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Adaptive Mutation and Sex

Three papers, two in the issue of 21 April (J. P. Radicella *et al.*, p. 418; T. Galitski and J. R. Roth, p. 421) and one elsewhere (*1*), show that adaptive mutation of a bacterial episome requires gene products that are also known to be involved in transfer of the episome during bacterial mating. Radicella *et al.* go further and imply, in their abstract and at other points in their report, that they have demonstrated an association between adaptive mutation and conjugal transfer of the episome, even though they have not actually tested that idea. They could have asked whether episomes that have been transferred are more likely to be mutant than those that have probably not been transferred; this would have been an easy experiment, but they apparently did not do it. They say that their experiments suggest, at the very least, "a requirement for the formation of mating aggregates" as the stimulus for mutation. If that were true, cells whose mating pili had been destroyed by prior exposure to the detergent SDS should be unable to undergo episomal mutations when diluted into top agar and put on selective plates. As it happens, this was the control for the experiment described in figure 4 of their report, and it showed that the episomes of SDS-treated cells seem to have a higher mutation rate than normal cells.

Seeking support for their ideas, Radicella *et al.* end their report by quoting Foster and Trimarchi (*1*) as having shown that mutation "requires that the *lac* allele be on the episome and is enhanced by the expression of conjugal functions." It would have been less misleading if they had included the next sentence, "However, actual conjugation is not required and, in our experiments, there is little evidence that episome transfer is mutagenic" (*1*, p. 5487).

The report by Galitski and Roth is more straightforward, and they offer a testable explanation why reversion of an episomal lac frameshift appears to be more frequent when it is adaptive. Unlike Radicella *et al.*, they do not imply that adaptive mutation requires conjugal transfer; indeed, Roth has written, "Our experiments do not suggest that the act of transfer is required for adaptive mutation" (*2*).

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2. J. R. Roth, personal communication.

Response: We have shown that about 10% of the post-plating revertants in the standard *lac* assay can be associated with the successful transfer of the episome carrying the revertible allele from the indicator cells to the scavenger cells (figure 1 in our report). Our experiments (note 15 in our report) and those of Foster and Trimarchi (*1*) show that only between 0.1 to 1% of the episomes are transferred to the scavenger under those conditions. We therefore suggest that the mutations can be associated with transfer. As to the question of whether episomes that have undergone transfer display an elevated incidence of mutation, we have not made this claim; but Kunz and Glickman (*2*) have reported substantial episomal marker mutability associated with conjugal transfer. Attention has been drawn to this observation by Taddei *et al.* in an accompanying letter.

As to whether or not cells grown in the presence of SDS should be able to undergo episomal mutations when put on selective plates in the absence SDS, we suggest that the leakiness of the *lacI33* allele is sufficient to allow reassembly of the pili required for the generation of conjugational signals during selection. The two- to threefold greater yield of revertants seen in the control experiment (our figure 4) on which Cairns comments is within the range of experimental variability evident among independent cultures and in reported studies (*3*, *4*).

We have not argued that conjugal transfer must always be successful. We acknowledge that, even in the presence of an excess of scavenger cells, the majority of the reversion events occur in the indicator cells. The importance of conjugation need not depend on the successful completion of conjugal transfer. For example, the transferred DNA could fail to replicate in the recipient cells (most likely scavenger) and therefore be lost. The issue may simply be how many times, during prolonged selection, the F′ plasmid of the indicator bacteria has experienced a replication as a consequence of the initiation of the conjugation process. The success or failure of the transfer need not matter.

The suggestion by Cairns that we intended to mislead in not quoting a conclusion we do not find compelling does not do him credit.

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4. R. S. Harris, S. Longerich, S. M. Rosenberg, *Science* **264**, 258 (1994).

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Mutation Rate of the F Episome

The mutation rate per replication of all DNA-based genomes (including viruses like M13 and lambda, bacteria, and yeast) appears to be constant (on the order of one per 300 genomes replicated—Drake's rule) (*1*). This constant likely reflects an optimal or minimal rate of mutation. There is one noticeable exception to this rule, the F episome. Although the F episome is not generally considered itself a microorganism,

rather a plasmid-like replicon, the various aspects of its life cycle—that is, vegetative growth, prolonged periods of nongrowth, and the ability to transmit its genetic material through conjugation—provide a basis for comparison with virions that are dependent on host factors for propagation.

The rate of mutation of the F episome depends on the stage of its life cycle. During vegetative growth, the mutation rate per base of the F episome is the same as its *Escherichia coli* host, which has a genome about 20-fold larger. Thus, during vegetative growth, more mutations accumulate in the host chromosome than in the F episome. However, during conjugal transfer, the mutation rate of the F episome increases at least 20-fold (*2*) compared to the host chromosome. Furthermore, Radicella *et al.* (21 Apr., p. 418) report that, when cells carrying an F episome bearing a frame shift mutation that prevents growth on lactose are incubated for a prolonged period, the reversion rate increases up to 50-fold as compared with the chromosomal locus. Therefore, if one takes into account the different aspects of the life cycle, the F episome fine tunes its mutation rate. The overall mutation rate per genome of the F episome thereby appears to conform to Drake's rule, rather than provide an exception to it.

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2. B. A. Kunz and B. W. Glickman, *Genetics* **105**, 489 (1983); *ibid.* **106**, 347 (1984); R. B. Christensen, J. R. Christensen, C. W. Lawrence, *Mol. Gen. Genet.* **201**, 35 (1985).

Response: Taddei *et al.* have overinterpreted recent reports on adaptive reversion of an F plasmid–borne mutant locus. Neither our report nor that of Radicella *et al.* allows conclusions to be made about mutation rates during F replication, either sexual or vegetative. Our reports suggest only that, under selective conditions, the observed reversion seen in nongrowing cells requires transfer replication. The point is that replicating DNA (the F plasmid) is more mutable than nonreplicating DNA (the chromosome). In order to determine rates of mutation for evaluation in terms of Drake's rule (*1*), one would need to know how many times the sequence was replicated; neither we nor Radicella *et al.* have assessed this. Also, the observed Rec dependence of reversion under selective conditions indicates a fundamental difference that may be related to the form of the replicated product. Standard F plasmid transfer experiments show no Rec dependence. Conclusions regarding whether F, or any other genetic entity, conforms to Drake's rule under conditions of strong selection and adversity are premature.

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Corrections and Clarifications

The ScienceScope item "Misconduct office gets mixed review" (7 July, p. 19) incorrectly stated that the Department of Health and Human Services' Office of Research Integrity has a budget of $50 million a year and employs 50 people. The correct figures are $3.8 million a year and 41 people.