

Research news

Stress, sex and evolution

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Despite the widespread view that the effects of mutations are exacerbated by environmental stresses, some stresses have been found to alleviate the effects of mutations in bacteria.

Open any textbook on evolutionary genetics and you are bound to find phrases such as 'stress reveals genetic variation'. The implication is that while many stresses have a slight effect on an 'average' organism, if the organism has a **mutation** then the **stress** can have greater impact. For bacteria this would be revealed in reduced growth. At the very least you would expect growth of mutated organisms to be inhibited by stress to the same extent as that stress inhibits the growth of the non-mutated progenitor strain.

Compare this with an engineering example and it makes obvious sense. A car drives faster down an urban road than across the stressful environment of rough terrain. If you 'mutate' the vehicle by removing a screw at random and it impedes the vehicle's ability to cope with the urban road, you would expect the effects of this 'mutation' to be similar, or exacerbated, when it is driven off-road. You would certainly not expect the deleterious effect to be reduced when driving off-road.

But according to results published in this issue of *Journal of Biology* [1], *Escherichia coli* appears not to have read the textbooks. Working in the Laboratory of Living Matter at Rockefeller

University, New York, Roy Kishony and Stanislas Leibler have found that if some stresses are applied to previously mutated organisms, the effect of each stress is less pronounced than when it is applied to wild-type bacteria (see 'The bottom line' box for a summary of their work). Kishony and

Leibler emphasize that these are bacteria with random deleterious mutations, not rare mutants that manage to do better than their wild-type parents; their intention was to see the effect of the average mutation as opposed to studying specific rare ones. The conclusion from this study

The bottom line

- Bacteria with deleterious mutations grow less well than wild-type organisms, but the difference in growth rates is reduced when the bacteria are placed in certain stressful conditions.
- For some stresses, the more an organism is stressed the less the effect of mutation is apparent.
- This result runs counter to conventional wisdom, which suggests that organisms with deleterious mutations will be especially susceptible to the negative effects of stress.
- The nature of the stress influences the outcome: acid stress aggravates mutational effects, while other stresses, such as the presence of antibiotics, alleviate the effects of mutations.
- The results of this study conflict with the prevailing theory that the advantage of sexual reproduction depends on the ability of genetic recombination to purge deleterious mutations from the population and, in particular, to counter the synergistic effects of multiple mutations.

is that if you were to pick a mutation at random, the chances are that some of its lost performance would be restored under particular stresses. This really is surprising. It is saying that if you take a damaged biological system and push it close to the extreme, somehow the damage becomes less deleterious.

There had been previous hints at this effect. "This is part of a growing body of data that shows that we don't understand mutational effects in different environmental conditions," says botanist Jeffrey Blanchard, who works at the National Center for Genomic Resources, Santa Fe, USA. "I am not very surprised by the results," adds James Fry, of the Biology Department at the University of Rochester, USA. "It goes against conventional wisdom, but then I wasn't very sure I believed the conventional wisdom in the first place. We had some results in our *Genetics* 2002 paper cited by Kishony and Leibler - it wasn't a major emphasis of the paper, but one of the implications is that there probably were mutations in which there probably were smaller proportional effects under stressful conditions." Fry's paper [2] gave an inkling, but Kishony and Leibler's has much more power to see what is going on. "Working with *Drosophila* means that our study was more crude than theirs," Fry notes.

A powerful method

Part of the power of Kishony and Leibler's work also comes from the technique and tools they developed, which allow them to run thousands of experiments in tightly controlled environments while making highly accurate measurements of cell growth. In addition, all the bacteria they used carried a plasmid bearing a luciferase promoter, so that they could accurately measure bacterial growth rates at very low cell densities using bioluminescence.

Kishony and Leibler started by creating 65 mutant strains and 12

controls, and then exposed them all to seven environments, performing at least two replicates of each trial. They created point mutations using the chemical mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; this method means that they don't know the exact number of mutations per organism, nor the location of the changes, but the authors are clear that this does not affect the interpretation of their findings. The experiment looked at the effect of those mutations, not on the likelihood of any specific mutation occurring, nor on the **relative fitness** of organisms (see the 'Background' box) if put in competition against each other. Another part of the power of their experimental system comes from testing a very diverse class of stresses. Some stresses, such as antibiotics, target specific cellular functions, while others, such as temperature and pH, have wider cellular impacts.

Reviewing the results showed that there was partial correspondence between the nature of the stress (specific or broad) and its influence on the average mutation effect. The stresses that target a specific cellular **module** (the antibiotics trimethoprim and chloramphenicol; see below) most powerfully alleviated the average mutation effects, while stresses with broad cellular impacts had all types of behaviors: for example, low pH aggravated the average mutation effect; high osmolarity had no average influence; and low temperature alleviated the average mutation effect.

Towards a mechanism?

When commenting about the mechanism underlying this apparent alleviation of the detrimental mutation, Kishony and Leibler inevitably enter the world of speculation because their work did not directly address this

Background

- There is a standard assumption that if an organism has any type of deleterious **mutation**, which negatively affects its growth under favorable conditions, and is then given an additional environmental **stress**, the mutant will do even less well under stress than under favorable conditions, and less well than would a wild-type organism under stress: that is, its **relative fitness** will be lower than that of wild-type.
- All cells can be thought of as being constructed of interconnecting **functional modules**, each made up of multiple **biochemical pathways**. Each module performs a specific function, for example during DNA replication.
- **Sexual reproduction** is thought to confer a benefit on organisms because the process of genetic recombination during sexual reproduction purges deleterious mutations from the population.
- A requirement of the prevailing theory to explain the advantage of sexual reproduction is that the harmful effect of having two deleterious mutations must be more than the sum of the effects of each on its own. This requirement is called '**synergistic epistasis**', by analogy with the genetic term 'epistasis' that describes the way that the function of a gene at one point in the genome influences the phenotype produced by another, distant gene.

issue. The explanation they most favor revolves around organisms having many different **functional modules** or **biochemical pathways**. When you apply a specific stress, such as an antibiotic, it targets a particular pathway. For example, trimethoprim disrupts folic acid biosynthesis and consequently inhibits DNA production; the reduced supply of DNA inhibits cell growth even though the rest of the cell's pathways could still work at full speed. If the mutation reduces the effectiveness of another pathway, it could be that that pathway can nevertheless operate sufficiently well that in the presence of the antibiotic it is not the rate-limiting step. The net effect will be that the stress substantially reduces the wild-type's ability to grow, but only marginally reduces the growth of the already slowed-down mutant.

"Where we are at is trying to figure out whether we can make any biological sense of these results in terms of particular biological pathways. For example, things that are activated by heat shock proteins - some type of pathway or genetic set of elements that might modulate mutational effects - that is the really interesting stuff," says Blanchard, who also points out that Fares and colleagues [3] recently provided evidence that the overexpression of a chaperone can compensate for the effects of deleterious mutations. "Thus, some of the results from Kishony and Leibler might be explained by the increased expression of heat shock proteins or other proteins that in turn modify (or buffer) the mutational effect," says Blanchard.

Sex – where a nice theory meets ugly facts

The new results have far-reaching implications. Kishony and Leibler's work is based on an assessment of what the average mutation achieves, and for various fundamental questions in evolution it really matters what happens on average. One critical

area is that of trying to make sense of the advantages conveyed by **sexual reproduction**.

There is an unchallenged dogma that sexual reproduction must have significant biological benefits: otherwise it would not be so widespread, particularly given that there are distinct costs associated with maintaining it within a population. The problem is determining exactly what that advantage is. All the current models look at sex as purely a process of genetic recombination, and consequently the question of why sex is advantageous comes down to one of why recombination is a good thing. The assumption is that any given population is not fully adapted to its environment, and that recombination will help it to adapt faster. There are two basic reasons why the population may not be fully adapted. First, the environment may have changed since the population last tweaked its genetic composition; and second, spontaneous deleterious mutations arise. So, it is argued, sexual reproduction allows genetic recombination to purge deleterious mutations from the population.

The theory that has tried to make biological sense of this is known as mutational determinism, and in 1998 Kondrashov [4] showed that for sex to confer an advantage, the effect of having two deleterious mutations must be more harmful than would be predicted from the effect of each alone - an effect called '**synergistic epistasis**'. A crude example of this is that if you had one 'mutation' that knocked out an organism's left eye and a second that knocked out the right eye, the effect of the two added together is considerably greater than either on its own.

For Kishony and Leibler this boils down to a question of what happens in the average situation. Is there a bias towards either positive or negative synergy, or no effect at all? Prior to their article, the most direct

measurement of epistasis between random mutations was in work conducted with bacteria by Elena and Lenski [5]. This showed no evidence for average epistasis between mutations, indicating that there is no synergy and thus starting to chip away at Kondrashov's basic requirement.

And now Kishony and Leibler's work strikes another blow at mutational determinism, this time showing that particular stresses can lessen the effect of the average mutation. Theoretically, if you added enough deleterious mutations together then the average multiply-damaged organism would perform better when put under the (antibiotic) stress than its wild-type progenitor - but this doesn't make sense. Kishony and Leibler explain that their data suggest either that diminishing-return epistasis occurs under favorable conditions or that synergistic epistasis would occur under mutation-alleviating stresses, but as yet the data do not allow them to distinguish between these two possibilities. This suggestion of epistasis, they stress, is by inference, rather than from direct observation.

Kishony and Leibler's work, therefore, does not directly contradict Lenski and Elena's 1997 paper [5]. Rather, it gives an argument to imply that average epistasis must exist, but at the same time that its existence may depend on environmental conditions and particularly on the presence of stresses that alleviate the average mutation effect. Kishony and Leibler's work could therefore motivate researchers to repeat Lenski and Elena's approach under various environmental conditions and, in particular, under environmental stresses that alleviate average mutation effects.

"This work adds to the growing body of data showing that we still don't have a handle on the environmental effects, and how environment changes mutational effects. That is a

wide open area of research and a difficult one to address. There is a need for novel approaches to assessing how the environment modulates mutational effect,” says Blanchard. Kishony and Leibler may have added such an approach to the evolutionary biologist’s toolkit, and at the least they have opened some new avenues for exploration.

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Editor’s note

Roy Kishony and Stanislas Leibler chose not to be quoted directly on their views within this article.