EVOLUTION

In Evolution, the Sum Is Less than Its Parts

Laboratory experiments with bacteria shed light on how epistatic interactions influence the pace of evolution.

Sergey Kryazhimskiy,[1,2] Jeremy A. Draghi,[1] Joshua B. Plotkin[1]

Propagating bacteria in a lab for thousands of generations may seem tedious, or even irrelevant, to most evolutionary biologists. Nonetheless, such experiments provide an opportunity to deduce quantitative principles of evolution and directly test them in controlled environments. Combined with modern sequencing technologies, as well as theory, recent microbial experiments have suggested a critical role for genetic interactions among mutations, called epistasis, in determining the pace of evolution. Two papers in this issue, by Khan *et al*. on page 1193 (*1*) and Chou *et al*. (*2*) on page 1190, present precise experimental measurements of these epistatic interactions.

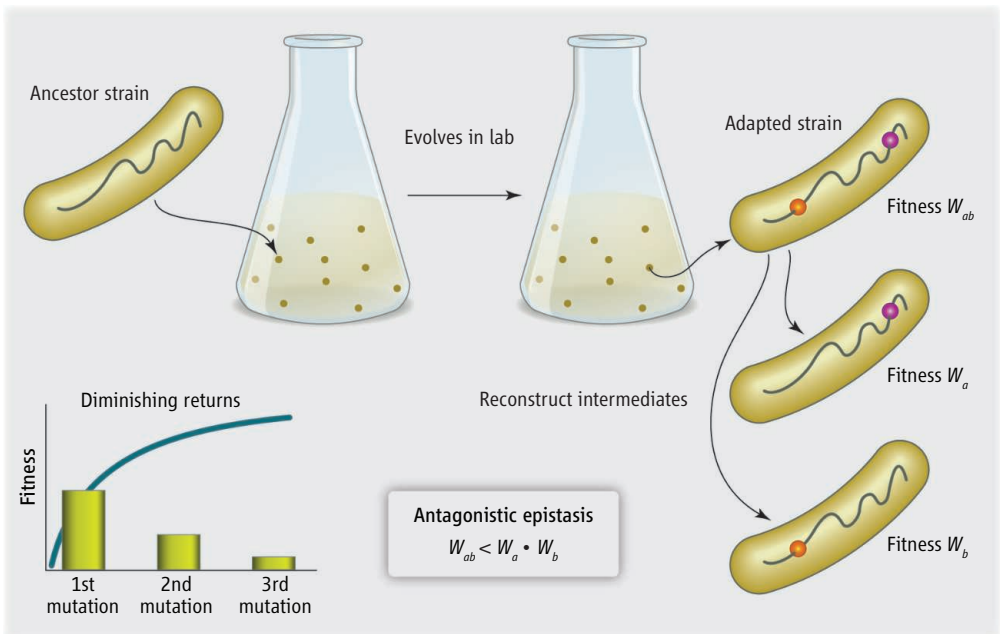
Microbial evolution experiments in a simple, constant environment reveal a characteristic pattern: At first, a population rapidly acquires beneficial mutations, but then adaptation progressively slows so that thousands of generations pass between subsequent beneficial substitutions (*3*). Unexpected outcomes, however, can and do occur even in these simple experimental conditions. Populations evolve a dramatically elevated mutation rate (*4*), discover rare phenotypic innovations (*5*), or diverge into distinct lineages that either coexist (*6*) or compete vigorously as each strain races to acquire more adaptive mutations (*7*). Recent theory suggests that a common cause underlies all these phenomena: the structure of epistatic interactions among mutations.

Epistasis describes how the fitness consequence of a mutation depends on the status of the rest of the genome. In one extreme example, called sign epistasis, a mutation may be beneficial if it arises on one genetic background, but detrimental on another. Although interactions among genes may seem an obvious fact of biology, the myriad possible forms of epistasis have made it difficult to formulate predictive evolutionary models or to infer such interactions from empirical data. Nevertheless, epistasis is at the heart of classical theories, such as the evolution of sex (*8*), and also of modern concepts such as robustness and evolvability (a population's ability to evolve) (*9*). Moreover, recent theoretical work (*10*) suggests that the overall dynamical pattern of adaptation observed in long-term microbial experiments can be explained by a prevalence of what is called antagonistic epistasis, in which beneficial mutations confer less benefit in combination than they do individually.

To quantify epistasis among beneficial mutations and to test these theoretical predictions, both Khan *et al*. and Chou *et al*. examined the initial substitutions that occurred in populations of bacteria adapting in the laboratory. The researchers identified the handful of mutations across the genome that had substituted in an evolved strain, and then constructed intermediate strains containing combinations of these mutations. By measuring the fitness benefits conferred by these mutations, individually and in combination, the researchers were able to directly quantify the extent and form of epistasis (see the figure).

Both studies found a predominance of antagonistic epistasis, which impeded the rate of ongoing adaptation relative to a null model of independent mutational effects. Chou *et al*. further interpreted the prevalence of antagonistic epistasis in terms of metabolic costs and benefits. The concordance of results from the two studies is noteworthy, especially because Khan *et al*. analyzed *Escherichia coli* populations [from the long-term experiments of Lenski (*3*)], whereas Chou *et al*. studied an engineered strain of *Methylobacterium extorquens*. The remarkable precision with which both studies quantified epistasis among beneficial mutations was made possible only by leveraging whole-genome sequencing combined with the ability to reconstruct mutational combinations and assay them in the same environment in which the mutations first arose.

The view of epistasis across a genome that emerges from this work contrasts sharply



Antagonistic epistasis. Bacteria adapt to a laboratory environment by acquiring beneficial mutations. Khan *et al*. and Chou *et al*. identified the mutations that accrued in an adapted strain, and measured their fitness benefits (growth advantage relative to the ancestor). The mutations conferred smaller marginal benefits in combination than they did individually. This antagonistic epistasis causes progressively slower rates of adaptation over time.

CREDIT: ADAPTED BY P. HUEY/*SCIENCE*

[1]Department of Biology, University of Pennsylvania, Philadelphia, PA 19103, USA. [2]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA. E-mail: jplotkin@sas.upenn.edu

Published by AAAS

with the type of epistasis found among adaptive mutations within a single protein (*11*). Notably, Weinreich *et al*. studied mutations in an antibiotic resistance gene, β-lactamase, and found a prevalence of sign epistasis, which limits the number of genetic paths that evolution can follow (*11*). In contrast, the epistasis documented by Khan *et al*. and Chou *et al*. exerts less constraint on the order of substitutions that increase fitness, so that the specific path that evolution will take is less predictable. At the same time, the prevalence of antagonistic epistasis measured by the two groups ensures a predictable tempo of adaptation characterized by diminishing marginal returns (*10*).

Although these new experiments suggest a consistent principle of how epistasis shapes the pattern of adaptation, many questions must be answered before their results can be extended to evolution outside the laboratory. It remains unclear, for instance, whether these results would be altered by changing fundamental evolutionary parameters, such as population size, rate of mutation, and rate of recombination. Likewise, it is unclear whether experiments in simple environments, with only one or a few niches for coexisting strains, will reflect the pattern of adaptation in more complex ecologies, such as *Pseudomonas fluorescens* in structured environments (*6*). Nonetheless, the compelling consistency between these two studies should inspire efforts to test the generality of their findings, by measuring epistasis in a wide range of experimental and even natural systems.

These studies, and the long-term laboratory evolution experiments from which they derive, represent a resounding achievement for the reductionist approach to studying biology. The mechanistic picture they paint of evolution is complex but not incomprehensible; although epistatic interactions lead to surprising phenomena, the advantages of a frozen "fossil record" of laboratory-raised isolates, and the ease of manipulating—and, now, fully sequencing—evolved strains enables researchers to tease apart and examine the underlying causes of these phenomena. Moreover, the theory and concepts developed to explain these simple experiments may have broad payoffs. Already, epistasis has been implicated in the evolution of drug resistance in influenza viruses (*12*) and in bacterial pathogens (*13*). Ultimately, populations of bacteria tediously propagated in the lab may be key to predicting the next moves of the most mutable and dangerous human pathogens.

References

1. A. I. Khan, D. M. Dinh, D. Schneider, R. E. Lenski, T. F. Cooper, *Science* **332**, 1193 (2011).
2. H.-H. Chou, H.-C. Chiu, N. F. Delaney, D. Segrè, C. J. Marx, *Science* **332**, 1190 (2011).
3. S. F. Elena, R. E. Lenski, *Nat. Rev. Genet.* **4**, 457 (2003).
4. P. D. Sniegowski, P. J. Gerrish, R. E. Lenski, *Nature* **387**, 703 (1997).
5. Z. D. Blount, C. Z. Borland, R. E. Lenski, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 7899 (2008).
6. P. B. Rainey, M. Travisano, *Nature* **394**, 69 (1998).
7. R. J. Woods *et al*., *Science* **331**, 1433 (2011).
8. A. S. Kondrashov, *Nature* **336**, 435 (1988).
9. G. P. Wagner, L. Altenberg, *Evolution* **50**, 967 (1996).
10. S. Kryazhimskiy, G. Tkačik, J. B. Plotkin, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 18638 (2009).
11. D. M. Weinreich, N. F. Delaney, M. A. Depristo, D. L. Hartl, *Science* **312**, 111 (2006).
12. J. D. Bloom, L. I. Gong, D. Baltimore, *Science* **328**, 1272 (2010).
13. S. Trindade *et al*., *PLoS Genet.* **5**, e1000578 (2009).

10.1126/science.1208072

GENOMICS

Behavior and the Dynamic Genome

Does behavior evolve through gene expression changes in the brain in response to the environment?

Alison M. Bell[1,3] and Gene E. Robinson[2,3]

When circumstances change, an organism's first response is often behavioral. But how does adaptive behavior evolve, given that it requires constant and often instantaneous interactions between an individual and its environment? The dominant view emphasizes new random DNA mutation as the starting point. This may lead to behavioral variation. If the resulting variants have different fitness values, then natural selection could result in behavioral evolution through changes in allele frequencies across generations. An alternative theory proposes environmentally induced change in an organism's behavior as the starting point (*1*), and "phenotypic plasticity" that is inherited across generations through an unspecified process of "genetic assimilation" (*2*). Despite numerous examples (*3*), the latter as a driver of behavioral evolution has never been widely accepted, perhaps as a reaction against Lamarckianism—the idea that characteristics acquired by habit, use, or disuse can be passed on across generations. However, behavioral genetics and genomics, especially for animals in natural populations, lend some plausibility to the phenotypic plasticity view.

The ability to analyze genome-wide gene expression through "transcriptomics" has shown that the genome responds dynamically to stimuli (*4*). One illustrative example is the honey bee. The African honey bee (*Apis mellifera scutellata*) responds much more fiercely when its hive is attacked than do other subspecies of honey bee. Evolutionary changes in brain gene expression may have resulted in an increase in responsiveness to alarm pheromone (the chemical bees use to alert each other to danger) for African honey bees (*5*). About 10% of the same genes regulated in the brain by alarm pheromone are also differentially expressed between African and the less aggressive European honey bees. These genes, acting over both physiological and evolutionary time scales, provide a possible mechanism for how behavioral plasticity might drive rapid behavioral evolution through changes in gene regulation. In an environment with more predators, colonies producing more bees with lower thresholds for responding to alarm pheromone would have fared better, which would then result in a population with patterns of gene expression whose output was an "aroused" behavior, even in the absence of alarm pheromone. Although this view does not rule out the possibility that these differences in aggression arose through new mutation, the transcriptomics agrees with the idea of "genetic accommodation" (*3*), the modern, more inclusive version of genetic assimilation, which could involve either evolutionary increases or decreases in plasticity. In certain environments, plastic genotypes might be favored, but in other environments, nonplastic genotypes might be preferred instead. Future studies will determine whether differences in honey bee aggression can be explained by selection on regulatory regions of the

[1]Department of Animal Biology, University of Illinois, Urbana-Champaign, IL 61801, USA. [2]Department of Entomology, University of Illinois, Urbana-Champaign, IL 61801, USA. [3]Neuroscience Program, Program in Ecology, Evolutionary Biology and Conservation, Institute for Genomic Biology, University of Illinois, Urbana-Champaign, IL 61801, USA. E-mail: generobi@life.uiuc.edu