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Dear Dr. Dougherty:

Thank you for your reprints and your letter of the 21st.

The experiment that you propose has been considered, and, to a degree, attempted. There are some difficulties both in execution and interpretation, but the results do suggest the occurrence of intracellular recombination.

I do not believe that there are any pairs of auxotrophic mutants for which the experiment would be feasible, owing to the relatively low rates of induced reversions. Mutants that were inherently revertible enough to be useful would probably never have been characterized as auxotrophs in the first place. If they were available, however, they would be best handled by using replica plating (rather than relying upon syntrophic maintenance) along the lines of the following experiment:

Both Witkin (Cold Spr. Harbor Symp. 1951, p. 357) and Newcombe (1952, Genetics, 38:134) have studied UV-induced mutations from Lac+ to Lac-, and from V_1^s to V_1^r, and their coincidence, in E. coli strain B. Their technique was to select for V_1^r mutant clones, by means of phage T1, on an EMB lactose agar on which coincidental Lac- mutants could also be detected. Both authors found an appreciable fraction of V_1^r clones that contained both Lac+ and Lac- components, and the question is how to explain them. Neither Witkin nor Newcombe appear to have favored the suggestion (see the CSH paper discussion, p.372) that they may arise by recombination between Lac+V_1^r and Lac-V_1^s nuclei arising by mutation in an originally Lac+V_1^s cell, or by the other possible "cross" of a double mutant by wild type. If this possibility is barred, one probably has to accept some sort of delayed or fractional effect of the UV, so that it would be a matter of some interest to settle it. The technique that was used precluded the isolation of V_1^s components in these clones: if it could be shown that many of the clones already known to have Lac+V_1^r and Lac-V_1^r also contained Lac-V_1^s individuals, the suggestion of recombination would be verified. Actually, it should not be too difficult to look for such cells by replica plating: UV-treated bacteria would be plated to give about 1000 survivors per plate, and the colonies replicated (while still small) to EMB lactose+ phage. If any Lac-V_1^r show upon the replicas, Lac-V_1^s can be rather easily looked for in inocula from the homologous sites on the original. The same design can be used, of course, for

other markers. If the coincidences were too infrequent, the experiment could still be done, using larger numbers of the surviving bacteria, but the definition of single clones would be less reliable.

When this work was first done, we all believed that E. coli B was an infertile strain; it has since been found that B is an F- strain, that can be crossed with other F+ strains, or made F+ (by contagion) and self-compatible.

I must add that we have had other tests for recombination in transitory heterokaryons (e.g., immediately after crossing) and that the results, generally, do not support its occurrence; it would not surprise me at all if uv treatment had something to do with it.

Unfortunately, both Witkin and Newcombe are working on new problems, and this particular problem is still left hanging. Could you be interested in going into it? Technically it ought not to be especially difficult or tedious, but would of course take some time. If you wanted to visit us, say for a month, that ought to wrap it up-- or if I could be of any assistance to your doing it at Berkeley, please let me know.

It is easy to at least guess what you're driving at in this matter- whether this process represents a stage in the evolution of sexuality. But as the B story illustrates, it will be impossible to decide whether intracellular recombination developed first, or whether strains that show this to the exclusion of intercellular sexuality are simply degenerate or defective in their ability to mate. You must have faced the same problem in evaluating Pontecorvo's "parasexual" recombination in fungi.

(By the way, I fear that Pontecorvo has muddied the field, and the impact of his own brilliant studies, by his terminology of "parasexuality". If it were possible to start again, I would have thought "parasexual" to be an excellent term to describe the external paraphernalia of sexuality, reserving this for the fundamental process of karyogamy. Then "aparasexual" would describe "vegetative" fusion and recombination, etc. As matters stand, sexual vs. parasexual is an artificial cleavage, separating some processes of karyogamy on the one hand, from other karyogamies plus an assortment of quite different mechanisms of genetic recombination on the other. I would have thought that any logical classification would have separated karyogamy as a unique category. What do you think about this? Can anything practical be done? How did you handle it in your review-- or would a terminological appendix be out of the question? There are still a lot of prefixes not yet preempted, but I am sorry about "para".)

Yours sincerely,

Joshua Lederberg
Professor of Genetics