AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Bacteriology

EDITORIAL

Classic Spotlight: Look, Max—No Math Required!

John S. Parkinson

Biology Department, University of Utah, Salt Lake City, Utah, USA

In 1952, Joshua and Esther Lederberg published a paper in the *Journal of Bacteriology* (1) that fully legitimized bacteria as genetic organisms, no different in principle from pea plants or fruit flies. In all cases, mutations are precipitous events that can generate stepwise changes in phenotype. The Lederbergs' report first described the method of replica plating, now a venerable microbiological technique. But more importantly, the Lederbergs used their replica plating method to resolve a long-standing controversy about the nature of bacterial "variants": when a bacterial culture is exposed to a virulent phage or a lethal antibiotic, do the rare resistant survivors arise from preexisting resistant mutants present in the population before the challenge or are the resistant mutants formed *de novo* in response to the challenging agent?

Let's call these opposing ideas about the origin of bacterial mutants the prechallenge and postchallenge hypotheses. Prechallengists claimed that bacterial mutants arose through random, spontaneous mutational events prior to the selective challenge that served to reveal the phenotypic consequences of the mutations. Postchallengists claimed that the strong selection itself elicited or directed heritable adaptive changes in the treated organisms. The Lederbergs' graphic experiment capped a decade of work on the origin of bacterial mutants, highlighted by two important earlier studies. The first of those studies relied on a more mathematical approach.

In 1943, Sal Luria, a microbiologist, and Max Delbrück, a physicist by training, teamed up to study culture-to-culture variation in the number of phage-resistant mutants appearing in independent, identical cultures of *Escherichia coli* (2). Luria did the experiments; Delbrück provided the mathematical theory. They claimed that "a decision [between these two hypotheses] can only be reached by a more subtle quantitative study than has hitherto been applied in this field of research." Accordingly, they pointed out the quantitative implications of the two alternative possibilities. If phage-resistant variants arise postchallenge, then the number of resistant individuals per culture should follow a Poisson probability distribution. However, if resistant variants arise as spontaneous prechallenge mutants, occasional cultures should produce a mutant jackpot, a large clone of resistant cells descended from a mutant that happened to arise early during culture growth. The experimental outcome was clear: resistant bacteria appeared "not as random samples, but in groups of varying sizes." Luria and Delbrück concluded that "resistance to virus is due to a heritable change of the bacterial cell which occurs independently of the action of the virus."

Although the Luria-Delbrück fluctuation test was conceptually simple, its interpretation relied on rigorous quantitative analysis. Somewhat later, in 1949, Howard Newcombe reported a simpler experimental approach to the prechallenge-versus-postchallenge issue (3). Using the same phage and bacteria as Luria and Delbrück, Newcombe seeded several plates with phage-sensitive cells and let them grow for a few hours. He then redistributed the cells on some plates by spreading and left other plates undisturbed. Both sets of plates were then challenged with phage. The results were striking: the respread plates yielded many more resistant colonies than did the undisturbed control plates. Newcombe reasoned that the original plates must have contained a few microcolonies formed when preexisting resistant mutants divided several times. Redistribution scattered cells from those microcolonies around the plate, where each gave rise to a resistant colony after phage challenge. In contrast, each microcolony on the unspread plates produced only one resistant colony. Newcombe's experiment provided strong, but nevertheless circumstantial, evidence that the phage-resistant mutants existed before the challenge.

A more direct demonstration that mutants could arise in the absence of a selective treatment was needed to solidify the prechallenge case. That was the key contribution of the Lederbergs' paper. They grew a lawn of cells on a nonselective plate and created a replica plate that was challenged with phage or an antibiotic. After locating phage- or antibiotic-resistant colonies on the challenged replica plate, they looked for resistant cells in the corresponding locations on the original master plate. After enrichment by several replica test cycles, they isolated resistant cells that had never been exposed to the selective agent. In the Lederbergs' words, "These observations . . . [confirm] previous evidence for the participation of spontaneous mutation and populational selection in the heritable adaptation of bacteria to new environments."

The selections in these early experiments were lethal and absolute. The only survivors seemed to be organisms that were already fully resistant, genotypically and phenotypically, at the time of the challenge. In fact, a weakness of these classic experiments is that they could not have detected postchallenge mutations. They show that some mutations arise prechallenge but do not eliminate the possibility that another fraction of mutability is stress induced. In recent years, experiments with less stringent selections, for example, demanding growth on a novel carbon source, have demonstrated an increase over time in the number of mutants that appear on a selective plate (4). These results suggested that, contrary to the classic experiments, selective challenges might cause mutations (5). Such observations resurrected the adaptive mutation controversy (6) but may yield to orthodox mutational explanations, given that weaker selections can favor multistep responses (5, 6). In the prophetic words of the Lederbergs (1), "no unequivocal

Citation Parkinson JS. 2016. Classic spotlight: look, Max—no math required! J Bacteriol 198:2281–2282. doi:10.1128/JB.00525-16.

Address correspondence to parkinson@biology.utah.edu.

case of a mutation specifically directed by and adapting cells to a chemical agent has yet been defended, despite numerous attempts of varying clarity."

ACKNOWLEDGMENT

Many thanks go to John Roth for insightful comments and editorial advice.

REFERENCES

1. **Lederberg J, Lederberg EM.** 1952. Replica plating and indirect selection of bacterial mutants. J Bacteriol **63:**399–406.
2. **Luria SE, Delbrück M.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:**491–511.
3. **Newcombe HB.** 1949. Origin of bacterial variants. Nature **164:**150–151. http://dx.doi.org/10.1038/164150a0.
4. **Cairns J, Foster PL.** 1991. Adaptive reversion of a frameshift mutation in *Escherichia coli*. Genetics **128:**695–701.
5. **Maisnier-Patin S, Roth JR.** 2015. The origin of mutants under selection: how natural selection mimics mutagenesis (adaptive mutation). Cold Spring Harb Perspect Biol http://dx.doi.org/10.1101/cshperspect.a018176.
6. **Maisnier-Patin S, Roth JR.** 2016. Adaptive mutation controversy, p 26–36. *In* Kliman RM (ed), Encyclopedia of evolutionary biology, vol 1. Academic Press, Oxford, United Kingdom.