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*SIBLING RECOMBINANTS IN ZYGOTE PEDIGREES OF ESCHERICHIA COLI**

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Until recently, the sexual process in *Escherichia coli* was inferred only from the genetic analysis of recombination, without morphological corroboration. Cavalli's discovery[1] of a highly fertile mutant (*Hfr*) has made it possible to relate genetic exchange to a pairwise conjugation of the mating bacteria. Lederberg[2] observed and isolated conjugal pairs under phase-contrast microscopy and showed a high incidence of recombinant genotypes among their progeny. Anderson[3] has made striking electron micrographs of conjugal pairs, in which one parent was labeled by the pre-adsorption of bacteriophage. The significance of conjugal pairing has

been underlined by recent experiments on the timed interruption of the sexual process by mechanical shearing or by chemical inhibition.[3]

The present report amplifies a preliminary account[2] of the segregation of various markers in single-cell pedigrees of isolated zygotes. Particular interest attaches to the occurrence of complementary recombinants, that is, whether crossing over is reciprocal, as in *Neurospora* and *Drosophila*, or not, as has been suggested for bacteriophage.

Cultures and Methods.—The parental strains were chosen as a highly fertile combination, differing in a number of easily scored markers which had been introduced by a sequence of mutations. W-3011, an *Hfr* mating type derived from strain K-12, is motile and relatively slender; W-2401, an F^- mating type derived from another line (wg28A) of *E. coli*, is nonmotile and plumper. The conjugal pairs are thus quite characteristic under the microscope. The isolation of pairs and of cell lineages followed de Fonbrune's techniques of micromanipulation.[2, 4] After isolation, the mating pair was left undisturbed for 1 or 2 hours, by when it usually separated spontaneously. The exconjugants were then isolated to separate microdroplets and allowed to form large clones either directly or after re-isolation of the daughters for one to six fissions to establish cell lineages. These clones were then studied for their genotypic content by platings on various indicator media.

Markers and Notation.—Nine markers were scored in this cross (Table 1).

TABLE 1

CHARACTERIZATION OF 75 EXCONJUGANT CLONES

A. Sequence of loci according to incidence of clones containing the 1 allele (from *Hfr* parent).** The 1 allele is indicated below, the 0 allele above.

−	*s*	−	*r*	−	−	−	+	−	000	0000	00	F^-
Lac	V_1	*Ara*	*S*	*Mal*	*Xyl*	*Mtl*	*Gal*	*Fla*				
+	*r*	+	*s*	+	+	+	−	+	111	1111	11	*Hfr*
70	29	25	5	6	5	2	1	2				

B. ××× 0000 00 clones* (total 65)

I. Clones with One Recombinant		II. Total Incidence of This Type†	III. Clones with More than One Recombinant‡	
100*	37	45	111 & 011	7
111	6	18	111 & 100	1
110	5	8	110 & 010	1
001	2	4	100 & 010	1
011	1	8	001; 110; 100; 010	1
101	1	1	001; 100; 010	1
010	0§	5	110; 100; 010	1
000	♀	♀		

C. ××× ×××× 00 clones‖ (represented individually)

1. 111 0010
2. 111 1000; 111 1111
3. 111 1000; 111 0000
4. 100 0100; 000 0100
5. 100 0110; 100 0000; 000 0110; 000 0000‡
6. 111 1111; 111 0000; 000 1111; 000 0000‡
7. 100 0000; 100 1100; 000 1100; 000 0000‡
8. 100 1100; 100 0010; 000 1100; 000 0010

D. 000 0000 ×× clones

1. 000 0000 10
2. 000 0000 01

* The terminal group 0000 00 is to be understood. Thus 100 should be read 100 0000 00.
† This item includes the occurrences in parts B and C.
§ This class may not always have been ascertained.
‖ The terminal group 00 is to be understood. Thus 111 1000 should be read 111 1000 00.
‡ The 000 0000 00 class (♀ parent) was present in every exconjugant clone. It is written explicitly to complete possible tetrads.
** The sequence *S Mal Xyl* is preferred as minimizing apparent multiple crossing-over.

Lac, *Ara*, *Mal*, *Xyl*, *Mtl*, and *Gal* refer to the fermentation of lactose, L-arabinose, maltose, D-xylose, mannitol, and galactose, respectively; V_1 and *S* refer to the reactions (sensitive versus resistant) to phage T1 and to streptomycin. *Fla* refers to flagellation, scored by motility. However, for the purpose of this analysis, the phenotypic status of each recombinant is less important than the parental origin of the marker alleles. For simpler exposition, the allele from the F^- parent is designated 0, and its alternative from the *Hfr* parent, 1. For a marker not segregating or for another reason not specified in a given genotype, the designation will be x. Deleted segments are indicated as □□. The loci are written in the order specified in Table 1. This convention having been established, the genotypes are written as binary numbers; for example, 000 1111 00 represents Lac^- V_1^s Ara^- S^s Mal^+ Xyl^+ Mtl^+ Gal^+ Fla^-, the first three and the last two of the markers having stemmed from the F^- parent, the middle group of four (*S*, *Mal*, *Xyl*, *Mtl*) from the *Hfr*. Specific segments may be marked off by underscoring; thus 000 0000 00 refers to the *Ara-S* segment.

Hfr versus F^- represents another marker difference but was not systematically scored. However, some dozens of recombinants were all F^- with the single exception of D1 (000 0000 10) which was *Hfr*, in accord with Cavalli and Jinks's demonstration of linkage of *Hfr* to *Gal*.[5]

Both parents were sensitive to the temperate phage lambda, except that the Mal^- marker was associated with poor adsorption of the phage.[6]

Experimental Results and Discussion.—Table 1 exhibits the recombinant progeny from 75 conjugal pairs. About 125 other pairs that were isolated and gave a viable F^- exconjugant produced no detectable recombinants. The *Hfr* exconjugant was usually viable as well; however, all the recombinants appeared in the progeny of the F^- exconjugant. This asymmetry implies the unilateral transfer of genetic material from the *Hfr* to the F^- cell and justifies the description of the two mating types as ♂ and ♀, respectively.[7] However, *Hfr* × *Hfr* crosses are also fertile, though F^- × F^- are not. The *Hfr* is therefore ambivalent, but in crosses with F^- (obligate ♀) it can function only as a ♂. By analogy with the mating systems of other fungi, obligate ♂♂ may be supposed to exist but have not yet been noticed in *E. coli*.

In addition to recombinants, each F^- exconjugant contained a proportion, usually more than half, of the F^- parental genotype, viz., 000 0000 00. In pedigreed clones, at the second fission, usually three of the cells were pure 000, etc., while the fourth was still generating both parental and recombinant genotypes, these becoming pure in cells at the third or fourth fission. Figure 1 shows an instance of prolonged delay in segregation, with recombinants in three of the subclones at the four-cell stage. These results are readily understood by the regular assortment of three unfertilized from one fertilized nucleus and its meiotic products in the lineage of the multinucleate F^- exconjugant, complicated by occasional slippage and irregular assortment of nuclei. No persistent heterokaryons were observed in this study, nor could they be expected in a short bacillary form, whereas they are expected and are found in the filamentous streptomycetes.[8]

The various markers in Table 1 are arranged according to their segregation ratio, i.e., the relative incidence of the 1 allele among the exconjugant clones. As shown, this incidence varied from 1 each for *Gal* and *Fla* to 70 for *Lac*. This disparity con-

tradicts the assumption of random segregation of unselected markers which was postulated in the initial analysis of segregation and linkage in *E. coli*.[9] The disparity does accord with the bias found in unselected progenies from persistent heterozygotes[10, 11] and is now explained by the loss of segments from the ♂ chromosome, either before fertilization,[3] or after,[11] or both.

The 1 allele of each of the 9 markers of these parents has been recovered at least once, i.e., the ♂ parent *can* transmit any element. The ♂ exconjugants of exceptional zygotes such as D1, D2, and others were examined for a persistent alteration in the segregation pattern of further crosses, but none was found. Within this series, there was no instance (barring one creditable to a possible contamination) of the reappearance of the whole 111 1111 11 genotype in the progeny of a ♀ exconjugant. Whether or not the entire 111, etc., genotype is transmitted to the zygote, it therefore experiences either obligatory recombination or elimination of a chromosome segment at some stage, be this before or after fertilization.

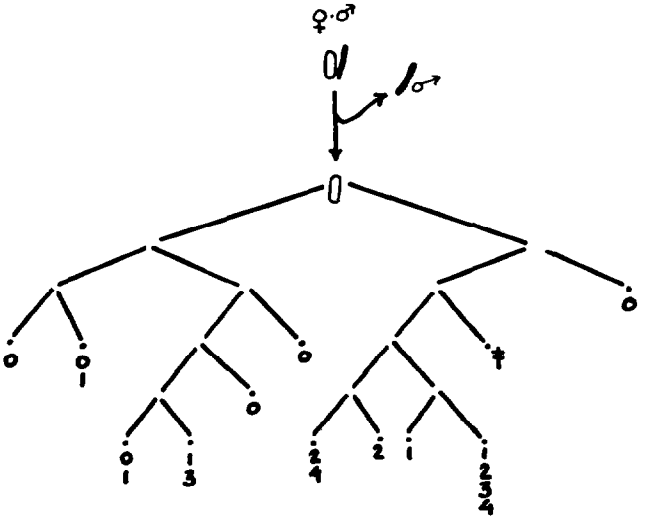

FIG. 1.—Cell lineage from a conjugal pair (C8). The lineage is abbreviated: where a subclone was pure, only the pure parent cell is shown. Genotypes (see Table 1): ♂ = 111 1111 11. ♀ = 0 = 000 0000 00. *1* = 000 1100 00; *2* = 100 0010 00; *3* = 100 1100 00; *4* = 000 0010 00. † = died.

Most of the clones contained only one detectable recombinant. However, as some pedigreed cells were inviable from technical mishaps or from no overt injury, this class may be overestimated. The incidence of these single-recombinant clones tells against the frequent occurrence of double fertilizations. The mating complexes chosen for isolation are, however, selected as simple pairs, and multiple fertilizations could still occur in the more complicated clumps which are also seen.

The distribution of types in part BIII suggests considerable coincidence of interchanges. Thus we find six clones with 111 alone, just one with 011, but seven with both together. Part C also points to an interaction between recovery of 111 and of further markers. That is, crossing over in the 000 0000 00 region is correlated with crossovers in other regions. This type of coincidence has been stressed in earlier reports[5] and can again be interpreted as a result of sporadic pairing, either

from poor synapsis in a complete heterozygote, or from the prezygotic loss of some chromosome segments.

Twenty clones had from two to four recombinants, none more than four, which suggests a regular mechanism of meiosis with a four-strand stage. Clones C5 through C8 are especially striking, as they comprise four different tetratype tetrads with partly complementary elements. For example, C6 includes the complementary set: 111 1111; 000 0000; 111 0000; and 000 1111. Another example of complementarity, viz., the reciprocal crossovers S^s *Mal*$^+$ *Xyl*$^-$ and S^r *Mal*$^-$ *Xyl*$^+$, is seen in C8. However, the suggestion that crossing over is inherently reciprocal must be qualified in view of the small numbers and the possible role of coincidence.

The frequency of clones with three or four recombinants poses an additional problem. Of the four tetratypes, three show the same crossover, $\underline{100}$, etc., in two combinations. For example, C8 has $\underline{100}$ 1100 and $\underline{100}$ 0010. Three possibilities are forwarded: a four-stranded heterozygote, with intense coincidence of crossing over; crossing over at a two-strand stage for some markers, followed by typical four-strand crossing over for others; or reiterated meiosis. As the last formulation also accounts for another anomaly, it will be set forth in more detail.

The abbreviated notation of Table 1 should not obscure the fact that no one clone was segregating for every marker. Even C6 is pure *Gal*$^+$ *Fla*$^-$, while C5 is pure for *Ara*, V_1, and *Mtl* as well; C7 for *Ara*, V_1, *Xyl*, and *Mtl*; and so on. Some mechanism must be postulated to furnish 0 alleles for these recombinant genotypes. One scheme may be illustrated with C8 as follows:

$$111\ 1111\ 11 \times 000\ 0000\ 00 \rightarrow \text{primary heterozygote } \frac{111\ 1111\ 11}{000\ 0000\ 00} \rightarrow \text{first meiosis} \rightarrow$$

$$100\ 1100\ 00 \text{ plus } 000\ 0010\ 00 \text{ plus } 111\ 0011\ \square\square \text{ plus } 011\ 1111\ \square\square.$$

The last two are inviable on account of the terminal deficiency, which stems from breakage proximal to the two markers. The two viable products rejoin to give the secondary heterozygote $\frac{100\ 1100\ 00}{000\ 0010\ 00}$. Further reduction, with crossing over at a four-strand stage in the region $100\ \underline{1100}\ 00$, gives the four products: 100 1100 00; 000 0010 00; 100 0010 00; and 000 $\overline{1100}$ 00.

The secondary heterozygote can be formulated by an alternative scheme, on the supposition of prezygotic loss of segments to give a fractional heterozygote (merozygote[3]):

$$\underline{111}\ \ 1111\ \underline{11} \times 000\ 0000\ 00 \rightarrow \frac{1\square\square\ 111\square\ \square\square}{000\ 0000\ 00}.$$

The merozygote would then be completed by partial replication from the 000, etc., template to give

$$\frac{100\ 1110\ 00}{000\ 0000\ 00}$$

followed, or accompanied, by crossing over to give $\frac{100\ 1100\ 00}{000\ 0010\ 00}$,

which is then reduced by a regular four-strand meiosis. However, as partial replication can be posited as the mechanism of crossing over, the two versions are formally similar and cannot be distinguished in the present experiments. On both versions, crossing over takes place between *Mal* and *Xyl* during the primary cycle.

The formation of secondary heterozygotes has been postulated previously[10] to account for demonstrable homozygosity of *Lac* in some persistent diploids and for "twin prototroph" recombinants. Sporadic automixis is characteristic of persistent diploids[10] and heterogenotes.[12] Iterated meiosis is therefore a recurrent feature of recombination in *E. coli*.[13]

Summary.—Individual mating pairs were isolated from highly fertile matings of *E. coli*, *Hfr* × F^-. All the recombinants were found in the progeny of the F^- exconjugant, which is therefore designated as ♀. The segregation of markers in the cell lineage indicated (1) the loss of certain chromosome segments, before or after fertilization or both; (2) coincidence of crossing over, suggestive of sporadic pairing; (3) possible correlation of reciprocal crossovers; (4) recycling of meiosis; and (5) an interval of nuclear assortment from a temporary heterokaryon.

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