CONJUGAL FERTILITY ASSOCIATED WITH RESISTANCE FACTOR R IN *ESCHERICHIA COLI*

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ABSTRACT

SUGINO, YOSHINOBU (Osaka University, Osaka, Japan) AND YUKINORI HIROTA. Conjugal fertility associated with resistance factor R in *Escherichia coli*. J. Bacteriol. **84**:902–910. 1962.—The introduction of the contagious drug-resistance factor, R, into an F^- strain of *Escherichia coli* allows the R^+F^- strain to mate with F^-R^- strains. The chromosome fragment transferred from the R^+ cell is relatively large, comparable to the conjugation between F^+ or Hfr male and F^- female bacteria. The complex R factor has been analyzed by transduction with phage Pl. Within the R factor, the fertility determinant is inseparable from the determinant responsible for its infectivity, but can be separated from the loci for drug resistance. R thus resembles the category of complex F factors (F-primes) previously analyzed.

Male fertility of *Escherichia coli* K-12 is usually determined by the presence of the F factor (Lederberg, Cavalli, and Lederberg, 1952).

Subsequently, a drug-resistance factor, R, has been discovered as the agent responsible for multiple drug resistance in *Shigella*. This agent has many properties similar to those of the F factor. It can be propagated among many enteric bacteria by direct cell contact, independently of the transfer of chromosomal markers (Ochiai et al., 1959; Akiba et al., 1960; Harada et al., 1960). It can replicate autonomously within a cell, and, like F (Hirota, 1960), may be eliminated by treatment with acridine dyes (Mitsuhashi et al., 1961*b*; Watanabe and Fukasawa, 1961*a*).

We have since found that the R factor also confers on F^- strains of *E. coli* the ability occasionally to transfer chromosome to other F^- cells. The present report will document this fertility effect mentioned in a preliminary report (Sugino and Hirota, 1961). In addition, the R factor has been analyzed by transduction with phage Plkc.

Terminology. *R* or *R factor* is a general term for the infectious drug-resistance factors (Mitsuhashi, 1960; at the Meeting of Microbial Genetics at Mishima, Japan, it was agreed by investigators in this field to use the term "R" for the multiple drug-resistance factor). *R-infection* is the contagious transmission of an R factor by cell contact. *R-mating* and *F-mating* denote chromosome transfer from the donor cell during a mating process mediated by an R or an F factor, respectively.

Designations. There are many forms of R factor, differing in the pattern of drug resistance which they confer on the bacteria carrying them (Mitsuhashi et al., 1961*a*).

R factors, originating from bacteria independently isolated from clinical sources, are designated by subscripts. Further derivatives of these R factors are denoted with additional suffixes (e.g., $R_{100\text{-}1}$) indicating that this R is derived from R_{100} but differs from it in some respect.

Bacteria possessing the R factor are generally designated as R^+ or more specifically as R_{11}^+, etc. Bacteria carrying no R factor are designated as R^-.

MATERIALS AND METHODS

Bacterial strains. *Shigella flexneri* strains carrying various kinds of R factor (Table 1) were used as initial donors of the R factor to *E. coli* K-12. *S. flexneri* 2b 222, originally isolated by R. Nakaya, was donated by T. Watanabe. Other *Shigella* were donated by R. Egawa of Gunma University.

Various mutants of *E. coli* K-12 (Table 2) were used in the mating and transduction experiments. As for the notation of genotypes of K-12, Lederberg's reports were followed (Lederberg et al., 1951; Lederberg, 1957).

Infection with R factor. R factors were trans-

TABLE 1. *Original Shigella flexneri carriers of R factors*

Strain	Name of R	Pattern of drug resistance*
2b 222	R_{100}	(SM, CM, TC, SA)
3a S86-1	R_{11}	(SM, CM, TC, SA)
2a 1214	R_{12}	(SM, CM, SA)
3a S86-1	R_{13}	(SM, TC, SA)
3a S86-1	R_{14}	(CM, TC)
3a S86-1	R_{15}	(SM, SA)
3a S86-1	R_{16}	(CM)
3a S86-1	R_{17}	(TC)
3a S86-1	R_{18}	(SM)

*SM, CM, TC, and SA refer to streptomycin, chloramphenicol, tetracycline, and sulfanilamide, respectively. The levels of resistance to each drug conferred by R are: over 20 μg/ml for SM, 25 μg/ml for CM or TC, and 200 μg/ml for SA.

TABLE 2. *Strains of Escherichia coli K-12 used*

Strain	Genotype*
W678	$T^-L^-B_1^-Lac_1^-Gal^-$
W3630	$Hfr_3Mal_5^-Lp^sT_1^r$
W3637	$M^-Sm^rLp^s$
W3659	$Lac_1^-Hfr_3Mal_5^-Lp^sT_1^r$
W3876	$Lac_{11D_3}^-Hfr_3Mal_5^-Lp^sSm^r$
W4172	Lac^-Pro^-
W4573	$Lac^-Gal^-Ara^-Xyl^-Mtl^-Sm^r$
W4617	Lac^-Hfr_{13}
JE52	$M^-B_1^-Mtl^-Xyl^-T_1^rT_6^r$
JE67	M^-Hfr_{12}
JE189	M^-
AB91 (JE53)	M^-sfa_2

*For the notations of genotypes of *E. coli* strains, see Lederberg et al. (1951) and Lederberg (1957). All of these genotypes were of the F^- mating type.

ferred from a donor *Shigella* or *E. coli* to a recipient by means of mixed culture in broth and appropriate selection on media containing one of the four drugs (Table 1).

Mating. Cultures of two mutants of *E. coli* K-12 in the exponential growth phase, at a concentration of about 10^8 cells per ml, were mixed and, after 2 hr of incubation at 37 C, plated on an appropriate selective medium where neither of the parents could grow. As a control, the parent strains were always separately plated. Unselected markers of recombinants were scored, after purification, by replica plating (Lederberg and Lederberg, 1952).

Disinfection of R by acridine treatment. The R^+ culture was grown from an inoculum of 100 to 1,000 cells in broth containing 30 μg per ml of acridine orange or 5 μg per ml of acriflavine. After overnight growth at 37 C, the culture was plated out. The colonies formed were replica-plated to drug agar to locate the drug-sensitive R^- clones.

Transduction by the phage P1kc. The method described by Lennox (1955) was followed. EMB-sugar agar or EM-sugar agar (Lederberg, 1950; Hirota, 1960), plus one of the four drugs, was used as the selective medium.

RESULTS

Fertility effects of different R factors. Two kinds of F^- strains were used for the demonstration of R-mating: standard F^-, e.g., JE189, and special ♀3 strains, e.g., W3876. An R factor was transferred to these F^- strains either directly from a *Shigella* or through an F^- *E. coli*. These R^+F^- parents were crossed with R^-F^- testers on minimal medium. All the crosses gave at least 10^{-8} Lac^+M^+, Mal^+M^+, or other selected recombinants (Table 3). When plated singly, the parents gave no more than 10^{-9} colonies on the selective medium, as was the case with the mixed cultures of the original $R^- \times R^-$ strains. Clones of F^- strains reisolated from mixed culture with the R donor strain without having acquired drug resistance were also infertile.

Thus, the fertility effect seems to be a general property among the R factors, independent of the pattern of resistance to drugs.

Richter (1961) developed strains, designated ♀3, which are especially suitable for the diagnosis of F and other contagious mating factors. These strains carry a locus (Hfr_3) inseparable from Mal_5, with a high affinity for F, so that infection with F is readily followed by the formation of highly fertile, Hfr_3 male derivatives.

The transfer of R_{15} and R_{18} to ♀3 testers gave recombination ratios as high as 10^{-4}, compared with 10^{-7} to 10^{-6} for other R factors, and 10^{-3} for F. A mutant of R_{100} ($R_{100\text{-}1}$) obtained by Egawa in our laboratory also gave relatively high fertility in ♀3 bacteria. $R_{100\text{-}1}$, R_{15}, and R_{18} have many properties in common, which will be discussed in a separate report.

Effect of disinfection of R. Cells can be disinfected of the R factor by treatment with acridine dyes (Mitsuhashi, Harada, and Kameda, 1961*b*;

TABLE 3. *Markers transferred through R mating**

R^+ strain			R^- strain		Selection	Rate (approx)
Original strain	R factor present	Relevant markers	Strain no.	Relevant markers		
JE189	R_{100}	M^-Lac^+	W4573	M^+Lac^-	M^+Lac^+	10^{-7}
JE189	R_{100}	M^-Gal^+	W4573	M^+Gal^-	M^+Gal^+	10^{-7}
JE189	R_{100}	M^-Ara^+	W4573	M^+Ara^-	M^+Ara^+	10^{-7}
JE189	R_{100}	M^-Xyl^+	W4573	M^+Xyl^-	M^+Xyl^+	10^{-7}
JE189	R_{15}	M^-Lac^+	W4573	M^+Lac^-	M^+Lac^+	10^{-6}
W4573	R_{15}	M^+Lac^-	JE189	M^-Lac^+	M^+Lac^+	10^{-8}
W678	R_{100}	$M^+T^-L^-$	W3637	$M^-T^+L^+$	$M^+T^+L^+$	10^{-7}
W3637	R_{100}	$M^-T^+L^+$	W678	$M^+T^-L^-$	$M^+T^+L^+$	10^{-7}
W3637	R_{15}	M^-Lac^+	W4573	M^+Lac^-	M^+Lac^+	10^{-7}
JE67	R_{100}	$M^-Lac^+sfa_{12}$	W4573	M^+Lac^-	M^+Lac^+	10^{-6}
JE53	R_{100}	$M^-Lac^+Pro^+sfa_2$	W4172	$M^+Lac^-Pro^-$	$M^+Lac^+Pro^+$	10^{-6}
W4617	R_{100}	M^+Lac^-	W3637	M^-Lac^+	M^+Lac^+	10^{-7}
W3876	R_{100}	$Hfr_3M^+Lac^-$	W3637	M^-Lac^+	M^+Lac^+	10^{-6}
W3876	R_{11}	$Hfr_3M^+Lac^-$	W3637	M^-Lac^+	M^+Lac^+	10^{-7}
W3876	R_{12}	$Hfr_3M^+Lac^-$	W3637	M^-Lac^+	M^+Lac^+	10^{-7}
W3876	R_{13}	$Hfr_3M^+Lac^-$	W3637	M^-Lac^+	M^+Lac^+	10^{-7}
W3876	R_{14}	$Hfr_3M^+Lac^-$	W3637	M^-Lac^+	M^+Lac^+	10^{-7}
W3876	R_{15}	$Hfr_3M^+Lac^-$	W3637	M^-Lac^+	M^+Lac^+	10^{-4}
W3876	R_{16}	$Hfr_3M^+Lac^-$	W3637	M^-Lac^+	M^+Lac^+	10^{-7}
W3876	R_{17}	$Hfr_3M^+Lac^-$	W3637	M^-Lac^+	M^+Lac^+	10^{-7}
W3876	R_{15}	$Hfr_3M^+Mal^-$	W3637	M^-Mal^+	M^+Mal^+	10^{-4}
W3659	R_{15}	$Hfr_3M^+Mal^-$	JE52	M^-Mal^+	M^+Mal^+	10^{-4}
W3630	R_{14}	$Hfr_3M^+Sm^s$	JE52	M^-Sm^r	M^+Sm^r	10^{-6}
W3630	R_{15}	$Hfr_3M^+Mal^-$	JE52	M^-Mal^+	M^+Mal^+	10^{-4}
W3630	R_{18}	$Hfr_3M^+Mal^-$	W3637	M^-Mal^+	M^+Mal^+	10^{-4}
W3630	R_{100}	$Hfr_3M^+Mal^-$	W3637	M^-Mal^+	M^+Mal^+	10^{-6}

* Selection for sugar fermentation-positive and nutritionally independent recombinant was made on EM sugar agar, which corresponds to the EMS sugar agar of Lederberg except for the omission of succinate. Selection for prototrophic recombinants was made on either EM sugar agar or Davis minimal agar (Lederberg, 1950). "Rate" was calculated by dividing the number of recombinants by the number of R^+ cells plated. Selection for SM^rM^+ recombinant was made on EM sugar agar plus 100 μg/ml of streptomycin.

Watanabe and Fukasawa, 1961*a*). When W3876 R_{100}^+ was treated in this way, the loss of drug resistance was accompanied by loss of fertility (tested by crossing with W3637 on EM lactose agar).

Segregation pattern of unselected markers among recombinants and polarity of chromosome transfer in R-mating. The segregation patterns of unselected markers among Lac^+M^+ recombinants from the reciprocal crosses, W4573 × JE190 (JE189 R_{15}^+) and JE151 (W4573 R_{15}^+) × JE189, are shown in Table 4. Table 5 shows the result of another cross. Tables 7 and 8 show results of crosses involving ♀3 strains. Tables 6 and 9 show results of analogous F-mating for comparison.

The occurrence in single clones of many unselected markers from both parents cannot be accounted for by spontaneous mutation, and is decisive evidence for the occurrence of genetic recombination. Furthermore, this fact shows that the recombinants are formed by mating, or transfer of comparatively large segments of chromosome by the conjugation process, not by transduction or transformation. The latter mechanisms of recombination transfer only a limited size of chromosomal segment in a single transfer.

The predominance of the alleles from the R^- parent among the recombinants in Tables 4, 5, and 7a suggests a polarity of transfer from R^+ to R^- cells, analogous with the case of F-mating.

The occurrence of R^- recombinants is in agree-

TABLE 4. *Pattern of segregation in R-mating* ($F^-R^+ \times F^-R^-$)*

R_{15}	M	Lac	Ara_2	Gal_2	Mtl	Xyl_2	No. of recombinants
			a. JE190 (JE189R_{15}^+) × W4573				
1	0	1	0	0	0	0	57 (61%)
1	0	1	1	0	0	0	29 (31%)
1	0	1	0	1	0	0	6 (6%)
1	0	1	1	1	0	0	1 (1%)
0	0	1	0	0	0	0	1 (1%)
					Total		94
			b. JE151 (W4573R_{15}^+) × JE189				
1	1	0	0	0	0	0	45 (88%)
1	1	0	0	0	1	0	2 (4%)
1	1	0	0	0	1	1	2 (4%)
1	1	0	1	1	1	1	1 (2%)
1	1	0	1	0	1	1	1 (2%)
					Total		51

JE190: $F^-R_{15}^+M^-Lac^+Ara^+Gal^+Mtl^+Xyl^+$: 1 1 1 1 1 1 1
W4573: F^-R^- $M^+Lac^-Ara^-Gal^-Mtl^-Xyl^-$: 0 0 0 0 0 0 0
JE151: $F^-R_{15}^+M^+Lac^-Ara^-Gal^-Mtl^-Xyl^-$: 1 1 1 1 1 1 1
JE189: F^-R^- $M^-Lac^+Ara^+Gal^+Mtl^+Xyl^+$: 0 0 0 0 0 0 0

* Lac^+M^+ recombinants were selected on EM lactose agar plates. The ratio of recombinants to initial R^+ (or F^+) cells was ca. 10^{-6} for JE190 × W4573, ca. 10^{-7} for JE11 × W4573, and ca. 10^{-8} for JE151 × JE189 or for JE214 × JE189. Recombinant colonies were picked at random, purified, and scored for unselected markers. Lac, Ara, Gal, Mtl, and Xyl refer to fermentation of lactose, arabinose, galactose, mannitol, and xylose, respectively. M refers to the ability to grow without external supply of methionine. The allele from the R^- (or F^- in Table 7) parent is designated 0, and the alternative from the R^+ (or F^+) parent, 1 (Lederberg, 1957).

ment with the idea that recombinant cells are produced by the transfer of chromosome from the R^+ to the R^- cells. If the direction of transfer were from the R^- to the R^+ cells, the resulting recombinants should be all R^+. An R^- recombinant can only be derived from an R^- cell, since the probability of spontaneous loss of R is low. There seems to be no linkage between the transfer of R and any specific chromosomal markers.

The results in Table 7b may seem aberrant. A possible explanation is as follows: R_{15} is first transferred during mixed culture to W3876,

TABLE 5. *Pattern of segregation in R-mating of JE11* (JE189R_{100}^+) × W4573*

R_{100}	M	Lac	Ara	Gal	Xyl	Mtl	No. of recombinants
1	0	1	0	0	0	0	17 (35%)
0	0	1	0	0	0	0	3 (6%)
1	0	1	0	1	0	0	8 (16%)
0	0	1	0	1	0	0	3 (6%)
1	0	1	1	0	0	0	7 (14%)
0	0	1	1	0	0	0	1 (2%)
1	0	1	1	1	0	0	4 (8%)
0	0	1	1	1	0	0	1 (2%)
1	0	1	1	0	1	0	1 (2%)
1	0	1	1	1	1	1	4 (8%)
						Total	49

JE11: $F^-R_{109}^+Ara^+Lac^+Gal^+Xyl^+Mtl^+M^-$: 1 1 1 1 1 1 1
W4573: F^-R^- $Ara^-Lac^-Gal^-Xyl^-Mtl^-M^+$: 0 0 0 0 0 0 0
* See footnote to Table 4.

TABLE 6. *Pattern of segregation in F-mating of JE214* (W4573F^+) × *JE189**

M	Lac	Ara_2	Gal_2	Mtl	Xyl_2	No. of recombinants
1	0	0	0	0	0	16 (53%)
1	0	0	0	0	1	2 (7%)
1	0	0	0	1	1	3 (10%)
1	0	1	0	0	0	8 (27%)
1	0	1	0	1	0	1 (3%)
				Total		30

JE214: $F^+M^+Lac^-Ara^-Gal^-Mtl^-Xyl^-$: 1 1 1 1 1 1
JE189: $F^-M^-Lac^+Ara^+Gal^+Mtl^+Xyl^+$: 0 0 0 0 0 0
* See footnote to Table 4.

inducing high fertility in the new host cell, which in turn transfers chromosomal markers to the original R_{15}^+ parent. This accounts for the lower rate of recombination in comparison with the reverse cross, i.e., JE95 (W3876 R_{15}^+) × JE52.

The segregation patterns of unselected markers among recombinants in a cross, R^+F^- × R^-F^-, as well as the ratio of recombinants to initial R^+ cells, seem to vary with the kind of R factor present.

In general, the linkage pattern in R-mating seems quite similar to that known from F-mating.

The results shown in Tables 4b and 7a are similar to those of Tables 6 and 9 respectively, probably reflecting quite similar mechanisms of chromosome transfer.

Chloroform treatment. To show that intact

TABLE 7. *Pattern of segregation in R-mating**

R_{15}	M	Lac	B_1	Mal	Mtl	Xyl	T_6	T_1	No. of recombinant colonies
				a. JE95 (W3876R_{15}^+) × JE52					
1	1	0	1	0	0	0	0	0	167 (94%)
1	1	0	1	0	1	0	0	0	2 (1%)
1	1	0	1	0	1	1	0	0	9 (5%)
								Total	178
				b. JE115 (JE52R_{15}^+) × W3876					
1	0	1	0	1	1	1	1	1	92 (92%)
1	0	1	0	1	0	0	1	1	6 (6%)
1	0	1	0	1	1	0	1	1	2 (2%)
								Total	100

JE95: $F^-Hfr_3R_{15}^+M^+B_1^+Mal_5^-Mtl^+Xyl^+T_6^sLac^-T_1^s$: 1 1 1 1 1 1 1 1 1
JE52: F^- R^- $M^-B_1^-Mal^+$ $Mtl^-Xyl^-T_6^rLac^+T_1^r$: 0 0 0 0 0 0 0 0 0
JE115: F^- $R_{15}^+M^-B_1^-Mal^+$ $Mtl^-Xyl^-T_6^rLac^+T_1^r$: 1 1 1 1 1 1 1 1 1
W3876: $F^-Hfr_3R^-$ $M^+B_1^+Mal_5^-Mtl^+Xyl^+T_6^sLac^-T_1^s$: 0 0 0 0 0 0 0 0 0

*Selection was made of Lac^+M^+ recombinants on EM lactose agar plates supplemented with 10 μg/ml of thiamine. The ratio of recombinants to initial R^+ cells was ca. 10^{-4} for JE95 × JE52, ca. 10^{-6} for JE115 × W3876 or JE 51 × JE52. The ratio of recombinants to initial JE116 cells is ca. 10^{-3} for the cross JE116 × JE52. Recombinant colonies were picked at random, purified, and unselected markers were scored. Lac, Mal, Mtl, and Xyl refer to the fermentation of lactose, maltose, mannitol, and xylose, respectively. M and B refer to the ability to grow without an external supply of methionine and thiamine, respectively. T_6^r and T_6^s or T_1^r and T_1^s refer to resistance or sensitivity to phage T_6 or T_1. The alleles from the R^- (or F^- in Table 9) parent are designated 0, and the alternative from the R^+ (or F^+) parent, 1.

TABLE 8. *Pattern of segregation in R-mating of JE51 (W3876R_{100}^+) × JE52**

R_{100}	M	Lac	B_1	Mal	Mtl	Xyl	T_6	T_1	No. of recombinant colonies
1	1	0	1	0	0	0	0	0	34 (50%)
0	1	0	1	0	0	0	0	0	4 (6%)
1	1	0	1	0	0	0	0	1	1 (1%)
1	1	0	1	0	0	1	0	0	4 (6%)
0	1	0	1	0	0	1	0	0	1 (1%)
0	1	0	0	0	1	1	0	0	2 (3%)
1	1	0	1	1	1	1	0	0	3 (4%)
1	1	0	1	1	0	0	0	0	3 (4%)
0	1	0	1	1	0	0	0	0	2 (3%)
0	1	0	1	1	0	0	1	0	1 (1%)
1	1	0	1	1	0	1	0	0	1 (1%)
1	1	0	1	0	1	0	0	0	2 (3%)
1	1	0	1	0	1	1	0	0	8 (12%)
0	1	0	1	0	1	1	0	0	2 (3%)
								Total	68

JE51: $F^-Hfr_3R_{100}^+M^+B_1^+Mal_5^-Mtl^+Xyl^+T_6^sLac^-T_1^s$: 1 1 1 1 1 1 1 1 1
JE52: F^- R^- $M^-B_1^-Mal^+$ $Mtl^-Xyl^-T_{6r}Lac^+T_1^r$: 0 0 0 0 0 0 0 0 0
*See footnote to Table 7.

cells are necessary for recombinant formation, and to help further eliminate the possibilities of transduction or transformation, mixing experiments were performed in which either the R^+ or R^- strain was treated with chloroform before mixing. The strains used for this experiment were JE95 (W3876 R_{15}^+: Lac^-) and W3637 (M^-).

TABLE 9. *Pattern of segregation in F-mating of JE116 (W3876 F+) × JE52**

M	Lac	B_1	Mal	Mtl	Xyl	T_6	T_1	No. of recombinants
1	0	1	0	1	0	0	0	72 (89%)
1	0	1	0	1	1	0	0	8 (10%)
1	0	1	0	0	1	0	0	1 (1%)
						Total		81

JE116: $F^+R^-Hfr_3M^+B_1^+Mal_5^-Mtl^-Xyl^+T_6^sT_1^sLac^-$: 11111111

JE52: F^-R^- $M^-B_1^-Mal^+Mtl^+Xyl^-T_6^rT_1^rLac^+$: 00000000

* See footnote to Table 7.

To the cultures in the exponential growth phase, a few drops of chloroform were added and the mixtures were shaken vigorously. Chloroform was evaporated completely by shaking at 37 C for 20 min and the treated culture was mixed with untreated culture. From the mixtures of chloroform-treated R^+ and untreated R^- cells, or conversely, of untreated R^+ and chloroform-treated R^- cells, no Lac^+M^+ recombinants could be detected, although the mixture of untreated R^+ and R^- cells, run in parallel for control, yielded a normal number of recombinants.

Sensitivity to the phage tau. The temperate bacteriophage *tau* is known to form plaques only on F^- cells, not on F^+ or Hfr strains of *E. coli* K-12 (Hakura, *unpublished data*; Zinder, 1961). These fertile F^-R^+ strains were found to form as many plaques of this phage as the original F^-R^- strains.

Sensitivity to the phage f_1. The phage f_1 is known to attack F^+ or Hfr mutants of *E. coli* K-12, but cannot be adsorbed to F^- cells (Loeb, 1960). F^-R^+ cells are resistant to the phage, as are the original F^-R^- cells.

Transduction with P1kc. The R factor can be transduced with the phage Plkc (Nakaya, Nakamura, and Murata, 1960; Watanabe and Fukasawa, 1961*b*). Resistance to one or the other drug is sometimes lost during the course of the transduction, in contrast with the transfer by cell contact where resistance to different drugs usually behaves as a unit. Now, if the fertility determinant is really located within the R factor, it may be jointly transduced by Plkc with the genetic determinants for drug resistance. Furthermore, rare transductants may be found which have not received the fertility determinant. By studying the correlation between loss of the fertility determinant and loss of resistance to some drug, it may be possible to determine the degree of linkage of the fertility determinant with the drug-resistance markers.

To confirm these expectations, transduction experiments were carried out using a $♀_3$ strain, W3630, as recipient, and the same strain possessing $R_{100\text{-}1}$ (CM, SM, TC, SA), JE177, as donor. This R factor was isolated by Egawa from R_{100}. The original R factor, R_{100}, when present in Hfr cells such as W1895, lowers the efficiency of recombinant formation by 100-fold (Nakaya et al., 1960). From the strain W1895 R_{100}^+, a variant clone was found which recovered high fertility but retained resistance to the four drugs. Egawa and Hirota (1962) showed that a mutation at a locus *i* on the R factor is involved. Incidentally, this variant R factor induced high fertility when transferred by infection to $♀_3$ mutants. This system would permit efficient scoring of the fertility of transductants by replica plating onto an EM maltose plate seeded with F^-M^- bacteria.

The multiplicity of infection of the phage Plkc in the transduction experiment was kept low (ca. 0.1) to exclude multiple infection or lysogenization of the phage (Lennox, 1955). Selection was made by each of the four drugs, and the transductants were scored for unselected markers, i.e., resistance to other drugs and fertility.

Almost all of the transductants were of the donor type (Table 10). This is additional evidence that the R factor is indeed responsible for the fertility of our F^-R^+ strains, since only very closely linked genetic elements can be transduced jointly by Plkc (Lennox, 1955).

In other words, the fertility determinant, which we shall designate *m*, is located on a particle linked to the drug-resistance markers.

Rare exceptions which are infertile, but drug resistant, indicate the separability of these determinants, the fertility determinant perhaps being closest to the TC-locus.

The infective transmissibility of the R factor in all the exceptional transductants, as well as random samples of the donor types, was examined.

The infertile transductants were unable to transfer drug resistance in mixed culture, while other types of transductants could transfer resistance as efficiently as the donor strain (Table 11). Thus, the mediation of chromosome transfer and infectivity of drug resistance by R may be

TABLE 10. *Results of transduction of R_{100-1} by P1kc**

Drug used for selection	Rate of transduction	No. of transductants examined	Markers lost from the R factor
SM	10^{-7}	9	Fertility (1)
SA	10^{-7}–10^{-6}	460	TC (3)
TC	10^{-6}–10^{-5}	320	Fertility (1) CM (1)
CM	10^{-6}–10^{-5}	800	Fertility (2) Fertility and TC (1)

*About 10^9 recipient bacteria with ca. 10^8 Plkc were plated on EMB maltose agar plus 20 μg/ml of SM, 30 μg/ml of TC, or 20 μg/ml of CM, or on EM lactose agar plus 200 μg/ml of SA. The second column gives the approximate number of cells which have acquired drug resistance divided by the number of phage added. The third column shows the number of clones examined for unselected markers. The majority were of the donor type, i.e., resistant to SM, SA, TC, and CM, and fertile in crosses with F^- cells. The number of clones different from the donor type are shown in the fourth column together with the markers with respect to which they are different from the donor type. When selected with SM, the number of transductants is very small for some unknown reason. This is in accord with a previous report (Watanabe and Fukasawa, 1961*b*).

controlled by the same determinant, although these events are to some extent independent. In a control experiment, none of 2,000 single colonies of donor bacteria showed spontaneous loss of fertility.

Drug resistance could be retransduced by Plkc from infertile transductants to W3630, and the new transductants were similarly infertile and noninfective. This proves that the change (i.e., loss of fertility effect) had occurred in the R factor and not in the host bacteria. When transduced into the Hfr_1 mutant, W1895, this R factor did not inhibit F-mating.

Another interesting point with the defective R is that it can be eliminated by treatment with acridine dyes (Table 11). This suggests that its state in the cell is cytoplasmic, replicating autonomously, independently of the host chromosome. This point should be confirmed by analysis of linkage with chromosomal markers by F-mating.

When such a defective R and a normal R are present in one and the same F^- cell, the cell possesses fertility as well as infective transmissibility of R. This aspect is now under investigation and will be discussed in a separate report.

TABLE 11. *Characteristics of R after losing the fertility effect through transduction**

Characteristic	R factor that has lost fertility effect	Original R (R_{100-1})
Fertility effect	−	+
Infectivity	−	+
Accessibility to disinfection by AO	+	+
Inhibition of F-mating	−	−

*The transductants lacking fertility were rechecked for recombinant formation, with W3637 (F^-M^-) as the tester. Selection was made for M^+Mal^+ recombinants. No recombinants could be detected per 10^9 R^+ cells, while the original donor strain gave ca. 1 recombinant per 10^3 R^+ cells. Infectivity was checked by mixing with JE189 (F^-R^-). Inhibition of F-mating was checked in W1895, where the "defective" R factors had been transduced by Plkc.

DISCUSSION

The conclusions that can be drawn from the above results may be briefly summarized as follows. (i) The R factor, when introduced into an F^- cell of *E. coli* K-12 either by infection or transduction by the phage Plkc, induces fertility, i.e., the capacity to transfer a comparatively large chromosomal segment by conjugation. (ii) When the R factor is eliminated from an F^-R^+ strain, loss of fertility ensues. (iii) Intact cells are necessary for chromosome transfer, caused by the R factor, to occur. (iv) The fertility determinant on R is either identical with or at least extremely closely linked to the genetic determinant of infectivity of the R factor. (v) Autonomous replication of R is not affected by loss of fertility and the infectivity determinant. (vi) Joint transduction by Plkc of the fertility determinant and drug resistance shows them to be closely linked.

From these conclusions, it can be said that the R factor has a structure analogous to F-prime factors, although no evidence is yet available for the chromosomal origin of the resistance markers (Adelberg and Burns, 1959; Hirota, 1961; Jacob, Schaeffer, and Wollman, 1960).

Analogous to F and R, colicinogenic factors are reported to confer fertility on *Salmonella* and *E. coli* (Stocker, 1960; Ozeki, Haworth, and

Clowes, 1961), suggesting that these factors might be related in their origin.

The R factors resemble the cinogens but differ from F or F-prime with respect to their effect on the resistance of the host cell to phage f_1 and the inhibition of growth of phage *tau*.

The drug-resistant strains obtained by transduction, lacking fertility and infective transmissibility of resistance, are especially interesting. It is suggested that the same mechanism, probably a specific surface structure, is involved in the transfer of host chromosome and in the infective transmission of the R factor itself. The remarkable fact that sex determinants so far known in bacteria are usually infectious or episomic might be related to analogous situations (Holloway and Fargie, 1960; Bhaskaran, 1960). On the other hand, infectious transmissibility and autonomous replication, which are usually linked in F or R, have been separated in these defective R factors.

Several strains of *Shigella* and *E. coli* isolated by Ochiai (*personal communication*) and Nakaya (1962) carry noninfectious multidrug resistance. They could carry defective R factors which had become defective in their transmissibility through spontaneous deletions or through a transductional process by a phage.

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