A STABLE PARTIAL DIPLOID STRAIN OF ESCHERICHIA COLI

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IN 1949, LEDERBERG reported the discovery of an unusual partial diploid strain in *Escherichia coli* K-12. Later studies (LEDERBERG, LEDERBERG, ZINDER and LIVELY 1951; LEDERBERG 1951) with other partial diploid strains revealed that reduction to the haploid state always occurred in one step and at a high rate (5 to 10 percent per bacterium per generation). Recombination with retention of the partial diploid state occurred infrequently and yielded strains which were homozygous at one or more loci. These partial diploid strains made it possible to analyse dominance and permitted functional analysis of phenotypically similar mutations (LEDERBERG *et al.*, 1951).

In a preceding study (CURTISS 1964a), several partial diploid strains were isolated in crosses involving phage-resistant mutants of *E. coli* K-12. In contrast to the partial diploid strains isolated by LEDERBERG (1949, 1951), one of these was stable on complete medium as well as on synthetic medium and was, therefore, selected for further study. This communication reports on the isolation and general properties of this strain and also on the mechanism of transfer of the partial chromosome from partial diploid donors to haploid recipients. A preliminary report of this work has been made (CURTISS 1962a). The partial diploid strain described in this paper is currently being employed in studies on gene expression and control and on the mechanism of genetic recombination.

MATERIALS AND METHODS

The media, bacteriophages and all basic techniques were described previously (CURTISS 1964a).

Bacteria: The haploid strains are listed in Table 1 and the partial diploid strains in Table 2. Partial diploid strains are transferred on Penassay agar slants at two month intervals after growth in minimal media lacking proline to reduce the frequency of haploid segregants. For some experiments with partial diploid strains in which homogeneity of the population was necessary, individual colonies were picked from minimal agar lacking proline.

Isolation of bacterial mutants: Auxotrophic mutants induced by ultraviolet light (UV) were isolated by the penicillin enrichment procedure of LEDERBERG (1950a), using the minimal medium of DAVIS and MINGIOLI (1950). Fermentation, valine-resistant, azide-resistant and phage-resistant mutants were isolated by selection on appropriate media (LEDERBERG 1948; MANTEN and ROWLEY 1953; LEDERBERG 1950b; and CURTISS 1964a; respectively).

Mating procedure: In $F^+ \times F^-$ crosses the ratio of donor to recipient cells was between 1:5 and 1:1. Otherwise the procedures were as described by CURTISS (1964a).

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TABLE 1

Escherichia coli *K-12 haploid strains*

Strain Number	Mating type	Genetic markers*															Derivation†
		thr	*ara*	*val*$_1$	*leu*	*azi*	*T1*	*pro*$_1$	*pro*$_2$	*lac*	*T6*	*ade*$_3$	*try*	*his*	*str*	*met*	*thi*	
χ11	HfrC	+	+	s	+	s	s	+	+	+	s	+	+	+	s	—	+	W1895
χ15	F+	+	+	s	+	s	s	+	+	+	s	+	+	+	s	+	+	W1485
χ42	F+	+	+	s	+	s	s	+	+	+	s	+	+	—	s	+	+	K-12-112
χ44	F−	+	+	s	+	s	s	+	+	+	s	+	—	—	s	+	+	K-12-112
χ85	F−	—	+	s	—	s	r	—‡	—‡	—y	s	+	+	+	r	+	—	C600
χ131	F−	+	—2	r	—	r	r	—1	+	—2	r	+	+	+	s	+	—	χ12
χ134	F−	—	+	r	—	r	r	—‡	—‡	—y	r	+	+	+	r	+	—	χ85
χ137	F−	—	—1	r	—	r	r	—‡	—‡	—y	r	+	+	+	r	+	—	χ134
χ148	F−	+	—2	s	—	r	r	+	+	—2	r	—	—	+	r	+	—	χ114
χ152	F−	+	+	r	—	s	r	—‡	—‡	—y	s	+	+	+	s	+	—	C600
χ153	F−	—	—2	r	—	r	r	—‡	—‡	—2	r	+	—	+	r	+	—	χ114
χ206	F−	+	—2	s	—	s	s	—	—	—2	s	+	+	+	s	+	—	χ12

* The markers are arranged in the order in which they occur on the chromosome. The following abbreviations are used: *thr*—threonine; *ara*—arabinose; *val*—valine; *leu*—leucine; *azi*—axide; *T1*, *T3* and *T6*—bacteriophages T1, T3 and T6 (*T1*^{r} mutation also confers resistance to phage T5); *dap*—diaminopimelic acid; *B6*—pyridoxal; *pan*—pantothenic acid; *pro*—proline; *lac*—lactose; *ade*—adenine; *gal*—galactose; *ura*—uracil; *try*—tryptophan; *shi*—shikimic acid; *his*—histidine; *lys*—lysine; *phe*—phenylalanine; *tyr*—tyrosine; *aro*—phenylalanine—tyrosine-p-aminobenzoic acid—tryphtophan; *cys*—cysteine; *ser*—serine; *str*—streptomycin; *mal*—maltose; *xyl*—xylose; *mtl*—mannitol; *ile*—isoleucine; *ilv*—isoleucine-valine; *met*—methionine; *cyc*—cycloserine; *thi*—thiamine; *arg*—arginine; +—ability to synthesize or utilize; – — inability to synthesize or utilize; s—sensitive; and r or /—resistant. Numbers and letters for *ara*$^-$, *pro*$_1^-$ and *lac*$^-$ mutations are isolation designations. χ11, χ42 and χ44 are lysogenic for bacteriophage λ.

† Intervening steps in derivations omitted (see CURTISS 1964a).

‡ All of these *pro*$_{1,2}^-$ strains have */3,7,λ,pro*$_{1,2}^-$ pleiotropic deletion-mutations which confer resistance to bacteriophages T3, T7 and λ. The *pro*$_1$ and *pro*$_2$ loci are 1 or 2 minutes apart on the *E. coli* K-12 chromosome (CURTISS 1964a), and therefore, 1 or 2 percent of the genome is missing in strains with */3,7,λ,pro*$_{1,2}^-$ mutations. χ85, χ134, χ137 and χ152 all have the same */3,7,λ,pro*$_{1,2}^-$ mutation.

TABLE 2

Escherichia coli *K-12 partial diploid strains*

Strain Number		Genetic markers											Mating type	Derivation
		thr	*ara*	*val*$_1$	*leu*	*azi*	*T1*	3,7,λ, *pro*$_{1,2}$	*lac*	*T6*	*str*	*thi*		
χ98Ex1	exogenote		+	s	+	s	s	s$^+$						
	chromosome	—	+	s	—	s	r	r$^-$	–y	s	r	—	F−	χ11 × χ85
χ98Ex11	exogenote		+	s	+	s	s	s$^+$						
	chromosome	—	+	s	—	s	r	r$^-$	–y	s	r	—	F+	χ42 × χ98Ex1
χ134Ex4	exogenote		+	s	+	s	s	s$^+$						
	chromosome	—	+	r	—	r	r	r$^-$	–y	r	r	—	?	χ98Ex11 × χ134
χ137Ex2	exogenote		+	s	+	s	s	s$^+$						
	chromosome	—	—1	r	—	r	r	r$^-$	–y	r	r	—	F−	χ206Ex6 × χ137
χ152Ex46	exogenote		+	s	+	s	s	s$^+$						
	chromosome	+	+	r	—	s	r	r$^-$	–y	s	s	—	F+	χ98Ex11 × χ152
χ206Ex6	exogenote		+	s	+	s	s	s$^+$						
	chromosome	+	—2	s	—	s	s	r$^-$	–2	s	s	—	F+	χ98Ex11 × χ206

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Acridine orange curring: The F particle was removed from F+ bacteria by growth in beef-peptone broth (pH 7.60) containing 0.4 percent NaCl and 50μg acridine orange per ml (HIROTA 1960).

Mating type determinations: The mating types of recombinants were determined after purification by restreaking on the selective medium used to isolate them. The isolates to be tested were then grown in 2 ml of Penassay broth and, after growth had ceased, were cross streaked from left to right against a suitable F⁻ tester strain on streptomycin containing minimal agar deficient for a nutritional requirement of the F⁻ tester strain. In such tests, F⁻ × F⁻ crosses did not yield any recombinants while F+ × F⁻ crosses gave about 50 to 100 recombinants to the right of the F⁻ streak. In experiments in which all of the recombinants were *str*^{r}, tests were done on media deficient in growth requirements for both the F⁻ tester and the recombinants. Frequently, F+ tester strains were also employed.

Fluctuation tests: The fluctuation test of LURIA and DELBRÜCK (1943) was used to test for mutations from the autonomous state of F to the attached state according to the procedures described by JACOB and WOLLMAN (1956).

RESULTS

Isolation of the partial diploid strain: In a cross between the Cavalli Hfr and an F⁻ with a */3,7,λ,pro$_{1,2}$⁻* pleiotropic deletion mutation, one *pro$_{1,2}$*+ recombinant was observed which appeared to be both stable and heterozygous. When a culture of this *pro$_{1,2}$*+ recombinant was streaked against T7 on EMB containing 0.1 percent glucose (CURTISS 1964a), about one percent of the cells survived phage infection. These T7-resistant survivors required proline. A broth culture of this *pro$_{1,2}$*+ recombinant was diluted, plated on Penassay agar, and after incubation, individual colonies were picked into Penassay broth, and after growth were streaked from left to right against T7. Out of 100 colonies tested, 89 yielded cultures which contained 99 percent T7-sensitive cells, while 11 cultures contained only T7-resistant cells. When these same cultures were streaked against T7 on minimal agar lacking proline the 89 cultures grew to the left of the phage streak but yielded no proline-independent, T7-resistant survivors to the right of the T7 streak. The other 11 cultures did not grow on this medium. Since phage sensitivity and ability to synthesize amino acids are dominant to phage resistance and inability to synthesize amino acids (LEDERBERG 1949), these results indicated that this *pro$_{1,2}$*+ recombinant was heterozygous for the */3,7,λ,pro$_{1,2}$* region. Further testing indicated that segregation was also occurring at the *T1* and *leu* loci.

In this partial diploid strain, the chromosomal fragment from the Cavalli Hfr will be referred to as the exogenote and the complimented region of the F⁻ chromosome will be referred to as the endogenote (MORSE, LEDERBERG and LEDERBERG 1956). Genetic markers in the remainder of the F⁻ chromosome will be referred to as chromosomal markers. Data obtained on UV-induced genetic recombination has been used to calculate that the mean number of exogenotes per endogenote is 1.1 (CURTISS 1963, 1964b). This validates referring to this strain as a partial diploid.

Complementation of mutant alleles by the exogenote: The original partial diploid strain, χ98Ex1, was F⁻, which necessitated its infection with the F particle to obtain conjugation transfer of the exogenote. χ98Ex1 was therefore grown with χ42 and several F+ partial diploid strains were isolated. Because of the low

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frequency of recombination obtained with these strains, UV-irradiation and the replica plating method used by JACOB and WOLLMAN (1956) to isolate Hfr mutants from F+ cultures, were employed to isolate a partial diploid strain that would transfer the exogenote at a high frequency. Repeated attempts to isolate such a strain were completely unsuccessful.

Various F+ partial diploid strains were used as donors of the exogenote to F- strains possessing different mutant alleles. Most of the mutants were isolated from χ134 (*leu⁻ T1ʳ /3,7,λ,pro$_{1,2}$⁻ strʳ*), χ148 (*leu⁻ T1ʳ strʳ*) and χ153 (*leu⁻ T1ʳ /3,7,λ, pro$_{1,2}$⁻ strʳ*). The results of these experiments are presented in Table 3.

Genetic structure of the partial diploid strain: There were 12 phenotypic classes of mutations complemented by the exogenote (Table 3). Most of these mutations were mapped with respect to each other by time of entry experiments with Hfr H as the genetic donor (CURTISS 1962b, 1964a). The genetic structure of the partial diploid strain which was based on the results of these time of entry experiments is diagrammed in Figure 1. Since the */3,7,λ,pro$_{1,2}$⁻* mutation is a deletion (CURTISS 1964a), the exogenote has to form a loop to pair with the homologous regions on either side of the deletion (Figure 1).

The partial diploid strain has an exogenote which is about 9.5 minutes long. This is about 9 percent of the *E. coli* K-12 genome (see JACOB and WOLLMAN 1961). This estimate is based on the distance between the *ara* and *pro$_2$* loci (CURTISS 1962b, 1964a) and the failure of the exogenote to complement *thr⁻* and *lac⁻* mutations (Table 3). To make sure that the partial diploid strain was hemizygous instead of homozygous diploid for the *thr⁻* and *lac⁻* mutations, *thr⁺* and *lac⁺*

TABLE 3

*Complementation of mutant alleles by the exogenote from a partial diploid strain**

Mutant type	Dominant allele	Number tested	Number complemented
ara⁻	*ara+*	10	8
val$^r_1$	neither	8	8
leu⁻	*leu+*	2	2
azi^r	neither	18	18
T1^r	*T1^s*	9	9
dap⁻	*dap+*	1	1
dap-met⁻	*dap-met+*	1	1
peptide⁻	*peptide+*	3	1
B6⁻	*B6+*	3	2
pan⁻	*pan+*	6	6
pro$_1$⁻	*pro$_1$+*	5	5
pro$_2$⁻	*pro$_2$+*	9	9
/3,7,λ,pro$_{1,2}$⁻	*(/3,7,λ)^{s}pro$_{1,2}$+*	4	4

* Most strains tested had the markers *leu⁻ T1ʳ strʳ* or *leu⁻ T1ʳ /3,7,λ,pro$_{1,2}$⁻ strʳ*, in addition to having the mutant allele being tested. After 60 minutes of mating with χ152Ex46 or χ206Ex6, plating was done on streptomycin containing media lacking leucine (proline) and the growth requirement of the mutant. If recombinant colonies were observed, they were picked, purified and tested for segregation at the *T1* locus (*/3,7,λ,pro$_{1,2}$* locus). The following mutant alleles and number of each tested in parenthesis were not complemented by the exogenote: *lac⁻* (2), *pro$_3$⁻* (9); *T6ʳ* (2); *ade⁻* (5); *gal⁻* (1); *ura⁻* (1); *try⁻* (2); *shi⁻* (2); *his⁻* (2); *lys⁻* (3); *phe-tyr⁻* (4); *aro⁻* (1); *met-cys⁻* (1); *ser⁻* (1); *strʳ* (4); *T3ʳ* (1); *mal⁻* (2); *xyl⁻* (1); *mtl⁻* (1); *val$_2$ʳ* (7); *ile⁻* (1); *ilv⁻* (3); *met⁻* (1); *cycʳ* (3); *val⁻* (1); *thi⁻* (1) *arg⁻* (1); and *thr⁻* (1).

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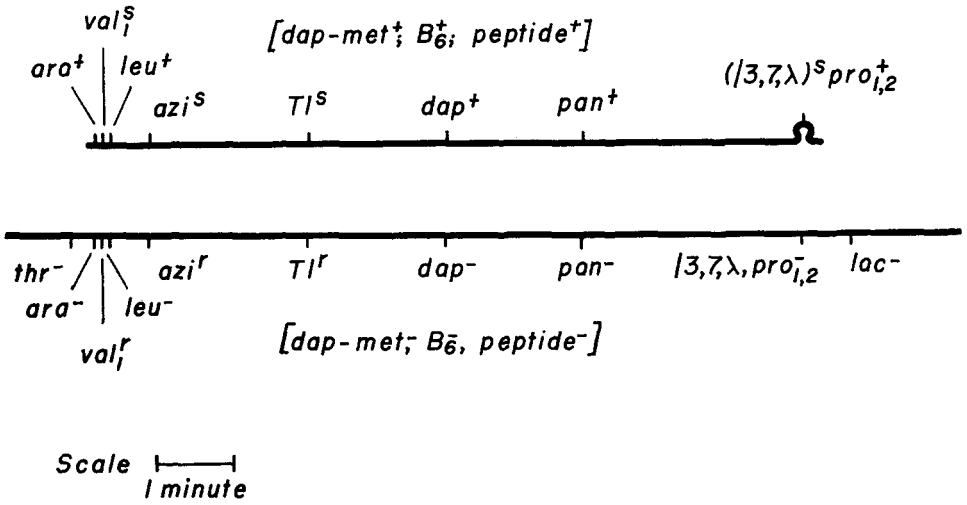

FIGURE 1.—Genetic structure of the partial diploid strain. The top of the figure depicts the exogenote with the wild-type alleles. The bottom shows the region of the chromosome that was complemented by the exogenote. The *thr*$^-$ and *lac*$^-$ alleles were not complemented by the exogenote. The markers in brackets were shown to be linked between the *ara* and *pro*$_1$ loci, but their order with respect to other markers was not determined. This diagram is figurative in that no one partial diploid strain was heterozygous for all of the indicated markers.

revertants were isolated and tested for recovery of the *thr*$^-$ and *lac*$^-$ alleles among haploid segregants. No *thr*$^-$ or *lac*$^-$ segregants were obtained.

Rate of haploidization: When the partial diploid strain underwent haploidization it almost always lost the (/3,7,λ)^s*pro*$_{1,2}^+$ exogenote allele. (Only 2 out of 217 haploid recombinants, obtained by random picking of colonies grown on Penassay agar, had inherited the (/3,7,λ)^s*pro*$_{1,2}^+$ exogenote marker.) In so doing, the ability to grow in the absence of proline was lost and resistance to T3, T7 and λ was expressed. It was thus possible to determine haploidization rates by plotting the increase in the percentage of T3 resistant survivors against the number of generations grown in a complete medium. Control experiments demonstrated that the partial diploid strain and its haploid segregants had essentially the same growth rates in Penassay broth. An example of one rate of haploidization determination is given in Figure 2. In this experiment the calculated rate of haploidization was 0.4 percent per bacterium per generation. Comparable haploidization rates were obtained in repeats of this experiment and in experiments employing two other methods (CURTISS 1962b).

If the exogenote was transferred to a haploid recipient having a *pro*$_1^-$ point mutation, then the resulting partial diploid strain, like those isolated by LEDERBERG (1949, 1951), underwent haploidization at rates of 5 to 10 percent per bacterium per generation.

Recombinant types resulting from haploidization: The results in Table 4 show that haploidization was not accomplished by the complete loss of the exogenote. The *ara*$^+$-*leu*$^+$ end of the exogenote was incorporated in almost 100 percent of

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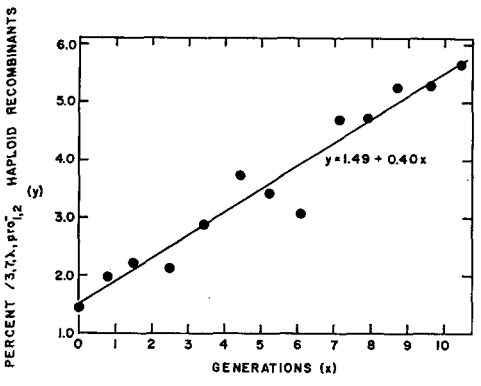

FIGURE 2.—Rate of haploidization for a partial diploid strain. χ98Ex1 was grown in minimal media lacking proline for about 20 generations to reduce the frequency of haploid segregants. Then Penassay broth was inoculated to contain 10^4 cells per ml and the culture was aerated at 37°C. After 90 minutes to reach log phase, samples were taken at various times, diluted and plated on EMB containing 0.1 percent glucose for total count and on this same medium spread with T3 for /3,7,λ,pro$_{1,2}^-$ haploid segregants. The generation time was 34.3 minutes. The equation was calculated by the method of least squares.

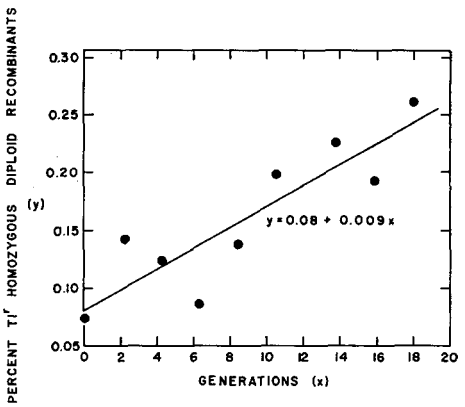

FIGURE 3.—Rate of recombination with retention of the partial diploid state. χ134Ex4 was grown in minimal medium lacking proline with aeration at 37°C. The initial concentration of cells was 10^6 per ml and the culture was twice diluted during the course of the experiment to maintain the log phase of growth. At intervals samples were taken, diluted and plated on minimal agar lacking proline and on this same medium spread with purified T1 phage. The generation time was 57 minutes.

the haploid recombinants, whereas the *T1^s* exogenote allele was incorporated in 66 and 72 percent of the haploid recombinants from χ98Ex1 and χ137Ex2, respectively. Only four out of several thousand haploid segregants analysed had lost the entire exogenote with no detectable recombination. One example of this is listed in the χ98Ex1 data, but it should be noted that with χ137Ex2, there were two recombinants that received only the *ara*$^+$ exogenote allele. Therefore, it is doubtful that loss of the exogenote occurs without genetic recombination since recombination in regions without genetic markers could not be detected.

Rate of recombination with retention of the partial diploid state: When a partial diploid strain heterozygous at the *T1* locus was streaked against T1 on minimal agar lacking proline, T1-resistant, proline-independent colonies were obtained. These were picked, purified and tested and found to be partial diploids that had become homozygous for the *T1^r* allele. These partial diploid recombinants continued to segregate at the *ara*, *leu* and */3,7,λ,pro$_{1,2}$* loci. A low frequency of these *T1^r* homozygous partial diploid recombinants had also become homozygous for the *leu*$^-$ allele or for both *ara*$^-$ and *leu*$^-$ alleles.

Figure 3 shows a plot of the data from an experiment designed to determine the rate of recombination with retention of the partial diploid state. The rate of 0.009 percent per bacterium per generation did not reflect the total rate of recombina-

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TABLE 4

Recombinant types resulting from haploidization in partial diploid strains

	leu+		*T1*^{s}		*pro*$_{1,2}$+	
χ98Ex1*	1–2		3	4		
	thr−	*leu*−		*T1*^{r}	*/3,7,λ,pro*$_{1,2}$−	*lac*−
Genotype of recombinant haploids		Recombination in regions			Number	Percent
leu+ *T1*^{s} */3,7,λ,pro*$_{1,2}$−		1–2 and 4			169	65.0
leu+ *T1*^{r} */3,7,λ,pro*$_{1,2}$−		1–2 and 3			88	33.8
leu− *T1*^{s} */3,7,λ,pro*$_{1,2}$−		3 and 4			2	0.8
leu− *T1*^{r} */3,7,λ,pro*$_{1,2}$−		none			1	0.4
Total					260	100.0
	ara+	*leu*+	*T1*^{s}		*pro*$_{1,2}$+	
χ137Ex2†	1	2	3	4		
	thr− *ara*−	*leu*−	*T1*^{r}		*/3,7,λ,pro*$_{1,2}$−	*lac*−
ara+ *leu*+ *T1*^{s} */3,7,λ,pro*$_{1,2}$−		1 and 4			140	70.0
ara+ *leu*+ *T1*^{r} */3,7,λ,pro*$_{1,2}$−		1 and 3			54	27.0
ara+ *leu*− *T1*^{r} */3,7,λ,pro*$_{1,2}$−		1 and 2			2	1.0
ara− *leu*− *T1*^{s} */3,7,λ,pro*$_{1,2}$−		3 and 4			3	1.5
ara− *leu*+ *T1*^{r} */3,7,λ,pro*$_{1,2}$−		2 and 3			1	0.5
Total					200	100.0

* Conditions as for Figure 2. T3-resistant colonies were picked into 2 ml Penassay broth containing antiserum to T3 at a 1:50 dilution. After growth each individual culture was streaked against T1 and T7 and tested for leucine and proline requirements.

† χ137Ex2 was grown in minimal medium lacking proline for about 20 generations and then was plated on Penassay agar spread with T3. T3-resistant colonies were treated and tested as above.

tion with retention of the partial diploid state at the *T1* locus since those recombinants homozygous for the *T1*^{s} allele were excluded. Random picking and testing of colonies obtained by plating partial diploid strains which were heterozygous at the *T1* locus indicated that *T1*^{r} homozygous partial diploid recombinants (2/2331) were less frequent than those homozygous for the *T1*^{s} allele (7/2331). This observation has been confirmed in studies on spontaneous and UV-induced partial diploid recombinants (CURTISS 1964b). Therefore, the rate of 0.009 percent per bacterium per generation (Figure 3) must be increased by a factor of four to five to account for the exclusion of *T1*^{s} homozygous partial diploid recombinants.

Frequency of haploid and partial diploid recombinants in partial diploid populations: Table 5 presents data on the frequency of recombinant types in cultures of partial diploid strains grown for extended periods in proline-deficient minimal medium. The value of 0.5 percent for total haploid recombinants was in fair agreement with their rate of formation. Haploid recombinants could not grow in this medium for more than a generation or two, since the ability to synthesize the first enzyme(s) involved in proline biosynthesis was lost. Thus, the number of haploid recombinants recovered should have been approximately equal to the number formed in the previous two or three generations, or about 1.0 percent (based on the results shown in Figure 2). This estimate should be reduced, how-

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TABLE 5

*Frequency of haploid and partial diploid recombinants recovered from partial diploid strains grown in proline-deficient minimal medium**

Bacterial type	Percent
A. Partial diploid	98
B. Total haploid recombinants	0.51
C. *T1r* homozygous partial diploid recombinants	0.15
D. *T1r* homozygous partial diploid and *T1r* haploid recombinants	0.30
E. *T1r* haploid recombinants (D minus C)	0.15(29)†
F. *T1s* haploid recombinants (B minus E)	0.36(71)†

* The data are the average of three experiments with χ134Ex4 and χ137Ex2. The bacteria had grown for at least 20 generations in minimal medium lacking proline with aeration at 37°C at the time of sampling. The percentage of each bacterial type was calculated from the following ratios × 100: A. titer on minimal agar minus proline divided by titer on Penassay agar; B. titer on Penassay agar spread with T3 divided by titer on Penassay agar; C. titer on minimal agar minus proline spread with purified T1 divided by titer on Penassay agar; and D. titer on Penassay agar spread with T1 divided by titer on Penassay agar.

† Relative frequencies of *T1r* haploid *vs.* *T1s* haploid recombinants.

ever, to account for the phenotypic lag in expression of resistance to T3. Therefore, the value of 0.5 percent was about what would be expected. The percentages of haploid recombinants in the three experiments were 0.42, 0.52 and 0.58.

The frequencies of *T1r* homozygous partial diploid recombinants varied greatly from experiment to experiment and this variation was reflected in the total number of *T1r* partial diploid and haploid recombinants observed. The relative frequencies of *T1r* and *T1s* haploid recombinants calculated from the data in Table 5 were in good agreement with those in Table 4.

With partial diploid strains having *leu*⁻ and *pro*$_1$⁻ point mutations in the endogenote, the frequency of haploid segregants in cultures grown in minimal medium lacking proline and leucine, was between 20 and 80 percent. About 80 percent of these haploid segregants had incorporated the *leu*⁺ and *pro*$_1$⁺ exogenote alleles by recombination and could therefore grow without leucine or proline.

Conjugation transfer by F⁺ partial diploid donors: The remaining sections of this paper pertain to studies on the mechanism of exogenote and chromosome transfer by F⁺ partial diploid donors. Table 6 presents data from one experiment on conjugation transfer of exogenote and chromosomal markers. Chromosomal markers are those which are not in the exogenote or in the region of the chromosome complemented by the exogenote (endogenote). The only seemingly unusual result in Table 6 is the occurrence of two *thr*⁺ *leu*⁺ *str*^{r} recombinants. This recombinant class was observed on several occasions. Since *leu*⁺ haploid recombinants occurred at a frequency of 5×10^{-3} in partial diploid cultures grown in proline-deficient minimal medium (see Tables 5 and 4), the frequency of *thr*⁺ *leu*⁺ recombinants should be the product of this frequency and the frequency of *thr*⁺ recombinants. Since in other experiments *thr*⁺ recombinants were formed at a frequency of 10^{-5}, then the frequency of *thr*⁺ *leu*⁺ *str*^{r} recombinants should be about 5×10^{-8}. This is not far from the observed frequency of 1.8×10^{-8} (Table 6).

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TABLE 6

*Conjugation transfer by an F+ partial diploid strain**

Donor:	χ152Ex46	F+	*thr*+	*leu*+	*pro*$_{1,2}$+			
Recipient:	χ153	F-	*thr*-	*leu*-	/3,7,λ,*pro*$_{1,2}$-	*T6*^{s}	*try*+	*str*^{s}
					/3,7,λ,*pro*$_{1,2}$-	*T6*^{r}	*try*+	*str*^{r}

Recombinant type	Concentration (cells/ml)	Frequency†
leu+ *str*^{r}	3.4×10^3	3.1×10^{-5}
pro$_{1,2}$+ *str*^{r}	1.6×10^3	1.5×10^{-5}
leu+ *pro*$_{1,2}$+ *str*^{r}	1.4×10^3	1.3×10^{-5}
thr+ *leu*+ *pro*$_{1,2}$+ *str*^{r}	0	0
thr+ *leu*+ *str*^{r}	2	1.8×10^{-8}
try+ *str*^{r}	1.2×10^3	1.1×10^{-5}

* χ152Ex46 and χ153 were grown in proline-deficient minimal medium and in appropriately supplemented minimal medium, respectively. The mating was for 60 minutes in minimal mating medium at 37°C and was interrupted with UV-irradiated T6. The unadsorbed T6 was neutralized with antiserum to T6.

† Based on the initial cell concentration of χ152Ex46 which was 1.1×10^8/ml. The titer of χ153 in the mating mixture was 5.7×10^8/ml.

When *thr*+ *str*^{r} recombinants were selected in a cross between χ152Ex46 and an F- *thr*- *leu*+ *pro*+ *str*^{r} strain, almost all the *thr*+ recombinants were *leu*- and fifty percent were /3,7,λ,*pro*$_{1,2}$-. The data from this cross and the one analysed in Table 6 indicated that the exogenote and endogenote were transferred independently. Data obtained from numerous conjugation experiments with F+ partial diploid and F+ haploid donors indicated that the exogenote had no effect on the transfer of chromosomal markers. The mean frequency of *try*+ recombinants in thirteen experiments with F+ partial diploid donors was 2.5×10^{-5}. With F+ haploid donors the mean frequency of *try*+ recombinants in nine experiments was $2\,3 \times 10^{-5}$. Similar results were obtained when comparing recombination frequencies for other markers.

In crosses with F- strains having a /3,7,λ,*pro*$_{1,2}$- marker, all *leu*+ *pro*$_{1,2}$+ recombinants isolated were partial diploids (Table 7). In these crosses 50 percent of the *leu*+ recombinants were *leu*+ /3,7,λ,*pro*$_{1,2}$- haploids and 50 percent were *leu*+ *pro*$_{1,2}$+ partial diploids. All *pro*$_{1,2}$+ recombinants were partial diploids. After conjugation transfer of the exogenote to F- strains having a /3,7,λ,*pro*$_{1,2}$- mutation, a large amount of recombination for the *T1*^{r} marker took place (Table 7).

Similar conjugation experiments with F+ partial diploid donors were done with F- strains possessing *pro*$_1$- point mutations instead of the /3,7,λ,*pro*$_{1,2}$- marker. The recombination frequencies were the same as obtained with F- strains with the /3,7,λ,*pro*$_{1,2}$- mutation, but the recombinant types were different. Table 8 presents data on the analysis of these recombinants. Approximately 70 percent were haploid upon initial isolation while 30 percent were partial diploids. These partial diploids were unstable in broth and segregated several different types of haploid recombinants at a rate of 5 to 10 percent per bacterium per generation.

Time of entry for exogenote markers: Figure 4 shows the time of entry for the

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TABLE 7

*Recombinant types after conjugation transfer of the exogenote to recipients having a /3,7,λ,pro$_{1,2}^-$ mutation**

Transferred exogenote:	*leu*+	*T1*^{s}	*pro*$_{1,2}$+
Endogenote:	*leu*−	*T1*^{r}	*/3,7,λ,pro*$_{1,2}^-$
Recombinant type		Number	Percent
leu+ *T1*^{s} *pro*$_{1,2}$+ / *leu*− *T1*^{r} */3,7,λ,pro*$_{1,2}^-$		145	85
leu+ *T1*^{s} *pro*$_{1,2}$+ / *leu*− *T1*^{s} */3,7,λ,pro*$_{1,2}^-$		12	7
leu+ *T1*^{r} *pro*$_{1,2}$+ / *leu*− *T1*^{r} */3,7,λ,pro*$_{1,2}^-$		13	8
Total		170	100

* The data are the pooleed results from crosses between χ98Ex11 and χ152, χ152Ex46 and χ134 and χ206Ex6 and χ153 in which *leu*$_{1,2}$+ recombinants were selected.

leu+ and *pro*$_1$+ exogenote alleles from an F+ partial diploid strain. Both markers began to enter at 5 minutes, which showed that there was no oriented order of transmission for the exogenote. Recombinants which had received both the *leu*+ and *pro*$_1$+ exogenote alleles appeared first after 16 minutes of mating. From these results it could be concluded that the exogenote was about 11 minutes long. This

TABLE 8

Recombinant types after conjugation transfer of the exogenote to recipients having a pro$_1^-$ *point mutation**

Transferred exogenote:		*ara*+	*leu*+	*T1*^{s}	*pro*$_1$+
Region:	1	2	3	4	5
Endogenote:		*ara*−	*leu*−	*T1*^{r}	*pro*$_1^-$
Type	Recombination in regions		Number	Percent of total	Percent of haploids
Haploid recombinants					
ara+ *leu*+ *T1*^{s} *pro*$_1$+	1 and 5		102	51.0	74.5
ara+ *leu*+ *T1*^{r} *pro*$_1$+	1, 3, 4 and 5		19	9.5	13.9
ara− *leu*+ *T1*^{s} *pro*$_1$+	2 and 5		11	5.5	8.0
ara− *leu*+ *T1*^{r} *pro*$_1$+	2, 3, 4 and 5		5	2.5	3.6
Total haploid recombinants			137	68.5	100.0
Partial diploid recombinants					
ara+ *leu*+ *T1*^{s} *pro*$_1$+ / *ara*− *leu*− *T1*^{r} *pro*$_1^-$	none		63	31.5	
Total recombinants			200	100.0	

* The data are the pooled results from crosses between χ98Ex11 and χ131 and between χ152Ex46 and χ131 in which *leu*+ *pro*$_1$+ recombinants were selected. The recombinant colonies were carefully picked into minimal medium lacking leucine and proline and after growth were tested for T1 sensitivity and arabinose fermentation. If *leu*+ recombinants were selected, 41 percent were *pro*$_1$+ and if *pro*$_1$+ recombinants were selected, 63 percent were *leu*+.

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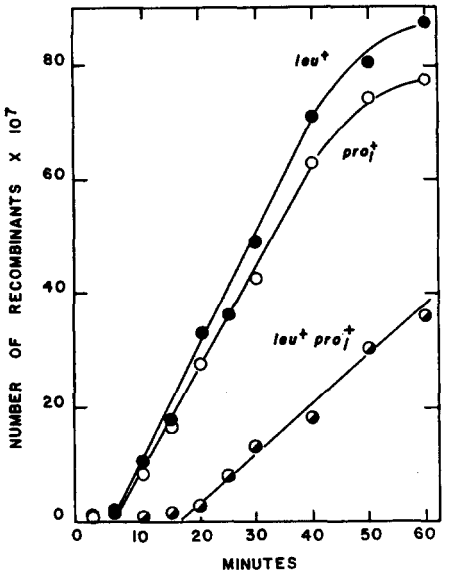

FIGURE 4.—Time of entry for exogenote markers. χ98Ex11 was grown in proline-deficient minimal medium and the mating with χ131 was in minimal mating medium. A cycloserine-resistant mutant of χ131 was used and cycloserine at a final concentration of 20 μg per ml was incorporated into selective minimal agar. The *cyc*^{r} mutation is linked to *met*$_1$ (HARRIS and CURTISS, unpublished). χ98Ex11 and χ131 *cyc*^{r} were at concentrations of 2.1 × 10^8/ml and 2.9 × 10^8/ml in the mating mixture. The mating was interrupted with UV-irradiated T6.

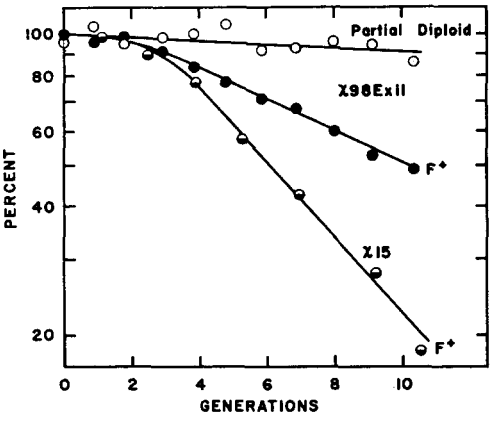

FIGURE 5.—Effect of acridine orange on the loss of F from a partial diploid strain. Samples from the cultures were taken periodically and plated on Penassay agar (χ98Ex11 and χ15) and on proline-deficient minimal agar (χ98Ex11). The titer of χ98Ex11 on proline-deficient minimal agar divided by the titer on Penassay agar × 100 gives the percentage of partial diploids in the culture (top curve). The decline in the frequency of F+ bacteria in the χ98Ex11 culture is plotted in the middle curve and in the χ15 culture in the bottom curve. The generation times for χ98Ex11 and χ15 were 87 and 69 minutes.

agreed with the estimate of 9.5 minutes which was based on complementation studies and map distances (see Table 3 and Figure 1).

Mating type of exconjugants in crosses with F+ partial diploids: In crosses between F+ partial diploids and F- recipients, 70 percent of the *leu*$^+$ *pro*$_1$$^+$ *T6*^{r} recombinants became F+ (335 out of 476 tested). In these experiments the ratio of donor to recipient cells was 1:5 and the donor strains were killed with T6 to prevent infection of recombinant colonies with F on the plates. Data on the mating type of nonrecombinants were obtained by growing equal concentrations of F+ partial diploid and F- bacteria together for 2 hours and then diluting and plating on media that would only support the growth of the F- strain. Only 18 percent (57 out of 318 tested) of the nonrecombinants were changed to F+. (The transfer of F by partial diploid strains was not as rapid as by F+ haploid strains. Under similar conditions χ42 transferred F to 70 to 90 percent of the nonrecombinant F- population.) The results of these experiments indicated that the exoge-

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note could be transferred without the simultaneous transfer of F and vice versa.

Effect of acridine orange on an F+ partial diploid strain: The lack of oriented transfer of the exogenote and the observed independent transfer of F and the exogenote, suggested that the F particle was not attached to the exogenote. An attempt was thus made to cure F+ partial diploid strains of the F particle by acridine orange treatment (HIROTA 1960). The results of this experiment are presented in Figure 5. Acridine orange did not bring about the loss of the exogenote, but did cure the partial diploid strain of F. The haploidization rate in this experiment was one percent per bacterium per generation. The difference in the rate of removal of F from χ98Ex11 as compared to χ15 was reproducible.

Fluctuation tests on the transfer of exogenote and chromosomal markers: JACOB and WOLLMAN (1956) showed that in a F+ × F− cross, the recombinants formed were due to mutations from the autonomous state of F to the attached state. To prove this point they used the fluctuation test of LURIA and DELBRÜCK (1943) and observed a large fluctuation in the number of recombinants arising from matings between F− bacteria and samples from individual F+ cultures.

Line 1 of Table 9 presents data from a fluctuation experiment with χ98Ex11 to determine whether the autonomous F particle attached to the exogenote. There was no fluctuation for the transfer of the entire exogenote and the mean number of *leu*+ *pro*$_1$+ recombinants per plate was approximately equal to the variance. Likewise, there was no fluctuation in the number of *leu*+ or *pro*$_1$+ recombinants observed from separate matings. (The low value of P calculated for the *pro*$_1$+ recombinants does not reflect a significant fluctuation since no correction was made for the observed variation of 100 percent in the final cell concentrations among the individual F+partial diploid cultures.) These results indicated that

TABLE 9

*Fluctuation experiments**

	Donor	Recipient	Recombinant type selected	Number of cultures	Number of recombinants: Range Low	Range High	Mean	Variance	Chi square	P
(1)	χ98Ex11	χ131	*leu*+ *pro*$_1$+	19	23	42	31.0	24.6	15.1	0.7
			leu+	19	62	100	79.8	105.1	25.0	0.15
			pro$_1$+	19	19	40	27.4	47.7	33.1	0.03
(2)	χ98Ex11	χ44	*try*+ *his*+	62	0	12	5.0	5.5	68.6	0.4
(3)	χ42	χ148	*leu*+ *str*^{r}	20	0	171	17.2	1647	1915	<0.001
			ade$_3$+ *str*^{r}	20	0	75	8.4	444	1058	<0.001
			try+ *str*^{r}	20	0	87	9.8	580	1184	<0.001
(4)	χ45 F+	χ148	*leu*+ *str*^{r}	20	0	227	25.2	3586	2848	<0.001
			ade$_3$+ *str*^{r}	20	0	185	14.7	1878	2555	<0.001
			try+ *str*^{r}	20	0	324	24.6	5747	4673	<0.001

* Cultures of donor strains were grown in appropriately supplemented minimal media (partial diploid strains in proline-deficient minimal media). Each 1 ml culture was descended from about 400 cells and after growth had ceased, 0.1 ml samples were mixed with 0.4 ml of F− cells in minimal mating media in Wasserman tubes at 37°C. Mating was for 80 minutes.

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the F particle did not attach as a mutation-like event either to the ends or to the middle of the exogenote to aid in its transfer.

The striking feature of the χ98Ex11 × χ44 cross (line 2, Table 9) was the absence of fluctuation in the number of *try*$^+$ *his*$^+$ recombinants. Of the many fluctuation tests done with F$^+$ partial diploid strains no significant fluctuations were observed for the transfer of exogenote or chromosomal markers. In some of these experiments the donor cells were killed with T6 and streptomycin to make sure that the absence of a fluctuation was not due to mating on the plates. In other experiments mating was interrupted after 20 minutes instead of 80 minutes. Others were done with cells grown and mated in broth and others with log phase F$^+$ partial diploid donors grown either in broth or in proline-deficient minimal media. Since these results with F$^+$ partial diploid strains were at variance with the scheme of JACOB and WOLLMAN (1956), several types of control experiments were done. The data from a fluctuation experiment with the F$^+$ strain which had been used to infect the original F$^-$ partial diploid strain with F, supported JACOB and WOLLMAN'S (1956) model (line 3, Table 9). JACOB and WOLLMAN (1956) noted that the types of recombinants formed in each individual mating were usually the same. The point was confirmed in this experiment.

To determine whether the F particle had been altered during its sojourn in χ98Ex11, it was returned to χ45, an F$^-$ strain isolated from χ42 by acridine orange treatment. These χ45 F$^+$ strains gave large fluctuations in the number of recombinants obtained from matings with individual F$^+$ cultures (line 4, Table 9). Thus the F particle had not been modified.

In order to determine whether the exogenote prevented attachment of F to the chromosome, two types of experiments were done. The first of these employed haploid recombinants isolated from χ98Ex11. For the other type of experiment, all F$^-$ strains which had chromosomes homologous to the chromosomes of partial diploid strains were infected with the F particle from χ42. No fluctuations in the number of recombinants were observed in these two types of experiments. These results indicate that in some F$^+$ strains Hfr mutants do not account for the recombinants formed when they are mated with F$^-$ strains. This conclusion, based on different kinds of experimental evidence, has also been made by LEDERBERG (1958), REEVES (1960) and HAYES (1960).

DISCUSSION

The partial diploid strain described in this report is diploid for about 9 percent of the *E. coli* K-12 genome. Its stability can be ascribed to the presence of the pleiotropic deletion mutation, */3,7,λ,pro$_{1,2}^-$* (CURTISS 1964a) in the endogenote. This conclusion is based on the observation that the rates of haploidization per bacterium per generation were 0.4 percent for partial diploid strains which had the */3,7,λ,pro$_{1,2}^-$* mutation in the endogenote and 5 to 10 percent for partial diploid strains which had *pro$_1^-$* point mutations in the endogenote. A further indication of the stabilizing effect of the */3,7,λ,pro$_{1,2}^-$* mutation was evident in experi-

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ments in which the exogenote was transferred to F⁻ recipients. When the F⁻ had the */3,7,λ,pro$_{1,2}$⁻* mutation, all of the *pro$_{1,2}$*⁺ recombinants were partial diploids. By contrast, only 30 percent of the *pro$_1$*⁺ recombinants were partial diploids when the F⁻ had a *pro$_1$*⁻ point mutation.

The data in Table 4 indicate that recombination is obligatory to obtain haploidization. This conclusion has been confirmed in experiments on UV-induced haploidization with χ137Ex2 (CURTISS 1964b). To explain this, it is logical to postulate that the exogenote can only be lost by being physically consumed in the process of recombination. This explanation is supported by the finding that UV-induced genetic recombination occurs by a conservative mechanism (CURTISS 1963, 1964b).

Since the mean number of exogenotes per endogenote is about 1.1 (CURTISS 1963, 1964b) and loss of the exogenote without recombination is not observed, it must be concluded that the exogenote replicates synchronously with the endogenote. To obtain synchronous replication, it is postulated that the exogenote is normally synapsed with the endogenote. This synapsis can not be caused by any physical attachment since the exogenote and endogenote are transferred independently in conjugation experiments. Synapsis of the exogenote with the endogenote in the region of the *ara* and *leu* loci should be normal and consequently recombination could occur freely. The */3,7,λ,pro$_{1,2}$⁻* deletion mutation should interfere with synapsis between the *pro$_{1,2}$*⁺ end of the exogenote and the endogenote. This effect in preventing synapsis, and thus recombination, should decrease proportionately with increasing distance from the */3,7,λ,pro$_{1,2}$⁻* marker. This reasoning is supported by the observation that exogenote alleles are incorporated into haploid recombinants at frequencies which increase in direct proportion to the distance from the *pro$_{1,2}$*⁺ end of the exogenote.

With F⁺ partial diploid strains the exogenote was transferred independently of endogenote and chromosomal markers. Since recombination frequencies obtained for chromosomal markers donated by F⁺ partial diploid and F⁺ haploid donors were the same, it can be concluded that the presence of the exogenote did not interfere with the transfer of the chromosome. This is quite different from the behavior of the resistance transfer factor which does interfere with the transfer of chromosomal markers (WATANABE and FUKASAWA 1962). This difference is not too surprising however, since the resistance transfer factor can act as its own vector in conjugation, whereas the exogenote in χ98Ex1 cannot.

In regard to the mechanism of exogenote transfer, it can be concluded that the F particle and the exogenote are always separate entities in the cell and that the principal function of F is to permit effective conjugal pairs to form. This conclusion is based on a number of observations. First, time of entry experiments indicated that the exogenote entered the F⁻ recipient in an unoriented manner. This finding is just the opposite of the situation found for an exogenote attached to an F particle (HIROTA and SNEATH 1961). They found that the F-attached exogenote was transferred in an oriented manner with the attached F particle entering the recipient last. Second, the exogenote could be transferred without transfer of F and vice versa. Third, acridine orange could be used to cure the

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partial diploid strain of F without affecting the exogenote. Fourth, F^+ partial diploid strains which had become haploid by recombination remained F^+ and F agent could still be cured by acridine organge. Lastly, all fluctuation experiments to test for attachment of F to any part of the exogenote gave results which indicated that this attachment did not occur.

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SUMMARY

A stable partial diploid strain of *Escherichia coli* K-12 was isolated which was diploid for the *ara* to pro_2 region of the chromosome (9 percent of the genome). The rate per bacterium per generation for haploidization was 0.4 percent, and for recombination with retention of the partial diploid state was 0.04 percent. Recombination was obligatory to obtain haploidization. The stability of this strain was due to the nature of the $/3,7,\lambda,pro_{1,2}^-$ deletion mutation in the endogenote since the same exogenote in a strain with a pro_1^- point mutation in the endogenote underwent haploidization at a rate of 5 to 10 percent per bacterium per generation.

Since the original partial diploid strain was F^-, it was infected with the F particle for conjugation experiments. Conjugation transfer of the exogenote occurred at frequencies of about 10^{-5}, and if the F^- recipient had a pro_1^- point mutation, 70 percent of the leu^+ pro_1^+ recombinants were haploid and 30 percent were partial diploids. If the F^- recipient had a $/3,7,\lambda,pro_{1,2}^-$ mutation, all leu^+ $pro_{1,2}^+$ recombinants were partial diploids. Exogenote and chromosomal (or endogenote) markers were transferred independently of each other and the exogenote did not affect the frequency of chromosomal marker transfer.

In time of entry experiments both leu^+ and pro_1^+ recombinants began to appear 5 minutes after mixing, thus indicating that the exogenote was transferred in an unoriented manner. The exogenote could be transferred independently of the F particle and vice versa. Acridine range cured the partial diploid strain of F but had no effect on the exogenote. Fluctuation experiments to determine whether F attached to the exogenote to aid directly in its transfer indicated that F did not attach. From these experiments it was concluded that F was necessary only to permit conjugal pairing.

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