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CORRELATED SELECTION FOR MOTILITY AND SEX-INCOMPATIBILITY IN ESCHERICHIA COLI K12

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Sexual fertility in *Escherichia coli* is influenced by a number of factors, environmental and genetic.[1] One of the latter is *F*, which is diagnosed by the following rules of compatibility: $F^+ \times F^+$ and $F^+ \times F^-$ are fertile, but $F^- \times F^-$ is sterile. The wild-type K12 strain is F^+, but "self-incompatible" F^- mutants have appeared sporadically in laboratory cultures. Among the genetic elements of *E. coli*, *F* is remarkable for its high contagiousness: F^- cells become F^+ when the two types are grown in mixed culture. Furthermore, the progeny of $F^+ \times F^-$ crosses are uniformly F^+. Therefore, a reliable method of obtaining F^- derivatives of various stocks would be desirable as a technical help[2] as well as for its possible clarification of the nature of *F*.

In the course of immunogenetic studies of *E. coli*, various strains were *motilized*, that is, passed through semisolid agar to select for the highest motility and development of flagellar antigens. Many of the selected clones proved to be F^-. The experiments reported here indicate that this is a result of a selective advantage of F^- mutants rather than a direct induction of them by the technique.

Experimental.—The motilization medium (NGA) consists of nutrient broth plus 8 per cent gelatin and 0.4 per cent agar.[3] Comparable results were obtained in the same medium without gelatin. In these media, nonmotile bacteria grow only at the site of inoculation, while motile bacteria eventually swarm through the medium. After an initial lag, swarms appear from which faster swarms may arise in turn. The fastest rate of swarming observed on this medium (at 37° C.) was 3 mm/hr. The succession of faster swarms upon subcultures on fresh NGA medium,

together with preliminary genetic analyses, indicate that motility is under polygenic control.

For motilization, the bacteria are inoculated at the top of a culture tube containing a column of NGA about 6 cm. deep. After swarmers reach the bottom, the top 5-cm. layer is melted off and a loopful from the bottom layer streaked on nutrient agar.[4] Single-colony cultures are tested for their F status by established methods.[5]

Many F^+ substrains of *E. coli* K12 have been motilized and tested for their F status. About half of several hundred of these motilizations have given F^- clones after a single tube passage. A few dozen cultures, which had not yielded F^- progeny at first, were examined further, and it was always possible to obtain an F^- culture after a few additional passages.

Most of the isolates so obtained are identical with standard F^- cultures in the following respects: (1) their compatibility pattern, which is clonally stable; (2) their reconversion to F^+ when grown with F^+ cells; (3) the clonal stability of the reconverted F^+; and (4) the production of F^- derivatives when these are again motilized. Self-incompatible mutants were also obtained from another mating type, *Hfr*, including some novel types. These included refractory F^- (not reconvertible to F^+ by mixed culture) and stocks of intermediate fertility; they require further study.

The incompatible phenotype has also been secured by growing F^+ cultures with heavy aeration. However, this is an F^- *phenocopy*. Unlike the F^- mutants, these incompatible cells produce only compatible F^+ clones when they are grown out under standard conditions. In contrast, some motilized F^- substrains have been subcultured for four years without manifesting any change in this trait. The motilization technique has been applied successfully to all of the principal crossing stocks in use in this laboratory.[2, 5, 6]

There remains the problem whether motilization induces or selects the F^- mutants. During the procedure, the bacteria are continuously exposed to fresh medium and are therefore growing rapidly under chemostatic conditions. The conditions might be analogous to those for the attenuation of kappa in *Paramecium*.[7] To explore this analogy, various F^+ cultures were grown in chemostats[8] in a defined medium. Although the first run did give an F^- isolate (perhaps by an accident of periodic selection), no further trials were successful, nor was a trial in which a dilute *Hfr* culture was transferred for 100 fissions at intervals of 4 hours (10 fissions). These results were therefore indecisive.

F^+ cultures generally, including those obtained by reconversion of motilized F^- isolates, are not strikingly less motile than their F^- counterparts. Different stocks show wide differences in their initial motility, independently of their F status. However, the competitive status of F^+ and F^- cells could be assessed directly in NGA, as follows. To minimize genetic heterogeneity, closely related stocks were developed. A Gal^- (galactose- negative) F^+ stock was motilized, and a Gal^-F^- isolate recovered. From this, Gal^+F^-, Gal^-F^+, and Gal^+F^+ derivatives were secured by reverse mutation (selected on EMB galactose agar), F reconversion, or both. Pairs of cultures were then mixed in equal proportions and inoculated in broth and in NGA plates. After overnight incubation, the broth cultures were plated on EMB galactose agar for relative counts of Gal^+:Gal^-. The NGA plates were incubated for 24 hours, at which time samples were cored out from swarms

(about 7 cm. from the point of inoculation), dispersed in water, and plated likewise. As shown in Table 1, the Gal^+ marker is not entirely neutral and has an advantage

TABLE 1*

COMPETITION OF Gal^+/Gal^- AND F^+/F^-

Mixture		Inoculum Ratio		Ratio after Passage in Broth		Ratio after Passage in NGA		Samples from Replicate NGA Passages		
Gal^+	Gal^-	Gal^+	Gal^-	Gal^+	Gal^-	Gal^+	Gal^-	Pure Gal^+	Pure Gal^-	Mixed $Gal^{+,-}$
F^+	F^+	246	194	226	186	393	4	0	0	10
F^+	F^-	229	155	370	154	0	595	0	9	1
F^-	F^-	187	142	355	127	606	400	0	0	10
F^-	F^+	247	240	317	193	310	0	7	0	1

* The counts are from two plates each of EMB galactose agar. The total density of the inoculum was about 10^9/ml. Its growth in the broth passage would correspond to about 10 fissions. In NGA this would be more variable, but some cells at the frontier might have a history of up to 50 fissions if they were not inhibited by metabolic products diffusing from the population behind them. In the replicate NGA passages, one sample from each motilization plate was streaked on EMB galactose agar.

over Gal^-, but this is superimposed on, and can be overridden by, an advantage of F^- over F^+ during passage in motility agar. The consistency of the selective differences was shown by replicate tests with the same mixtures. However, this experimental design still suffers from the possibility of random mutational drift (with respect to motility polygenes) in the few isolated clones.

These findings encouraged attempts to measure differences in the motility of comparable F^- and F^+ isolates. Exponential phase cultures of a motilized isolate of W945 ($T^-L^-Th^-Gal_2^-F^-$) and of K12 (Gal^+F^+) were mixed in broth at a ratio of 1:10. After 1 hour's incubation, when about half the F^- cells should have been converted to F^+, the mixture was plated on EMB galactose agar. Gal^- colonies were picked to broth. These cultures were tested for their compatibility by appropriate crosses and, at the same time, loopfuls were inoculated into NGA tubes. The progress of the swarm front was measured after 16 hours' incubation. Twenty-four isolates proved to be still F^-; seventeen were F^+. As shown in Figure 1, there

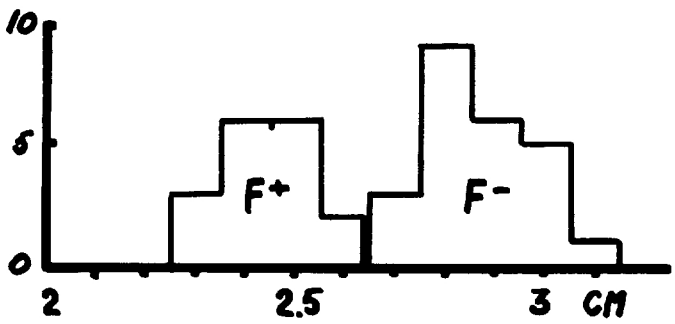

Fig. 1.—Relative motility of F^+ and F^- isolates. *Ordinate:* frequency. *Abscissa:* progression of swarm front after 16 hours' incubation of NGA tubes.

was a clear assortment of these classes in respect to motility ($t_{39} = 13$; $p < .01$), although the intraclass variation (random drift) is equally plain. The same experiment has been repeated with other F^- stocks which were inherently more variable, with similar if less striking results.

Discussion.—The multistep response to selection suggests that motility in bacteria is under polygenic control, as it is also in *Drosophila.*[9] Bacterial flagella are also subject to oligogenic mutations[3] which, however, are not involved in these *E. coli* stocks. On this polygenic background, the F^- mutation appears to have a further effect in augmenting motility, which is a sufficient explanation of the correlated selection. This assumes the occasional occurrence of spontaneous F^- mutations. Whether the sporadic mutants already cited,[5, 6] were "spontaneous" or related to incidental mutagenic treatments would be different to verify without more convenient methods of detecting rare F^- mutants. The same obstacle makes it difficult to rule out further direct effects of the motilization procedure on the rate of F^- mutation.

An effect of F status on motility is not unexpected if the compatibility phenotype is to be associated with the cell surface. F^- cells are reportedly more electronegative[10] and hence less readily agglutinated in saline and certain antiserums than F^+. Unfortunately, *E. coli* K12 is so "rough" as to be particularly unsuited to serological studies by agglutination techniques. Further work is therefore needed to test such speculations as the identification of a hypothetical F substance with a surface layer that mechanically impedes motility. Immunogenetic studies with more suitable strains are in progress.

The inference that motilization has only a selective effect limits its interest for the general problems of the nature of F, whether it is a nuclear or extranuclear element, and the manner of its transmission in reconversion experiments. On this hypothesis, the procedure has no bearing on the mutational event $F^+ \rightarrow F^-$, but only on its secondary phenotypic effects.[11]

Summary.—In the course of selection for high motility, F^+ (sexually compatible) cultures of *E. coli* frequently produce F^- (self-incompatible) clones. This technically useful effect can be explained by a higher motility of F^- genotypes, superimposed on a polygenic control of motility.

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[1] See review by J. Lederberg and E. M. Lederberg, *Symposia Soc. Development and Growth,* **14,** 101, 1956.

[2] T. C. Nelson and J. Lederberg, these PROCEEDINGS, **40,** 415, 1954.

[3] The history and application of this medium are discussed by J. Lederberg, *Genetics,* **41,** 845, 1956.

[4] This simple procedure (cf. P. R. Edwards and W. H. Ewing, *Identification of Enterobacteriaceae* [Minneapolis, Minn.: Burgess Publishing Co., 1955]) has perils for the uninitiated. To avoid an explosion, the plug of agar should be broken with a long wire loop or by spinning the tube while it is being heated. An improvised socket clamp for the tube, with an extension rod to rotate between the fingers is helpful. Many complicated methods, including U-tubes and concentric tubes, for reaching the same ends have been described, and NGA plates may also be used where convenient.

[5] J. Lederberg, L. L. Cavalli, and E. M. Lederberg, *Genetics,* **37,** 720, 1952.

[6] W. Hayes, *Cold Spring Harbor Symposia Quant. Biol.,* **18,** 75, 1953, has mentioned an F^- mutant stock he received from C. C. Spicer. Dr. Spicer has informed us, in retrospect, that the parent culture had in fact been motilized and put in cold storage before being sent to Dr. Hayes.

[7] J. R. Preer, Jr., *Genetics,* **33,** 349, 1948.

[8] A. Novick and L. Szilard, *Cold Spring Harbor Symposia Quant. Biol.,* **16,** 337, 1951; B. Rot-

man, *J. Bacteriol.*, **70**, 485, 1955. We are indebted to these authors for their help with these experiments.

[9] H. L. Carson, *Genetics*, **41**, 636, 1956 (abstr.).

[10] G. A. Maccacaro and R. Comolli, *J. Gen. Microbiol.*, **15**, 121, 1956.

[11] Y. Hirota, *Nature*, **178**, 92, 1956, has reported that exposure of F^+ cells to Co^{++} solutions induces the formation of F^- types in certain strains.