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An Infective Factor Controlling Sex Compatibility in *Bacterium coli*

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SUMMARY: Incompatibility may occur in *Bacterium coli* strains which were previously considered homothallic. A cross between two incompatible strains is completely sterile. Such strains are termed F −. Strains which are self-compatible are termed F + and are productive when crossed either with other F + strains or with F − strains. The F + state is transmissible by infection due to a virus-like agent (F) which is not readily separable from the cells. Thus, both in vegetative and sexual reproduction, infection must be mediated by cell to cell contact. No changes other than those of compatibility have been correlated with the F + state. F is independent of λ, the latent phage of the K-12 strain of *Bact. coli*. A small proportion of other strains of *Bact. coli* are fertile when mated with K-12 and, among these, F + and F − strains are found with about equal frequency. In K-12, extreme variations in fertility are found which are only partly associated with F and partly depend on the residual genotype. The cross between two F + strains is usually less fertile than F + × F − and in such a cross one of the two strains behaves predominantly as F −, the other as F +. The F + state has a definite effect on segregation in the sense that the genetical contribution of the F + parent to the zygote, or at least to the resulting recombinants, is less than that of the F − parent.

The introduction of methods permitting the selection of rare recombinants from a mass of non-recombinant parental types enabled Lederberg & Tatum (1946) to demonstrate genetic recombination in mixtures of suitably 'marked' mutants derived from the K-12 strain of *Bact. coli*. From the genetic point of view the most typical aspect of recombination in this strain is that exchange of some genetically determined characters involves, at the same time, the exchange of other physiologically unrelated characters with a high probability. Moreover, whole cells have so far been found necessary for recombination to occur, in contrast with transformations or genetic transfers in other bacterial species, such as pneumococcus (Avery, MacLeod & McCarty, 1944; Griffith, 1928; Hotchkiss, 1951), *Haemophilus* (Alexander & Leidy, 1951) and *Salmonella typhimurium* (Zinder & Lederberg, 1952), which have been demonstrated by means of cell-free preparations. Thus, transfer of genetic material in *Bact. coli* K-12 occurs under conditions which imply that some sort of fertilization is taking place, although proof of this must await a morphological demonstration of cell fusion.

Apart from the occurrence of λ phage (see Lederberg & Lederberg, 1953), no infective agent was known to be harboured in *Bact. coli* K-12 until Lederberg, Cavalli & Lederberg (1952) and Hayes (1953) reported the occurrence of a peculiar example of infective inheritance mediated by an agent called F which controls a system of sex compatibility in this strain.

While the original K-12 strain and most of its descendants, obtained by mutation from the wild-type, show no mating limitations, so that this strain was originally described as truly homothallic (Lederberg, 1947; Lederberg, Lederberg, Zinder & Lively, 1951), a few derivative strains have more recently been found which will not cross with one another. These cross-incompatible strains (which are also self-incompatible) have been called F −, while strains showing the normal, apparently homothallic condition have been termed F +. Experiments have shown that while F − × F − crosses are completely infertile, F − × F + and F + × F + crosses are usually fertile. In practice, the term 'fertility' implies a yield of one recombinant/10^6 viable parental cells, though the yield is rather lower in the case of F + × F + crosses and varies with the markers used for selecting recombinants. The term 'incompatibility' means that when *c.* 10^9 cells of both parental types are mixed together and plated in media which would normally allow the detection of recombinants, no recombinants arise. Although this, by itself, might merely indicate a frequency of recombination of less than 10^{-9}, in fact no true recombinants have been isolated from hundreds of crosses between incompatible strains. It is remarkable that one of the standard strains frequently used in crosses, i.e. Y-10 (threonine-, leucine- and thiamine-dependent), as well as all its vegetative descendants, were found to be F −, a fact which must be borne in mind when using such strains for genetical experiments. The acquisition of the F − property by Y-10 arose during the second of the three mutational steps whereby this strain was derived from K-12 and has been preserved in several dozen of its descendants throughout innumerable transfers. So far only three other F − clones arising from F + strains, either spontaneously or after mutagenic treatment, have been recorded. A search for F − mutants in F + cultures has proved consistently negative, but the tediousness of the technique has prevented the testing of large numbers of cells.

The finding that all recombinants tested from F + × F − crosses, irrespective of the type of cross, were F + at once classed the inheritance of F + as different from that of all other markers. Moreover, it was shown that transformation of F − cells into F + could be achieved by contact with F + cells under conditions in which recombination was excluded, as shown by recovery of F + cells having all the marker characters of the F − strain from a mixture of the two types. The term transduction has been employed to indicate this type of transfer of heritable properties (Zinder & Lederberg, 1952), the agent of transfer in the present instance being the infective factor F. The F + property thus acquired by transduction is stable. Loss of newly acquired F + has been noted on only one occasion.

MATERIALS AND METHODS

Crossing techniques. Either of two procedures were used.

(1) Selection of prototrophs (i.e. cells which can form colonies on minimal medium). This method is applicable to the mating of two auxotroph strains, i.e. strains requiring one (monoauxotrophic) or more (polyauxotrophic) growth factors. Fresh overnight nutrient agar slope cultures of the strains to be crossed are harvested in saline, washed repeatedly in saline and 10^8–10^9 cells of each strain plated together on a minimal agar medium. Each strain is plated separately as a control, under the same conditions. On the plates spread with the mixture prototroph colonies appear after 24–36 hr. at 37°. No colonies should arise on the control plates. When, as is usual, the number of cells plated of each strain is equal, the yield of recombinants is usually expressed as $\frac{\text{no. prototroph colonies}}{\text{total no. cells plated of one parent}}$.

The more commonly used auxotrophic strains were:

Strain 58-161 and its derivatives. These are biotin- and methionine-dependent (BM−). The M− marker is excellent even when used alone because of its negligible back-mutation rate to M+.

Strain Y-10 and its derivatives, requiring threonine, leucine and thiamine (TLB_1-).

(2) Selection of streptomycin-resistant prototrophs (SRP selection) (Lederberg, 1951). In this method an auxotrophic streptomycin-resistant strain is crossed to a prototrophic streptomycin-sensitive strain. The mixture of the parents is incubated in broth prior to plating on minimal agar + streptomycin (500–100 μg./ml.). Prior incubation may be omitted if the streptomycin-sensitive (S^s) strain is the F+ mate, but even in this case it may increase the yield 100-fold. Time is saved and the prototroph yield increased by aeration of the mixture during incubation in broth. Thus the maximum production of prototrophs was achieved in 4–6 hr. with aeration carried out by rolling, as compared with 12 hr. or more if aeration was omitted.

The differentiation of F+ and F− strains. To determine whether a strain was F+ or F− it was crossed with a suitable standard F− strain, using the prototroph selection method described above. Since a large number of colonies had to be analysed for F behaviour, the technique was simplified by omitting to wash the strain under test. Two or three loopfuls of growth from an agar slope culture of the test strain (*c.* 2×10^9 cells) were suspended in 0·2 ml. saline. A drop of this suspension was then added to 10^9 washed cells of the standard F− strain and the mixture plated on minimal agar. Provided fresh agar slope cultures were used, no interfering background due to residual growth was observed under these conditions. F+ strains yielded 200–400 prototrophs while F− strains gave none. When the F behaviour of TLB_1- strains was under investigation, the standard F− strain employed was W-1607 (BM−).

Determination of high frequency of recombination (Hfr) behaviour. A derivative of 58-161 was isolated during selection for resistance to nitrogen mustard

which displayed a remarkably high frequency of recombination as compared with standard M – F + × TLB_1 – F – crosses (Cavalli, 1950). In order to test a strain for Hfr behaviour, 0·05 ml. of suspension of growth from a fresh agar slope culture (*c.* 10^8 cells/ml.) is plated on minimal agar, together with 10^9 washed TLB_1 – cells. An Hfr strain will give 200–400 prototroph colonies under these conditions, while a normal strain of the same biochemical constitution will give not more than one or two colonies.

Unselected fermentation marker characters of prototrophs were tested on EMB medium (Lederberg, 1950*a*; Cavalli, 1950; Lederberg *et al.* 1951).

EXPERIMENTAL

F + *transmission*

It has been stated in the introduction that F + behaviour, as judged by ability to mate with an F – strain, can be transduced from F + to F – cells by means of an infective process (for details see Lederberg *et al.* 1952). The factor F behaves like a virus in its capacity to infect cells which lack it and to be propagated indefinitely in the new host, but it has no obvious pathological activity.

Since centrifugation removes virtually all transducing activity from the supernatant fluid of F + cultures, it is evident that the majority, at least, of F agents must remain bound to the cells. Cell-free filtrates lend themselves to a more sensitive test of transduction since the F – cells can be left in contact with them for a much longer time and the whole culture subsequently tested for F + behaviour instead of having to rely on the isolation and individual testing of a relatively small number of colonies of the initially F – strain. Despite this, Seitz, Mandler, sintered Pyrex or collodion filtrates of growing and saturated F + broth cultures, of cultures grown in the presence of certain inhibitors (arsenate, citrate, dithionite) of enzymes which might possibly destroy the F agent, penicillin lysates, aqueous extracts of cells ground with alumina and ultrasonic lysates were all ineffective in transduction. It would seem, therefore, that it is the whole cells which are infective. Cells killed with heat (60° for 30 min.) have lost their transducing activity, though a preliminary experiment suggests that this activity may be lost at a proportionately lower rate than viability as judged by colony counts.

While little or no transduction was found to occur in minimal medium, and none in saline or in broth at 4°, when suitable numbers of living F + cells were present under conditions of rapid multiplication in broth, infection is remarkably efficient. For example, if logarithmic phase cells of F + Lac + and F – Lac – are mixed in equal proportions in broth at a concentration of *c.* 5×10^7 cells/ml. and incubated, and at intervals thereafter samples are removed, diluted and plated on EMB-lactose medium so that the originally F – cells can be selected from the mixed culture and tested for F + capacity, it will be found that 50 % of the F – cells have been infected after slightly more than 2 hr. incubation. If the same mixed culture is aerated by rolling

which increases the growth rate and, probably more important, the chance of contact between cells, the same result will be achieved in less than 1 hr.

The ratio of F + and F − cells seems to play an essential role in determining the efficiency of transduction. The results of an experiment demonstrating this are given in Table 1. The effect of variation in the F + : F − ratio is clearly seen in the table and is statistically significant. When F + donor cells are in

Table 1. *Effect of varying the ratio of F + to F − cells on the efficiency of F + transduction*

Concentration of F− cells maintained constant. F+ and F− cells mixed in the logarithmic phase and culture tubes rolled throughout the experiment. *Medium* = Difco 'Penassay' broth.

Concn. of F− (cells/ml.)	Concn. of F+ (cells/ml.)	Ratio, F− : F+	Ratio of no. of F− cells transformed to F+ : no. cells tested after ½ hr.	1 hr.	2 hr.
4×10^6	10^8	1:25	20/20	10/10	19/20
4×10^6	4×10^6	1:1	10/20	13/20	6/20
4×10^6	10^6	4:1	2/20	0/19	3/20

large excess, all or almost all F − cells become infected; in general, the fraction of infected cells approximates $(1-e^{-n_a/n_b})$, n_a being the number of infective and n_b being the number of susceptible cells. This would in fact be the expectation if every F + cell could infect but one F − cell, within the limits of the experiment.

A remarkable feature of this experiment is the lack of correlation between the degree of F + transduction and the duration of contact of F − and F + cells. Such lack of correlation has only been observed in rolled cultures and does not arise in static mixtures. Two possible explanations are (1) that every active F + cell can effect some transfers after which it becomes non-infective for a period of 2 hr. or more, and (2) that the newly infected cells do not transmit F to both daughter cells, thus compensating, at least in a gross way, for increase in frequency of infections with time.

F + and *fertility in* Bact. coli

The yield of recombinants obtainable from crossing two strains is here called the fertility of the cross. This fertility is dependent on a number of conditions, some of them inherent in the strains, which often make comparison of the fertilities of different crosses impossible. Among the most important of these conditions are:

(*a*) The genetic linkage relationships of the markers chosen for the selection of recombinants. If such markers are more closely linked, the yield is proportionately lower.

(*b*) The physiological nature of these markers which may determine the occurrence of a variable degree of syntrophic growth or inhibition of growth, or of growth resulting from the presence of traces of growth factors, when the mixture is plated on minimal agar.

(*c*) The age of the cultures, those in the logarithmic phase being more fertile than older cultures, both for F+ and F− strains.

(*d*) Whether or not the mixture has been incubated in broth prior to plating. This factor is particularly important when SRP selection is practised.

The effect of extraneous markers can be excluded in comparing the relative fertilities of F+ × F+, F+ × F− and F− × F+ crosses, however, if F− strains of both parents are available as is the case with BM− and TLB_1− strains. These F− strains can readily be transduced to the F+ state, so that F+ and F− cultures of both auxotrophs could be used. With these strains the fertility of F+ × F+ crosses was about ten times lower than that of the other two crosses. On crossing another F+ auxotroph, W1678, which is proline- and glycine-dependent (PG−) with TLB_1−, the F+ × F+ cross was 100 times less fertile than the F+ × F− cross; unfortunately, an F− strain of PG− was not available for the reverse cross. This relative decrease in fertility was not so marked, however, when PG− F+ was mated with BM− F+. In some other cases the decrease in the fertility of F+ × F+ crosses was less marked or absent. For example, in the case of TLB_1− S^r and TLB_1+ (the latter obtained by back-mutation from TLB_1−), using strains transduced to F+ and the SRP method of prototroph selection, no marked difference was noted between the fertility of F+ × F+, F+ × F− and F− × F+ crosses. Moreover, crosses between an independent occurrence of BM− F− (strain #8) and TLB_1− F+ and other F+ auxotrophs were found to have a very low fertility, thus showing that factors other than F must be taken into account in considering aberrant fertility behaviour.

Temporary phenotypic F+ to F− alteration due to environment (F− phenocopy). One other case must be mentioned in which the effect of F+ was manifested in a reduced yield from F+ × F+ crosses. This was observed by making use of BM− F− obtained, not by hereditary loss of the F agent, but by temporary suppression of its activity in BM− F+ which had been grown to saturation under conditions of aeration, either by rolling or bubbling air through the cultures. Under these special physiological conditions, BM− F+ behaves as F−, although subsequent growth under ordinary conditions causes a return to the normal F+ state. The effect, therefore, is not heritable. Moreover, it is not observed in aerated logarithmic phase cells nor when N_2 or CO_2 are bubbled through the cultures instead of air. It seems likely, though not proven, that a metabolite accumulating in fully grown aerated cultures is involved. This aeration effect is especially marked in strains related to BM−, while aerated cultures of other F+ strains yield at least some prototrophs when crossed with standard F− strains.

The fertility of Hfr strains. A most interesting variation in fertility has been observed in a strain, isolated from 58-161 after selection for nitrogen mustard resistance (Cavalli, 1950), which showed a remarkably high frequency of recombination (Hfr) when crossed with TLB_1− F−. This Hfr strain cannot be distinguished on morphological or biochemical grounds from the 58-161 (BM−) strain from which it was derived and which shows a normal frequency of recombination (Nfr). The Hfr strain is unstable, reverting to Nfr. This

instability has been observed repeatedly at Cambridge and Milan (Cavalli), and at least once at Madison, but the frequency of back-mutation has not yet been established. Hfr seems to be fairly rapidly outgrown in mixed culture with its parent (Nfr) strain, but no infective transfer of Hfr or Nfr behaviour has been noticed under such conditions. Because of the instability of Hfr with reversion to the Nfr state, the strain may be lost unless the isolation and testing of single colonies is carried out at intervals. Moreover, recently isolated subcultures of this strain may be necessary for experimental work as older cultures may contain both the Hfr and the reverted types.

When plated with $TLB_1 - F -$ on minimal agar, Hfr is 100 to 1000 times more fertile than its BM – F + (Nfr) parent strain. Most Hfr × F + crosses give a prototroph yield lower than Hfr × F – (though this is not clear-cut with $TLB_1 -$ transduced to F +) but usually higher than the equivalent F + × F – cross. Hfr × Hfr crosses, employing various auxotrophs (and/or other recombination markers such as drug and virus resistances) from Hfr, give fertilities varying from $1/10^4$ downwards. That the high fertility of Hfr is due to a high frequency of recombination, and not to syntrophic interaction with $TLB_1 -$ strains on minimal agar, has been shown by experiments in which selection for recombinants was carried out by the use of non-nutritional markers (e.g. streptomycin and azide resistance) (Lederberg, 1950*b*). Yields of recombinants 100 times as great as those given by Nfr were obtained.

The relationships between Hfr and F + are not clear. Hfr originated from an F + strain. Nevertheless, Hfr strains do not transduce F + in infection experiments. In conditions under which normal F + strains would give 100 % infections, no infections were ever observed using Hfr strains as F + donors. No transfer of F + has been observed even in recombinants, Hfr × F – crosses giving only F – recombinants which can, however, be transduced to F +. On the other hand, Hfr × Hfr crosses yield only Hfr recombinants. When Hfr reverts to Nfr it displays the normal F + state as has been shown both by its fertility with F – (several independent reversions of Hfr and its derivatives tested) and by its ability to transduce F + to F – (two presumably independent reversions tested as F + donors). Thus, in spite of its capacity to yield F + after back-mutation to Nfr, Hfr should be classed as F – so far as its activity in infection experiments is concerned, and as strongly F + in relation to its activity in recombination.

Occurrence of the F + agent in Bact. coli *strains other than K-12*

The occurrence of F + in other strains of *Bact. coli* has not been investigated extensively enough. In the survey by Lederberg (1951), of the capacity of about 2000 *Bact. coli* strains to cross with K-12, the K-12 indicator strain mostly used was $TLB_1 - S^rF -$, so that some potentially fertile strains may have been missed because of their F – state. Nevertheless, this survey yielded over fifty new strains which were fertile when crossed with K-12 strains by the SRP selection method. In seven out of thirty of these strains F + has been detected by transduction to BM – F – or $TLB_1 - F -$. Preliminary results indicate that the number of crossable strains which were missed in

the first survey, because of the use of an F − tester stock, may have been nearly as large as the number found. Some preliminary results seem to prove, however, that the system of compatibilities may be more complex than is indicated simply by the F state of such strains.

Cavalli & Heslot (1949) reported a strain of *Bact. coli* (NCTC 123) which was fertile in crosses with BM − F +. The original growth requirements of this strain are complex; good growth can only be obtained with casein hydrolysate, the addition of amino-acid mixtures alone being less satisfactory. Starting from reversions of this original strain which were either fully prototrophic or grew in the presence of methionine + lysine, a series of auxotrophic mutants was made by which it was shown that the strain was self-incompatible. Strain NCTC 123 behaves as F − in crosses with K-12 stocks (with some inconsistencies) and F + can be effectively transduced to it from K-12.

A search for the F agent in several infertile strains of *Bact. coli* and in *Salm. typhimurium* did not reveal the presence of this factor by the criterion of transduction. The strain ATCC 9637 (Davis, 1950) was originally thought to be infertile but was then found to be F −. This strain was subsequently shown to be crossable, but with an extremely low frequency of recombination.

F + and segregation

For genetical analysis two types of marker characters are used: (*a*) fixed or selected markers, such as growth factor requirements or, less frequently, drug resistances, which are used to select recombinants, and (*b*) markers such as, usually, ability to ferment lactose, galactose, maltose, etc., resistance to viruses and drugs or any other differential character between the strains crossed which are not directly selected in recombination (free or unselected markers). Each marker is obtained by one-step mutation in one of the two strains to be crossed, so that the two strains differ with respect to each marker. The segregation of unselected markers shows well reproducible ratios of the two parental types among recombinants selected on the basis of the fixed markers. This has been taken to demonstrate that reduction occurs soon after fertilization, thus allowing both parental types, as represented by one or more unselected markers, to reappear in the immediate progeny. The diploid state may exceptionally last for a number of generations (see later).

Ratios observed for almost all markers among recombinants differ significantly from 1:1, the ratio to be expected for unselected markers segregating independently of the fixed markers. This forms the experimental basis for linkage. A more refined analysis suggests that all the markers so far employed are linked together, i.e. that they fall into the same 'linkage group'. The evidence for linkage would not be complete, however, on this basis alone. An essential requirement of genetic linkage is that reversal of the segregation ratio is observed when an unselected marker is switched over from one to the other of the two parents (Lederberg, 1947). This type of evidence, which is the distinctive element of Mendelian inheritance, can be expressed in general terms as follows: if, in a cross between two strains A − B + C + and A + B − C −, recombinants selected for A + B + are found to be p% C + and

$(100-p)\%$ C−, then in the reversed cross A−B+C− × A+B−C+, $p\%$ of C− and $(100-p)\%$ of C+ recombinants should occur. This has actually been observed, (Lederberg, 1947; Rothfels, 1952; Cavalli, 1952) apart from minor deviations which do not throw any doubt on this major point (Bailey, 1951).

The evidence for linkage suggests that genetic transfer is effected by something more complex than a bag of transforming principles; the contents of the bag must at least be arranged in an orderly way. The next problem, therefore, was to determine the nature of the order. Linear order was assumed by Lederberg on the basis of his early results (1947), but the extension of analysis to other markers (Lederberg, 1949; Newcombe & Nyholm, 1950*a*, *b*; Cavalli, 1950; Lederberg *et al.* 1951) seemed to show that these later findings could not be reconciled with the original, simple assumption. Among recent contributions, however, that of Rothfels (1952) in favour of linearity may be quoted. It is clear that the problem of linearity will have to be reviewed in the light of the facts given in this paper.

Another, probably related, difficulty arose in the analysis of segregation of diploids. In the case of certain parent strains, known as Het (Lederberg, 1949), some zygotes do not undergo immediate reduction but form relatively stable, persistent heterozygotes which continually segregate out not only the two parental types but also recombinant types, thus showing that crossing-over (assuming this is the genetic basis of the observed exchanges) occurs. Analysis showed, however, that the heterozygotes were not truly diploid in that no segregation of many of the genes closely linked with methionine (e.g. streptomycin, maltose) was observed, so that, with respect to these genes, they carried the gene contribution of one parent only. This is not universal, however, since rare heterozygotes are found from which these genes do segregate (Lederberg *et al.* 1951).

The impact of F+ on the problems of segregation has been twofold. First, it was found that when heterozygotes are formed in a cross between HetF+ and F− strains, it is the contribution from the F+ parent which is partly lost. Such incomplete heterozygotes are thus diploid for a portion of markers contributed by both parents and haploid, or more precisely, hemizygous for the remaining portion contributed by the F− parent alone. The missing portion is always confined to the markers maltose (Mal) and streptomycin (S).

Secondly, it was observed that the pattern of segregation of recombinants is markedly affected by the F types of the parents. This is clearly shown by the data presented in Table 2 which are derived from crosses between TLB_1-S^r and TLB_1+, reversed with respect to the five markers, lactose, galactose, maltose, xylose and arabinose (for which the symbols Lac, Gal, Mal, Xyl, Ara respectively are used). Only the two crosses

Lac − Gal − Mal − Xyl − Ara − × Lac + Gal + Mal + Xyl + Ara +

and

Lac + Gal + Mal + Xyl + Ara + × Lac − Gal − Mal − Xyl − Ara −

have been considered out of the possible thirty-two (i.e. 2^5) crosses which might have been set up because the parents were obtained from a TLB_1 − Lac GalMalXylAra+ and a TLB_1 − LacGalMalXylAra− strain by mutations

for S^r and for prototrophism (TLB_1+) (in three steps). Both these parent strains were F− so that by transducing each to F+, F+ and F− strains of each were available for the examination of all three combinations, F− × F+, F+ × F− and F+ × F+. Since all the marker differences between these strains originated either through ultraviolet induced mutations (for the sugar fermentation deficiencies) or through spontaneous mutations, the chance that chromosome mutations might inadvertently be included, and thus create unwanted complications, is less than if such powerful mutagenic agents as X-rays had been employed. The data in Table 2 comprise the pooled results of a variety of crosses in some of which F+ had been transduced to one or other of the parents from different sources (K-12, BM−, W-705). The details of these transductions are irrelevant, however, since F+ transductions to the same F− strain either from different sources or from the same source on different occasions were found to yield the same segregation results. All crosses were carried out by SRP selection after 3 hr. incubation of the mixture in broth in rolled tubes. Some slight heterogeneity was found between identical crosses on different days. This was probably due to lack of close standardization of the age of the cultures to be crossed. In view of this, but more especially because some of the prototroph colonies, which were all scored without isolation, were found to be mixed with respect to some of the sugar markers, a detailed statistical analysis of the data has not been undertaken. In spite of the rawness of the data they provide clear evidence of the effects of F+ on segregation. It is noteworthy that the F+ × F+ cross tends to resemble more or less strongly one of the two F+ × F− crosses rather than to be intermediate between the two. Numbers are too small, especially in the final column to allow a closer comparison.

The details of a preliminary genetic analysis of these data will not be included here. It may be enough to say that the B_1 marker was found, by crosses not reported here, to fall outside the S^r – TL region, while T and L were found to be closely linked, in agreement with earlier results of the M− × TLB_1− cross (Lederberg, 1947). The five sugar markers (which all belong to strain W-945) seem to be located between S and TL, in the probably linear order: S – Mal – Xyl – Gal – Lac – (Ara – TL). Similar results concerning the effect of F on segregation of prototrophs had been obtained in crosses between BM− and TLB_1 – F− (parental) or TLB_1 – F+ (filial), published by Lederberg *et al.* (1951, table 5), but had, at that time, been interpreted in a different way.

DISCUSSION

Infective inheritance was first described in micro-organisms 25 years ago (Griffith, 1928). It has been found since in a few cases in higher organisms where its role cannot, however, be assessed with certainty. In some species of micro-organisms, e.g. in pneumococcus (Ephrussi-Taylor, 1951; Hotchkiss, 1951) and in *Salm. typhimurium* (Zinder & Lederberg, 1952), infective inheritance seems to have taken over the role of hybridization which is carried out in other organisms through fertilization.

Table 2. *Effect of F state on segregation*

The numbers of prototrophs which showed the combination of markers given on the left

Phenotype of	Lac	Gal	Mal	Xyl	Ara	F condition of parents		
TLB_1- S^r parent	−	−	−	−	−	−	−	+
TLB_1+ parent	+	+	+	+	+	+	−	+
TLB_1+ S^r recombinants	−	−	−	−	−	1	1	2
	−	−	−	−	+	290	25	315
	−	−	−	+	−	.	.	.
	−	−	+	−	−	.	.	.
	−	+	−	−	−	.	1	.
	+	−	−	−	−	.	.	3
	−	−	−	+	+	60	1	37
	−	−	+	−	+	8	1	10
	−	+	−	−	+	5	3	14
	+	−	−	−	+	192	8	214
	−	−	+	+	−	.	.	.
	−	+	−	+	−	.	.	.
	+	−	−	+	−	.	.	.
	−	+	+	−	−	.	.	.
	+	−	+	−	−	.	.	.
	+	+	−	−	−	.	.	.
	−	−	+	+	+	28	1	21
	−	+	−	+	+	2	3	.
	+	−	−	+	+	34	4	46
	−	+	+	−	+	.	.	.
	+	−	+	−	+	4	1	1
	+	+	−	−	+	208	276	139
	−	+	+	+	−	.	.	.
	+	−	+	+	−	.	.	.
	+	+	−	+	−	.	.	.
	+	+	+	−	−	.	.	.
	−	+	+	+	+	2	1	.
	+	−	+	+	+	22	.	32
	+	+	−	+	+	57	253	53
	+	+	+	−	+	4	14	5
	+	+	+	+	−	1	.	.
	+	+	+	+	+	27	175	24
Total nos. of recombinants tested						945	768	916

Phenotype of	Lac	Gal	Mal	Xyl	Ara	F condition of parents		
TLB_1- S^r parent	+	+	+	+	+	−	+	+
TLB_1+ parent	−	−	−	−	−	+	−	+
TLB_1+ S^r recombinants	+	+	+	+	+	1	.	.
	+	+	+	+	−	30	.	1
	+	+	+	−	+	.	.	.
	+	+	−	+	+	.	.	.
	+	−	+	+	+	.	.	.
	−	+	+	+	+	.	.	.
	+	+	+	−	−	12	.	1
	+	+	−	+	−	10	.	.
	+	−	+	+	−	8	2	.
	−	+	+	+	−	19	2	.
	+	+	−	−	+	1	.	.
	+	−	+	−	+	1	.	.
	−	+	+	−	+	.	.	.
	+	−	−	+	+	.	.	.
	−	+	−	+	+	.	.	.
	−	−	+	+	+	.	.	.
	+	+	−	−	−	9	2	1
	+	−	+	−	−	5	.	1
	−	+	+	−	−	5	.	1
	+	−	−	+	−	3	.	.
	−	+	−	+	−	6	.	.
	−	−	+	+	−	32	181	14
	+	−	−	−	+	.	.	.
	−	+	−	−	+	.	.	.
	−	−	+	−	+	.	.	.
	−	−	−	+	+	.	.	.
	+	−	−	−	−	11	.	.
	−	+	−	−	−	9	.	.
	−	−	+	−	−	12	140	26
	−	−	−	+	−	10	14	3
	−	−	−	−	+	.	.	.
	−	−	−	−	−	14	156	24
Total nos. of recombinants tested						198	497	72

The present case concerns a bacterial species in which genetic transformations induced by means of cell-free preparations have not been satisfactorily demonstrated but in which, on the other hand, a system of genetic transfer by typical hybridization has been described. Oddly enough, the factor showing infective inheritance controls hybridization through control of sex compatibility. Of the several problems which have had to be freshly formulated, or have arisen *de novo*, as a consequence of this finding, none has been solved in an entirely satisfactory way. The first problem is related to the nature of the F agent itself. It behaves like a virus in its capacity to infect without, however, producing any obvious pathological manifestations. The separate occurrence and independent transmission of F and λ phage show beyond doubt that these two agents are quite distinct (Lederberg *et al.* 1952).

The F agent cannot easily be separated from the bacterial cells, but some preliminary experiments suggest that this may eventually be achieved. This difficulty of extracting the virus-like agent or plasmid (Lederberg, 1952) from the cells, or of finding it spontaneously free, may be taken to imply that under natural conditions infection is mostly, or only, due to cell-to-cell contact. This would lend support to the idea that recombinants arise from intimate contacts between cells since all recombinants from F + × F − crosses are F +. The hypothesis that the F plasmid itself plays a direct role in recombination, being, for instance, the vector of the genetic material which is transferred or an essential part of a 'gamete', cannot be accepted because Hfr × F − crosses give only F − recombinants. On the other hand, the hypothesis that recombination is the result of some sort of conjugation needs direct, visual support which it is hoped some experiments now planned may supply.

Hfr forms an apparent exception to the rule that, in K-12, the presence of F (i.e. of the F + state) in one of the mates is essential for recombination to occur. This rule would mean that F is, in some direct or indirect way, the determiner of the formation of cells with gametic activities (at least for one sex). For such hypothesis to hold, however, one must be ready to assume that Hfr does contain F, though in a bound, non-infective form. That this may be so is implied by the fact, now under closer study, that Hfr can revert to F +. As to the origin of Hfr, the hypothesis that it may be the consequence of mutation of the F agent, resulting in increased gametic activity and loss of infectivity, cannot be discarded, even if it may seem preferable to think that Hfr results from a gene mutation at a locus situated in the proximity of markers which are commonly eliminated in crosses with F − strains (to account for the fact that Hfr does not reappear from crosses with F −). The Hfr gene would then exert a control on the activity of the F particles. However, exceptions to the rule that F + is essential for gamete formation may be found in strains of *Bact. coli* other than K-12.

In F + × F − crosses, two effects described by Hayes (1952*a*, *b*) should be taken into consideration; the relative insensitivity to streptomycin of an F + streptomycin-sensitive strain, so far as prototroph forming capacity is concerned, and the ultraviolet stimulation of prototroph formation on F +

strains. These effects have probably not been studied extensively enough, and especially in sufficient F + and F − strains, to allow a superimposition of these effects on to the F + condition. There may still be room for these effects to be independent of each other, as well as of F, at least to some extent. Within these limitations, the two effects strongly suggest a physiological difference in the gametic activities of F + and F − strains, while the genetical consequence of the F + state, as revealed by segregation, demonstrates that the genetic contribution of the F + and F − parent is different. Whether there is also a morphological differentiation between the gametes of an F + and an F − strain in a cross, remains to be determined. If this were so it would imply true sexuality. For the moment it seems valid to retain the use of the term 'sex' in relation to *Bact. coli*, especially in view of the inadequacy of its present definitions.

The role of F + in fertility is still less clear. Variations in fertility are found, ranging from the extreme of F − × F − which has zero fertility, to that of Hfr × F − which may reach values much higher than the standard rate (i.e. 10^{-6}) of F + × F − crosses. It seems necessary to assume that residual genotype (i.e excluding F) plays an important role in fertility. Both the fertility data and the genetical data suggest that in a cross between two F + strains, one of the two behaves mostly as F + and the other as F −, the relative role in a cross being determined by the residual genotype. There are some indications that various strains have different F + strengths, and that the relative behaviour of the two strains depends on the difference of F + strength between the two; the greater the difference the higher the fertility, while the stronger F + will behave as F + and the weaker as F − in the cross. The weakest F + would be BM −, with a gradation through TLB_1 − and PS − to Hfr which would be top F +. This hypothesis demands a considerable body of data, both on fertility and on segregation, for experimental analysis. Data based merely on fertility would not seem enough to support or discard it since the hypothesis would not readily yield quantitative predictions of fertility behaviour. An important question would be how much of the fertility in a cross between two strains is predetermined and how much is the consequence of direct interaction between the strains. Experiments testing competitive mating of three or more strains may throw light on this point.

As to the effects of F + on segregation, it is obvious that further analyses of linearity of the chromosome (the physical basis of the linkage group) in *Bact. coli* K-12 will have to take them into consideration. At least one hypothesis, based on Mendelian theory, can be put forward to account for them: the elimination of a specific segment of the chromosome contributed by the F + parent may take place regularly at every fertilization. There is at present no definite evidence to suggest whether such elimination might occur during formation of the F + gametic cell, during fertilization, or at the ensuing reduction.

Another possibility is that there is a different degree of effective ploidy of the F + and F − gametic cells, the F − gametic cell having a higher degree of ploidy (or, possibly, more nuclei) than the F +. In such a case, segregating

markers closely linked with the marker excluded in recombination (e.g. BM −) would have a lower chance of being represented in recombinants when they are carried by the relatively F + parent, than when carried by the F − parent. There is a formal resemblance between this hypothesis and the situation arising in bacteriophage recombination, when a different multiplicity ratio is used for the two parents. This second interpretation, however, does not agree well with some features of the data in Table 2 so that, at the moment, the hypothesis of segmental elimination remains the more attractive.

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