CLASSICS

Though bacterial sexuality was discovered in 1946 by Lederberg and Tatum, the evidence was entirely based on genetics. The experiment essentially involved the isolation of prototrophic recombinants by mixing two cultures of auxotrophs. Though the involvement of cell-cell contact was confirmed by the U-tube experiment of Bernard Davis, the evidence provided by this experiment was also circumstantial. The elegant short paper reproduced below is an attempt by Joshua Lederberg to identify mating pairs of bacteria microscopically, to find cytological evidence for bacterial conjugation. This is one of the early publications that demonstrated the cellularity of bacteria.

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CONJUGAL PAIRING IN *ESCHERICHIA COLI*[1]

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Previous attempts to correlate genetic with cytological evidence of bacterial sexuality have been indecisive (Lederberg and Tatum, Science, **118**, 169, 1953). Further progress has been achieved with a pair of *Escherichia coli* cultures which are highly interfertile and morphologically distinguishable. They are a motile K-12 Hfr (Cavalli *et al.*, J. Gen. Microbiol., **8**, 89, 1953) and an F- mating type of another strain whose cells are plumper and nonmotile. They are also marked by mutations for Lac, Mal, S, Xyl, Mtl, Ara, V_1 and Gal_2. Both are lambda-sensitive.

Sixteen hour cultures were mixed in the ratio one Hfr to ten F-, diluted in 10 volumes of penassay broth, and incubated 1 hour at 37 C. Reservoir drops of the mixed culture were then prepared as prescribed by de Fonbrune (*Technique de micromanipulation*, Masson, Paris, 1949); however, micropipettes were prepared by hand. The cultures were studied under darkfield at 150 × and under dark phase contrast at higher powers. When the reservoirs were joined to drops of fresh broth, a few per cent of the Hfr cells would carry F- mates, oriented as in figure 1, as they swam away. Other motile cells were stuck in large clumps.

The pairs were isolated to individual droplets. After about an hour, they would disjoin spontaneously, sometimes dividing meanwhile. The

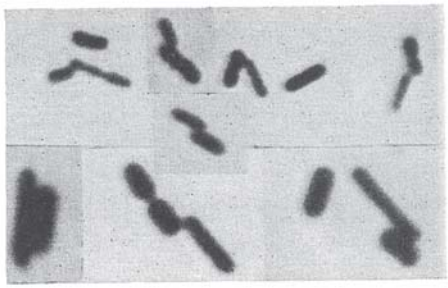

Figure 1. Conjugal pairs, from smear stained with crystal violet. Figures 1, 2, and 3 are at various magnifications; the transverse diameter of the bacteria is about 1 μ.

exconjugant cells were then reisolated and allowed to form larger clones; sometimes, successive daughters were separated first. The following day, the clones were transferred for further propagation and scoring of genetic markers.

Altogether, 279 pairs were isolated. Of these, 222 gave viable progeny from the Hfr exconjugant, none of which showed recombinations; 190

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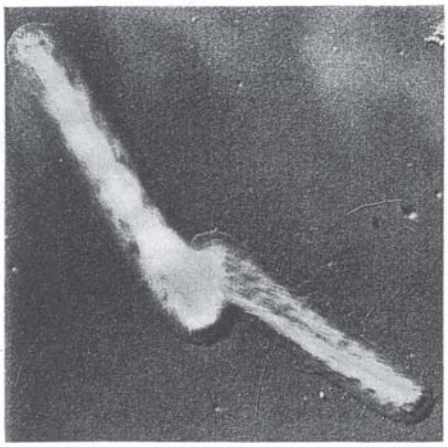

Figure 2. A conjugal pair? Electronmicrograph, chromium-shadowed, of formalin-fixed cells. The assistance of Dr. Paul Kaesberg and Dr. L. L. Cavalli in preparing this photograph is gratefully acknowledged.

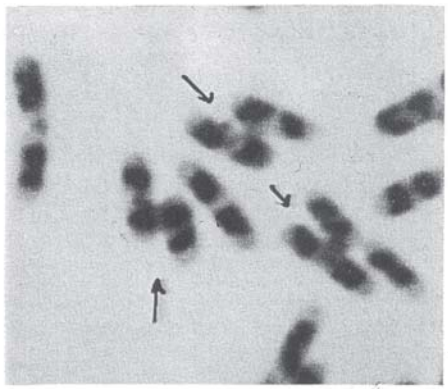

Figure 3. Conjugal pairs? Giemsa stain after acid hydrolysis and osmic fixation. The F- culture in this mating is also from strain K-12.

of the pairs gave viable clones from the F- exconjugant, of which 66 included recombinants as well as the original F- parental combination; 51 of these 66 pairs also engendered viable (and unaltered) clones from the Hfr exconjugant. Control platings of the whole mating population showed that fewer than 1 per cent of the total F- cells were Lac + S^r recombinants (Lac + from Hfr; S^r from F-). The distribution of recombinant types was strongly biased in favor of the F- parent. While 63 of the clones included Lac + S^r, among other recombinants, only one (still Lac- S^r) carried the Gal_2 and Hfr markers of the Hfr parent. As discussed elsewhere (Science, **122**, 920, 1955), haploid segregation data are ambiguous as to the mechanism of the bias. Further details are being collected and analyzed for fuller presentation.

The distinctive motility of the joined pairs cannot be displayed in a still photograph, and figure 1 is intended only to illustrate typical orientations. Owing to difficulties of resolution, the mode of union has not been visualized in living cells. On the other hand, figures 2 and 3 from fixed preparations merely invite apt questions, still being studied, as to the control of preparative artefacts. The principal conclusion that can be justified at present is that recombination is correlated with cell-to-cell pairing, that both exconjugants usually remain viable, and that the recombinants segregate only from the F- parent. These findings are consistent with the postulated transfer of a gametic nuclear element from one multinucleate parental cell to the other.

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