

stumbling blocks to the effectiveness of chemotherapy.

Several different models for the basic process of mismatch repair have been proposed, all of which conceivably include a 'signalling' element. In bacteria, MutS binds to a mispair and then interacts with MutL, signalling the activation of at least two other proteins<sup>2</sup>. One of these proteins makes a break in the DNA strand containing the incorrect base. The other unwinds this segment of the DNA strand so it can be destroyed. The resulting gap in the strand will then be repaired. A similar process could be at work in eukaryotes, although it has also been suggested that the eukaryotic MutS complexes function as mobile signalling molecules<sup>9</sup>. These signalling cascades appear to rely on protein-protein interactions and conformational changes in proteins, which is why it is so important — in terms of understanding mismatch repair — that the structure of the first protein in the process has now been unveiled<sup>4,5</sup>.

Obmolova *et al.*<sup>4</sup> have been studying the structure of the MutS dimer from the bacterium *Thermus aquaticus*, bound to an unpaired base in a DNA strand. Lamers *et al.*<sup>5</sup>, meanwhile, looked at the *Escherichia coli* MutS dimer, bound to a mismatched base pair. Remarkably, even though the two MutS proteins that make up each dimer are identical, the dimer assumes an asymmetric conformation on binding the mispair, forming a ring around the DNA. Only one of the subunits contacts the mismatch, although both subunits have specific contacts with DNA. Recognition of the mispair by the dimer leads to a bending of the DNA.

Each MutS protein has a region that binds and hydrolyses ATP, producing ADP. It seems<sup>4,5</sup> that these regions also differ between the two MutS proteins. The ATP-binding domains interact with each other and with the DNA-binding regions of both proteins, allowing the effects of ATP/ADP binding and DNA binding to be transferred across the protein. These details provide a structural basis for the biochemical observation that the binding and hydrolysis of ATP results in conformational changes in the MutS protein and in the modulation of protein-DNA interactions.

An intriguing question is how the MutS proteins bind specifically to DNA containing mismatched bases. These proteins have only about a 20-fold higher affinity for mispaired bases relative to normal base pairs, and mispaired bases are not all that common — they occur probably only once in every one million to ten million base pairs<sup>10</sup>. The new structures suggest one possible answer: that only binding to a mispair induces the conformational changes seen in both MutS and the bound DNA. If these conformational changes are required for the MutS proteins to interact with other proteins during mis-

match repair, then these changes and subsequent protein-protein interactions may effectively increase mispair specificity.

The eukaryotic mismatch-recognizing dimers — each of which consists of two different MutS-related proteins — are also asymmetric, in that the two subunits are related but different. The structures of the eukaryotic dimers have not yet been resolved. But the obvious parallels between the eukaryotic and bacterial complexes offer the opportunity for comparative structure-based biochemical studies, and may also be useful in the clinic.

We know that some heritable mutations in the human MSH2 gene predispose patients to a cancer known as hereditary non-polyposis colorectal carcinoma, or HNPCC. But a vexing problem is how to interpret the variety of amino-acid-altering mutations that have been found in the MSH2 gene in people suspected of having this syndrome. The available biochemical, clinical and population data are often insufficient to allow researchers to state with confidence that a particular sequence variation indeed causes disease, or is instead simply a natural variation in the human population<sup>11</sup>. In general, it has not yet been possible to test directly the effect of a particular amino-acid-changing mutation on the function of the MSH2 protein. So, not surprisingly, clinicians are reluctant to make decisions about treatment on the basis of finding a

given variation in MSH2 in either cancer patients or healthy individuals suspected of having HNPCC.

But the two new crystal structures<sup>4,5</sup> will provide a valuable starting point for molecular models of the effects of mutations in the MSH2 gene. The results of the simulations will guide studies of MSH2 function, and help researchers to work out whether a particular mutation actually affects mismatch repair. Conversely, the mutations in MSH2 detected in patients by genetic testing will be important tools in probing the mechanism of mismatch repair. This is a wonderful example of how basic science and clinical care can help each other. ■

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Evolutionary biology

## Use it or lose it

Robert D. Holt

On page 736 of this issue Cooper and Lenski<sup>1</sup> tackle an enduring question in evolutionary biology — the origin and maintenance of ecological specialization<sup>2–4</sup>. There are two different theories of how specialization can arise. Cooper and Lenski's aim was to test them using laboratory cultures of bacteria allowed to evolve over many generations.

First, however, step back and consider the striking fauna of caves, including fish and

other organisms in which the eyes are absent or greatly reduced (Fig. 1)<sup>5,6</sup>. Blindness increases vulnerability to predation and decreases competitiveness in well-lit surface environments. So these species typically remain confined to caves.

Two processes can generate functional degeneration and habitat specialization of this sort. Mutations may arise that degrade the optical system but do not have other effects on the organism's function. In caves,



Figure 1 Eyeless example — the blind cave fish *Astyanax fasciatus mexicana*.

such mutations are neutral as far as natural selection is concerned, being neither beneficial nor harmful, and can drift to fixation (that is, become an enduring part of the genome). The gradual accumulation of neutral mutations could erode previously adaptive structures and indirectly lead to habitat specialization. Alternatively, natural selection may actively re-sculpt the organism, reallocating resources from eyes which are useless in the dark to more useful structures (a pattern of correlated effects that geneticists call 'antagonistic pleiotropy' and ecologists call 'trade-offs'). But it is difficult to tell the difference between these causes of specialization in most natural populations<sup>6</sup>. This is why Cooper and Lenski have explored the problem not in a cave, but in a laboratory study of microbial evolution.

The short generation time and high abundance of microbes, and the experimental control possible in lab conditions, make microbes good subjects for evolutionary study. Lenski and colleagues<sup>7</sup> have been carrying out a long-term analysis of evolution in cultures of the bacterium *Escherichia coli*, and their system provides an ideal forum for investigating the genetic basis for a loss of ecological function — in this case, a decline in the ability of bacteria to use a variety of carbon sources for nutrition.

In their study<sup>1</sup>, Cooper and Lenski confined replicate, genetically homogeneous lineages of bacteria to a simple environment (a minimal nutrient with glucose added). The lineages originated from an ancestral population sustained in a rich nutritive medium, with a smorgasbord of carbon substrates. Over 20,000 generations (taking about ten years in time), mutation resulted in increased adaptation of bacteria to glucose, with an accompanying decay in their ability to use alternative foodstuffs. In effect, *E. coli* growing on just glucose evolved to use a narrower diet, specializing to the available resource at the expense of their potential ability to live on alternative resources. This is comparable to cave fish which have lost a functional ability (sight) that is potentially useful in surface habitats.

Cooper and Lenski use several strands of evidence to argue that antagonistic pleiotropy has occurred. Mutations arise at random. So if functional loss occurs because of an accumulation of neutral mutations, one would expect an exponential decay in diet breadth. Instead, the decay was initially rapid but then slowed (mirroring the temporal dynamics of improved adaptation to glucose). Second, the pattern of functional loss was similar between the replicates. Because mutations arise independently in separate lineages, under the mutation-accumulation theory different functions should be knocked out first in different lineages. By and large, this did not happen.

Third, for one alternative resource

(ribose), the molecular mechanism coupling the improved use of glucose to the reduced use of the alternative is understood: a separate study showed that the mutations that lead to a loss of capacity on ribose provide a selective advantage on glucose. Finally, if the mere accumulation of mutations leads to reduced ability to use a varied diet, higher mutation rates should result in higher rates of loss of that ability. Fortuitously, three lineages evolved changes that impaired their capacity for repairing damaged DNA, and so had much higher genome-wide mutation rates. But this elevated mutation rate had no significant effect on the rate of change in diet use.

Another, similar study<sup>8</sup> has also examined evolution in a microbial population with high mutation rates, but with an experimental regime leading to severe 'bottlenecks'. This is the situation when a population is reduced to very few members, which enhances random processes of genetic evolution at the expense of selection, and in particular facilitates the fixation of deleterious mutations. In this study<sup>8</sup>, reduction in bacterial diet breadth was exponential in time, and unpredictable in pattern between replicate lineages — the patterns expected according to the mutation-accumulation theory. The contrast between this result and that of Cooper and Lenski<sup>1</sup> (where populations were large, greatly reducing the impact of random changes in genetic composition) also bolsters the case for trade-offs (antagonistic pleiotropy) being involved in ecological specialization in the latter experiment.

Nonetheless, many questions remain unanswered, both about Cooper and Lenski's experiments and their broader implications. A more quantitative characterization of the ancestral environment of the bacteria would be desirable, as would a mechanistic understanding of the metabolic constraints underlying antagonistic pleiotropy. Because Lenski's project is ongoing, further adaptive decay in diet breadth might emerge that is consistent with the mutation-accumulation hypothesis<sup>9</sup>. It would also be intriguing to examine evolutionary reversals. Can a lineage specialize to feed on a single nutrient and then re-evolve to use a generalized diet? If so, does this reversal become less likely, the longer the period of specialization? From comparative studies it seems that transitions from generalist to specialist occur more readily than in the other direction<sup>10</sup>. The lab system allows experimental assessment of such evolutionary asymmetries.

Microbes potentially provide solutions to environmental problems ranging from biological control of agricultural pests to cleaning up oil spills. So there are good practical reasons to understand the evolutionary constancy of microbial niches in natural environments, so as to assess the reliability and risks of such solutions. Generalizations

Daedalus

## David Jones

David Jones, author of the *Daedalus* column, is indisposed.

to natural populations should of course be made with caution, given the simplified conditions of lab cultures. The bacterial strains in Lenski and Cooper's cultures are strictly asexual, whereas most natural populations can sexually exchange genetic material. This matters if the availability of genetic variation is a rate-limiting factor in evolution. Sexuality could also alter the expression of antagonistic pleiotropy and the rate of evolution towards ecological specialization.

Moreover, evolution in this study was driven only by selection on the ability to live on abiotic resources in lab environments that are relatively homogeneous in space and time, and occupied by a single bacterial species. In natural communities, specialization occurs in heterogeneous arenas<sup>11</sup> seething with other species, including competitors, predators and parasites. Also, the functional degradations assessed by Lenski and Cooper reflect changes in relative ability to use resources, not absolute losses of functions. So these results may better predict the exclusion of a particular lineage through indirect competition for resources (where exclusion may emerge from slight differences in two strains' relative abilities to use resources), than population persistence in competitor-free habitats (where exclusion may require the nearly complete absence of function).

Despite these caveats, the study by Cooper and Lenski provides valuable insights into the evolutionary dynamics of ecological specialization. These dynamics are central to the evolution of species diversity<sup>12</sup>, and understanding them may even shed light on evolution in the stygian depths occupied by blind cave organisms. ■

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