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SEX COMPATIBILITY IN ESCHERICHIA COLI[1]

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GENETIC recombination in bacteria was first successfully studied in strain K-12 of *Escherichia coli* (TATUM and LEDERBERG 1947; LEDERBERG 1951). Since the nutritional mutants used in the crosses were derived directly from this strain under clonal propagation, their compatibility implied a homothallic or self-compatible sexual system (*cf.* WHITEHOUSE 1949). The inference that crossing was genetically unrestricted was supported by the absence of marked hereditary mating preferences among the segregants of a variety of crosses (LEDERBERG 1947, 1949; *cf.* LEUPOLD 1950). More recently, evidence has been secured for a system of sexual compatibility which was previously obscured by its unique inheritance via an infective agent.

METHODS AND CULTURES

Experimental techniques differed slightly in Madison and Milan, but the concordance of the results obviates a detailed comparison. Crossing procedures, genetic symbols and the detailed pedigrees of many stocks are given elsewhere (CAVALLI 1950; LEDERBERG 1947; LEDERBERG *et al.* 1951). Most of the experiments have involved crosses of the original K-12, of stocks clonally related to 58-161, a double mutant requiring biotin and methionine (B^-M^-), and stocks related to Y-10, a triple mutant requiring threonine, leucine, and thiamin ($T^-L^-Th^-$). (Since the original isolation of 58-161 and early crossing experiments (TATUM 1945; LEDERBERG 1947) its biotin requirement appears to have been lost. The methionineless stock is, fortunately, so stable that back mutations to prototrophy are undetectable under the conditions of crossing experiments.) Stocks carrying similar markers which have been extracted from crosses (LEDERBERG 1949; LEDERBERG *et al.* 1951; ROTHFELS 1952) will be designated *filial*.

Auxotrophic parents were crossed in the usual way, by plating washed suspensions into or on the surface of minimal agar to select for prototrophic recombinations. For example, 58-161 ($M^-T^+L^+Th^+$) cells plated with Y-10 ($M^+T^-L^-Th^-$) gave $M^+T^+L^+Th^+$ prototrophs in yields approximating one per million of the parents inoculated. The occurrence of prototrophs, thus

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selected, from platings of thoroughly investigated auxotroph parents has been taken as *prima facie* evidence of crossing, a conclusion supported by other experiments in several laboratories.

Prototrophic streptomycin-sensitive (S^s) stocks were crossed to S^r (streptomycin-resistant) auxotrophs by plating the parents on minimal-streptomycin agar. This selection for S^r prototrophic recombinants has been abbreviated *SRP*. To avoid confusion, some familiar expressions will be used in a restricted sense for this paper. Two stocks are termed *crossable* if the selection of recombinant genotypes is technically possible, that is, if they carry non-overlapping nutritional requirements, or if they are amenable to the *SRP* method. *Fertility* will refer to the experimentally observed production of recombinants from crossable parents. *Compatibility* will be reserved for the status of a stock with respect to the system to be described in this paper.

Most of the genetic studies with *E. coli* K-12 have involved the 58-161 (M^-) and the Y-10 ($T^-L^-Th^-$) lines, and other auxotrophs were used to a limited extent. All crossable stocks appeared to be fertile with each other, as expected in a homothallic system. It was not readily feasible, however, to test self-fertility, and in view of the common clonal origin of the various lines directly from K-12, this did not appear to be urgent. Occasional deviations in quantitative yields of prototrophs were ascribed to linkage relationships affecting the probability of detectable recombinations, and to secondary, perhaps indirect, modifiers of productivity in the test system.

RESULTS AND CONCLUSIONS

Self-incompatibility. The first evidence of a compatibility difference was recognized when a routine single-colony isolate, W-1607, of an M^- stock, unlike all others previously tested, was found to be completely infertile with "standard" $T^-L^-Th^-$ parents such as Y-10. At the time, W-1607 was not analyzed further, but in view of later work will be labelled F^-, in contrast to the type F^+. Although W-1607 was not fertile with any clonal derivative of Y-10 it was fertile with other auxotroph testers and with *filial* $T^-L^-Th^-$. Another M^- stock isolated after selection for resistance to nitrogen mustard was later recognized as a second recurrence of F^-.

In order to explain certain peculiarities in linkage behavior, it has been suggested that 58-161 and Y-10 differed from each other in chromosome structure, possibly as a result of the many kiloroentgens of X-rays to which they had been exposed in their history. To obtain crossable stocks which might be free of such differences, spontaneous prototrophic reversions from $T^-L^-Th^-$ (Y-10 line) to $T^+L^+Th^+$ were selected, factor by factor. Two such prototrophic reversions were secured, and were tested with $T^-L^-Th^-S^r$ (W-1177, a clonal derivative of Y-10) by the *SRP* method. Unlike the original K-12 or various other prototrophs from recombination experiments, the reversions proved to be completely infertile with W-1177. However, the reversion prototrophs were fully fertile with M^-S^r and with *filial* $T^-L^-S^r$ stocks in comparable *SRP* crossing tests, suggesting that the Y-10 line was

self-incompatible but cross-fertile. The similar compatibilities of the aberrant M^-, W-1607, and of the reversion prototrophs from the Y-10 line, when they were tested with a variety of K-12 derivative stocks made it apparent that W-1607 and the Y-10 line carried a similar " mutation," F^-.

Compatibility may be experimentally defined by the following properties:

$F^- \times F^-$	sterile
$F^+ \times F^-$	fertile
$F^+ \times F^+$	fertile

for which W-1607 and W-1177 serve as standard F^- testers. The application of this test (fertility in crosses $\times$ W-1607) to the ancestry of W-1177 has indicated that the F^- mutation in this line probably occurred during the isolation of T^-L^- (F^- strain, 679-680) from T^-L^+ (F^+ strain 679; TATUM 1945).

Test for an F^+ hormonal stimulus. The compatibility conditions may be summarized by the requirement that at least one parent must be F^+ to give a productive cross. This suggested that F^+ cells might secrete a hormone required for sexual reproduction. The following experiment was therefore set up to determine whether F^+ cells could stimulate an otherwise incompatible cross. $M^-Lac^+S^sF^+$ (58-161) was grown together with $M^-Lac^-S^rF^-$ (W-1607) in broth. The cell mixture was then crossed with $T^-L^-Th^-Lac^+S^sF^-$ (Y-10) and the prototrophs were characterized for *Lac* and *S*. All prototrophs from the only compatible pair, $M^-Lac^+S^sF^+ \times T^-L^-Th^-Lac^+S^sF^-$ should be uniformly Lac^+S^s. Prototrophs carrying either Lac^- or S^r markers would indicate an illegitimate crossing of $M^-Lac^-S^rF^- \times T^-L^-Th^-Lac^+S^sF^-$. These markers were, in fact, observed in about one fourth of the progeny, a large number of which must then have originated from the incompatible parents. In further experiments of similar design, it was shown that either of the F^- parents, W-1607 or W-1177, could be stimulated to cross with an otherwise incompatible partner by previous growth with F^+ cells. This stimulation occurred only when the F^- and F^+ cells were grown together prior to plating on minimal medium. Cell-free filtrates of F^+ or of mixed F^- with F^+ cultures were not effective, nor was the F^+ stimulus transmitted across a bacteria-tight filter through which the medium supporting F^+ and F^- cells was flushed from one compartment to another (DAVIS 1950a). At first sight, these experiments appeared to support the concept of an F^+ hormone, but without the substantiation of a cell-free preparation.

Inheritance of F^+/F^-. It has already been indicated that all *filial* stocks which have been tested have reacted as F^+, *i.e.*, no segregation of F^- has been observed in $F^- \times F^+$ crosses. This might be interpreted as an incident of linkage, a direct effect of sexual reproduction, or a form of non-Mendelian transmission. As a partial discriminatory test, the exceptional prototrophs of the previous experiment were examined. These presumably issued from $F^- \times F^-$ under the influence of an F^+ stimulus, and might be expected to inherit the F^- quality, but when a number of such prototrophs were crossed by the *SRP* method to F^- testers, all proved to be compatible. The " hormonal " influence

of F^+ cells in the stimulation experiments was therefore inherited by the presumed $F^- \times F^-$ progeny. The large proportion of exceptional prototrophs suggested the feasibility of a direct test of the parents.

Transmission of F^+. The same marked pairs of F^- and F^+ stocks used in the stimulation experiments were grown together in the same way. Each mixed culture was then streaked out on EMB lactose or streptomycin agar to re-extract the component that was originally F^-. Single colonies were then propagated in broth, and the cultures crossed to F^- testers. The cultures were also re-checked for purity, especially to rule out contaminating F^+ cells. In every experiment, well over half of the originally F^- cells had become F^+; in some instances, all of the colonies tested. In a further check of purity, the prototrophs in the test crosses were found to carry no other markers from the original F^+ donor. The new F^+ cultures retained this transduced trait through numerous single colony isolations, and appear to be as stable as the standard F^+. F^+ was also repeatedly transmitted in series to F^- carriers. Each of a number of F^+ and F^- pairs tested behaved in the same way. No evidence of the transduction of any other markers was observed in these experiments.

This transmission is remarkably efficient. Lac^+F^+ and Lac^-F^- cells were mixed in broth at an initial concentration of 10^8 per ml. each. After one hour at 37°C the mixture was streaked out on EMB lactose agar. Individual Lac^- colonies were tested; about 10 percent had become F^+ during this brief exposure. Mixtures in saline or minimal medium, or held in broth at 4°C for one hour evinced no F^+ transductions. Cultures grown together overnight in synthetic medium manifested only a limited degree of transduction. Whether prototrophs from $F^- \times F^+$ crosses become uniformly F^+ by a more efficient route than inter-cellular transduction remains to be determined.

In contrast to the efficiency of intact cultures, cell-free preparations have so far been inactive. Following ordinary, by no means exhaustive, centrifugation, the sedimented cells of F^+ cultures have retained all of the F^+ transducing activity, and left none in the supernatant. Other preparations from F^+ cultures that have given negative or inconclusive results include autolysates, penicillin lysates, sonic lysates, aqueous extracts of dried and alumina-ground cells, and heat-inactivated cells. Further kinetic studies have been hampered by technical difficulties in testing the compatibility of large numbers of isolates.

In many experiments, it is desirable or necessary to test the transduction of F^+ without spending the time and effort required to reisolate the exposed clones. For such experiments, a *filial* $M^-T^-L^-F^+$ stock has been isolated as a segregant from a persistent heterozygote M^-/T^-L^- (LEDERBERG 1949). This stock can function as a source of F^+ in combinations with M^- or $T^-L^-Th^-$ parents without itself participating in effective recombinations, owing to the overlapping nutritional requirements which prevent the development of prototroph offspring. That is to say, it can be used as an otherwise inert source of the F^+ agent for these experiments. It should be feasible to study the kinetics of F^+ transduction by direct measurements of the yield of

prototrophs from crosses of M^-F^- with $T^-L^-Th^-F^-$ cells that have been cultured with $M^-T^-L^-F^+$ far varying intervals of time. This possibility is based upon two findings: 1) platings of washed cells of $M^-T^-L^-F^+$ with an incompatible cross on minimal agar gave no prototrophs, presumably owing to the limited transduction of F^+ under these conditions, as previously demonstrated, and 2) platings of $T^-L^-Th^-F^-$ with washed cells of a mixture of M^-F^- and $M^-T^-L^-F^+$ that had been held in broth for one to two hours gave considerable yields of prototrophs. The latter seem to show that F^+ is phenotypically effective immediately after its transfer, but it must be admitted that only the first step is blocked on minimal agar, and that the subsequent development of the compatibility phenotype may proceed on the crossing plates.

Relationship of the F^+ agent to latent virus. The infective transmission of F^+ at once calls to mind the latent bacterial viruses, especially since strain K-12 itself is lysogenic for such a phage, λ. However, the filtrability of bacteriophage at once differentiates it from the F^+ agent, as well as from the gametes themselves. A re-examination of existing stocks at once showed no correlation between lysogenicity and F^+. Nonlysogenic as well as lysogenic stocks are freely intercrossable (E. M. LEDERBERG 1951). In further experiments, F^+ was readily transduced from a nonlysogenic, λ-sensitive donor to F^- stocks, whether lysogenic or not. Finally, the transmission of λ and of F^+ from a lysogenic F^+ to a sensitive F^- occurred independently and separately to give derived λ^+F^- and λ^-F^+ stocks. The cell-to-cell transmission of F^+ in short term experiments is far more efficient than that of λ. These agents share the property of infectious transmission, but there is no evidence that λ is either a determinant or an agent of genetic recombination, and it is sharply delineated from F^+ in many other respects.

An incompatibility phenocopy. During the early investigation of genetic recombination it was noted that crosses of 58-161 (M^-F^+) and Y-10 ($T^-L^-Th^-F^-$) that had been grown to high densities (*ca.* 10^{10} cells per ml) with the help of vigorous aeration were markedly unproductive. It has now been shown that this low fertility is related to incompatibility. Aerated cultures of 58-161 (genetically M^-F^+) behave phenotypically as if they were F^-, giving few or no prototrophs with F^- testers, while remaining fertile with the same testers transduced to F^+. This behavior is a " phenocopy "; several dozen clones isolated from these suspensions, but re-grown without aeration, behaved again as F^+.

The physiological basis of the phenocopy is poorly understood. The substitution of CO_2 or N_2 for air or oxygen abolished the development of the phenocopy, but did not reverse it in fully grown suspensions. The modification also occurred when cells were aerated at 26°, although the lower temperature reduced the growth rate (crudely estimated) to the level of unaerated cultures at 37°. Cells harvested from partly grown aerated cultures at low cell density were phenotypically F^+, although the inoculum had been a previously aerated phenocopy. When the cells later reached saturation density, they again reacted as F^-. The effects were similar whether aeration was accomplished by bub-

bling air (Madison) or rolling the cultures (Milan). An accumulated metabolite is possibly involved, but has not yet been directly demonstrated.

Only those cultures derived from 58-161 have responded to aeration. The reaction is inherent in the cells and not in the F^+ agent, as determined by the responses of stocks made up from F^- cells and F^+ agents transduced from various sources. In particular, Y-10 transduced by F^+ from 58-161 has not shown any definite response to aeration. Aeration might be thought to affect the F^+ agent itself, or to interfere at some other stage of the development of the compatibility phenotype. Most of the limited evidence supports the latter view. As already mentioned, aeration does not seem to influence the genetic stability of the F^+ potentiality, as might be expected if it were inactivating the F^+ agent, and the response to aeration is an attribute of the residual genotype, rather than of the transmissible F^+ agent. In addition, aerated 58-161 cells which were phenotypically F^- successfully transmitted the F^+ agent to W-1607 in mixtures plated out after a short time.

Hfr (*High frequency of recombination*). In the course of selection experiments for resistance to nitrogen mustard (CAVALLI and VISCONTI 1948), a derivative of 58-161 was isolated which displayed a high frequency of recombination and was designated *Hfr* (CAVALLI 1950). M^-Hfr crosses with $T^-L^-Th^-F^-$ appear to produce one hundred to one thousand times as many prototrophs as M^-F^+ crosses, but a proper comparison of mating efficiency requires a detailed kinetic analysis (NELSON 1951) which remains to be applied here.

In compatibility tests, *Hfr* resembles the F^+ phenotype. However, efficient attempts to demonstrate the transduction of an F^+ agent from *Hfr* to F^- stocks have been entirely unsuccessful. Similarly, all progeny of $Hfr \times F^-$ have been F^- (with very rare exceptions). These progeny resemble the F^- parent in every material respect; they are compatible with F^+ testers, but not with F^-; they fail to transduce F^+ to standard F^- testers; they can accept an F^+ agent from standard F^+ stocks to become compatible. The progeny of $Hfr \times F^+$ are usually F^+.

An F^+ agent may be present in the *Hfr* stocks in a masked or bound form. One old stock of *Hfr* was observed to have lost its exceptional productivity. It proved to have become F^+, as judged both by mating efficiency and by the capacity to transmit the agent.

The *Hfr* behavior affords another instance of the separability of the compatibility phenotype and the transmissible F^+ agent.

It has been suggested (*cf.* HAYES 1952) that the F^+ agent acts directly as a vehicle of genetic recombination. Since the propagation of the F^+ agent persists in incompatible phenotypes (aerated 58-161) and conversely recombination occurs without the transmission of an F^+ agent to the progeny ($Hfr \times F^-$), this hypothesis may be inadequate to account for all of the evidence. We suggest instead that the F^+ agent is one causal element which determines sexual compatibility in conjunction with the residual genotype and environmental conditions.

Compatibility preferences. The infective transmission of F^+ permits a com-

parison of $F^+ \times F^-$ with $F^+ \times F^+$ against a uniform residual genotype. In all the combinations tested: $M^- \times T^-L^-Th^-$, $M^- \times P^-G^-$ (requiring proline and glycine, W-1678), and $T^-L^-Th^- \times P^-G^-$ plated on minimal agar, $F^+ \times F^+$ has been less productive than the corresponding $F^- \times F^+$. The effect has differed in different combinations, being most striking in $T^-L^-Th^- \times P^-G^-$, where the $F^- \times F^+$ cross has been more than one hundred times as productive as $F^+ \times F^+$. Unfortunately, no $P^-G^-F^-$ is yet available for the reverse cross. The differential is also apparent (about a tenfold increment) in comparisons of $T^-L^-Th^-F^+ \times$ aerated and unaerated M^-F^+ (58-161). In this instance, the F^- status is phenotypically impressed rather than genetically determined. These results are readily compatible with the concept of relative sexuality (HARTMANN 1929). F^- would have a grade of zero, 58-161 (unaerated) would be 2^+, Y-10F^+ ($T^-L^-Th^-F^+$) would be 3^+, W-1678 ($P^-G^-F^+$) would be 4^+, and *Hfr* the highest $^+$. The fertility of different combinations would be proportional to the difference in their relative grade. Although a number of stocks so far tested have agreed with this scale, many more must be tested and the productivities of different crosses must be quantitatively expressed to provide a basis for final judgment. It cannot be said whether differences in grade stem from the nutritional mutations directly, or from other genetic differences inherent in the various stocks and not otherwise detectable.

Compatibility and linkage. The limitations that are imposed on linkage analysis by the necessity for selective isolations of sexual progeny have been discussed at length elsewhere (LEDERBERG *et al.* 1951). Nevertheless, the linear arrangement of genes in *E. coli*, K-12 has strong experimental support (LEDERBERG 1947; ROTHFELS 1952). The most specific evidence of deviations from the familiar pattern of chromosomal segregation is the structure of persistent heterozygous diploids, which occur in certain crosses (LEDERBERG 1949, 1951). As a rule these diploids are heterozygous for all of the differential markers of the parents except for *S* and *Mal* (streptomycin response, maltose fermentation). The latter has been proved to be hemizygous in the otherwise heterozygous diploid, from which it has been concluded that the diploids are regularly deficient for a chromosomal segment which includes the linked *Mal* and *S* markers. This deficiency also perturbs the segregation of other markers which although digenic are adjacent to the segmental deficiency. If one assumes that a similar deficiency is the rule in the ordinary, transient zygotes, one must expect that normal linkage relationships will be obscured not only for *Mal* and *S*, but for other markers in accordance with their proximity, exactly as has been found for *Mtl* and *Xyl* (mannitol, xylose fermentation) (NEWCOMBE and NYHOLM 1950; CAVALLI 1950; LEDERBERG *et al.* 1951). Although deficiency for the *Mal-S* segment occurs with great regularity, diploids have been isolated which are heterozygous for each one of the ten or more differential markers of the parents, including *Mal* and *S*. These intact diploids, although rare, are compelling evidence that the underlying chromosomal mechanisms in *E. coli* are regular although complicated by modifications not yet thoroughly understood.

A hitherto enigmatic question has been the determination of which parental genotype would suffer the segmental elimination. Some regularities had been observed previously: that is, in crosses that would now be symbolized $M^-Mal^+F^+ \times T^-L^-Th^-Mal^-F^-$ the diploids were usually hemizygous Mal^-. When it was observed that a *filial* (*i.e.*, F^+) $T^-L^-Th^-Mal^-$ parent gave the opposite result, hemizygous Mal^+, in a comparable cross, this was taken as evidence for structural heterozygosity of the original parents. These differences are also reflected in the distribution of markers to haploid prototroph recombinants. The detailed segregation differences between parental (F^-) and *filial* (F^+) $T^-L^-Th^- \times M^-$ cited in a previous paper (LEDERBERG *et al.* 1951) are reproducible with $T^-L^-Th^-$ made F^+ by transduction. These experiments are therefore immaterial to the cited hypothesis of structural difference.

These crosses have been studied more closely since the compatibility situation was better understood. $M^-Mal^+F^+ \times T^-L^-Th^-Mal^-F^-$ gives diploids which are mostly hemizygous Mal^-. $M^-Mal^+F^+ \times T^-L^-Th^-Mal^-F^+$ gives diploids mostly hemizygous Mal^+. Unfortunately, material is not available for diploid analysis of a comparable cross $M^-Mal^+F^- \times T^-L^-Th^-Mal^-F^+$. If one accepts the higher F^+ potency of the $T^-L^-Th^-Mal^-F^+$ over $M^-Mal^+F^+$, as postulated earlier on the basis of productivity comparisons, we can generalize that segmental elimination tends to occur in the contribution of the parent with the higher relative potency. This is borne out by the segregations in haploid prototrophs, comparing the various combinations: A, $M^-F^+ \times T^-L^-Th^-F^-$; B, $M^-F^+ \times T^-L^-Th^-F^+$; and C, $M^-F^- \times T^-L^-Th^-F^+$. (For more elaborate tabulations, see LEDERBERG *et al.* 1951. Cross A is given in table 5A, Cross B in 5B, and Cross C gives segregations indistinguishable from B. Further documentation and discussion is held in abeyance pending a substantiation of the concept of relative potency.)

Occurrence of the F^+ agent. Some forty new strains of *E. coli* have been found that are fertile with K-12 testers. These have been screened from over 2000 separate isolates by means of *SRP* selection. Unfortunately, W-1177, F^-, was used as the tester for most of the screening experiments, so that potentially fertile strains which behave as F^- were passed over. A re-screening of a limited number of *E. coli* strains with the use of an F^+ tester has produced a crop of F^- fertile strains about equal to the number of fertile strains detected with W-1177 as a tester (DOROTHY C. GOSTING, unpublished). In at least seven new strains, F^+ has been detected by its transduction to W-1607 and W-1177, but some of the foreign agents appear to be unstable in the K-12 line. One other new strain that is compatible but F^- by the criterion of transduction has been infected with the F^+ agent from K-12. However, its mating behavior was not altered, and the latent F^+ agent could be detected only by its re-transfer back to W-1607. In view of this diversity of behavior, and possible biasses in the collection of this sample of cross-fertile strains, no generalizations can be stated as to the role of the F^+/F^- compatibility system in strains other than K-12.

Salmonella typhimurium and a number of infertile strains of *E. coli* have

been tested for $F+$ with negative results by the criterion of transduction. However, the " Waksman " strain of *E. coli,* ATCC 9637 (DAVIS 1950b) which had previously been thought to be infertile, carries an $F+$ agent. Upon re-examination, this strain was found to be self- and inter-fertile (with K-12), but at a low rate which barely permitted an unequivocal demonstration. The extremely low productivity of this strain must be ascribed to other, unknown causes, and not a deficiency with respect to the compatibility factor.

DISCUSSION

The inference of a sexual mechanism in *E. coli* has rested almost entirely on indirect genetic evidence. No one has succeeded so far in verifying or controverting this conclusion by a morphological demonstration of the biology of genetic exchange. Efforts in this direction had been discouraged by the apparently sporadic occurrence of such exchanges in mixed cultures. In an unanalyzed homothallic system, the rare occurrence of sexual fusions must be ascribed to chance. With a probability as low as one per million that a given pair of cells would mate, there is little likelihood of successful and verifiable microscopic observations. For this reason, the research of the past five years has been devoted largely to the exploitation of cross-breeding for physiological and formal genetic analysis, rather than to studies on the sexual mechanism itself.

The present studies have opened an avenue for further work depending on physiological and genetic control of mating. Hitherto, morphological studies were based on the concept of homothallism. One would have anticipated that sexual fusions occurred equally among the cells of a given parental culture and in cross-matings. Possibly relevant morphological processes that occurred uniquely in a mixed culture, and not in the parents alone might have been overlooked. The more recent recognition of self-incompatibilities necessitates a reversal of this point of view. Studies on the morphology of self-incompatible cultures are now seen to provide a necessary control on observations in mixed cultures where the results of a sexual process are genetically detectable. In addition, it is now and should become more and more feasible to plan experiments on a rational basis to provide the maximum frequencies of sexual fusions. It may therefore be hoped that this limitation to microscopic verification will be overcome.

Despite these hopeful auguries, the incompatibility experiments have raised more questions than they have answered. The physical basis of the transduction of the $F+$ agent is especially paradoxical, for the effect is transmitted from one cell to another, and in heredity, with very high efficiency, but no evidence of a material agent in transit has been secured. It is possible, of course, that the ambulant factor is highly unstable, or that it is communicated by direct contact. Some irony may also be seen in the fact that the one ambulatory gene (excluding λ from such consideration) to be detected in *E. coli* so far is decisive for the entire process of sexual recombination, and that its extra-nuclear transmission was responsible for its having been overlooked. By

contrast, each of the genetic factors of *Salmonella typhimurium* appears to be transducible by means of phage particles (ZINDER and LEDERBERG 1952). The Salmonella system differs from *E. coli* not only in the filtrability of the agent of genetic exchange, but in the individual transmission of each factor, no linkages of any kind having been detected. As discussed elsewhere, however, the fragmentation of the genotype in transduction does not necessarily exclude a chromosomal basis for normal hereditary transmission (LEDERBERG 1952). Further work may be expected to clear up the now obscure relationships of the distinctive processes of recombination now known for different bacteria, sexual recombination involving genetic linkage groups, and transduction of individual genes or very small fragments.

The F^+/F^- relationships suggest speculations on heterogametic differentiation. Evidence supporting a physiological differentiation of F^+ and F^- parents is provided by HAYES' experiments (1952) which imply that streptomycin-" killed " cells can function as F^+ but not as F^-. Morphological correlates of this differentiation have not been demonstrated. The effects of this differentiation on the segregation mechanism are still *sub judice,* but analogies are not difficult to find in the higher organisms, *e.g.,* in the selective elimination of paternal sex chromosomes in *Sciara coprophila* (METZ 1938).

Whether the incompatibility relationships are evidence of a rigorously heterothallic system remains to be settled by further work. The decision rests on the interpretation of $F^+ \times F^+$ crosses. If cells of identical F^+ status are actually compatible with each other, we should not speak of heterothallism, but rather of a genetic requirement for fertility. The low productivity of many $F^+ \times F^+$ crosses is the main argument for a more complex situation. We have seen, however, that the compatibility phenotype of 58-161 (F^+) is subject to modification by environmental effects. One might then argue that, to different degrees, F^+ cultures are heterogeneous with respect to compatibility phenotype. On this basis, crosses of $F^+ \times F^+$ cultures may actually involve $F^+ \times F^-$ cells. Differences in apparent potency might then refer to the fractions of phenotypic variants. Alternatively, F^+ may exhibit different grades of expression, depending on the genotype of the culture and on the environment. Although the latter interpretation seems more likely, a definite conclusion is not yet possible, and we are not even entitled to insist on the premise that an effective opposition of F^+ to F^- is a prerequisite of mating.

SUMMARY

Fertility of *E. coli* crosses has previously been thought to be homothallic or genetically unrestricted. This view has been altered with the discovery of self-incompatible stocks, designated as F^- mutations. Thus, $F^- \times F^-$ is completely infertile. $F^- \times F^+$ and $F^+ \times F^+$ are both fertile, but the latter combination is less productive in such a way as to suggest a gradient of relative sexual potencies among various F^+ stocks.

Self-compatibility is determined by an ambulatory or infective hereditary factor that is readily transduced from F^+ to F^- cells in mixed culture. The

phenotypic expression of F^+ is subject to environmental control (aeration) in some stocks.

The polarity of crosses with respect to compatibility status influences the segregation mechanism in an orderly way, not yet well understood, but interpretable on the basis of a sexual process underlying recombination in *E. coli*.

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