

# MicroCommentary

## Adjusting to alien genes

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### Summary

**From the perspective of a bacterium, higher eukaryotes are oversexed, unadventurous and reproduce in an inconvenient way. Sex, or recombination following horizontal gene transfer (HGT) events, to be less provocative, is a rare event for a bacterium, but a potentially profound one. Through HGT a bacterium can acquire DNA from distant as well as closely related species and, thereby, instantly obtain genes that encode novel functions or replace its existing genes with better ones. While there is an abundance of retrospective evidence for HGT in bacteria, there has been little consideration of the dynamics of the process. In this issue of *Molecular Microbiology* Lind *et al.* explore these dynamics theoretically, and then experimentally by substituting *Salmonella* Typhimurium ribosomal genes with orthologues from various microbial origins. The authors show that the majority of these newly acquired ribosomal proteins reduce fitness in *S. Typhimurium*, but within short order (40–250 generations) subsequent evolution will mitigate the fitness costs of the alien alleles. The presented results suggest that that at least the initial phase of adapting to alien genes of this informational core ilk is not by changing them but rather by increasing their level of expression by gene amplification. Lind *et al.* argue that their results provide an explanation as to why duplicated genes are overrepresented among horizontally transferred genes.**

Do we believe in evolution? Believe in it? We've seen it, as has everybody else with the good taste to do research with bacteria. Indeed, even the more intelligently designed Creationists accept the kind of evolution we see.

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Small changes in the genetic composition of populations over relatively short periods of time, so-called micro-evolutionary events, are readily observed, whether we want to see them or not. But, what about evolutionary events that occurred before we appeared on the scene; can we do more than just make up stories about the genetic and ecological (selection) processes responsible and illustrate them with equations and sequences of As, Ts, Cs and Gs extracted from extant or very recently extinct organisms? The answer is yes. With bacteria that can be cultured in the lab, we can do experiments to test evolutionary hypotheses. To be sure, evidence gathered in this way only supports or refutes the evolutionary hypotheses being tested, but that's all that Science (inductive inference, if you prefer) can do anyhow. We cannot say with absolute assurance that's how evolution proceeded, and couldn't even if we were eyewitnesses to that evolution.

The study by Lind *et al.* (2010) in this issue of *Molecular Microbiology* addresses the evolutionary consequences of inter species horizontal gene transfer (HGT) of 'informational core genes', more specifically those coding for ribosomal proteins. As we recently said, 'Bacteria may not have sex often, but when they do it can be really good, evolutionarily' (Johnsen *et al.*, 2009). The reason is not solely because the rate of evolution can be increased by recombination-mediated shuffling of homologous genes among members of the same species (Cooper, 2007; Baltrus *et al.*, 2008; Levin and Cornejo, 2009), but more because HGT enables bacteria to acquire genes and clusters of genes (pathogenicity and nicer islands) from phylogenetically quite distant species. By exploiting these genetic aliens, evolution in bacteria need not proceed by the slow pace of mutation, selection and occasional recombination within a 'species'. In single events bacteria can acquire novel genes and operons that have already passed through the trials and tribulations of natural selection in some other species and thereby, leap tall evolutionary buildings in single bounds' (jump between adaptive peaks to use Evolutionary Biology jargon).

Although there are an abundance of sequence gazing and other retrospective evidence for HGT in bacteria (Ochman *et al.*, 2000), there has been little to no consideration of the population and evolutionary dynamics of the

HGT process. Most importantly, how do genes acquired from external sources, alien genes, evolve and become established in bacterial populations? The study by Lind *et al.* is important because it addresses this question theoretically as well as experimentally and provides a reasonable, if not comprehensive, answer.

The theory, the formal part of which (the equations) is relegated to an appendix, sets the stage by postulating that alien genes are unlikely to become established by chance alone; i.e. by genetic drift. This is especially so if they engender a fitness cost. While a Population Geneticist (BRL to be more specific) might quibble with some of the details and completeness of their analyses and choice of parameters, their argument is generally compelling. If genes acquired from without engender a fitness cost to the bacteria that receive them, they will be lost. For these genes to ascend to dominance in the recipient population, this initial cost must either not exist or be overcome by subsequent compensatory evolution. Following that, the population has to confront an environment where these alien genes (or gene clusters) impart a fitness advantage to those bearing them in competition with those that do not.

The experiments in this report provide evidence in support of the hypothesis that even when the alien gene is orthologous to informational core genes, bacteria acquiring them are likely to be at a fitness disadvantage relative to otherwise isogenic bacteria bearing the aboriginal gene. They also demonstrate that if by some mechanism bacteria with burdensome alien genes ascend to dominance in a population, in short order the fitness costs of these genes are likely to be mitigated by subsequent compensatory evolution. While this might not restore fitness completely, it buys some time before the alien gene is ousted, thereby increasing the possibility of its establishment in the recipient population.

Using the  $\lambda$ -red recombination system, Lind *et al.* replaced three *S. Typhimurium* genes coding for different ribosomal proteins with, respectively, 7, 9 and 10 orthologues from close and distantly related species of bacteria and one from a yeast. Although they have the same function and some almost identical DNA sequences, bacteria bearing these alien ribosomal protein genes had significantly lower exponential growth rates than otherwise isogenic *S. Typhimurium* with the native allele. In general, the more phylogenetically distant the source of the ribosomal protein gene transferred, the greater this fitness burden.

The evidence for mitigating these costs of the alien ribosomal protein genes, compensatory evolution, came from experiments where populations of bacteria bearing these costly genes were maintained by serial passage. Within 40–250 generations, the initial cost of carrying foreign ribosomal protein genes *rpsT* (from *Haemophilus influenzae*) as well as *rplA* (from *Sulfolobus acidocal-*

*darius*) were mitigated. DNA sequencing, Southern and Western blot analyses data suggested that for at least three strains for which subsequent evolution reduced the fitness burden, the mitigation of these costs was achieved by increasing the amount of the ribosomal protein by two- to three-fold. The increased dosage was achieved by amplifying the genomic regions that included the ribosomal proteins (from 2.2 to > 200 kb). Using a very elegant, if somewhat classical, complementation assay, Lind *et al.* provided evidence in support of the hypothesis that increased dosage of suboptimal ribosomal proteins through amplification was responsible for the mitigation of the fitness costs of these genes. Taken at large, the authors provide compelling evidence that, by increasing the quantity of suboptimal proteins, gene amplifications can extensively compensate for the initial fitness cost of harbouring these ‘loser’ ribosomal proteins.

The results of this study are interesting and important in ways beyond increasing our understanding of the dynamics of HGT. First, they explain why informational core genes are conserved. Albeit unlikely, their results suggest that a bacterium might acquire a core gene that is or will become better than the highly evolved one it got from its ancestor. Second, the data raise some intriguing questions about the evolution of ribosomes. What are the selective pressures responsible for maintaining structural constraints on these fundamental particles? What is more important in protein synthesis, speed or fidelity, and under what ecological conditions are these generally opposing attributes favoured? The methods Lind *et al.* used here, i.e. replacing existing ribosomal proteins with those of organisms from different environments, are a promising way to address these questions about the evolution of ribosomes and the constraints of their structure experimentally. Finally, as Lind and his collaborators point out, and as seen by experimental evolutionists of an earlier generation (Horiuchi *et al.*, 1963; Betz *et al.*, 1974), the first step in evolving a new gene from an existing one is an increase in the expression level of the old gene.

While we won't harp on it to avoid reducing the apparent intellectual purity of this evolutionary – molecular genetic story, it is worth pointing out that the phenomenon of compensatory evolution and amplification has practical implications. This is particularly so for antibiotic resistance genes. Although these genes commonly engender a fitness cost in the absence of the selecting antibiotic, those costs are likely to be ameliorated by second site compensatory mutations (e.g. see Schrag and Perrot, 1996; Björkman *et al.*, 2000). Gene amplification enables bacteria to increase the level of resistance to antibiotics without acquiring new or modifying existing structural genes (see Sandegren and Andersson, 2009 for an excellent review). Moreover, amplifications increase the mutational targets of these genetic regions, thereby increasing

the likelihood of an adaptive change in these structural genes (Sun *et al.*, 2009).

In closing, we want to say that the experimental approach in this report, the construction of strains with genes from other species, is an appealing general way to study the population dynamics of HGT. Particularly interesting to us will be the sequels to this study in which the alien genes are not essential informational core genes, but rather genes that are truly novel to that species and could provide an immediate fitness advantage in the suitable environment. How will these genes fair and hang around until the recipient population confronts the right environmental conditions? We conjecture that, once again, compensatory evolution making up for their fitness costs will be necessary, perhaps even by amplification.

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