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DOI: 10.1016/j.cub.2009.11.018

Genetic Adaptation: A New Piece for a Very Old Puzzle

Does stress create mutations or only serve as an agent of natural selection? New experiments reveal effects of transcription and temperature on the response to growth limitation and could help resolve a 150-year-old debate.

John R. Roth

In many biological situations, growth limitation leads to rapid genetic change. Examples include the adaptation of pathogens to hosts, the acquisition of cellular resistance to antibiotics or cancer therapies, and the adaptation of Galápagos finches. In other situations, natural selection is effectively blocked — our own somatic cells accomplish about 10^{14} acts of cell division per year for 90 years, yet two-thirds of us avoid the strongly-selected escape of cells from growth control that causes cancer. What accounts for this difference? Rapid adaptation could reflect stress-induced increases in mutation rate [1–3]. Alternatively, a rapid response could occur whenever selection can detect small-effect mutations that arise frequently under all conditions [4]. Evidence has accumulated for and against each view, but has not resolved the question. In effect, the same puzzle pieces are being assembled into two distinct but conflicting pictures. Despite the importance of the puzzle to biology and medicine, it is not clear which picture will ultimately fill the frame. New work by Cohen and Walker [5], reported in this issue of *Current Biology*, reveals roles for transcription and effects of temperature that promise to liven the debate and help solve a puzzle that has persisted since the time of Darwin.

The new experiments employ a bacterial genetic system developed by John Cairns and Pat Foster [6] and used for much recent work on the origin of mutations. In this system, 10^8 cells of a *lac* frameshift mutant (+1) are plated on lactose medium, where the parent population cannot grow and about 100 revertant Lac^+ colonies appear over five days. Each revertant Lac^+ colony includes cells with a compensating (–1) frameshift. Are these colonies initiated when stress induces a rare, large-effect mutation (–1) in a non-growing cell? Or are the revertants initiated by common small-effect mutant cells that pre-exist selection but grow and improve rapidly under growth-limiting conditions? The two views of this elephant are diagrammed in Figure 1. To see the world of stress-induced mutation, ignore the small-effect mutants (in the top part); to see the world of selection, focus on the small-effect mutants (in the top part). The effect of selection stringency is seen by comparing the top and bottom parts.

In considering the effects of selection, it is important to note that the commonest mutation types have the smallest effect on phenotype. This is true for both loss-of-function mutations (dashed line low in Figure 2) and gain-of-function mutations (solid line high in Figure 2). Note that gain-of-function mutations form at rates that vary over a 10^6-fold range, because copy-number variants (duplications and amplification steps) arise at vastly higher rates than conventional point mutations.

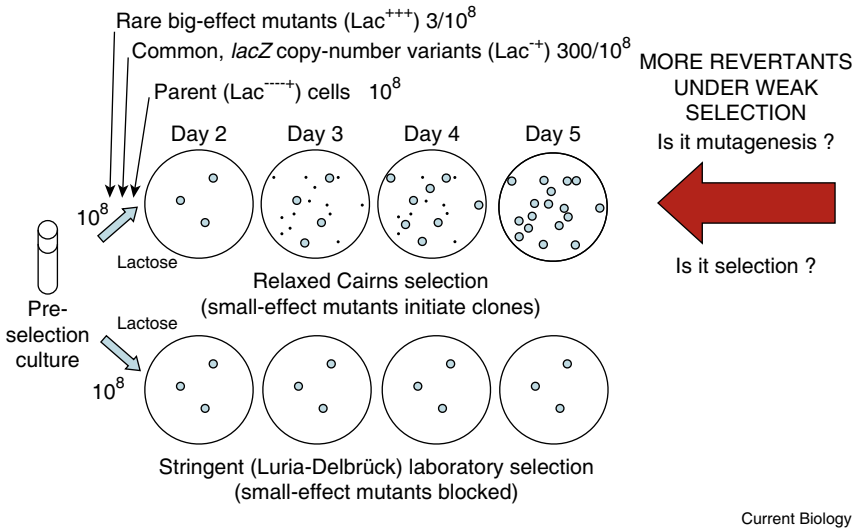

Figure 1. Selection stringency affects mutant frequency.

(Top) A non-stringent selection, like that in the Cairns system, allows common pre-existing small-effect mutants to initiate colonies within which selection drives rapid improvement in growth ability. Models for stress-induced mutation propose that mutations are induced in non-growing cells under selective conditions and are predominantly large-effect types. (Bottom) Stringent positive selection conditions used in standard lab genetics of bacteria, allow only pre-existing large-effect mutants to grow.

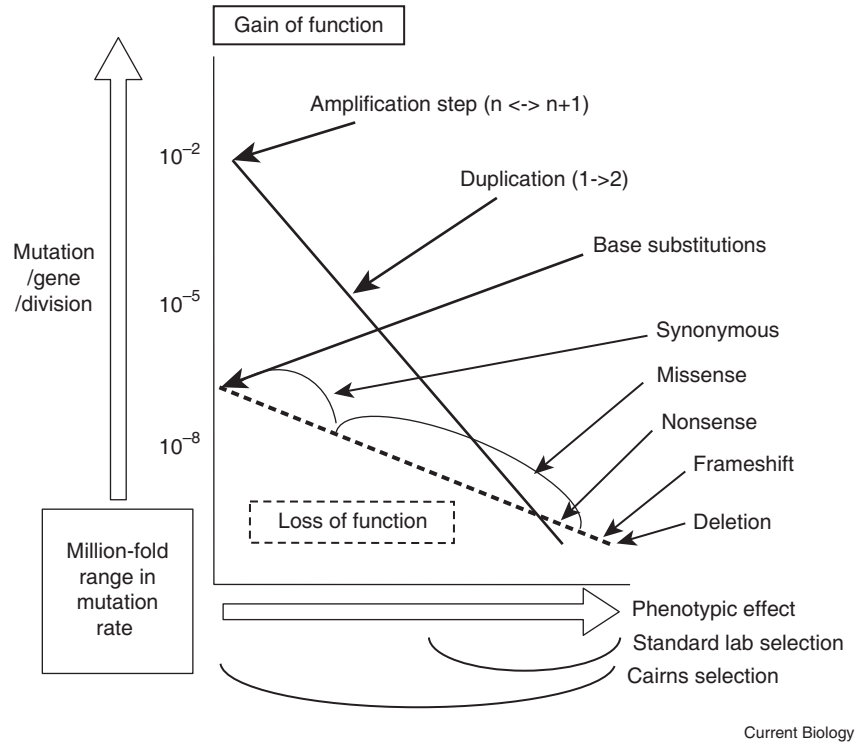

Figure 2. Distinct mutation types arise at characteristic frequencies.

In general, mutation types that arise at the highest rate have the smallest effect on phenotype. Formation rates are expressed per gene per cell division. Gain-of-function mutations include copy-number variants, which arise at extremely high rates, and rare point mutations that increase gene function by altering coding sequence or promoter quality.

The intensity of selection dictates the fate of the several mutation types. In laboratory bacterial genetics, stringent selective conditions are routinely used to detect pre-existing large-effect mutants. These (positive selection) conditions prevent growth of both the parent cells and the frequent copy-number variants. Classic experiments of Luria, Delbrück and Lederberg [7,8] showed that strong lab selections detect mutants without affecting their frequency (diagrammed in lower part of Figure 1). In contrast, natural populations grow and adapt rapidly to limitation, because natural selection can detect the small phenotype differences caused by the commonest mutations and then drive an exponential increase in their frequency.

In the Cairns system used by Cohen and Walker [5], the stringency of selection is intermediate between that of a standard positive selection used in laboratory genetics and that of natural selection as it appears in the wild. Cairns' conditions are stringent enough to prevent growth of the parent mutant cells, but weak enough to allow slow growth of common copy-number variants, which have a few extra copies of the partially-functional mutant *lac* allele. Supporters of stress-induced mutation emphasize that parent cell growth is prevented and conclude that revertant colonies are initiated by normally rare (–1) mutants induced *de novo* in non-growing cells by stress on the selection plate. Supporters of selection models emphasize that common copy-number variants can initiate colonies under this selection and cells in these colonies can improve their growth ability by successive amplification, reversion and loss of mutant alleles within the developing colony (Figure 1).

Cohen and Walker [5] add a new member (*nusA*) to the list of genes whose mutations reduce the yield of revertants in the Cairns system (Table 1). The NusA protein is known to help extend transcription through terminators in the development of phage lambda and to prevent unscheduled termination of some bacterial gene transcripts including *lac* [9]. Thus, NusA brings transcription into the discussion of genetic adaptation. The possibility that NusA might contribute to mutation was suggested by the observation that NusA protein binds directly to the error-prone repair polymerase DinB [10] and over-expression of *dinB* corrects the lethal effects of a *nusA* mutation. To understand possible roles for NusA in the origin of mutations, one must first know a bit about DinB.

The DinB protein is induced as part of the 'SOS' DNA-damage repair response. It allows repair replication to bypass obstacles — damaged or missing bases in the template — and thereby contributes to survival at the cost of some mutagenesis. DinB often makes frameshift mutations [11] and contributes to formation of deletions [12]. Up until now, a DinB deficiency caused a two- to five-fold reduction in revertant yield in the Cairns system — an effect used as a major support for models of stress-induced DinB mutagenesis [13,14]. Selection supporters suggest that this small contribution is an artifact generated when the *dinB* gene co-amplifies with *lac* under selection (the genes happen to be close together) [15]. Even without *dinB*, selective conditions still enhance revertant yield 25-fold.

Cohen and Walker report that a *nusA* mutation reduces revertant yield nearly 500-fold, perhaps the largest effect ever reported for this system. In their hands, a *dinB* mutation also caused a large drop in revertant yield (75-fold) — much larger than the two- to five-fold reported previously. These large effects are seen at low temperature. Because NusA is essential for life, a temperature-sensitive *nusA* mutation was used. This mutation is lethal at high temperature, but can be used at the permissive temperature (30°C) to test the effect of reduced NusA on reversion. At this low temperature, revertant yield in the normal parent strain increases about three-fold and becomes heavily dependent on both DinB (the mutagenic

Table 1. Interpreting mutations that reduce revertant yield.

Mutation that reduces yield	Known activity of normal protein/gene	Role of function in each model	
		Stress-induced mutagenesis of non-growing cells	Selective improvement of pre-existing common variants
dinB	Error-prone polymerase	Makes mutations when induced by stress	Makes mutations when *dinB* and *lac* co-amplify under selection
recA	Recombination and SOS induction	Provides recombinational replication in non-growing cells	Allows *lac* amplification during growth before and during selection
rpoS	Stationary phase sigma factor	Helps induce *dinB* during growth limitation	Optimizes growth and survival during strong growth limitation
str^{R} (makes coding more stringent)	Standard leakiness of mutant *lac* gene	Leakiness of *lac* allele provides energy for mutagenesis in non-growing cells	Leakiness allows *lac* amplification to support faster growth
galETK	Allows use of lactose-derived galactose	Helps leaky *lac* allele provide energy for mutagenesis in non-growing cells	Doubles growth yield provided by each copy of mutant *lac* allele
nusA	Helps prevent transcription termination	Brings mutagenic DinB to site of stress-induced transcription block	Prevents transcript termination in genes that support growth under selection

polymerase) and NusA (the extender of transcription). These effects are not limited to the *lac* system, but are also seen when selection favors reversion of a mutant drug resistance gene. What does all this say about the ancient puzzle?

In interpreting the new results, one should note that all previous observations can be interpreted in terms of either of the two general models. The table below lists mutations that reduce revertant yield under selection and how each model uses the normal function to explain reversion under selection. In general, stress-induced mutation models attribute reduced mutant yield to impaired activity of the mutagenic DinB polymerase. Selective amplification models attribute reduction either to reduced ability of mutant *lac* allele to support growth (more stringent selection) or to impaired ability to amplify gene copy number (less frequent small-effect mutations).

Cohen and Walker [5] interpret their new results in terms of stress-induced mutation and suggest that NusA may sense starvation-induced transcription problems and direct DinB to the offending site to solve the transcription problem mutationally. Their model predicts a NusA/DinB-mediated increase in general mutation rate during strictly limited growth, especially at 30°. While this idea is attractive, it seems equally likely that the *nusA* defect impairs transcription of genes needed for slow growth under starvation. The *nusA* mutation is shown here to cause a seven-fold reduction in residual expression of the mutant *lac* allele. Lowered expression of genes that limit growth rate would make selection more stringent, demanding higher amplification or even making it impossible for any copy-number variants to grow. The reconstruction experiments are not telling since they ignore improvement of small-effect mutants during growth under selection. So far the new results can fit with either model.

Regardless of how this all turns out, the new results require both sides of the argument to demonstrate the basis of the effects of low temperature and the role of the NusA protein. It looks like we are in for another round of experiments. There is a very good reason that this important puzzle has remained unsolved for 150 years — it is difficult to separate the effects of selection and mutation. Let us hope that these new results will point the way to a resolution.

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DOI: 10.1016/j.cub.2009.11.043