

Using test tube experiments to study how bacterial species evolve antibiotic resistance

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Given a critical change in the environment, how exactly, do species adapt? Professor Tom Vogwill and colleagues wanted to get at the heart of this evolutionary question by measuring the growth rates and DNA mutations of 8 different species of *Pseudomonas* bacteria. They controlled a single but vital variable during growth, the dose of the antibacterial drug rifampicin.

Overall, they challenged 480 populations from 8 different strains of *Pseudomonas* (3840 total) with adapting to the minimal concentration of rifampicin that is needed to completely inhibit the growth of the ancestral strain of each species. They carried out the experiment over 30 generations of bacteria. Next, the authors selected 75 randomly chosen rifampicin-resistant mutants from 8 different clonal [bacterial strains](#) and sequenced the *rpoB* gene in all 600 mutants, identifying 47 different mutations. They measured both the growth rates of the clones in the presence of rifampicin, and the DNA mutations of a gene, *rpoB*, a known mediator of drug resistance.

They found that most of these *rpoB* mutations occurred only once, some occurred multiple times in a single strain, and others occurred multiple times in multiple strains. However, in agreement with the prevailing hypothesis, populations of the same strain tended to evolve in parallel. Despite that fact the 8 bacterial strains are genetically very different from each other, they also found that the same mutations have different effects on fitness in the different strains, indicating that the genetic make-up is an important fitness factor. Finally, the authors demonstrated that the growth rates varied more within species than between species.

"Antibiotic resistance often evolves by mutations in genes that are conserved across bacteria, raising the possibility that resistance evolution might follow

similar paths across bacteria," said colleague Craig MacLean. "Our study provides good evidence that the rest of the genome influences which resistance mutations are observed, and how these mutations influence Darwinian fitness. These findings imply that we need to be cautious when trying to extrapolate our understanding of the genetics of [antibiotic resistance](#) between bacterial strains or species."

The breadth of the study shows how the powerful new tool of experimental evolution can provide important insights into the relationships between DNA [mutations](#), growth and evolution.

The study was published in the advanced online edition of *Molecular Biology and Evolution*.

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