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GENE TRANSFER BY F′ STRAINS OF *ESCHERICHIA COLI*

IV. Effect of a Chromosomal Deletion on Chromosome Transfer

JAMES PITTARD[1] AND T. RAMAKRISHNAN[2]

Department of Microbiology, Yale University, New Haven, Connecticut

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Abstract

Pittard, James (Yale University, New Haven, Conn.), and T. Ramakrishnan. Gene transfer by F′ strains of *Escherichia coli*. IV. The effect of a chromosomal deletion on chromosome transfer. J. Bacteriol. **88**:367-373. 1964.—Evidence is presented that in an F′ strain of *Escherichia coli*, AB1206, the chromosomal region corresponding to the merogenote of F_{14} is deleted. AB1206 differs from all other F′ strains in its stability and in its relative inability to transfer chromosomal markers under conditions in which transfer of the merogenote occurs at a frequency of 100%. Its stability can be accounted for by the fact that the loss of the F merogenote, which contains approximately 10% of the genetic information of the cell, would be a lethal event. The failure of AB1206 to transfer chromosomal markers, except at very low frequencies, can be explained in terms of the crossover model proposed to explain chromosome transfer by F′ strains, since the homologous chromosomal region is missing in this strain. The conclusion that the chromosomal region is deleted is strengthened by the finding that the different enzymes coded for by the genes on F_{14} are present in AB1206 at the level found in haploid strains, but have double this activity in homozygous diploids carrying F_{14}.

In a previous communication (Pittard, Loutit, and Adelberg, 1963), a brief analysis was presented of the differences between an F′ strain, AB1206, and a number of related F′ strains which were formed when strain AB1206 transferred its F merogenote (F_{14}) to suitable recipients. Strains which had received F_{14} from AB1206 were termed "first generation males." Such strains are unstable merodiploids, segregating F^- females at a very high rate. Relatively pure cultures of first-generation males can be effectively maintained only by growth in minimal medium, in which selection is made for cells retaining the wild-type alleles of F_{14}. When acting as donors, first-generation males transfer chromosomal markers with the same polarity and with approximately one-tenth of the frequency obtained in the corresponding cross with the Hfr strain (AB313) from which the F merogenote was initially derived. Strain AB1206, on the other hand, is very stable and does not spontaneously segregate F^- females at a detectable rate. In addition, strain AB1206 transfers chromosomal markers at a frequency that is 10^{-3} to 10^{-4} times less than the frequency observed in crosses with first-generation males.

Analyses of chromosome transfer by F′ strains of *Escherichia coli* (Pittard et al., 1963; Pittard and Adelberg, 1963, 1964; Scaife and Gross, 1963) have demonstrated that the transfer of chromosomal markers requires recombination between the F merogenote and its homologous chromosomal region. Such an event has been termed a donor crossover. It is the purpose of this paper to establish that in strain AB1206 the chromosomal region corresponding to the merogenote of F_{14} is missing. We believe that the failure of strain AB1206 to transfer chromosomal markers at normal frequencies is a direct consequence of the inability of F_{14} to pair with the chromosome and thus undergo donor crossovers.

Materials and Methods

Organisms. The strains used in this work are described in Table 1. They are all derivatives of *E. coli* K-12.

Media and culture methods. The media and culture methods used in this work were described previously (Adelberg and Burns, 1960).

Mating conditions. The mating conditions described by Adelberg and Burns (1960) were used. When necessary, unstable F′ strains were grown in liquid minimal medium prior to mating but were diluted into complete medium when mixed with the recipient strain.

[1] Present address: Department of Bacteriology, University of Melbourne, Victoria, Australia.

[2] On leave from Pharmacology Laboratory, Indian Institute of Science, Bangalore, India.

TABLE 1. *List of strains**

Strain no.	Auxotrophic characters										Energy source utilization					Response to		Sex
	ile	*ilva*	*arg*	*met*	*thi*	*thr*	*leu*	*pro*	*try*	*his*	*gal*	*lac*	*mal*	*xyl*	*tpa*	T6	Str	
AB 1171	+	7	3	+	1	+	+	2	+	4	2	1	+	5	+	S	S	♀
AB 1203	+	7	3	+	1	+	+	+	+	+	2	1,4	+	5	+	R	S	♀
AB 1276	+	+	+	+	1	+	+	+	+	4	2	+	+	+	+	S	S	♀
AB 1440	+	+	+	+	1	+	+	+	+	4	+	+	+	+	+	S	R	♀
AB 1450	+	16	1	1	2	+	+	+	+	1	1	8	1	4	+	R	R	♀
AB 1457	+	+	+	+	1	+	+	2	+	4	2	1	+	+	+	R	R	♀
AB 1469	+	7	3	+	1	+	+	2	+	4	2	1	+	+	+	S	S	♀
AB 2147	+	+	192	1	1	2	+	+	+	+	+	8	1	4	1	R	R	♀
AB 1206	+	+	+	+	1	+	+	2	+	4	2	1	+	+	+	S	R	♂ F14
AB 1516	+	+/+	+/7	+/3	+/1	+	+	2	+	4	2	1	+	+	+	S	R	♂ F14
AB 1278	+	65	+	+	1	+	+	2	+	4	2	1	+	+	+	S	S	♂ F′
AB 313	+	+	+	+	1	+	+	2	+	+	—	—	+	+	+	S	R	♂ Hfr
AB 1234	+	+	+	+	1	+	+	2	+	4	2	1	+	+	+	S	R	♂ Hfr
AB 1461	+	7	+	+	1	+	+	2	+	4	2	+	+	+	+	S	R	♂ Hfr

* The following abbreviations have been used in the table and the text: *ile*, isoleucine; *ilva*, isoleucine and valine; *val*, valine; *arg*, arginine; *met*, methionine; *thi*, thiamine; *thr*, threonine; *leu*, leucine; *pro*, proline; *try*, tryptophan; *his*, histidine; *ser*, serine or glycine; *xyl*, xylose; *mtl*, mannitol; *mal*, maltose; *lac*, lactose; *gal*, galactose; T6, bacteriophage T6; *str*, streptomycin; R, resistant; S, sensitive; *tpa*, tryptophan synthetase. Numbers refer to allele numbers that have been allotted to mutant loci in these laboratories; — = not done.

Transduction. In general, the methods described by Lennox (1955) were used. The bacteriophage used was P1Kc. Bacteriophage and cells were mixed in the proportions of approximately 2:1, and 30 min were allowed for adsorption at 37 C.

Preparation of cell-free extracts. Cells harvested at 14 to 15 hr, while still in the logarithmic phase, were suspended in 5 volumes of 0.03 M phosphate buffer (pH 7.0) and subjected to ultrasonic oscillation in the cold in a MSE ultrasonic disintegrator (Instrumentation Associates, New York, N.Y.) for 2 min. The solution was centrifuged at 0 C for 20 min at 11,000 × *g* in a Servall superspeed centrifuge.

Enzyme assays. The assay for the condensing enzyme was performed according to the method described by Radhakrishnan and Snell (1960). The substrate used was sodium pyruvate, and the reaction was carried out at pH 8.0. Reductoisomerase was assayed according to the method of Armstrong and Wagner (1961). Dihydroxy acid dehydrase was assayed according to the method of Myers (1961). Transaminase B (Rudman and Meister, 1953) was assayed according to the method of Umbarger (*personal communication*); α-ketoglutarate and L-valine were used as substrates, and the formation of α-ketoisovalerate was measured by dinitrophenylhydrazine after extraction with toluene to separate it from α-ketoglutarate. Threonine deaminase was assayed according to the method of Umbarger and Brown (1957). Acetylornithinase was assayed according to the method of Vogel and Bonner (1956). Argininosuccinase was assayed according to the method of Ratner (1955).

RESULTS

Isolation and properties of strain AB1206. Strain AB1206 was isolated as an *ilva*$^+$ *arg*$^+$ recombinant when Hfr strain AB313 was mated with F$^-$ strain AB1171. The mating was interrupted at 70 min by blending and by killing the male parent with bacteriophage T_4. The genotypes of strain AB1206 and of each of the parent strains are shown schematically in Fig. 1. It can be seen that AB1206 possesses the proximal markers *xyl*$^+$ and *str-r* and the distal markers *arg-3*$^+$, *ilva-7*$^+$, and sex factor of the Hfr strain AB313, but still retains the *thr*$^+$, *leu*$^+$, *his-4*, and *pro-2* alleles of the recipient. (For a description of symbols used, see Table 1.) Because the ratio of males to females used in this mating was greater than one, however, we cannot be sure

that the zygote which gave rise to AB1206 received both the proximal and distal genes from the same Hfr cell.

As shown in Fig. 1, the distal markers *arg-3*$^+$ and *ilva-7*$^+$ of the Hfr parent did not become integrated into the chromosome of strain AB1206, but are carried on the F merogenote, F_{14}. This F merogenote carries a number of genetic loci concerned with the biosynthesis of methionine (*met*), arginine (*arg*), and isoleucine-valine (*ilva*). [A detailed description of the markers carried by F_{14} was published previously (Pittard et al., 1963).] During conjugation, the markers on F_{14} are transferred to the recipient at high frequencies and in a linear order, *ilva*$^+$ and sex factor (F) always being transferred last.

Stability of strain AB1206. By analogy with other known F′ strains, it was initially assumed that AB1206 was diploid for the F_{14} region and that it possessed at least two copies of the loci carried on F_{14}. Since the female parent, AB1171, had carried the mutations *ilva-7* and *arg-3*, strain AB1206 was expected to be heterozygous $+/-$ for both of these genes. However, attempts to demonstrate the presence of these mutant alleles were unsuccessful. No spontaneous or acridine-induced *ilva-7* or *arg-3* segregants were found (Pittard et al., 1963). When a population of cells of strain AB1206 was treated with acridine orange, however, 70% of the clones that were recovered now behaved as *ilva*$^+$ *arg*$^+$ Hfr males transferring markers with the same frequencies and in the same linear order as did Hfr strain AB313. Table 2 shows the recombination frequencies for the markers *xyl*$^+$ and *ilva*$^+$ when one of these Hfr males, AB1234, and the original F′

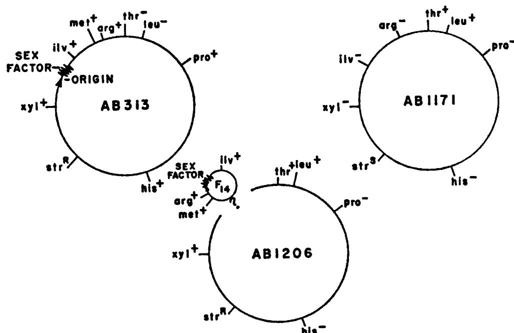

FIG. 1. *Schematic representation of the genotypes of strain AB1206 and of each of the parent strains.*

TABLE 2. *Comparison of the recombination frequencies for xyl*$^+$ *and ilva*$^+$ *when Hfr strain AB1234 and F′ strain AB1206 are the donors*

Cross	No. of *xyl*$^+$ recombinants per ml	No. of *ilva*$^+$ recombinants per ml
AB1234 × AB1203	10^6	3×10^4
AB1206 × AB1203	10^3	4×10^6

strain, AB1206, are mated with the *xyl*$^-$ *ilva*$^-$ female, AB1203.

The recovery at high frequency of clones of isoleucine-valine-independent, arginine-independent Hfr males from a culture of strain AB1206 that had been treated with acridine orange was unexpected. Later in this paper we shall return to these Hfr strains and to the problem of their formation from strain AB1206.

Attempts to form a stable first-generation male. In contrast to the behavior of strain AB1206, first-generation males which are heterozygous $(+/-)$ for *ilva-7* and *arg-3* segregate *ilva-7 arg-3* females at a very high rate; 50% or more of the clones recovered, after such a male has grown in complete medium for 18 hr, are *arg-3 ilva-7* females which have lost F_{14}. Treatment with acridine orange only further enhances this instability.

In an attempt to discover some of the factors affecting the stability of F_{14}, a variety of first-generation males were produced by the transfer of F_{14} from AB1206 to different recipients. The recipient strains which were chosen possessed different genotypes for the chromosomal region corresponding to F_{14}. Since attempts to remove F_{14} from strain AB1206 had failed, nothing was known about this chromosomal region in AB1206, but it seemed likely that the stability of this strain was in some manner related to a genetic aberration in this region. Some recipients were also chosen because they had received the region *Origin-str* from Hfr AB313. It will be remembered that strain AB1206 also received these genes from strain AB313, and it was thought possible that *Origin* might be exerting some stabilizing effect on F_{14}. The results (Table 3) show that no stable F′ strains were obtained with any of these recipient strains. It is of particular interest that even the female strain AB1457, derived from strain AB1234 by further treatment with acridine orange, gave rise only to un-

TABLE 3. *Description of different recipients used in crosses with strain AB1206 or with a tryptophan-requiring derivative of AB1206**

Recipient	Relationship to strain AB1234, AB1171, or AB1206	Stability of F′ males formed from these recipients
AB1203	*his*$^+$ *pro*$^+$ derivative of strain AB1171	Unstable
AB1469	*xyl*$^+$ *mal*$^+$ *str-r* derivative of strain AB1171	Unstable
AB1276	*ilv*$^+$ *arg*$^+$ derivative of strain AB1171	Unstable
AB1440	*Origin xyl*$^+$ *str-r ilva*$^+$ *arg*$^+$ derivative of strain AB1171	Unstable
AB1234	Hfr strain derived from F′ strain AB1206	No F′ males formed†
AB1461	*ilv*$^-$ derivative of strain AB1234	No F′ males formed†
AB1457	Female strain derived from Hfr AB1234 by treatment with acridine orange	Unstable
AB356	Female strain unrelated to strain AB1171	Unstable
AB1450	Female strain unrelated to strain AB1171	Unstable
AB1171		Unstable

* All the recipients except AB1234 and AB1461 became unstable first-generation males when they received F_{14}.

† When F_{14} was transferred to the Hfr males AB1234 and AB1461, no F′ males were formed. In the case of AB1461, it was possible to select for *ilva*$^+$ recombinants having received the wild-type *ilva*$^+$ allele from F_{14}. Although *ilva*$^+$ recombinants were recovered at a high frequency, none of 400 tested had retained the F merogenote F_{14}.

stable F′ males. AB1234, it will be recalled, is a stable Hfr derived directly from AB1206. It seems, therefore, that whatever is apparently stabilizing F_{14} in strain AB1206 is no longer present in either strain AB1234 or AB1457.

Isolation of ilva$^-$ *mutants from strains AB1206.* The structural genes for the enzyme threonine deaminase and for the four common enzymes involved in the biosynthesis of isoleucine and valine (condensing enzyme, reductoisomerase, dihydroxy acid dehydrase, and transaminase B), are all clustered together near the terminal end of F_{14} (Pittard et al., 1963). Strain AB1171 carries the *ilva-7* mutant allele and hence possesses no reductoisomerase activity. If strain AB1206 still retains the chromosomal genes of strain AB1171 for the F_{14} region, it should be heterozygous (+/−) for the reductoisomerase gene and homozygous (+/+) for the genes controlling the formation of condensing enzyme, threonine deaminase, dihydroxy acid dehydrase, and transaminase B. Under these circumstances, selection of *ilva*$^-$ mutants in strain AB1206 might be expected to yield mainly mutants that are deficient in reductoisomerase activity, since such mutants could arise as a result of a single mutation in the wild-type reductoisomerase gene carried on F_{14}. Mutants deficient in condensing enzyme, dihydroxy acid dehydrase, threonine deaminase, or transaminase B would be very rare, since they could arise only if mutations in the same cistron occurred simultaneously on both F_{14} and chromosome, or if mutations which occur on either F-merogenote or chromosome are transferred to the homologous structure by some "copy-choice" mechanism.

When strain AB1206 was treated with different mutagens, a number of *ilva*$^-$ derivatives were obtained. There was no preponderance of reductoisomerase-deficient strains, however, and strains deficient in threonine deaminase, reductoisomerase, dehydrase, and transaminase B were all isolated. These results indicate that strain AB1206 has not retained the *ilva-7* allele of strain AB1171. Furthermore, the fact that *ilva*$^-$ mutants can be readily obtained carrying mutations in the dehydrase, reductoisomerase, transaminase, or threonine deaminase loci can only mean that in strain AB1206 the wild-type alleles of these loci are present in the haploid condition. Since they are present on F_{14}, it would appear that this region of the chromosome is deleted in strain AB1206. (Despite exhaustive screenings, no mutants deficient in the condensing enzyme have yet been isolated in any strains of *E. coli* K-12.)

Reversion of ilva$^-$ *mutants of AB1206.* Further support for the conclusion that AB1206 has a chromosomal deletion of the *ilva* region comes from studies on the reversion of an induced *ilva*$^-$ mutant of AB1206.

Strain AB1278 is an *ilva*$^-$ mutant derivative of AB1206. Extracts of this strain lack dehydrase activity; the strain will grow on the keto acid

precursors but not the dihydroxy acid precursors of isoleucine and valine; and the F_{14} which this strain carries will, when transferred to F^- recipients, complement reductoisomerase mutants but not dehydrase mutants. Thus, the F_{14} of strain AB1278 carries a mutation (*ilva-65*) in the structural gene for the dehydrase enzyme.

If AB1206 had been diploid for the *ilva* region, induction of a mutation in the dehydrase locus on F_{14} could only have produced the auxotrophic phenotype of AB1278 if the chromosome also carried a mutation in the dehydrase locus. If the chromosomal locus were the same as *ilva-65* (perhaps resulting from some type of recombination between F_{14} and the chromosome), then reversion of AB1278 should in half the cases yield diploids of the type $ilva^+F/ilva$-*65* and in the other half of the cases diploids of the type *ilva-65* $F/ilva^+$. Only the former would produce $ilva^+$ diploids by the transfer of F_{14} to F^- dehydrase mutants. On the other hand, if AB1206 and its derivative, AB1278, had been haploid for the *ilva* region, carrying the *ilva* loci on F_{14} only, all of the revertants of AB1278 would produce $ilva^+$ diploids by the transfer of F_{14} to F^- dehydrase mutants.

Fourteen $ilva^+$ revertants of AB1278 were induced with diethylsulfate and were crossed with the dehydraseless F^- strain, AB1450. All 14 produced $ilva^+$ diploids at high frequency (about 30%), as predicted by the haploid model.

The above test depended on the assumption that, if diploid, AB1278 carried identical *ilva-65* mutations on both chromosome and on F_{14}. It still seemed possible (although highly unlikely) that AB1278 was a diploid carrying two different mutations in the dehydrase locus. When such a noncomplementing diploid is constructed, however, by transferring the F_{14} of strain AB1278 to a different dehydrase mutant such as AB1450, a diploid is obtained which gives rise to $ilva^+$ recombinants at an elevated frequency. For example, if strain AB1278 is crossed with strain AB1450 and the zygotes are plated on minimal agar, about 1% of the zygotes give rise to $ilva^+$ recombinant clones.

In contrast, AB1278 is exceedingly stable. Only one $ilva^+$ revertant was obtained when 5×10^9 cells were plated on minimal medium. It can thus be ruled out that AB1278 is a heterozygous diploid carrying two different dehydrase mutations.

Finally, it is evident that the behavior of AB1278 cannot be explained by assuming that this strain has retained the *ilva-7* (reductoisomerase) locus of AB1171. A diploid of the type *ilva-65* *F/ilva-7* has been constructed, and has been found to have the $ilva^+$ phenotype as the result of complementation. Since AB1278 is phenotypically $ilva^-$, this cannot be its genotype.

All of the above results are incompatible with any diploid model for AB1278, and can only be explained by assuming that AB1278, and hence AB1206, has a deletion of the *ilva* region of the chromosome.

Interruption of linkage between the tpa locus and the ilva loci in strain AB1206. The locus *tpa*, which codes for the structure of the enzyme tryptophanase, and the cluster of isoleucine-valine loci, *ilva*, are situated very close together on the chromosome of *E. coli* K-12. Transducing lysates of bacteriophage P1 prepared on a tpa^+ $ilva^+$ female cotransduce these two markers to a tpa^- $ilva^-$ female at a frequency of approximately 25%. In the male strain AB313 (the Hfr strain from which AB1206 was originally derived), sex factor is integrated into the chromosome between *tpa* and *ilva*. In F′ strains, however, the sex factor is no longer integrated into the chromosome, so that, in the case of first-generation males, the loci *tpa* and *ilva* are still very closely linked on the chromosome, even though the F merogenote, F_{14}, carries the *ilva* genes but not the *tpa* gene.

Phage prepared on such a first-generation male, AB1516, was used to transduce $ilva^+$ into the tpa^- $ilva^-$ female AB1247; 30% of the isoleucine-valine independent transductants were found to have integrated the *tpa* gene. When phage which had been prepared on AB1206 was used in the same experiment, no cotransduction of tpa^+ and $ilva^+$ was observed. In this case, approximately 20,000 tpa^+ transductants were tested for the inheritance of $ilva^+$ by use of the replica plating technique, and 100 isoleucine-valine independent transductants were tested for tryptophanase activity. In neither case were tpa^+ $ilva^+$ recombinants found. This failure to cotransduce these markers from AB1206 adds further support to the conclusion that the chromosomal region containing the isoleucine-valine genes is missing from AB1206.

Comparison of enzyme levels in haploid and F′ strains. Jacob and Monod (1961) showed that F′ cells carrying the F merogenote *F-lac* possess two to three times more β-galactosidase than do corre-

TABLE 4. *Comparison of specific activities of different enzymes in crude extracts prepared from AB1276, AB1206, and homozygous +/+ first-generation males**

Enzyme	Specific activities in crude extracts from		
	Haploid F⁻ AB1276	AB1206	Homozygous +/+ first-generation male
L-Threonine deaminase	1.72	1.70	3.50
Dihydroxy acid dehydrase	0.70	1.00	2.00
Transaminase B	1.35	1.30	2.40
Condensing enzyme	0.52	0.50	0.81
Reductoisomerase	0.22	0.20	0.39
Acetylornithinase	2.50	1.50	3.40
Argininosuccinase	0.31	0.30	0.62

* Specific activities are expressed as micromoles of substrate converted or product formed per milligram of protein per hour. The reaction mixtures for enzyme assay contained the following (μ-moles). L-Threonine deaminase contained in 1 ml: phosphate, 100; cysteine, 250; L-threonine, 400; cell-free extract, 0.8 mg of protein. Dihydroxy acid dehydrase contained in 1 ml: tris(hydroxymethyl)aminomethane (tris) buffer (pH 7.8), 200; cysteine, 250; ferrous sulfate, 50; DL-α,β-dihydroxyisovalerate, 50; cell-free extract, 0.8 mg of protein. Transaminase B contained in 1 ml: tris buffer (pH 7.8), 200; pyridoxal phosphate, 100; α-ketoglutarate, 125; L-valine, 250; cell-free extract, 0.8 mg of protein. Condensing enzyme contained in 1 ml: phosphate buffer (pH 8), 200; thiamine pyrophosphate, 4; manganous sulfate, 0.5; sodium pyruvate, 300; cell-free extract, 0.8 mg of protein. Reductoisomerase contained in 1 ml: tris buffer (pH 7.4), 200; β-mercaptoethanol, 5; magnesium sulfate, 2.5; α-acetolactate, 20; boiled enzyme, 0.1 ml; cell-free extract, 0.8 mg. Nicotinamide adenine phosphate type II (0.1 μmole) was used to start the reaction. Acetylornithinase contained in 0.5 ml: phosphate buffer (pH 7), 100; glutathione, 0.5; cobaltous chloride, 0.1; *N*-α-acethylornithine, 3; cell-free extract, 0.8 mg. Argininosuccinase contained in 1 ml: phosphate buffer (pH 7.4), 200; sodium argininosuccinate, 5; arginase containing 6 Van Slyke-Archibald units, 0.1 ml.; cell-free extract, 0.8 mg.

sponding haploid strains. Garen and Garen (1963) reported a similar increase in the levels of alkaline phosphatase in cells carrying the F merogenote F_{13}. This increase in the level of enzyme activity is thought to result directly from an increase in the number of copies of a given structural gene per cell, under conditions in which total cell protein is not significantly altered. In an attempt to elucidate further the ploidy of strain AB1206, this strain, together with certain first-generation males and a haploid prototrophic female (AB1276), were all examined for the levels of different enzymes coded for by the genes on F_{14}. The first-generation male that was used in a given experiment was always constructed so that it was homozygous +/+ for the allele under investigation. For example, for the assay of all the isoleucine-valine enzymes and for the enzyme acetylornithinase, the first-generation male that was used was made by transferring F_{14} into a *met-1 arg-1 ilva*$^+$ female. On the other hand, when the enzyme argininosuccinase was being measured, the first-generation male was formed by use of an *arg-3 ilva-7* female. The *arg-3*$^+$ allele codes for the enzyme acetylornithinase, whereas the *arg-1*$^+$ allele codes for the enzyme argininosuccinase. The results of these enzyme assays are shown in Table 4. It is at once apparent that, although the specific activity of each enzyme in extracts prepared from first-generation males is double that found in extracts from the haploid strain AB1276, there is no significant difference between extracts prepared from strain AB1276 and from strain AB1206. Since this finding applies also to the enzymes acetylornithinase and argininosuccinase, we conclude that the chromosomal deletion in strain AB1206 extends over most, if not all, of the F_{14} region.

DISCUSSION

F′ strain AB1206 differs from all other F′ strains in its stability and in its relative inability to transfer chromosomal markers under conditions in which transfer of the F-merogenote occurs at a frequency of 100%. Both of these properties are explained by the finding that this strain carries a chromosomal deletion for the region of chromosome homologous to the F merogenote. The loss of the F merogenote (F_{14}) from strain AB1206 would be expected to be a lethal event, since approximately 10% of the cell's genetic information is present only on the F merogenote, hence, the failure to find F_{14}^- segregants. The failure to reconstruct strain AB1206 by transferring F_{14} to recipient strains is also understandable, as none of the recipients used carried a chromosomal deletion of the F_{14} region.

The inability of strain AB1206 to transfer chromosomal markers, except at very low frequencies, further strengthens the crossover model proposed to explain chromosome transfer by F′ strains (Scaife and Gross, 1963; Pittard et al., 1963; Pittard and Adelberg, 1963, 1964). This model proposes that chromosome transfer by F′ strains always results from a crossover event between F merogenote and its homologous chromosomal region. Since the homologous chromosomal region is missing in strain AB1206, the model predicts that chromosome transfer by this strain should not occur. The very low frequencies at which strain AB1206 does transfer chromosomal markers can be readily explained in terms of very infrequent pairing events between F_{14} and one or more regions of the chromosome. Such pairings also explain the formation (by crossing-over) of the stable haploid Hfr males which are revealed when strain AB1206 is treated with acridine orange.

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