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ESCHERICHIA COLI

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## SINGLE-CELL ISOLATIONS OF DIPLOID HETEROZYGOUS ESCHERICHIA COLI

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In a previous report (Lederberg, 1949), certain cultures of *Escherichia coli*, strain K-12, were described as heterozygous diploids. This conclusion was developed from the following findings:

(1) Unlike the parent cultures, or recombinants usually isolated by means of nutritional or drug-resistance selection from mixtures of complementary types, these exceptional cultures were extremely unstable. They repeatedly threw off new cultural types exhibiting various combinations of nutritional requirements and fermentative characteristics. The segregated types remained stable upon further subculture.

(2) The presumed diploids were unstable only for characters in which the parents differed and were stable and similar to the parents for those in which the parents were alike. The characters studied included response to various bacteriophages as well as nutritional and fermentative qualities.

(3) Diploid cultures selected from parents differing in several characters afforded the opportunity to study their association or linkage. When a segregated colony proved to be stable or pure for any single character, it was found to be pure for all. That is, segregation involves a complete block of differential characteristics. The instability is thus best understood as the occasional separation of blocks of characters derived from the two parents, but more or less permanently associated.

(4) The intracellular level of this association is a necessary condition of their description as heterozygous diploids. The arguments previously offered were probably valid, but mostly indirect and genetic. Aside from the fact that the unstable, segregating cultures could be maintained indefinitely through judiciously selected single-colony isolations, the segregants included a significant proportion of different, new combinations (see table 1). Recombination during segregation suggests an intracellular association of factors, unless extracellular hereditary influences are assumed.

This last point is perhaps the least rigorous assertion of the previous work. The present investigation was undertaken to test this assertion by direct isolation of single cells under microscopic observation. In work of this kind, a closely attached pair of cells might be mistaken for a single cell, which possibility requires the most critical exclusion, because of the cultural behavior of the presumed diploids. Therefore, a method was adopted for following a pedigree from a single initial cell for several generations of fission. This technique has been used previously in a study of morphological variation in *Salmonella* (Zelle, 1942) and has been described in detail (Zelle, 1951).

TABLE 1  
*Fermentation tests on segregants isolated from individual Malv colonies*

151 MAL+ SELECTIONS				66 MAL- SELECTIONS			
No.	Mal	Mtl	Xyl	No.	Mal	Mtl	Xyl
141	+	+	+	61	-	-	-
6	+	-	+	4	-	+	-
3	+	-	-	1	-	+	+
1	+	-	+				

Individual *Malv* colonies were suspended in sterile water and restreaked on maltose agar. No more than one Mal+ and one Mal- colony was obtained from an individual streaking. The larger number of tests on Mal+ is due in part to the preponderance of Mal+ among segregants obtained in this way.

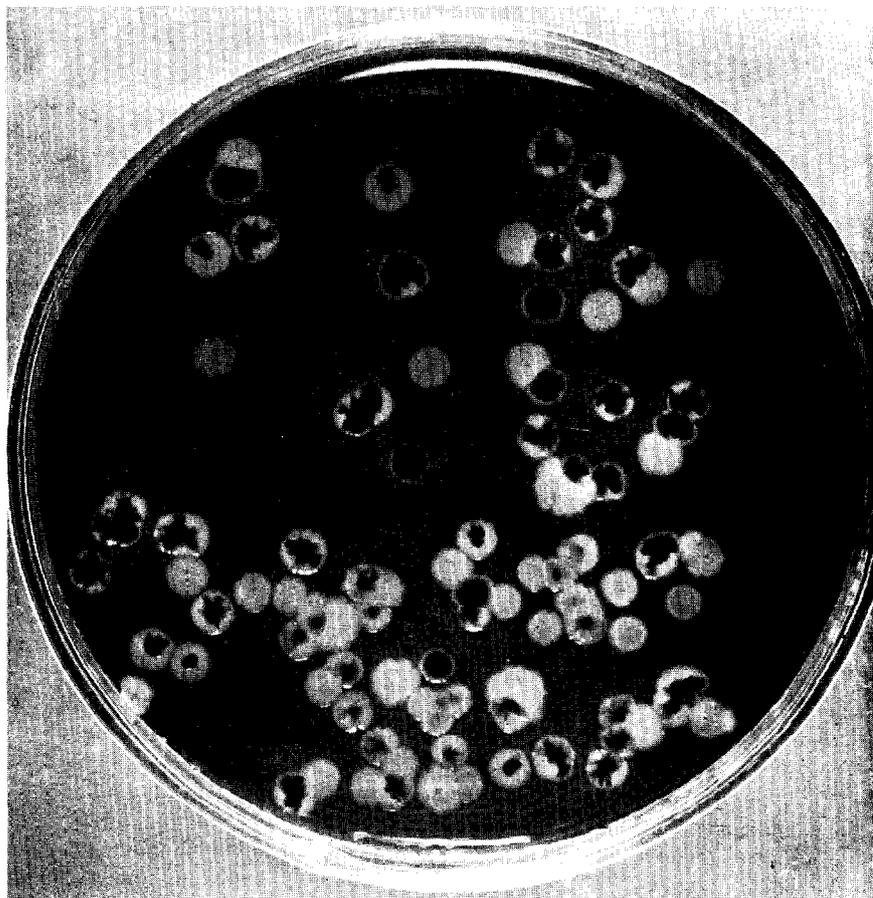


Figure 1. Appearance of *Malv* colonies from a heterozygous diploid culture plated on eosin methylene-blue maltose agar. Pure Mal+ and Mal- colonies are also observed.

## EXPERIMENTAL RESULTS AND CONCLUSIONS

Single-cell studies have been conducted on four different diploid cultures. The results obtained with one of these, H-226, will be recorded as typical. The parents of H-226 differ in the fermentation of maltose, xylose, and mannitol, as

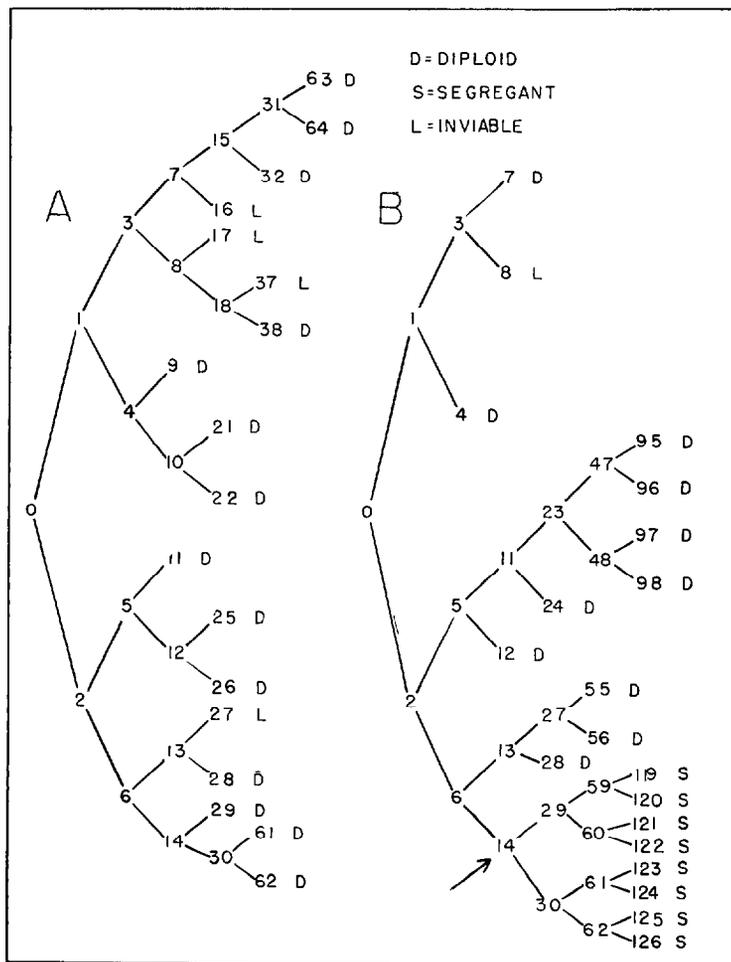


Figure 2. Single-cell pedigrees of diploid *E. coli* (H-226). A: A pedigree in which no segregation has occurred. B: A pedigree involving segregation. Cell number 14 (arrow) represents the point at which segregation occurred. The high incidence of lethals appears to be characteristic of pedigrees of diploid cells.

well as in a number of other characters (nutrition, phage responses), which for reasons of simplicity will not be detailed here. One parent was Mal<sup>+</sup>, Xyl<sup>+</sup>, Mtl<sup>+</sup>, whereas the other was Mal<sup>-</sup>, Xyl<sup>-</sup>, Mtl<sup>-</sup>. Parents and segregants are perfectly stable with respect to these characters under all ordinary cultural conditions. The nonfermenting characters were obtained independently of each other

as ultraviolet-induced mutations and are readily scored on eosin methylene-blue agar containing the respective sugars. In contrast to the uniform pure + or - colonies produced by the parents, H-226 gives variegated (*v*) colonies, like those illustrated in figure 1, when plated on agar containing any one of these carbohydrates. The *v* colonies are mosaics of pure + and - and still unsegregated cells, as is shown by replating them on the same medium.

The blockwise character of segregation from *v* into pure + and - types is shown in table 1, which gives the fermentative characteristics of 151 Mal+ and 66 Mal- colonies, each + or - colony having been recovered from a different *v* colony in order to sample a large number of different segregations. This table shows that most segregants retain a parental combination of fermentative characteristics (all + or all -), but a number show definitely new combinations. It should be emphasized that in these 217 tests, segregation was always complete, that is, no types like Mal- Xyl+ were recovered. Similar results are obtained from segregants first identified on mannitol or xylose agar and then tested on the three sugars.

Studies of pedigrees in the course of which segregation has occurred are still in progress and will be reserved for detailed report elsewhere. At this time we wish to report that several hundred single-cell isolations from diploid cultures have been made and analyzed. Most of these isolates, including 234 out of 257 from H-226, retained the instability pattern of the original diploid. The others (barring a few pedigrees from initial cells that were already segregants when picked) represent segregations that occurred during the development of the pedigree. In figure 2, two pedigrees are presented that demonstrate the persistence of the diploid (i.e., Mal+ Xyl+ Mtl+) condition throughout several iterated single-cell isolations. There can, then, be little doubt of the concurrence within a single cell of genetic traits originally contributed by two parents, and of their subsequent separation. Along the lines of previous discussions (Lederberg, 1947, 1949; Tatum and Lederberg, 1947), these data reinforce the experimental proofs for a sexual process in this bacterium.

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#### SUMMARY

Segregating diploid cultures of *Escherichia coli* were studied by a single-cell technique, involving the microscopic observation of the development of clonal pedigrees from single initial cells. The segregation pattern of these cultures was retained through the single-cell isolations so as to preclude the possibility that the diploids represent extracellular associations. The result further confirms the sexual basis of gene recombination in this bacterium.

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