosome of a clonal ancestor. The individual clonal segments, which are frequently shared among numerous strains, evidently originated via recombinational replacement between divergent isolates in nature, followed by vertical transmission to descendants in a growing clone.

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Given the assumption that these clonal segments arose by recombination, the question remains as to how these patches (on the order of several kilobases) came to be so short (Milkman and McKane Bridges 1993). One process, the successive separate overlapping incorporations of entrant fragments, often from close relatives, is of course inevitable over time. Another process, the incorporation of small discontinuous segments from large entrant molecules, is consistent with previous suggestions that restriction endonucleases might play an important role in bacterial recombination (Boyer 1964, 1971; Price and Bickle 1986; DuBose *et al.* 1988; see also Milkman 1997), and with observations that restriction-modification (R-M) systems are polymorphic in *E. coli,* often varying from isolate to isolate (Daniel *et al.* 1988; Janulaitis *et al.* 1988; Sharp *et al.* 1992; Barcus and Murray 1995; Barcus *et al.* 1995; Roberts and Macelis 1998, 1999). This also includes endonucleases that recognize methylated bases in certain DNA sequences (Raleigh 1987). Also, E. Raleigh (unpublished results) has noted extensive polymorphism in the ECOR strains for sensitivity to McrBC, which recognizes some sequences that include methylated cytosine. This observation complements the earlier finding of Povilionis *et al.* (1989) that the cognate methylases in three R-M systems, Eco 47II, Eco 47III, and Cfr10I, confer sensitivity to McrBC and have been found in a number of *E. coli* strains including clinical isolates (ECOR strains were not tested).

This work follows a paradigm (McKane and Milkman 1995) involving the transduction (Masters 1996) of three individual ECOR strains into K12, whose results supported the role of R-M systems in recombination in *E. coli* under natural conditions. The donor strains were chosen on the basis of known mutual sequence and restriction fragment length polymorphism (RFLP; Milkman and McKane Bridges 1990) differences, and the ability to make P1 phage lysates from them. From each member of each group of transductants, a set of 1500-bp PCR fragments was obtained and digested with commercial restriction endonucleases previously found to distinguish the respective fragments in donor and recipient. These high-resolution experiments indicated the frequent incorporation of multiple discontinuous donor DNA fragments. Next, two transductant strains were backcrossed to the original recipient strain. The transductants carried the known R-M genes of the recipient strain, located far from the transduced region. Thus, the backcross donor DNA was likely to be protected, at least to a considerable extent, against restriction by the recipient. The backcross progeny showed no fragmentation of their DNA. This result supported the importance of the role of R-M differences in the original crosses. Small patches of donor DNA with frequent discontinuities in the progeny of experimental transductants, together with the near absence of cutting and shortening in the progeny of backtransduction to the recipient strain, provided strong evidence of the participation of restriction endonucleases in natural recombination in *E. coli*.

This experimental paradigm has been extended to further transduction experiments and to a considerable variety of conjugational crosses. Here we present and compare their results and consider some emerging implications.

MATERIALS AND METHODS

Phage and bacterial strains (detailed in Table 1): Transduction was mediated by phage P1 strain *Cm clr100*. The initial transduction donor was ECOR 47 (Ochman and Selander 1984). The principal restricting recipient designated W3110 *trpA33* by C. Yanofsky was found to be *supF* and λ+; these markers were presumably introduced along with *trpA33* from a Ymel background (C. Yanofsky, personal communication; Bachmann 1996). The principal nonrestricting recipient was ER2476 (derived from W3110 *trpA33*), which lacks all known restriction systems. While ER2476 will be referred to as "restrictionless," this term should be understood to be tentative. The standard tests of a bacterial strain's restriction activity involve bacteriophages, which of course have relatively small genomes. Also, the variety and complexity of restriction mechanisms and defenses as presently understood (Bickle and Krüger 1993) suggest that not all contributors to the process are invariably detected. The same caveat applies to the "restrictionless" strain C1a, a faster-growing "adapted" version of strain C maintained for several years on Davis minimal medium (Sasaki and Bertani 1965).

Conjugation donors in earlier experiments include BW-7623, BW7622, and BW6160; these are Hfr derivatives of K12 carrying Tn*10*, which confers tetracycline resistance (Low 1996). Recipients were derivatives of ECOR 47, ECOR 56, and ECOR 72 selected for streptomycin resistance (presumably *rpsL* mutants) and/or for rifampin resistance (presumably *rpoB* mutants). Thus selection for both tetracycline resistance and streptomycin resistance, for example, yielded transconjugant colonies. More recent crosses involve tetracycline-resistant donors derived from K12 W3110 *trpA33*, ECOR 47, ECOR 72, and C1a: these all carry *zbi-29*::Tn*10* at 18 min (Singer *et al.* 1989; Berlyn *et al.* 1996) and a Broda 7 Hfr region, whose origin of transfer is near 31 min (Low 1996). The str^R recipients are corresponding strains. The K12 W3110 *trpA33* recipient acquired streptomycin resistance via P1 transduction of *rpsL700* from strain PK1206, and for this K12 strain a 4-hr delay in adding streptomycin (Miller 1992) was insufficient to overcome the segregation/expression lag. Eight hours worked well.

Strain construction: A major objective was to make Hfr derivatives of the strains that were also to be used as recipients. Each derivative was to acquire an F-prime plasmid that would incorporate, by virtue of homology, into a specific chromosomal position common to all the donor strains to be made. Also a selectable marker, common to all donors, was introduced by transduction. For this purpose, the plasmid would be introduced into a Rec^- master donor by conjugation and subsequently retransmitted to the several specific donors-to-be.

First (Figure 1), to produce a master donor, an F-prime plasmid had to be made that could be selected during construction and transmitted only as an F-prime until it had reached its target strain. It would then establish an integration-excision equilibrium sufficient to support a useful level of Hfr activity. Accordingly (Figure 1A), the transposon *trg-3120*::Tn*10kan* (Singer collection) was transduced by phage P1 into F621, an F-prime plasmid in strain PK1206. This transposon will then recombine with the last region to be transmitted by a corresponding Hfr strain. Next (Figure 1B), the transductant strain was mass-mated to strain DH5α, containing the genetic markers *gyrA* (nalidixic acid resistance) and *recA*, using a procedure modified to permit expression of kan^R. Selection for resistance to both kanamycin and nalidixic acid identified the master donor strain. In DH5α, the genetic marker *recA* kept the plasmid from integrating, thus permitting only the F-prime plasmid containing the transposon to be transmitted to the (eventual donor) F- strain.

Next, to produce the individual ECOR donors, *zbi-29*::Tn*10* at 18 min (also from M. Singer) was P1-transduced (Figure 1C) into each desired F- ECOR strain to provide a donor-selectable chromosomal tet^R marker for the eventual ECOR donor strain. The master donor was then mated to each modified ECOR strain (Figure 1D), selecting for both tetracycline and kanamycin resistance to produce each specific ECOR donor. The F-prime subsequently integrated reversibly (Figure 1E) in the 28-min/B7/*trg* region, with a standing frequency sufficient to produce transconjugants in the required numbers. Although the kan^R is very rarely transferred directly with the donor chromosomal DNA in an Hfr cross, it may be transmitted on an F-prime plasmid and subsequently integrate into the recipient chromosome (Firth *et al.* 1996; Heinemann *et al.* 1996; Ankenbauer 1997).

Crosses: Transductants were selected on minimal medium, indicating the replacement of *trpA33* by a normal allele. Further details are given in McKane and Milkman (1995). Transconjugants were selected in the same way or on Luria (L) medium containing tetracycline (15 μg/ml) and in one case (Table 10) kanamycin (150 μg/ml) as well. The recipient marker *rpsL* was selected by including streptomycin (100 μg/ml) in the medium. Typically conjugations began with over-

TABLE 1

Strains

Name	Relevant genotype; restricting state	Source/reference
K12 W3110 *trpA33*	*IN(rrnD-rrnE)1* Δ*(e14) trpA33* λ+; EcoK r^+ m^+, McrA$^-$, McrBC$^+$ Mrr$^+$	C. Yanofsky/McKane and Milkman (1995)
ECOR 47, 56, 72	Standard wild strains (unknown)	H. Ochman/Ochman and Selander (1984)
ER 2476	*IN(rrnD-rrnE)1* λ- *mcrA trpA33* Δ*(mcrC-mrr)114*::IS*10 trpA33*; EcoK r^- m^-, McrA$^-$, McrBC$^-$ mrr$^-$	This work
BW7622	Hfr (PO 44 of Hfr KL96) λ$^-$ *trpB114*::Tn*10*	ECGSC[a]/Wanner (1986)
BW7623	Hfr (PO 43 of Hfr Broda 7) *purE79*::Tn*10*λ$^-$	ECGSC[a]/Wanner (1986)
BW6160	Hfr (PO 118 of Hfr Broda 8) λ$^-$ *zdi-57*::Tn*10* λ^R	ECGSC[a]/Wanner (1986)
C	No. 122 of the National Collection of Type Cultures, London	Bertani and Weigle (1953)
C-1a	Derived from C (see text)	E. Six/Sasaki and Bertani (1965)
PK1206	F-plus (F621) *thi argE3 aroD6 rpsL700 galK2 lacY1 mtl-1 manA4*	Peter Kuempel/Low (1996)
DH5α	*recA gyrA*	ECGSC[a]/Hanahan (1983)
DCFP3	DH5α (F621 *trg-3120*::Tn*10kan*)	This work
DCHF1	BW7623 *zcj-3118*::Tn*10kan*	This work
DCHF2	ECOR 72 (F621 *trg-3120*::*Tn10kan*) *zbi-29*::Tn*10*	This work
DCHF3	K12 W3110 *trpA33* (F621 *trg-3120*::Tn*10kan*) *zbi-29*::Tn*10*	This work
DCHF4	ECOR 47 (F621 *trg-3120*::Tn*10kan*) *zbi-29*::Tn*10*	This work
DCHF5	C-la (F621 *trg-3120*::Tn*10kan*) *zbi-29*::Tn*10*	This work
DCHF7	HAZ-12 (K12 with ECOR 47 DNA from ~1276–1335 kb)	This work

[a] *E. coli* Genetic Stock Center, Yale University, New Haven, CT.

night L broth cultures of donor (unshaken) and recipient (shaken) strains. Donor cultures were diluted 25-fold in 5 ml L broth and kept at 37° for 2 hr without shaking. Recipient cultures were diluted 10-fold in 1 ml L broth. Then 0.2 ml of donor and 0.2 ml of recipient were mixed in 15-ml tubes at 37°; left 1.5 hr unshaken; shaken in 2-additional-ml L broth for 2 hr; and serially diluted for plating (Miller 1992, p. 249). Densities were determined at 540 nm and adjusted as desirable. Note that conjugational backcrosses are made possible by the reintroduction of the F-prime plasmid to a chosen transconjugant and by the recipient-parent strain's acquisition of a new selectable marker, usually *rpoB* for rifampin resistance (100 μl/ml), since the transconjugant now used as a backcross donor already contained the original recipient marker, *str*^{R}.

Analysis: The conjugation experiments required PCR fragments chosen over a broad range. Primers were based on known sequences (K12 with very few exceptions) from genes in desired locations according to Rudd (Berlyn *et al.* 1996) or the *E. coli* database collection (ECDC) map (Kröger and Wahl 1998), and most recently the K12 MG1655 genome sequence (Blattner *et al.* 1997; see also Berlyn 1998; Rudd 1998).

RESULTS

Conjugations: The results of a typical conjugational cross are illustrated in Table 2. In this and all conjugations described, transfer is counterclockwise: that is, donor DNA enters the recipient cell at a progressively greater counterclockwise distance from the origin of transfer. Here the Hfr donor strain is BW7622 (Table 1), whose origin of transfer is at 47 min; the recipient strain is ECOR 47. The map positions of the 1500-bp PCR fragments are given in minutes. Then below, the positions are given vertically in kilobases, as estimated from the *E. coli* K12 MG1655 sequence (Blattner *et al.* 1997). The PCR fragments themselves are represented by vertical two- or three-letter symbols and their DNA is labeled "D" (donor) or "-" (recipient). Occasionally a fragment is found to contain some donor DNA and some recipient DNA; this of course requires that two or more distinguishing restriction sites be present in the fragment. The selected donor (*****) and recipient (+) markers are also indicated. The donor marker at 28 min in Table 2 is thus about 19 min from the origin of transfer. The transconjugants are grouped according to the number of separate donor segments observed. Within each group, they are ordered according to the range over which donor DNA is present, irrespective of interruptions. Further details and conventions are given in the individual tables.

To explore strain differences, crosses were first made using the K12-derived donor BW7623 (origin of transfer, 27 min; donor marker, 12 min) and, as recipients, ECOR 47, ECOR 56, and ECOR 72. The resulting transconjugants gave evidence of abridgment similar to that seen in the transductants, but on a larger physical scale, as already illustrated in Table 2. Abridgment was greatest with ECOR 56 as recipient and least with ECOR 72 (detailed data not shown). All the donor fragments were much smaller than the "large chunks" inferred for ~80% of the transconjugants of a large set of K12 × K12 crosses compiled by Smith (1991), in which restriction would presumably have been absent.

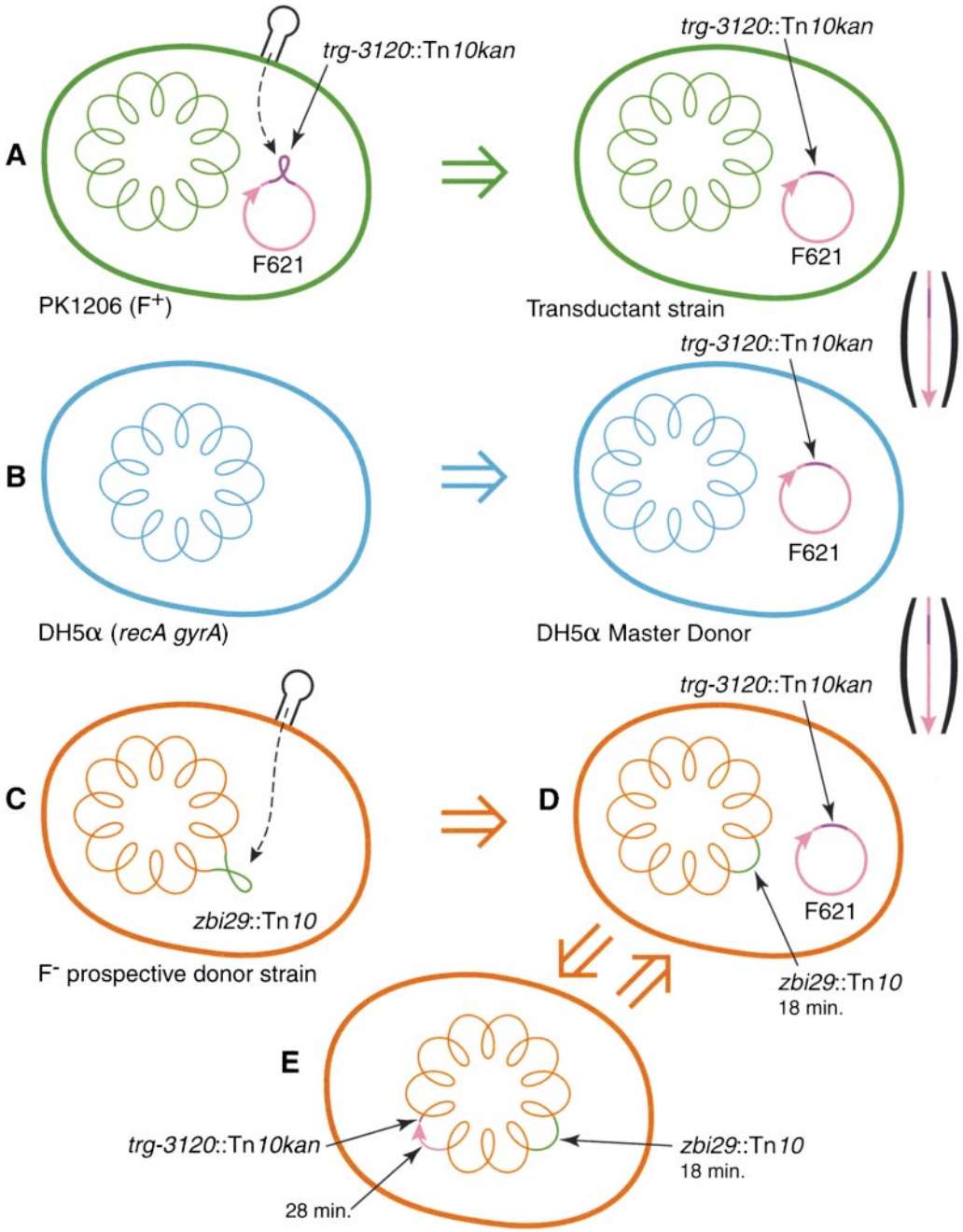

Figure 1.—Diagram of the construction of donor and recipient strains for conjugation experiments. See materials and methods.

The main experiments to follow fall into three groups: first, conjugations corresponding to the transductions whose results have been published; second, pairs of reciprocal conjugations involving K12, ECOR 47, and ECOR 72; and third, transductional and conjugational crosses and backcrosses involving ECOR 47 and either ER2476 or C1a, both putatively restrictionless K12 derivative strains.

Conjugations corresponding to transductions: The ECOR 47 → K12 W3110 *trpA33* transductions (McKane and Milkman 1995) were subsequently paralleled by conjugations (Table 3A) using the ECOR 47 donor DCHF4 and streptomycin-resistant derivatives of the K12 W3110 *trpA33* recipients. The DNA incorporation patterns in conjugation resembled those of the transductions, although, as expected, the total length of the donor fragments tended to be much longer. Next, a back conjugation employing as donor the transconjugant with the longest uninterrupted donor segment (Table 3B) showed the dramatic reduction in abridgment previously seen in back transductions.

Reciprocal conjugation crosses among K12, ECOR 47, and ECOR 72: To reveal possible abridgment in both directions, indicating that each parent contains at least one restriction endonuclease against which the other is not protected, we constructed a set of Hfr donors, and a corresponding set of marked F-minus recipients, derived from strains K12, ECOR 47, and ECOR 72.

TABLE 2

A representative data set. Conjugation: BW7622 → ECOR 47/Hfr origin 47 min

	Minutes																												
	75//	9	12	16	18	20	22	25	27.5	----28----														----29----				30	33	36	
kb	3						1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	4	4	5	7	8	9	0	1	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5	6	
	7	0	5	5	4	4	3	8	7	9	9	0	0	0	1	1	1	1	1	2	2	3	3	6	6	9	3	8		
	2	2	4	9	6	1	3	4	6	7	9	5	6	8	1	2	4	5	9	7	8	0	5	2	7	7	8	5		
	+	P H	C S G	S C	G L N	D M S	H Y A	P O T	N A	A D	O P	G O	G L	B U	L K	A L	S Q	*	E D	B S	S B	T A	A C	A H	P S P	N R	N Z	E F		
Trans																														#
6	+	-	-	-	D	D	D	D	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	x	-	-		(1)
22	+	-	-	-	-	-	-	D	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	x	D	-		
5	+	-	-	-	-	-	D	D	D	D	D	D	D	D	D	D	D	*	D	D	D	d-	-	-	-	-	-	-		
20	+	-	-	-	-	-	-	D	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	x	-	-		
8	+	-	-	-	-	-	-	D	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	x	-	-		
23	+	-	-	-	-	-	-	-	-	-	-	-	-	-	D	D	D	*	D	D	D	D	D	D	D	x	D	-		
11	+	-	-	-	-	-	-	D	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	-	-	-	-	-		
12	+	-	-	-	-	-	-	D	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	-	-	-	-	-		
7	+	-	-	-	-	-	-	D	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	-	-	-	-	-		
27	+	-	-	-	-	-	-	D	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	-	-	-	-	-		
2	+	-	-	-	-	-	-	-	D	D	D	D	D	D	D	D	D	*	-	-	-	-	-	-	-	-	-	-		
1	+	-	D	-	-	-	-	-	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	x	-	-		(2)
21	+	-	-	D	-	-	-	D	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	x	-	-		
25	+	-	-	-	-	D	D	-	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	-	-	-	-	-		
4	+	-	-	-	-	-	-	-	D	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	-	-	-		
19	+	-	-	-	-	-	-	-	D	D	D	D	D	D	D	D	D	*	D	-	D	D	D	-	-	-	-	-		
26	+	-	-	-	-	-	-	-	D	-	-	-	-	-	D	D	D	*	-	-	-	-	-	-	-	-	-	-		
28	+	-	-	-	D	D	-	-	-	d-	-	-	-	-	-	-	-	*	D	D	D	D	D	-	-	-	-	-		(3)
24	+	-	-	-	-	-	-	-	D	-	-	D	D	-	D	D	D	*	-	D	D	d-	-	-	-	x	D	-		(5)

Transconjugants are arranged in sets in order of number of donor DNA segments and (within these sets) according to range of donor DNA. Trans, transconjugant strain; #, number of donor segments; *, selected marker, Tn10 in *trpB*; +, counterselected marker, *rpsL*; d-, fragment contains donor DNA on left and recipient DNA on right. Further symbols are explained in the text. x denotes inability to make the PCR fragment. Hfr BW7622 cannot be amplified with the NR primers used, and all the fragments in NR have the ECOR 47 pattern. Thus, in this cross, it is the donor DNA that evidently cannot be amplified in the NR region.

TABLE 3

Distribution of donor and recipient DNA in transconjugants

A. Conjugation: ECOR 47 → K12 W3110 *trpA33*

	Minutes																							
	75//	20	22	23.5	25	27	-- 28 ----------																29	30	
kb	3		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	4	9	0	1	1	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	7	4	3	1	8	7	9	0	0	0	1	1	1	1	1	1	1	2	2	3	3	6	8		
	2	1	3	5	4	6	7	5	6	8	0	1	2	4	5	6	9	7	8	0	5	2	5		
	+	D M S	H Y A	H T R	P O T	N A	A D	G O	G L	B U	O N	L K	A L	S Q	*	C B	E D	B S	S B	T A	A C	A H	T Y R		
Trans																									#
12	+	-	-	-	-	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	-	-		(1)
4	+	-	-	-	-	-	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	-	-		
8	+	-	-	-	-	-	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	-	-		
11	+	D	D	D	D	-	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	-		
21	+	-	-	-	-	-	D	D	D	D	D	D	D	D	*	D	D	D	-	-	-	-	-		
3	+	-	-	-	-	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D		-		
7	+	-	-	-	-	-	-	D	D	D	D	D	D	D	*	D	D	D	D	-	-	-	-		
16	+	-	-	-	-	-	-	-	-	-	-	-	D	D	*	D	D	D	D	D	D	-	-		
13	+		-	-	-	-	-	-	-	-	-	-	-	-	*	D	D	D	D	D	D	-	-		
25	+	-	-	-	-	-	-	-	-	-	-	-	-	-	*	D	D	D	D	D	D	-	-		
29	+	-	-	-	-	-	-	D	D	D	D	D	D	D	*	D	-	-	-	-	-	-	-		
22	+	-	-	-	-	-	-	D	D	D	D	D	D	D	*	D	-	-	-	-	-	-	-		
6	+	-	-	-	-	-	-	-	D	D	D	D	D	D	*	-	-	-	-	-	-	-	-		
14	+	-	-	-	-	-	-	-	-	-	-	-	-	D	*	D	D	-	-	-	-	-	-		
24	+	-	-	-	-	-	-	-	-	-	-	-	-	-	*	D	D	-	-	-	-	-	-		
15	+	-	-	-	-	-	-	-	-	-	-	-	D	D	*	D	-	-	-	-	-	-	-		
26	+	-	-	-	-	-	-	-	-	-	-	-	-	-	*	D	-	-	-	-	-	-	-		
27	+	-	-	-	-	-	-	D	D	D	D	D	D	D	*	-	D	D	D	D	D	-	-		(2)
2	+	-	-	-	-	D	-	-	-	-	-	-	-	D	*	-	-	-	-	-	-	-	-		
28	+	-	-	-	-	-	-	D	D	-	D -	D	D	D	*	D	D	D	-	-	-	-	-		
17	+		-	-	-	-	-	-	-	D	d -	D	D	D	*	D	D	-	-	-	-	-	-		
18	+	-	-	-	-	-	-	-	-	D	d	D	D	D	*	D	D	-	-	-	-	-	-		
1	+	-	-	-	-	-	-	-	-	-	d-	-	-	D	*	-	-	-	-	-	-	-	-		
19	+	-	D	D	-	-	-	D	D	D	D	D	D	D	*	D	D	D	D	d-	D	-	-		(3)
30	+	D	D	D	-	D	-	-	-	-	-	-	D	D	*	D	-	-	-	-	-	-	-		
23	+	-	-	-	-	-	-	-	-	-	-	-	-	D	*	-	D	-	-	-d	-	-	-		
20	+	-	-	-	D	-	-	-	-	-	-	-	-	-	*	D	-d-	d-	-	-	-	-	-		(4)
10	+	-	-	-	-	-	-	D	D	D	D	D	-	D	*	d-	-d	-	-	D	-	-	-		
9	+	-	-	D	D	-	-	-	D	D	D	D	-	-	*	D	-d	D	D	d-d	-	-	-		(5)
5	+	-	-	-	-	-	-	m	m	m	m	m	m	m	*	m	m	m	m	m	m	-	-		

(*continued*)

TABLE 3

(Continued)

B. Backcross HAZ-12 (ECOR 47 → K12 W3110 *trpA*33) → K12

				Minutes																		
	90//	25	27	--28----------																	----------	29
	+	P	N	A	G	G	B	O	L	A	S	*	C	E	B	S	T	A	A		
		O	A	D	O	L	U	N	K	L	Q		B	D	S	B	A	C	H		#
Trans		T																				
[Donor HAZ-12:	+	-	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	-]		
JAZ-1-3, 5-28, 30	+	-	D	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	-		(1)
4	+	-	-	-	-	-	D	D	D	D	D	*	D	D	D	D	D	D	-		
29	+	-	-	-	-	-	-	-	-	-	D	*	D	D	D	D	D	D	-		

Symbols as in Table 2. *, selected on minimal medium for *trpA*+ at 28 min; -d, d-, -d-, d-d, fragment is mosaic as indicated; +, counterselected marker, *rpsL* (at 75 min, top) or *rpoB* (at 90 min, bottom); m, mixed donor and recipient bands (not compiled).

Results of three reciprocal pairs of crosses are detailed in Tables 4–6. In these three pairs, the donor marker is situated near minute 18, rather than the previous crosses' minute 28. Three related patterns of variation are evident: first, the results of reciprocal crosses are often strikingly different; second, different recipients vary in their abridgment of a common donor's DNA; and third, DNA from different donors is abridged differently by a common recipient.

Transduction and conjugation with "restriction$^-$" recipients: The ECOR 47 → ER2476 transductants (Table 7A) showed considerably less abridgment than the previously reported transductants of a restriction$^+$ recipient (McKane and Milkman 1995). This was particularly striking in the length of the donor fragments incorporated; nevertheless, 5 of the 20 transductants did reveal the presence of two discrete stretches of donor DNA. The 30 backtransductants (Table 7B) have longer stretches of original donor DNA than do the transductants, as summarized in Table 9. This, taken together with the presence of only one double incorporation in the backtransductants, suggests that ER 2476 may still contain one or more R-M systems. That is, strain K-12 W3110 may contain more restriction factors than were eliminated to make ER 2476.

In a parallel conjugation experiment (Table 8A), ECOR 47 was then crossed to the "restrictionless" ER2476. There was a sharp reduction in interruptions and an increase in donor segment length relative to ECOR 47 → K12 (Table 3A). The corresponding backcross (Table 8B; *cf.* Table 3B) produced a further decrease in abridgment. In summary, both transduction and conjugation experiments suggest the importance of known R-M systems, as well as possible additional ones that had not been detected by experiments with bacteriophage. A third conjugational cross-and-backcross combination involving the "restrictionless" strain C1a is only summarized in Table 9 (the abridgment of ECOR 47 DNA by the C1a recipients is striking); no transductional counterpart has been made.

Summaries of results (Table 9): The crosses are now regrouped to address a different perspective. For purposes of comparison, the following parameters are useful, though with the same limitations as any quantification of distances based on linked classical markers. The range over which the donor DNA is seen, whether interrupted or not, is measured in kilobases. The length of a stretch of donor DNA with no evident interruptions is taken as the distance in kilobases between its extremes; this crude estimate assumes that the regions between the PCR fragments analyzed contain no recipient DNA. When interruptions result in the presence of more than one discrete donor fragment, the largest is used in the compilations. The number of progeny displaying interruptions is counted, as well as the total number of interruptions. The collective measurements for the progeny of a cross produce *average donor DNA range* (R), *average maximum donor DNA stretch length* (S),

TABLE 4

Distribution of donor and recipient DNA in reciprocal transconjugants

Conjugation DCHF4 (ECOR 47) → DCRA33 (K12)

	Minutes									
	75	16	17		18	20	24	27	30	
Trans	+	SC	BIO	*	GLN	DMS	HTR	NA	NR	#
2, 4, 5, 7–30	+	-	-	*	-	-	-	-	-	(1)
3	+	-	-	*	D	-	-	-	-	
6	+	-	D	*	-	-	-	-	-	
1	+	-	-	*	-	D	-	-	-	(2)

Conjugation: DCHF3 (K12) → ECOR 47

	Minutes																					
	75	2	9	12	15	16	17		18	20	22	24	25	27	28 ------			-- 29 --			30	
Trans	+	ILV	PH	CSG	NAG	SC	MOA	*	GLN	DMS	HYA	HTR	POT	NA	BU	SQ	ED	TA	AH	TYR	NR	#
4	+	-	-	-	-	-	D	*	D	D	D	D	D	D	D	D	D	D	D	D	D	(1)
22	+	-	-	-	-	D	D	*	D	D	D	D	D	D	D	D	-	-	-	-	-	
30	+	-	D	D	D	D	D	*	D	D	D	D	-	-	-	-	-	-	-	-	-	
11, 29	+	-	-	-	D	D	D	*	D	D	D	D	-	-	-	-	-	-	-	-	-	
25	+	-	-	-	-	-	D	*	D	D	D	D	-	-	-	-	-	-	-	-	-	
14	+	-	-	-	D	D	D	*	D	D	D	-	-	-	-	-	-	-	-	-	-	
7	+	-	-	-	-	-	D	*	D	D	D	-	-	-	-	-	-	-	-	-	-	
5	+	-	-	-	-	-	-	*	D	D	D	-	-	-	-	-	-	-	-	-	-	
17, 21, 28	+	-	-	-	D	D	D	*	D	D	-	-	-	-	-	-	-	-	-	-	-	
18	+	-	-	-	-	-	D	*	D	D	-	-	-	-	-	-	-	-	-	-	-	
23	+	-	D	D	D	D	D	*	D	-	-	-	-	-	-	-	-	-	-	-	-	
12, 15	+	-	-	-	-	D	D	*	D	-	-	-	-	-	-	-	-	-	-	-	-	
19	+	-	-	-	D	D	D	*	D	-	-	-	-	-	-	-	-	-	-	-	-	
3, 13, 10, 20, 26	+	-	-	-	-	-	D	*	D	-	-	-	-	-	-	-	-	-	-	-	-	
10	+	-	-	-	-	-	D	*	D	-	-	-	-	-	-	-	-	-	-	-	-	
20	+	-	-	-	-	-	D	*	D	-	-	-	-	-	-	-	-	-	-	-	-	
26	+	-	-	-	-	-	D	*	D	-	-	-	-	-	-	-	-	-	-	-	-	
13	+	-	-	-	-	-	D	*	D	-	-	-	-	-	-	-	-	-	-	-	-	
9	+	-	-	-	D	D	D	*	-	-	-	-	-	-	-	-	-	-	-	-	-	
24	+	-	-	-	-	D	D	*	-	-	-	-	-	-	-	-	-	-	-	-	-	
8, 16	+	-	-	-	-	-	D	*	-	-	-	-	-	-	-	-	-	-	-	-	-	
1	+	-	-	-	-	-	D	*	D	-	D	D	D	-	-	-	-	-	-	-	-	(2)
27	+	-	-	D	D	D	D	*	-	-	-	-	D	D	D	-	D	-	-	-	-	
2	+	-	-	-	-	D	D	*	D	-	-	D	-	-	-	D	D	D	D	D	D	
6	+	-	-	-	D	D	D	*	D	D	D	-	-	D	D	-	D	D	D	-	-	(3)

*, selected Tn*10* marker near 18 min; +, counterselected marker, *rpsL*. Other symbols as in Table 2.

number of progeny with interrupted donor DNA (IP), and *total number of interruptions* (TI). Finally, in backcrosses (included in Groups A and B in Table 9), the extent of mismatched DNA is limited to the extent of the original donor DNA present in the backcross donor, and thus the backcross progeny can contain original donor DNA no more extensive than this *limiting length* (L).

Dual selection experiment: One striking aspect of the DNA incorporation patterns in conjugations is the frequent paucity of donor DNA in the direction of the origin of transfer. It is of course easy to understand the decrease in the direction of the recipient marker, but what has become of the proximal donor DNA, which has surely entered the recipient cell? An exploratory experiment employed DCHF1 (Table 1), a modified BW7623 donor now carrying *zci-3118*::Tn*10kan* near 28 min in addition to its tet^R in *purE79*::Tn*10* (Table 1), which is near 12 min. A cross to ECOR 47 produced

TABLE 5

Distribution of donor and recipient DNA in reciprocal transconjugants

A. Conjugation: DCHF2 (ECOR72) → DCRA33 (K12)

	Minutes																	
	90 //	2	9	12	15	16	18	20	22	24	25	27	----28-----			29	30	
	+	I L V	P H	C S G	N A G	S C	*	G L N	D M S	H Y A	H T R	P O T	N A	B U	E D	A H	N R	
Trans																		#
8	+	-	D	D	D	D	*	-	-	-	-	-	-	-	-	-	-	(1)
14	+	-	-	D	D	D	*	D	D	-	-	-	-	-	-	-	-	
4	+	-	-	D	D	D	*	D	-	-	-	-	-	-	-	-	-	
3	+	-	-	-	-	-	*	D	D	-	-	-	-	-	-	-	-	
6, 9	+	-	-	-	D	D	*	-	-	-	-	-	-	-	-	-	-	
5, 13	+	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	
1	+	D	D	D	D	D	*	-	-	D	-	-	-	-	-	-	-	(2)
10	+	-	-	-	-	-	*	D	-	-	-	-	-	D	D	-	-	
15	+	-	D	D	D	-	*	D	-	-	-	-	-	-	-	-	-	
11	+	-	-	-	-	-	*	D	-	-	-	-/d	-	-	-	-	-	
7	+	-	-	-	-	-	*	-	D	D	D	-	-	-	-	-	-	
12	+	-	-	-	-	-	*	-	-	D	-	-	-	-	-	-	-	
2	+	D	D	D	D	D	*	-	D	-	-	D	D	D	D	D	-	(3)

B. Conjugation: K12 *trpA33* Hfr → ECOR 72 str

	Minutes																		
	75 //	2	9	12	15	16	17	18	20	22	24	25	27	----28-----			29	30	
	+	I L V	P H	C S G	N A G	S C	B I O	*	G L N	D M S	H Y A	H T R	P O T	N A	B U	E D	A H	N R	
Trans																			#
F72- 1, 4, 8, 11	+	-	D	D	D	D	D	*	D	D	D	D	D	D	D	D	D	D	(1)
7	+	-	D	D	D	D	D	*	D	D	D	D	D	D	D	D	D	-	
5, 6	+	-	-	D	D	D	D	*	D	D	D	D	D	D	D	D	D	D	
2	+	D	D	D	D	D	D	*	D	-	-	-	-	-	-	-	-	-	
12	+	-	-	-	D	D	D	*	D	D	D	D	D	D	D	D	D	-	
10	+	-	-	-	-	D	D	*	D	D	D	D	D	D	D	D	D	D	
13, 14	+	-	-	-	-	-	D	*	D	D	D	D	D	D	D	D	D	D	
3, 9	+	-	-	-	D	D	D	*	D	D	-	-	-	-	-	-	-	-	
15	+	-	-	-	D	D	-	*	D	D	D	-	-	-	-	-	-	-	(2)

+, counterselected marker, *rpoB* (at 90 min, top), or *rpsL* (at 75 min, bottom). Other symbols as in Table 2.

large numbers of colonies on tetracycline (933) and large numbers on kanamycin (545), but very few on tetracycline + kanamycin (19). Ten transconjugants from each selection regimen were analyzed in detail (Table 10). The transconjugants selected for resistance to both tetracycline and kanamycin are of two comparably frequent types: those with long single donor fragments and those split into at least two donor fragments, each including a selected marker. In each of the singly selected groups, one of the *two-marker* split types happens to appear (DK08, DT04).

Clearly, the donor DNA appears near the respective selected marker(s) in the foregoing experiment, and there is no reason to believe that the donor DNA nearer the origin of transfer is incorporated only very rarely. Although exonucleases are likely to play a role, the discrete fragments generated by restriction cleavage may also be incorporated frequently into different nascent chromosomes and segregate in subsequent cell divisions. Note that the conjugational backcross data (Tables 3 and 8) show a smaller proportion of missing proximal donor DNA than do the original crosses. Thus, it seems likely that most of the missing donor DNA was actually lost after several cell divisions due to the lack of tetracycline resistance or kanamycin resistance. Indeed, sectoring at low frequencies has often been observed even in intrastrain crosses (*e.g.*, by Lloyd and Buckman 1995). This process would help explain the paucity of

TABLE 6

Distribution of donor and recipient DNA in transconjugants

A. Conjugation: DCHF4 (ECOR 47) → ECOR 72str

	Minutes																			
	75			15	16	17		18	20		22	24	25	27		29				
	+			NAG	SC	BIO	*	GLN	DMS	MUK	HYA	HTR	POT	NA		AH				
Trans																				#
16	+			-	-	-	*	D	D	D	D	D	D	-		-				(1)
30	+			-	-	-	*	D	D	D	D	D	D	-		-				
25	+			-	-	D	*	D	D	D	D	D	-	-		-				
18	+			-	-	-	*	D	D	D	-	-	-	-		-				
9	+			-	-	-	*	D	D	-	-	-	-	-		-				
21	+			-	-	-	*	D	D	-	-	-	-	-		-				
8	+			-	-	-	*	D	d-	-	-	-	-	-		-				
4, 6, 7, 10, 13–15, 17, 29	+			-	-	-	*	D	-	-	-	-	-	-		-				
1, 12	+			-	-	-	*	d-	-	-	-	-	-	-		-				
2, 3, 11, 19, 22, 23, 26–28	+			-	-	-	*	-	-	-	-	-	-	-		-				
5, 24	+			-	-	x	*	-	-	-	-	-	-	-		-				

B. Conjugation: DCHF2 (ECOR 72) → ECOR 47str

	Minutes																					
	75 //	2	9	12	15	16	17		18	20	22	24	25	27	- - 28 - - - -			29	30			
	+	ILV	PH	CSG	NAG	SC	BIO	*	GLN	DMS	HYA	HTR	POT	NA	BU	ED		AH	TYR	NR		
Trans																						#
9	+	D	D	D	D	D	D	*	D	D	D	D	D	-	-	-		-	-	-		(1)
1	+	D	D	D	D	D	D	*	D	-	-	-	-	-	-	-		-	-	-		
2	+	-	D	D	D	D	D	*	D	-	-	-	-	-	-	-		-	-	-		
6	+	-	-	D	D	D	D	*	D	-	-	-	-	-	-	-		-	-	-		
14, 15	+	-	-	-	D	D	D	*	-	-	-	-	-	-	-	-		-	-	-		
4, 7	+	-	-	-	-	D	D	*	D	-	-	-	-	-	-	-		-	-	-		
10	+	-	-	-	-	D	D	*	-	-	-	-	-	-	-	-		-	-	-		
13	+	-	-	-	-	-	-	*	D	-	-	-	-	-	-	-						
5	+	-	-	-	-	-	D	*	-	-	-	-	-	-	-	-		-	-	-		
12	+	-	D	D	D	D	D	*	D	-	D	-	-	-	-	-		-	-	-		(2)
3	+	-	-	D	D	D	D	*	-	D	D	-	-	-	-	-		-	-	-		
8	+	-	-	-	-	-	-	*	-	-	-	D	D	D	-	-		-	-	-		
11	+	-	-	D	-	-	D	*	D	-	-	-	-	-	-	-		D	D	-		(3)

x, PCR fragment could not be made. Hfr origins at 31 min. Other symbols as in Table 2.

TABLE 7

Distribution of donor and "restrictionless-recipient" DNA in transductants

Transd.	NA	AD	GO	GL	BU	ON	LK	AL	SQ	*	CB	ED	BS	SB	TA	AC	AH	PS	#
A. Transduction to restrictionless recipient: ECOR 47 → ER 2476																			
Minutes	27.5	28														29			
47D-(4)	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	D	(1)
47D-01	-	-	-	D	D	D	D	D	D	*	D	D	D	D	D	D	D	D	
47D-16	-	-	-	-	-	-	-	D	D	*	D	D	D	D	D	D	D	D	
47D-03	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	-	
47D-13	-	-	-	-	-	-	-	D	D	*	D	D	D	D	D	D	D	-	
47D-09	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D	-	-	
47D-04	-	-	-	-	-	-	-	D	D	*	D	D	D	D	D	D	-	-	
47D-07	-	-	-	-	-	-	-	D	D	*	D	D	D	D	D	-	-	-	
47D-08	-	-	-	-	-	-	-	-	D	*	D	D	-	-	-	-	-	-	
47D-18	-	-	-	-	-	-	-	-	D	*	D	d/	-	-	-	-	-	-	
47D-02	-	-	-	-	-	-	-	-	D	*	D	-	-	-	-	-	-	-	
47D-19	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	
47D-05	-	-	-	-	-	-	-	-	D	*	D	D	D	D	D	D	-	D	(2)
47D-17	-	-	-	-	-	-	-	-	D	*	D	D	D	/d	d/	-	-	z	
47D-20	-	-	-	D	D	D	D	D	D	*	d/	-	-	D	D	D	-	-	
47D-15	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	D	-	z	
47D-06	-	-	d/	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	
B. Backtransductants: ECOR 47D-10 → ER 2476																			
[Donor 47D-10	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	D]	#
Backtr.																			
9	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	D	(1)
4	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D	-	-	
4	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	-	-	-	
6	-	-	D	D	D	D	D	D	D	*	D	D	-	-	-	-	-	-	
1	-	-	D	D	D	D	D	D	D	*	D	d/	-	-	-	-	-	-	
2	-	-	D	D	D	D	D	D	D	*	D	-	-	-	-	-	-	-	
1	-	-	-	D	D	D	D	D	D	*	D	-	-	-	-	-	-	-	
2	-	-	D	D	D	D	D	D	D	*	-	-	-	-	-	-	-	-	
1	-	-	D	D	D	D	D	D	-	*	D	-	-	-	-	-	-	-	(2)

Transd., transductant; backtr., backtransductant; z, data missing. Other symbols and locations as in Table 2.

donor DNA near the origin of transfer, relative to a distant selected region. Additionally, in the present case, the relatively low yield of kanamycin-resistant transconjugants, despite the location moderately near the origin of transfer of the Tn*10kan* used here, may be due in part to the slower expression of kanamycin resistance (Miller 1992). A reversal of markers would provide a simple test.

DISCUSSION

Abridgment has now been demonstrated in a variety of conjugation experiments, including some that parallel the transduction crosses both in the strains used and in the region analyzed. Both reciprocal crosses and backcrosses to the respective recipient parental strains have also been made. Their results confirm and extend the evidence of the importance of restriction in natural recombination within *E. coli*, and they also have a bearing on the success frequency of more distant horizontal transfer.

The results of reciprocal crosses rule out DNA mismatch (at the 1–3% level) as a major cause of abridgment, since both crosses in a reciprocal set share a common mismatch (Worth *et al.* 1994; Milkman *et al.* 1998; Milkman 1999). The effects of different recipient strains and the effects of different donor strains suggest a common determinant of abridgment: the set of restriction activities in the recipient against which the donor DNA is not protected. This can be illustrated by a rectangular version of a Venn diagram, in which the subtraction of sets is illustrated (Figure 2). Each of three reciprocal pairs of subtractions is diagrammed; they are then superimposed below to illustrate that the same dimensions can be used consistently.

Recombination among laboratory derivatives of strain

TABLE 8

Distribution of donor and "restrictionless-recipient" DNA in transconjugants

A. Conjugation: ECOR 47 → ER 2476

	Minutes																						
	75 //	22	24	25	27.5	-- 28 ----------										---------- 29 ----------						-- 30 --	
	+	HYA	HTR	POT	NA	AD	GO	GL	BU	ON	LK	AL	SQ	*	CB	ED	BS	SB	TA	AC	AH	TYR	NR	
Trans																								#
15	+	-	-	-	-	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	D	D	(1)
2, 24	+	-	-	-	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	D	D	
12, 23	+	-	-	-	-	-	-	-	-	-	-	-	D	*	D	D	D	D	D	D	D	D	D	
13	+	-	-	-	-	-	-	-	D	D	D	D	D	*	D	D	D	D	D	D	D	D	-	
16	+	-	-	-	-	-	-	-	-	-	-	-	D	*	D	D	D	D	D	D	D	D	-	
27	+	-	-	-	-	-	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	-	-	
30	+	-	-	-	-	-	-	-	-	-	-	-	D	*	D	D	D	D	D	D	D	-	-	
3	+	-	-	-	-	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	-	-	-	
1, 22	+	-	-	-	-	-	-	-	-	-	-	-	D	*	D	D	D	D	D	D	-	-	-	
20	+	-	-	-	-	-	-	-	-	-/d	D	D	D	*	D	D	D	D	D	-	-	-	-	
21	+	-	-	-	-	-	-	-	-	-	-	-	-	*	D	d/-	D	D	D	D	-	-	-	
25	+	-	-	-	-	-	-	-	-	-	-	-	-	*	D	D	D	D	D	-	-	-	-	
5, 10	+	-	-	-	-	-	-	D	D	D	D	D	D	*	D	D	-	-	-	-	-	-	-	
17	+	-	-	-	-	-	D	D	D	D	D	D	D	*	D	-	-	-	-	-	-	-	-	
7	+	-	-	-	-	-	-	-	-	D	D	D	D	*	D	-	-	-	-	-	-	-	-	
18	+	-	-	-	-	-	-	-	-	-	-	D	D	*	D	d/-	-	-	-	-	-	-	-	
29	+	-	-	-	-	-	-	-	-	-	-	-	D	*	D	d/-	-	-	-	-	-	-		
19	+	-	-	-	-	-	-	-	-	-	-	D	D	*	D	-	-	-	-	-	-	-	-	
4, 6, 11	+	-	-	-	-	-	-	-	-	-	-	-	D	*	-	-	-	-	-	-	-	-	-	
9	+	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	
14	+	D	-	-	-	-	-	-	-	-	-	-	-	*	D	-	-	-	-	-	-	-	-	(2)
26	+	-	D	D	-	-	-	-	-	-	-	-	-	*	D	D	-	-	-	-	-	-	-	
8	+	-	-	D	-	-	-	-	-	-/d	D	D	D	*	D	D	D	D	D	D	-	-	-	
28	+	-	-	-	-	D	D	D	D	d/-	-	-	D	*	-	-	-	-	-	-	-	-	-	

B. Back Conjugation: (ECOR 47 → ER2476) → ER2476

	Minutes																						
	90 //		25	27.5	--- 28 ----------												--------- 29 ----------					-- 30 --	
	+		POT	NA	AD	GO	GL	BU	ON	LK	AL	SQ	*	CB	ED	BS	SB	TA	AC	AH	TYR	NR	
[Donor HLZ-15			-	-	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	D	D]	
Trans																							#
3, 5–8, 11–15	+			-	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	D	D	(1)
4	+			-	D	D	D	D	D	D	D	D	*	D	D	D	D	D	D	D	-	-	-
1, 9, 10	+			-	-	-	-	-	D	D	D	D	*	D	D	D	D	D	D	D	D	D	
2	+			-	D	D	D	D	D	D	D	D	*	D	D	-	-	-	-	-	-	-	(2)

*, selected marker, *trpA*+; +, counterselected marker, *rpsL* (at 75 min, top) or rpoB (at 90 min, bottom). Other symbols as in Table 2.

TABLE 9

Various comparisons of conjugations and transductions

Set		Table	Cross Backcross	Donor marker at	*N*	R (kb)	S (kb)	IP	TI	L (kb)	S/L
			A. Transductional crosses and backcrosses								
1		-	47→K12trpA33	28 min	18	12	8	8	12		
		-	47K-4→K12trpA33		15	23	23	0	0	24	0.96
		-	47K-9→K12trpA33		15	19	19	0	0	19	1.00
2	"R-"	7A	47→ER2476	28 min	20	35	30	5	5		
	"R-"	7B	47D10→ER2476		30	32	32	1	1	63	0.51
			B. Conjugational crosses and backcrosses								
3		3A	47→K12	28 min	29	51	16	12	21		
		3B	47K12#12→K12		30	57	57	0	0	59	0.97
4	"R-"	8A	47→ER2476	28 min	30	42	31	3	3		
	"R-"	8B	47KR#15→ER2476		15	90	90	0	0	100	0.90
5	"R-"	-	47→C1a	28 min	15	48	48	0	0		
	"R-"	-	47C1a#15→C1a		15	105	105	0	0	121	0.87
			C. Conjugational reciprocal crosses								
6		4A	47→K12	18 min	30	4	1	1	1		
		4B	K12→47		30	260	200	4	7		
7		5A	72→K12	18 min	15	342	224	7	8		
		5B	K12→72		15	686	686	0	0		
8		6A	47→72	18 min	30	57	57	0	0		
		6B	72→47		15	368	272	4	5		
9	"R-"	-	47→C1a	18 min	15	46	10	2	3		
			C1a→47		15	346	231	6	1		

N, number of progeny; R, average length of range of donor DNA; S, average maximum segment length; IP, number of progeny with donor DNA interruptions; TI, total number of interruptions; L, length of original donor DNA segment in backcross donor. Origin of transfer is at 31 min in all cases. In sets 1–5, initial crosses are placed above the line and backcrosses below. "R-" indicates a nominally restrictionless recipient, ER2476 or C1a.

K12 is not expected to be subject to restriction. Smith (1991) surveyed the results of conjugation among various derivatives of K12 (genetically marked Hfr substrains) and concluded that some 80% of all exconjugants contained a large continuous stretch of the DNA molecule introduced into the cell. Nevertheless, the possibility had already been recognized, as noted earlier, that restriction in bacteria might be important in natural gene transfer and its evolutionary consequences. It is now clear that incoming DNA can be incorporated after initial fragmentation by restriction endonucleases and any subsequent exonuclease activity. Also, a significant level of intraspecific polymorphism in R-M systems in nature has been demonstrated, as noted previously. Moreover, the degree of abridgment of incoming bacterial DNA by strain ER2476, from which all known restriction genes have been removed, and in the putatively restrictionless strain C1a, points to a potential set of as-yet-unidentified polymorphic R-M systems that target bacterial DNA, some of which may exist in some familiar strains.

In the protection of backcross donor DNA against restriction, the central question is whether R-M genes are transferred in the original cross. In the transductional backcrosses discussed here, only a small region near *trp* is transferred, leaving the transductants identical to the recipient strain in restriction properties. Thus a backcross to the recipient should not involve restriction. Restriction in conjugational backcrosses depends on the position of the origin of transfer, the selected marker, and the extent of the DNA transferred. In our conjugations, the known restriction (-modification) loci were not ordinarily transferred, again resulting in the protection of DNA backcrossed to the recipient. However, Boyer's (1964) early experiments involved Hfrs with origins of transfer and selected markers that made transfer of the R-M loci probable. Thus Boyer's backcrosses to the *donor* strain enabled him to conclude correctly that "the genetic loci responsible for restriction and modification of DNA in strains K-12 and B in *E. coli* are located between the *thr* and *pil* [now *fimBC*] loci." This region is about 2 min in length.

Finally, the evident general rarity of recombination in *E. coli* is likely to be compensated by the formation of multiple small recombinogenic DNA fragments. These tend to separate the beneficial elements from deleterious ones, and this operation is especially effective if several nascent chromosomes are available to incorpo-

TABLE 10

Conjugation: DCHf1 → ECOR 47/Hfr origin 31 min

	75	2		9	10	11		12	15	16	18	20	21	22	24	25	27	28		29					30			
	+	I	F	P	A	D	*	C	N	S	G	D	M	H	H	P	N	B	E	T	A	$	A	P	T		
		L	T	H	C	N		S	A	C	L	M	U	Y	T	O	A	U	D	A	C		H	S	Y		
Trans		V	S		R	X		G	G		N	S	K	A	R	T									P	R	#	
DK04	+	-		-	-	-	-	-	-	-	-	-	-	-	D	D	D	D	D	D	D	$	D	D	D	(1)	
DK03	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	D	D	D	D	D	$	D	D	D		
DK07	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	D	D	D	D	D	$	D	D	D		
DK02	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	D	D	D	$	D	D	D		
DK01	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-/d	D	D	$	D	D	D		DK
DK05	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	$	D	D	D		
DK10	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	$	D	D	D		
DK06	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	$	D	D	D		
DK09	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	D	-	-	-	D	$	D	D	-	(2)	
DK08	+	-		D	D	D	*	D	D	D	D	D	D	-	-	-	-	-	-	-	-	$	D	D	D		
DB01	+	D		D	D	D	*	D	D	D	D	D	D	D	D	D	D	D	D	D	D	$	D	D	D	(1)	
DB05	+	-		D	D	D	*	D	D	D	D	D	D	D	D	D	D	D	D	D	D	$	D	D	D		
DB09	+	-		-	-	-	*	D	D	D	D	D	D	D	D	D	D	D	D	D	D	$	D	D	D		
DB10	+	-		-	-	-	*	D	D	D	D	D	D	D	D	D	D	D	D	D	D	$	D	D	D		
DB04	+	-		-	-	-	*	D	D	D	-	-	-	-	-	D	D	D	D	D	D	$	D	D	D	(2)	DB
DB08	+	-		-	D	D	*	D	-	-	-	-	-	-	D	D	D	D	D	D	D	$	D	D	D		
DB03	+	-		-	D	D	*	D	-	-	-	-	-	-	D	D	D	D	D	D	D	$	D	D	D		
DB02	+	-		-	-	-	*	D	-	-	-	D	D	D	D	D	D	D	D	D	D	$	D	D	D		
DB07	+	-		-	D	D	*	D	D	D	-	D	-	-	-	-	D	D	D	D	D	$	D	D	D	(3)	
DB06	+	-		D	D	D	*	D	-	-	-	-	-/d	D	D	-	-	-	-	D	D	$	D	D	D		
DT05	+	-		D	D	D	*	D	D	D	D	D	D	D	D	D	-	-	-	-	-	-	-	-	-	(1)	
DT08	+	-		-	-	-	*	D	D	D	D	D	-	-	-	-	-	-	-	-	-	-	-	-	-		
DT06	+	-		-	-	-	*	D	D	D	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
DT03	+	-		-	-	D	*	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
DT09	+	-		-	-	-	*	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		DT
DT02	+	-		-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
DT04	+	-		D	D	D	*	D	D	D	D	-	-	D	D	D	D	D	D	D	D	$	D	D	D	(2)	
DT01	+	-		-	-	-	*	D	-	-	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
DT07	+	-		-	-	-	*	D	-	D	D	D	D	D	-	-	-	-	-	-	-	-	-	-	-		
DT10	+	-		-	D	D	*	D	D	D	-	D	D	-	-	D	-	-	-	-	D	-	-	-	-	(4)	

The DK strains were selected on kanamycin only; DB on both kanamycin and tetracycline; DT on tetracycline only. Subsequent screening revealed tetracycline resistance in exconjugant DK-08 and kanamycin resistance in DT-04. Trans, transconjugant strain; #, number of donor segments; *, tet^R donor marker; $, kan^R donor marker; +, recipient marker, *rpsL*. Other symbols as in Table 2.

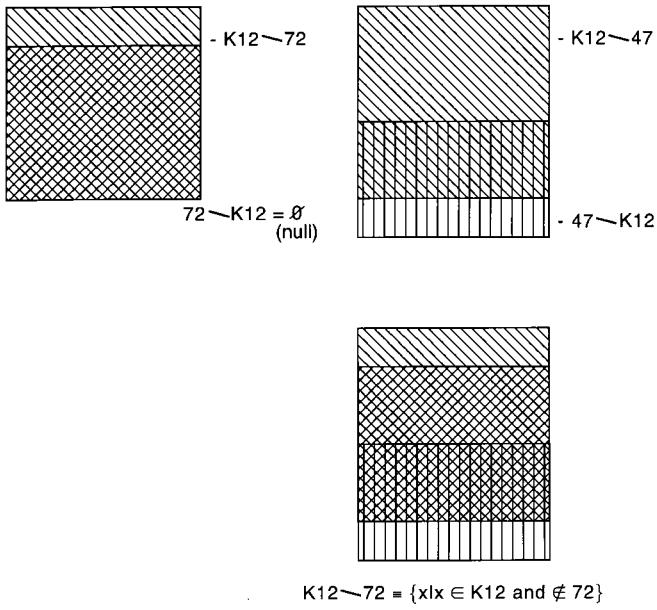

Figure 2.—Rectangles representing the respective restriction-modification systems of strains K12, ECOR 47, and ECOR 72 are partially superimposed in pairs to represent the differences between sets, which are labeled with standard symbols for subtraction of sets. The recipient's set is always given first, since the determinant of abridgment appears to be the subtraction of the shared donor-recipient set from the recipient's, leaving that portion of the recipient set against which the donor DNA is unprotected. The three pairs above can be superimposed, implying the consistency of the three schemes.

rate incoming DNA. If, for example, four alternative homologs are present and incoming DNA can invade in either of two directions, eight or more possible recombinant chromosomes can form, even if no subsequent recombination can take place before segregation. Note that the probability of any combination of incorporations is not the product of the probabilities of the respective individual incorporations. This is because all incorporations depend on a common prior event, the entry of donor DNA into the cell.

Separation of beneficial from deleterious elements should be especially important in lateral transfer of DNA from phylogenetically distant sources (Lan and Reeves 1996; Lawrence and Roth 1996; Lawrence and Ochman 1997). The isolation of a highly advantageous stretch from (likely) unfavorable DNA on both sides makes possible a vast increase in the all-important likelihood of the replacement's *retention* (Milkman 1997, 1999). This could compensate for the rarity of initial replacement by lateral transfer in cases like O-antigen genes and restriction-modification genes, whose variation in *E. coli* does indeed seem consistent with significant lateral transfer. The exploration of these possibilities should be informative.

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