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Genetic evidence for sex in bacteria. Joshua Lederberg, Jane Coffin Chilis Fellow in Medical Research, and E+L+Tatum, Professor of Microbiology, Yale U.

In the short time allotted, it will be impossible to discuss in any detail all the experiments that have led us to the conclusion that there is a sexual phase in the life history of a strain of Escherichia coli with which we have been working. It seems best to present a single experiment in detail, which most forcefully illustrates the kind of evidence that has been accumulated. The general thesis of these experiments is the same as that which was proposed by Sherman and Wing 10 years ago, that if there is a sexual phase in bacteria, it should be possible to show that when mixed cultures containing organisms differing in various characters are studied, new types will be found which are characterized by recombinations of those characters. The strains used by these authors were too variable for them to draw any conclusions as to the occurrence of sexual fusions, and other authors have had similar difficulties.

Several types of characters have been used in our investiagtions. They have all been obtained as mutations in a single strain of E. coli (strain K-12). The spontaneous variability of eachbof these characters has been studied intensively and it can be anticipated categorically that none of the phenomena which I am bout to describe can be accounted for on this basis.

Most of the characters used are nutritional requirements, i.e. the need for a specific growth factor, including such compounds as biotin, thiamine, threonine, leucine, and other vitamins and amino acids. They are sharply determinable, since optimal growth can be obtained in the presence of the specific requirement, whereas in its absence growth does not take place. The requirements ~~are found to originate~~ were obtained by mutation, one at each mutational step. By ultraviolet irradiation of a strain which is already a biochemical mutant, multiple mutants can be obtained, and these have been very useful

in these studies. Other characters which have been used are resistance to a specific bacteripphage, and the inability to ferment lactose (as indicated on Eosin-Methylene Blue-Lactose plates such as these.)

Character recombination in E. coli can be detected by the appearance of wild types in mixed cultures of biochemical mutants. Such wild types have never been found in the individual cultures. These wild types can be detected readily since they form colonies in minimal agar medium, whereas the original mutants are unable to proliferate in the absence of their specific requirements. Thus although only about 1 cell in 10 million in these mixed cultures is a wild type, they can be readily detected by plating about 500 million cells after careful washing, into a minimal agar plate.

If these wild types are the result of recombination of characters, these colonies should also show recombination of other character differences present in the 'parental strains+' This has been found to be the case. $B_1^+ M^+ T^- L^- B_1^- Lac^- T_1^S$ $B^- M^- T^+ L^+ B_1^+ Lac^+ T_1^R$

By plating Y-53, which is.. and Y-10 into agar containing thiamin, but lacking biotin, methionine, threonine or leucine, the colonies which appear should represnt those cells which have recombined with respect to B.M, T and L, as indicated on the boards. One should also expect to find that the other 3 characters should recombine also, namely B_1^-; Lac- and T_1^r. There are 8 possible recombinations of these characters, as indicated, and the ones which are part of the parental complexes are encircled. All 8 have been recovered, as can be demonstrated by this plate, and these tubes. The figures on the board represent the relative frequencies with which they have been obtained. The disparity in these numbers is of course, of great interest; some evidence has been obtained that this disparity may be explicable on the basis of the same kind of factorial linkage that characterizes the inheritance of higher organisms.

Evidence has been obtained that in mixed cultures containing more than 2 types, recombination takes place between only 2 types of cell at a time, strengthening the hypothesis that these data are only to be explained by a cell fusion and the orderly segregation of genes.