A COMMENT ON THE FERTILITY OF F_2 DONOR TYPES OF *ESCHERICHIA COLI* K12

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One of the characteristics of F_2 donor strains of *E. coli* K12 is that they appear to be about 1/20th as fertile as their Hfr counterparts when mated in broth (Adelberg and Burns, 1960). During a series of mating experiments involving a histidineless (*his*$^-$) derivative of Lederberg's Hfr4 strain (W4321), an F_2 donor type was identified by its reduced fertility in broth matings. The F_2 his$^-$ culture was purified by selecting single colony isolates. With the development of the membrane filter mating technique (Matney and Felkner, 1962) the question of Hfr vs F_2 fertility was re-examined.

The F_2 donor and either the stable Hfr4 his$^-$ male or the original methionineless (*met*$^-$) W4321 was mated with an F^- strain having a mutation in the arginine - 6 locus (*arg*$_6^-$). Overnight nutrient broth cultures of the parental strains were diluted into fresh broth (male, 1:20; female, 1:10) andthe incubation at 37 C with aeration continued for two hours. The resulting log phase cultures were chilled, mixed (1 volume of donor with 9 volumes of recipient), and the cells from 5 ml of mixture impinged on each of several membrane filters (approximately 1×10^8 donor cells and 2×10^9 recipient cells per filter). The cells were washed twice by flooding the membrane with saline (0.8% NaCl) and the membranes transferred to the surface

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of cold minimal agar plates (Vogel and Bonner, 1956). When the desired number of membranes were prepared, mating was initiated by rapidly transferring the membranes to pre-warmed (37 C) soft (0.75%) agar minimal plates supplemented with 10 μg of vitamin B_1 per ml. Mating was interrupted and the number of $\underline{arg}_6^+$ recombinants determined at the time intervals shown in Figure 1 by the following procedure. A membrane was removed from a warm plate and placed in an Erlenmeyer flask containing 10 ml of cold saline. The cells were eluted by shaking the flask vigorously, diluted, blended, and 0,1-ml aliquots spread onto minimal-B_1 agar plates.

The results shown in Figure 1 indicate that under these conditions of mating the F_2 donor gives rise to as many $\underline{arg}_6^+$ recombinants as does the stable Hfr male, but that the $\underline{arg}_6^+$ allele from the F_2 donor arrives about ten minutes late. The entrance times for $\underline{arg}_6^+$, $\underline{TL}^+$

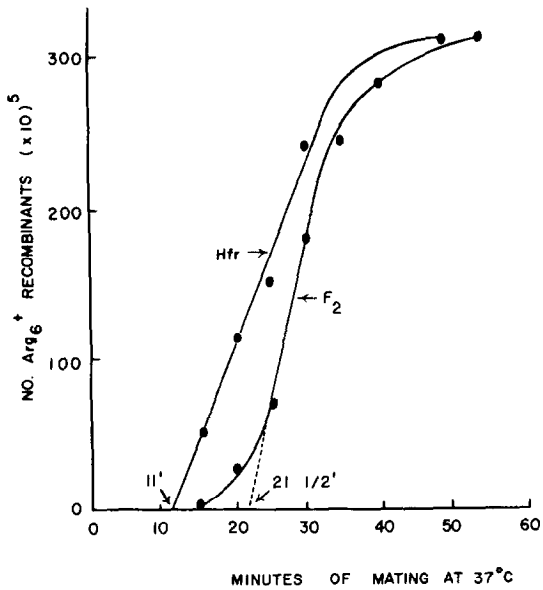

FIGURE 1. ENTRANCE OF $Arg6^+$ FROM A STABLE Hfr AND ITS F_2 COUNTERPART.

(threonine, leucine) and $\underline{arg}_1^+$ from both donor types are shown in Table 1. These were obtained by membrane filter matings with tree F^- strains containing the indicated mutations. The data in Table 1 indicate that chromosomal penetration is delayed about ten minutes in the case of the F_2 donor but that once initiated the rate of chromosomal transfer is the same as for the Hfr.

TABLE 1

COMPARISON OF ENTRANCE TIMES (IN MINUTES) BETWEEN A STABLE Hfr AND ITS F_2 DERIVATIVE

	Arg_6	TL	Arg_1
Hfr_4	11	44	102
F_2	21 1/2	57	112

The lower fertility of the F_2 donor in broth matings may be attributable to the rapid separation of mating pairs suffered during the ten-minute period of delay in chromosomal penetration, whereas positionally fixed conjugal pairs resting on the surface of membrane filters are not subjected apparently to such physical discomfort.

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