

SHORT PAPERS

**I sex factors and chromosomal recombination in
*Salmonella typhimurium***

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Colicin factor I (Col I) possesses a sex factor termed I which brings about chromosomal recombination in several species of enterobacteria including *Salmonella typhimurium* (Ozeki, Howarth & Clowes, 1961; Meynell, 1961; Smith & Stocker, 1962). A similar sex factor is also found in ColE1a (Meynell & Lawn, 1967). The mechanism by which the I factor enables its host to act as a genetic donor has been difficult to study, largely because the conjugating function of wild-type I factors is subject to repression (Meynell, Meynell & Datta, 1968). Mutant I factors have recently been isolated which do not become repressed (Edwards & Meynell, 1968) and now allow fluctuation tests to be done in order to test for the appearance of Hfr variants in the donor cultures. Such tests have proved to be uniformly negative (Meynell & Edwards, 1969).

The de-repressed I factors were I16*drd-1* and -2 from ColE1a-16, and IP9*drd-1* from ColIb-P9. The donor strain was *Salmonella typhimurium* LT2, *cysD-36 str-s* and the recipient *metA-22 trpB-2 str-r*. The donor also carried ColE1-30 which increased the rate of chromosomal recombination from 10^{-9} to 10^{-8} per donor cell, as with wild-type ColIb-P9 (Ozeki *et al.* 1961). Fluctuation tests were performed with 50 independent donor cultures grown in small tubes containing 1 ml Oxoid Nutrient Broth no. 2 inoculated with about 200 cells each, and a recipient grown in 100 ml of the same broth, all cultures being incubated overnight at 37°C without shaking. 1 ml of recipient was added to each tube of donor, incubated at 37°C for 60 min. and volumes of 0.2 ml then spread on supplemented glucose-salts medium containing 200 µg streptomycin/ml. Replicate samples of the donor culture consisted of 1 ml volumes taken from a 100 ml culture of the donor strain. These were treated in the same way to provide the 'dependent' cultures.

The results of four fluctuation tests are shown in Table 1. The magnitude of differences between the numbers of recombinant colonies derived from independent or dependent cultures is expressed by the ratio, variance/mean count, whose expected value is 1 if all differences arise purely by chance. Two points stand out on comparing the two sets of ratios. First, they are not markedly different, showing that the numbers of recombinants produced by independent cultures differed no more than those from dependent cultures. Secondly, the value of the ratio, although approximately 1 in five of the twelve crosses was as large as 2.6 in two instances. Considering that the ratio is often 1.6-1.9 when a highly diluted pure culture is counted on nutrient agar (see Snyder, 1947), it is hardly surprising that above-random values are sometimes obtained when two parental cultures are plated in high concentration on selective medium to isolate recombinants. The main point is that these values for the ratio are far less than those of 20-90 found in positive fluctuation tests involving known mutations (e.g. Luria & Delbrück, 1943).

There was thus no suggestion that unique stable donor clones arose at random during

growth of the I+ donor cultures. Negative fluctuation tests are likewise obtained in recombination experiments with certain F+ strains of *Escherichia coli* (Curtiss & Renshaw, 1965), although positive results are given by other strains shown to yield Hfr variants (Jacob & Wollman, 1961, p. 158). A positive test indicates that donors both

Table 1. *Fluctuation tests with Salmonella typhimurium carrying de-repressed I factors and colicin factor E1-30*

Sex factor	Selection for	Independent cultures			Dependent cultures		
		<i>n</i>	\bar{x}	σ_x^2/\bar{x}	<i>n</i>	\bar{x}	σ_x^2/\bar{x}
I16drd-1	<i>trp</i>	50	3.0	1.4	48	9.6	2.6
I16drd-1	<i>trp</i>	50	4.1	1.4	38	7.9	1.7
	<i>met</i>	50	2.9	1.0	44	6.9	0.9
I16drd-2	<i>trp</i>	50	5.9	2.6	46	5.5	1.3
	<i>met</i>	50	3.5	1.1	48	4.4	1.1
IP9drd-1	<i>met</i>	50	3.0	0.9	50	2.2	1.4

Donor: Idrd ColE1-30 *cysD-36 str-s*. Recipient: *metA-22 trpB-2 str-r*. *n*: number of cultures. \bar{x} : mean count of recombinants per tube. σ_x^2/\bar{x} : variance of the distribution of counts/mean count.

arise at random and are stable, and, if either condition is lacking, the test is negative. However, chromosomal recombinants produced by I sex factors are unlikely to result from even unstable Hfr clones, since Clowes & Moody (1966) showed that recombination frequencies in ColI+ strains of *E. coli* are not affected by whether the donor strain is *rec+* or *rec-*. Although this is not definitive, since the I sex factor might possess its own integration enzyme, the balance of evidence is therefore against the occurrence of integration.

SUMMARY

Colicin factors Ib-P9 and Ela-16 both convert their hosts to chromosomal donors. However, negative fluctuation tests suggest that chromosomal transfer does not result from the formation of stable Hfr variants.

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