Origin of mutants disputed

SIR—Cairns *et al.*[1] present experiments that lead them to the provocative conclusion that, in response to a particular environmental challenge, bacteria generate a class of mutations that specifically enhance their fitness in the novel environment. This conclusion is at variance with the neo-darwinian orthodoxy that mutations occur strictly at random with respect to any such challenge[2]. We here refer to these alternative possibilities as the 'directed mutation' model and the 'spontaneous mutation' model, respectively. Support for the directed mutation model was derived from the observation that mutation rates to variants with enhanced growth on a novel medium are apparently higher under exposure to the medium than without exposure. We suggest that the results as presented are consistent with both models, so that additional evidence is desirable before the spontaneous mutation model, so strongly supported by previous experiments[1,3], can be rejected.

The data presented by Cairns *et al.* are of two kinds. First, they use the statistical procedure of the fluctuation test[3-5] to estimate the distribution among replicate cultures of the numbers of mutations conferring the ability to ferment lactose, in an initially Lac⁻ strain of *Escherichia coli*. Second, they monitor the time of appearance of these Lac⁺ mutations after accumulation in the absence of lactose, followed by its addition. The mutations detected in these experiments are of two kinds, suppressors of a chain-terminating (amber) mutation in the *LacZ* structural gene, and mutations to the Lac⁺ phenotype which are due to loss of an inserted sequence upstream of *LacZ*.

In their interpretation of the data, the authors assume that all Lac⁺ mutations were neutral in the absence of lactose. This assumption implies that, on the spontaneous mutation model, the observed distribution of mutations should be the same whether or not the bacteria were previously exposed to medium containing lactose. If some of the Lac⁺ mutations were deleterious on medium lacking lactose, however, those mutations would have failed to propagate themselves as fast as neutral Lac⁺ mutations. In that case, the number of Lac⁺ mutations would be higher in bacteria exposed to lactose-rich medium because that medium would enable a class of spontaneous mutations to persist that would otherwise be lost. The data presented by Cairns *et al.* do not seem to permit us to reject this possibility, which is fully in line with the spontaneous mutation theory. One test of this idea would be to generalize the distributions of numbers of mutations derived by Lea and Coulson[4] for the fluctuation test to include selection on the mutants. This has been done by Koch[5], and Lenski, Slatkin and Ayala have shown (personal communication) that it can give a quantitative fit to the curves found by Cairns *et al.* In the second series of experiments, the delayed appearance of the Lac⁺ mutants in the absence of lactose is evidently consistent with their being at a selective disadvantage unless lactose is present.

In considering this interpretation, it should be borne in mind that because of the nature of the Lac⁻ mutations that were used, the majority of the Lac⁺ mutants in the experiments of Cairns *et al.* are probably not simple reversions to wild-type, that is they do not restore the ancestral Lac⁺ sequence of the *LacZ* region, but involve amber suppression or rearrangement of upstream regions. There is much evidence that the majority of spontaneous mutations with observable phenotypic effects are deleterious[2,6], and this is known to be the case for chromosome rearrangements without major loss of genetic material[7]; from the way in which suppression of chain-terminating mutations works, it is extremely likely that these would also have deleterious effects on fitness[8,9] and indeed there is some direct evidence for this[10-12]. This view of these experiments may also explain the difference noted by Cairns *et al.* between their results using nutritional markers and those previously obtained by others using phage and drug resistance markers[1,3]. Such mutations are known to show a delay of several cell generations in expression, after their initial occurrence[1], and it seems reasonable that this might apply to any deleterious effects that they may also have. If this were true, these mutations would indeed behave as truly neutral, at least until the time when their phenotypic effects begin to be expressed.

What evidence might be used to discriminate between the two models? If it is found that the mutations appearing only after exposure to the lactose-rich medium are selectively inferior to the ancestral Lac⁻ bacteria when tested on medium lacking lactose, the spontaneous mutation model would be supported. Highly sensitive techniques are available for measuring the relative fitnesses of different bacterial genotypes[6,7,12] and these could be applied to the Lac⁺ mutations in question. There even seems to be some internal evidence in Fig. 3 of Cairns *et al.* for a selective disadvantage to these mutations. In this figure, which shows the results of their second series of experiments, the plates that were overlaid with lactose-containing medium earliest can be seen to have the highest numbers of Lac⁺ cells at all later times, which suggests that the longer plates were kept without lactose, the lower the number of surviving Lac⁺ cells. Even if the late-appearing mutants do not exhibit such inferiority, the spontaneous mutation model can safely be rejected only if it can be demonstrated that natural selection among descendants of each late-appearing mutant could not have improved their fitness; such improvement would be likely to occur, given sufficient time[12].

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SIR—Loci at which directed mutation takes place (see Cairns *et al.*[1]) are likely to be 'heritable soma'. That is, although the contents are inherited, the information they contain is not potentially immortal, and so the loci are not germ plasm. Mutations at those loci will therefore have the same logical role in evolution as somatic adaptation in multicellular organisms, such as the strengthening of our muscles when we exercise.

Consider an example of directed mutation where the mechanism is well known. In the fission yeast, *Schizosaccharomyces pombe*, the contents of the locus *mat1* determine whether the mating-type of the cell is plus or minus[2]. The contents of this locus are regularly replaced by a copy of the contents of either *mat2* or *mat3*, loci which contain silent master copies of the alleles determining plus or minus mating-type respectively. The locus *mat1* is not germ plasm, because a point mutation at *mat1* will have a short-lived effect before the whole allele is replaced and subsequently degraded.

DNA is not only the repository of inherited and potentially immortal information — it is also just another biochemical tool that can be used in the service of the cell. No doubt cells switch states using a variety of mechanisms: protein concentrations, methylation of DNA, condensation of DNA, and sometimes the base sequence of DNA. No great theoretical

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