

Cryptic genes: Are directed mutations always beneficial?

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While the debate on the existence of 'directed', 'Cairnsian', 'adaptive' or 'post-selection' mutations still continues, the suggestion that such a feature will be strongly advantageous seems to be uncritically accepted. We modelled competition in a chemostat with the substrate alternating between a 'normal' and an 'alternative' nutrient. Simulations involving genotypes (1) with inducible operon for alternative substrate, (2) without any gene for the alternative substrate, (3) with a cryptic gene for the alternative substrate and the ability to bring about a directed mutation and (4) with a cryptic gene having mutational hotspot showed that a directed mutation conferred little selective advantage. The widely held belief is thus challenged and we need to look into the directed-mutation controversy with a new insight.

CRYPTIC genes are known to occur widely in bacteria. These genes are normally silent but specific mutations

Figure 1. Areas of selective advantage to the four strategies. The figure represents a horizontal and a vertical slice from a three-dimensional phase space representing three parameters, viz. the proportion of time spent in the alternative environment, mutational increment and the periodicity of the cycle. *a*, At high selective pressures (dilution rate 0.16, i.e. 80% that of the maximum growth rate of the highest-fitness genotype) the cryptic gene had a selective advantage over a wide range of conditions. Directed mutations, however, were advantageous only at unrealistically high mutational increments. Cryptic genes with a mutational hotspot were better competitors than the ones with directed mutations. *b*, At low selective pressures (dilution rate = 0.016) the advantage to cryptic genes is lost. Crp-dir gets selected at mutational increments of 10^{10} , which at this dilution rate means that at steady state the rate of directed mutations per cell generation is almost 1. Other parameters used here were $K = 1$; $L = 0.00001$, $SR_n/SR_a = 10$; $F(Opr) = 0.99875$, $F(Cn) = F(Hn) = 0.99975$, $F(Aal) = 0.9975$; $F(Cal) = F(Hal) = 0.9985$. For other ranges of parameter values used in simulations (Appendix I), the results were qualitatively similar. The areas of selective advantage to Crp-hot expanded or reduced depending upon the fitness costs assigned. However, Crp-dir was not selected at mutational increments of less than 10^8 .

can make them active. It has been suggested that cryptic genes are maintained in bacterial populations by alterations between the 'normal' environment, in which there is selection against the expression of the gene, and an alternative environment in which there is selection favouring the expression of the gene^{1,3}. Some of the cryptic genes studied have shown evidence of

environmentally 'directed' or 'adaptive' mutations⁴⁻⁶. Hall⁵ has suggested that mutations are subject to regulation by environmental factors and can be compared to physiological regulatory feedback loops.

When a substrate is only occasionally or rarely encountered, it would be costly for the cell to have constitutive enzymes for its utilization. Operons are well-known mechanisms which switch off the production when not necessary. It is further possible that having an active operon and keeping it repressed may be more costly than having a cryptic operon. Cryptic genes could be just another level of regulation of a gene sequence. Since all the three levels, viz. constitutive, physiologically regulated operons and mutationally regulated cryptic operons, are extant in nature, each can be expected to have selective advantage under some or the other environmental set-up. We have attempted to test this idea below.

Further, if we consider a cryptic operon to be environmentally regulated, it can be asked whether directed mutations are a necessary and indispensable part of the regulatory mechanism. It is generally assumed that any ability to predictive mutations will have selective advantages⁵. This axiom, however, has never been rigorously tested.

Mutations as studied in the laboratory in batch cultures may be far removed from those occurring under the natural growth conditions. Hall⁵ has criticized that mutations have been studied under unrealistic circumstances and in exponentially growing populations which represent only a small part of the life histories of microorganisms. The stationary phase in a Petri plate also may be an equally 'unnatural' condition. A chemostat is probably a better model to simulate the natural growth conditions. Chemostat models have been used to simulate microbial growth in the gut⁷, which is a natural environment for *E. coli*, in which 'adaptive' mutations have been reported. Chemostat models have also been used for evolutionary modelling⁹. Further, the chemostat model is flexible. At high dilution rates it simulates an active dynamic ecosystem with high selective pressures and at very low dilution rates it resembles the low selective pressure Petri plate experiments¹⁻⁶, in which directed mutations have been detected. We, therefore, use a chemostat model for the simulations.

In the chemostat modelled here the input periodically alternates between the 'normal' and the 'alternative' substrate. In this fluctuating environment, genotypes employing the following four alternative strategies compete for the available substrates:

(a) The first genotype does not possess any genetic machinery for the alternative substrate (Alt⁻). This genotype can grow only in the normal substrate. There is a weak possibility that a mutation that changes the specificity of some other enzyme may confer the

capability of utilizing the alternative substrate⁸ (Alt⁺). Such mutants are typically less efficient utilizers of substrate. Also the frequency of such mutations is usually very low.

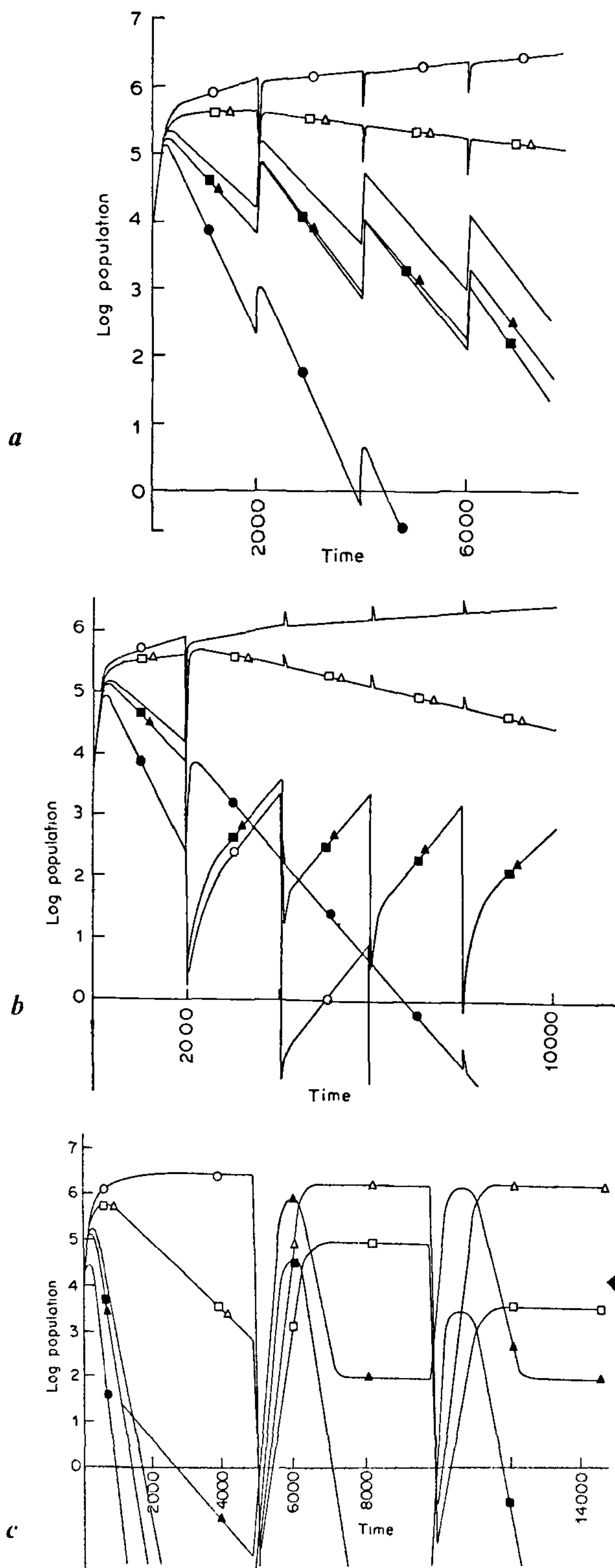
(b) *Active operon (Opr): Genotype having a regulated operon for the alternative substrate.* There will be a cost associated with the possession of such an operon and keeping it repressed in the absence of alternative substrate. Therefore, the growth rate of this genotype in the absence of the alternative substrate is expected to be lower than that of Alt⁻. Condit⁹ found the growth rate of lac⁻ mutant to be slightly higher than lac⁺ strains in the absence of lactose.

(c) *Genotype having a cryptic gene for the alternative substrate which can be activated by 'directed' mutations (Crp-dir).* In the presence of alternative substrate, specific mutations resulting in activation of the cryptic gene (Crp-dir⁻ to Crp-dir⁺) occur at increased rates, the mutational increments being several orders of magnitude. On the other hand, in the absence of alternative substrate, gene-inactivating mutations occur at similarly higher frequencies. It is assumed here that the cost of carrying a cryptic gene in the absence of the alternative substrate is substantially less than carrying an active operon.

(d) *Genotype having a cryptic gene for the alternative substrate and having a high rate of mutations that specifically activate or inactivate the cryptic gene, but without having a 'directed' component (Crp-hot).* Thus, both forward and backward mutations (i.e. Crp-hot⁻ to Crp-hot⁺ and vice versa) occur at high frequencies irrespective of the environment. The mutational increment is assumed to be identical to Crp-dir but bidirectional.

Other alternatives, viz. having constitutive enzymes for the alternative substrate, or having cryptic genes but a low or average rate of mutations, were considered initially but later eliminated from the simulations since they were at obvious disadvantage as compared to Opr and Crp-hot, respectively. The model also ignored mutations in Opr.

Competition among the seven genotypes (viz. Alt⁻, Alt⁺, Opr, Crp-dir⁻, Crp-dir⁺, Crp-hot⁻ and Crp-hot⁺) in an alternating substrate chemostat was modelled (see Appendix I for details). For ascribing relative fitness values, the following assumptions were made. Carrying an active operon involves the cost of carrying a few genes and the cost of synthesizing repressor protein to keep the operon repressed when not required. Considering the normal length of DNA and the number of polypeptides produced normally, the effect on the relative fitness would be of the order of 0.001. Cryptic genes presumably involve lower costs. Since no empirical estimates of the costs are available, a range of fitness values were used for each genotype without altering the rank order, which was as follows. In the



normal substrate, Alt^- had the highest fitness, followed by $Crp-dir^-$ and $Crp-hot^-$, Opr , $Crp-dir^+$ and $Crp-hot^+$ and lastly Alt^+ . In the alternative substrate, $Crp-dir^+$ and $Crp-hot^+$ had maximum fitness, followed by Opr and Alt^+ , the remaining three being unable to grow on the alternative substrate. We studied the outcome of the competition over several alteration cycles. Simulations were carried out over a wide range of relative growth rates, dilution rates, mutation rates, proportion of time spent in the alternative substrate and periodicity of alternations (Appendix I). Since most of the 'directed' mutations have been demonstrated in the stationary phase, the mutation rates were assumed to be time-dependent rather than generation-dependent.

All the four strategies had selective advantages over the others in some or the other parameters ranges (Figure 1) and stable coexistence was not observed. Since the number of parameters affecting the results was large, we present below the salient features of the results leading to qualitatively important conclusions. Quantitative predictions may become meaningful when empirical estimates of the costs of carrying operons and cryptic genes or the relative fitnesses are available.

At high dilution rates, i.e. at high selective pressures, having a mutationally activable cryptic gene was advantageous at higher periodicities, higher proportions of time in the alternative substrate and higher mutational increments (Figure 1a). At lower periodicities of the cycle and higher proportions of time in the alternative substrate, the Opr was selected for. This was expected since this genotype is equally fit in both the substrates. The Alt^- died out in the alternative environment, whereas both the cryptic genotypes failed to adapt

Figure 2. The chemostat competition gave rise to complex population dynamic patterns. Represented here are three examples of competitive interactions in which (a) Opr , (b) Alt^- and (c) $Crp-hot$ are the winners. *a*, Periodicity of cycle = 2000, duration of time in alternative substrate = 0.03. At higher proportions of time in the alternative environment, and lower periodicities of change, the Opr was at a clear advantage since it grew equally well in both the substrates. *b*, Periodicity of cycle = 2000, duration of time in alternative substrate = 0.0075. At lower proportions of time spent in the alternative substrate, the Alt^- had little difficulty in maintaining its population, and because of its highest fitness in the normal substrate, outgrew all other genotypes. *c*, Periodicity of cycle = 5000, duration of time in alternative substrate = 0.06. At higher periodicities of cycle and moderate time in the alternative environment, the cryptic gene was selected for. In the normal environment, a higher population of $Crp-hot^+$ was maintained due to mutation selection balance. This gave it a head start when the environment changed. Pre-adaptive mutations were thus more advantageous than post-adaptive ones. Complex competitive interactions are evident in all the three curves. For example, Opr can grow equally well in both the substrates. However, in (a) it shows transient peaks when the substrate changes, presumably because of sudden decline in the competing populations. $M_i = 10^9$, other parameters as in Figure 1a. 1, Population of Alt^- (—○—), 2, population of Alt^+ (—●—), 3, population of Opr (—□—), 4, population of $Crp-dir^-$ (—△—), 5, population of $Crp-dir^+$ (—■—), 6, population of $Crp-hot^-$ (—◇—), 7, population of $Crp-hot^+$ (—◆—)

quickly to the change (Figure 2a). With lower proportions of time in the alternative substrate and over a wide range of periodicities and mutational increments, the Alt^- was selected for (Figure 2b).

It was, however, surprising that except at unrealistically high mutational increments, Crp-dir was at no selective advantage over Crp-hot. In the normal substrate, Crp-dir⁺ was selected against, and so was the Crp-hot⁺. However, because of a high rate of mutation, Crp-hot⁺ maintained substantially higher levels of mutation-selection balance. This gave a head start to Crp-hot⁺ when the substrate changed (Figure 2c). A pre-adaptation was thus more beneficial than a post-adaptation. This advantage to pre-adaptation outweighed the disadvantage of accumulating unnecessary mutations. Only at mutational increments of 10^9 and above, this disadvantage became severe putting Crp-hot at a loss. Crp-dir was selected only above mutational increment of 10^9 which amounts to over one mutation in hundred cell generations. All reported rates of directed mutations are several orders of magnitude below the rate required to get selective advantage over Crp-hot.

Directed mutations have been demonstrated in low selective pressure Petri plate experiments, where the starving cells have ample time to mutate. Therefore, one might expect selective advantage to Crp-dir at low dilution rates. Reducing the dilution rates, however, proved to be more beneficial to Opr which took over most of the areas of advantage from both the cryptic genotypes (Figure 1b). At low selective pressures, the cost of carrying an active operon did not result in quick loss of Opr in the normal substrate; as a result, this genotype quickly took over when the substrate changed.

Effects of change in the relative fitness values were predictable and can be qualitatively summarized as follows. The advantage to Crp-hot was lost when the fitness cost of carrying a cryptic gene was assumed to be the same as that for an operon. On the other hand, when the fitness costs of carrying a cryptic gene were assumed to be none, the advantage areas of Crp-hot increased substantially. However, as long as the fitness costs and mutational increments of Crp-hot and Crp-dir were assumed to be identical, Crp-dir had no selective advantage except at mutational increments above 10^9 . The advantage to Crp-hot was lost only if its mutational increment or relative fitness was lower than that of Crp-dir.

Since the cryptic gene is a mutational hotspot in Crp-hot, other mutations will also accumulate, making the gene irreversibly inactive². This kind of inactivation will accumulate with time spent in the normal substrate. In the alternative substrate, however, there will be strong selection against these mutants. As a variation of the model, the size of mutable population of Crp-hot⁻ was made a function of the time spent continuously in the normal substrate (Appendix I). The results, however, were robust towards this change. In the case of random mutations, gene-activating mutations are usually an

order of magnitude less frequent than the gene-inactivating ones. The results were also robust towards this consideration. A 10-fold reduction in the mutation Crp-hot⁻ to Crp-hot⁺ did not affect the results qualitatively (other parameters being the same as in Figure 2).

The results indicate that contrary to the popular belief, directed mutations do not confer a universal selective advantage. In the present model, the advantage to directed mutations was only marginal and highly conditional. Cryptic genes confer an evolutionary advantage provided the cost of carrying a cryptic gene is less than that for carrying an active operon. However, an increased rate of mutation in cryptic locus is sufficient, the directed component being unnecessary.

In these simulations, we have given three distinct advantages to Crp-dir. First, the assumed rates of spontaneous mutations were very low, giving an advantage to directed mutations. Secondly, the range of mutational increments was very wide and at higher values the rates of directed mutations were unrealistically high. The selective advantage to Crp-dir was only in the range of these unrealistically high mutation rates. Thirdly, we have not assumed any cost associated with a mechanism, if any, required for directed mutations. In spite of this bias in favour of directed mutations, they failed to get selected almost throughout the parameters ranges.

Three classes of bacterial mutations have been largely studied, phage resistance, antibiotic resistance and utilization or synthesis of metabolites. Out of these, for phage and antibiotic resistance, post-selection mutations are of no use since these mutations are not expressed for several generations⁴. The only possible advantage of these mutations may be if they make the cell capable of utilizing a novel substrate. However, most of the experimental evidence of directed mutations involves cryptic genes or reversions of mutations that inactivate an inducible gene. The organisms are thus not facing a really novel substrate.

Our results are based on a chemostat competition model and one can possibly argue that although chemostats simulate the gut environments fairly well⁷, directed mutations may have evolved in some non-chemostat-like situations. It is necessary then to identify the set of conditions in which directed mutations can evolve. The present debate on directed mutations is mainly centred around two questions: the nature of evidence for directed mutations, and the possible mechanism of these mutations^{10,11}. The debate may take a different turn if we take into account its limited selective advantage.

Appendix I

The parameters used in the chemostat model were:

S_n = standing concentration of the normal substrate in the chemostat.

Sal = standing concentration of the alternative substrate in the chemostat.
 K = substrate constant, which is assumed to be identical for both the substrates.
 VAn = the maximum growth rate of Alt⁻ in the normal substrate.
 F = the relative fitness, or relative maximum growth rate of a genotype in comparison with VAn. The suffix denotes the particular genotype.
 An = population of Alt⁻ genotype.
 Aal = population of Alt⁺ genotype.
 Opr = population of the Opr genotype.
 Cn = population of Crp-dir⁻ genotype.
 Cal = population of Crp-dir⁺ genotype.
 Hn = population of Crp-hot⁻ genotype.
 Hal = population of Crp-hot⁺ genotype.
 M = spontaneous mutation rate.
 Mi = mutational increment over the spontaneous mutation rate obtained by Crp-hot and Crp-dir.
 SRn = concentration of the normal substrate in the reservoir.
 SRal = concentration of the alternative substrate in the reservoir.
 D = dilution rate.
 L = the specific rate of substrate consumption, which was assumed to be constant for all genotypes for both the substrates.

The growth of the seven genotypes was simulated using differential equations for the chemostat model as follows:

$$\begin{aligned} J_n &= S_n / (S_n + K), \\ J_{al} &= S_{al} / (S_{al} + K), \\ dA_n/dt &= V_{An} \cdot A_n \cdot J_n - D \cdot A_n + M \cdot A_{al} - M \cdot A_n, \\ dA_{al}/dt &= V_{An} \cdot F(A_{al}) \cdot A_{al} \cdot (J_n + J_{al}) - D \cdot A_{al} + M \cdot A_n - M \cdot A_{al}, \\ dO_{pr}/dt &= V_{An} \cdot F(O_{pr}) \cdot O_{pr} \cdot (J_n + J_{al}) - D \cdot O_{pr}, \\ dC_n/dt &= V_{An} \cdot F(C_n) \cdot C_n \cdot J_n - D \cdot C_n + M(C_{al}) \cdot C_{al} - M(C_n) \cdot C_n, \\ dC_{al}/dt &= V_{An} \cdot F(C_{al}) \cdot C_{al} \cdot (J_n + J_{al}) - D \cdot C_{al} + M(C_n) \cdot C_n - M(C_{al}) \cdot C_{al}. \end{aligned}$$

(In the normal substrate, $M(C_{al}) = M \cdot M_i$ and $M(C_n) = M$; and in the alternative substrate, $M(C_{al}) = M$ and $M(C_n) = M \cdot M_i$.)

$$\begin{aligned} dH_n/dt &= V_{An} \cdot F(H_n) \cdot H_n \cdot J_n - D \cdot H_n + M \cdot M_i \cdot H_{al} - M \cdot M_i \cdot H_n, \\ dH_{al}/dt &= V_{An} \cdot F(H_{al}) \cdot H_{al} \cdot (J_n + J_{al}) - D \cdot H_{al} + M \cdot M_i \cdot H_n - M(M_i) \cdot H_{al}, \\ dS_n/dt &= D \cdot S_{Rn} - D \cdot S_n - L \cdot V_{An} \cdot J_n \cdot (\sum F(i) \cdot \text{Pop}(i)), \end{aligned}$$

where $F(i)$ represent relative fitness and $\text{Pop}(i)$ the populations of all the seven genotypes.

$$dS_{al}/dt = D \cdot S_{Ral} - D \cdot S_{al} - L \cdot V_{An} \cdot J_{al} \cdot (F(A_{al}) \cdot A_{al} + F(O_{pr}) \cdot O_{pr} + F(C_{al}) \cdot C_{al} + F(H_{al}) \cdot H_{al}).$$

After specified periods, the change in the environment was simulated by changing the parameter values of SRn, SRal, Sn and Sal. In the normal substrate SRn = 10 mM and SRal = 0, and in the alternative substrate SRn = 0 and SRal = 10. Sal was set to zero at the beginning of the normal environment and Sn was set to zero at the beginning of the alternative

environment. This simulates a sudden change when organisms are carried from one habitat to the other. Simulation program was written in 'basic' using Euler's method¹². No lower limits were imposed on a minimum viable population and even fractional number of mutants were considered viable.

As a variation of the model to account for the accumulation of nonreversible mutants in the Crp-hot genotype, mutable population was considered to be $H_n \cdot (1 - T \cdot (H_n))$, where T is the time spent continuously in the normal substrate.

The ranges of parameter values used were:

1. The periodicity of alterations between normal and alternative substrates ranged between 200 and 20,000 time units, step 0.5 log cycle. At lower periodicities, up to 2000, the substrates changed before the most predominant population could reach a steady state. At higher periodicities the substrates changed long after apparent steady states were achieved.

2. The proportion of the time spent in the alternative substrate ranged from 0.001 to 0.5, roughly doubling at each step.

3. The dilution rates used were 0.008, 0.08, 0.016 and 0.16 per unit time.

4. The maximum growth rate of Alt⁻ (VAn) was kept constant at 0.2 per time unit. The relative fitnesses of other genotypes were as follows:

$F(O_{pr})$ ranged between 0.9975 and 0.9997.

$F(C_n)$ and $F(H_n)$ ranged between 0.995 and 0.99994.

$F(A_{al})$ ranged between 0.995 and 0.9994.

$F(C_{al})$ and $F(H_{al})$ ranged between 0.997 and 0.9996.

5. In all simulations, M was kept at 10^{-12} and M_i ranged between 10^3 and 10^{10} . With the dilution rates used it approximates the mutation rates to between 10^{-10} and 2×10^{-11} per cell generation. These rates were deliberately kept low so as to give additional advantages to directed mutations. Mutational increments between 10^3 and 10^6 result in realistic rates of mutations. In the higher range of mutational increments, practically every cell can mutate when the substrate changes.

6. $K = 1.0$ mM; $L = 0.00001$ g/mM; $S_{Rn} = S_{Ral} = 10$ mM. These values were constant for all the simulations.

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