

Adaptability

Fusing the study of microbial pathogens with evolutionary biology potentially provides a means for predicting emergent pathogens

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Scientists working on infectious diseases wonder about the evolution of virulence. Indeed, people want to know why new diseases appear, where they come from, and, perhaps most interestingly, what is coming next. Many researchers are working hard to answer those questions, particularly the last one. Figuring out what comes next depends on understanding what makes infectious agents change to become more successful at infecting hosts, transmitting between hosts, and avoiding a host's immune system.

Once we understand the factors involved in conferring virulence, can we use that information to predict and possibly prevent the emergence of novel disease-causing pathogens? An approach to understanding those issues that fuses the study of microbial pathogens with evolutionary biology provides an exciting way of tackling these questions. Studying how disease-associated traits evolve holds the potential of enabling us to predict accurately the emergence of infectious diseases.

Evolvability, the Capacity to Respond to Evolutionary Pressures

From the standpoint of natural selection, the evolvability of a trait is its capacity to change in response to evolutionary pressures. In terms of evolvability, it is not enough that a trait changes transiently in response to a stimulus. Changes must become permanent and transmissible from one generation to the next.

Evolvability was conceived and first studied by examining information processing in the human brain, and was first tested in the fruit fly *Drosophila melanogaster*. Those early studies focused mainly on physiology or developmental biology, and the traits were measured by studying inbred

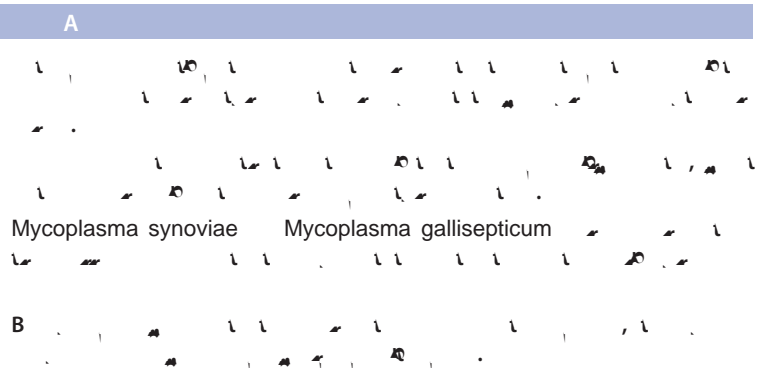
or outcrossed populations. Later, analyses included genetic diversity in the form of specific point mutations in DNA, and introduced evolutionary drivers, traits that change in direct response to selective pressure, and evolutionary passengers, traits that change in response to selection introduced by changes in their drivers.

Tumor cells are also used for characterizing evolutionary drivers and passengers as well as their evolvability. Some investigators are designing therapeutics to target traits that are presumed to be evolutionary drivers, while others are considering the value of targeting evolutionary passengers.

Other factors such as changes in gene expression, dominant and recessive forces, alternative gene splicing, and redundant functions add further complexity to the study of evolvability. However, by using bacterial systems, many of these potentially confounding factors can be more readily controlled.

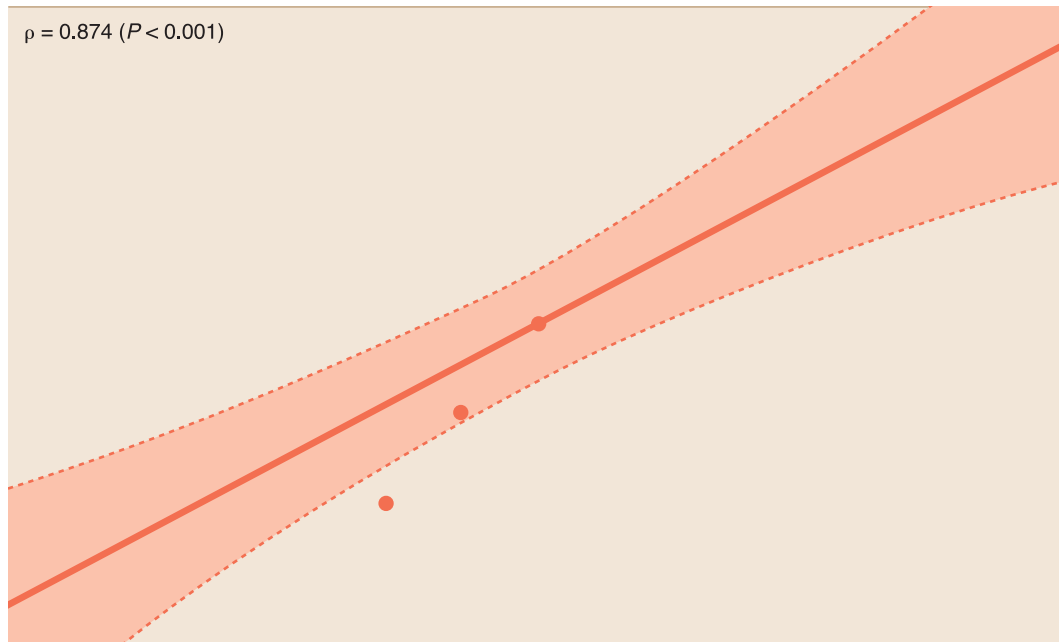
Examining Evolvability in Bacteria

Describing bacterial evolvability begins with considering selection outcomes. Selection can be





thought of as exerting either a •purifyingŽ or •di-



too, diversifies to escape host immune responses, related bacterial species *Streptococcus pneumoniae* and *Clostridium perfringens* unlike *VlsE* of *B. burgdorferi*, this specific adhesive function of *VlhA* is well known. This distinction is critical: a homologous gene comes from the same common ancestor, whereas an analogous gene is not related by descent, but performs the same function. We found that the analogous sialidases of *Streptococcus pneumoniae* and *C. perfringens* are largely conserved, and under global purifying selection, both the level of diversifying selection acting on *VlhA* and the mathematical relationship between diversify bacterial sialidases. Meanwhile, another question arises. Is there something unique about the *nanI* gene of *M. synechococcus* that makes it particularly prone to evolve? To address this question, we examined another species of *Mycoplasma* that parasitizes birds, *M. gallisepticum*. These two species frequently co-infect the same animal, creating opportunities to share genes by horizontal transfer and enabling the same gene to be in two different species simultaneously. *nanI* is one such shared gene, but To address the broader question of evolvability, we measured selection acting on analogous *nanI* in *M. gallisepticum* indicates that it is under purifying rather than diversifying selection.

Moreover, the strength with which *M. syn-* binds host cells depends on which variants of *VlhA* are being expressed. Some variants cling tenaciously, while others bind only weakly. Because of the predicted functional balance between sialidase activity and attachment, we assessed the level of diversifying selection acting on *VlhA* and the mathematical relationship between the two traits. Not only is *VlhA* also under significant ($P = 0.01$) diversifying selection, but there is also a striking, statistically significant ($P = 0.001$) correlation between sialidase activity level and adherence (Fig. 1).

Evolvability Is Not Universally Favored

These traits and the genes encoding them do not make their evolvability universally favorable. To address the broader question of evolvability, we measured selection acting on analogous *nanI* in *M. gallisepticum* indicates that it is under purifying rather than diversifying selection.



This critically important finding suggests that form an indispensable function: host cell attachment. No feature of the gene itself makes it evolvable. For a parasitic organism that attaches to its host surface, this capacity is tantamount to survival. But as variants of parasitic organisms may differ in their capacities to escape the responses of the host immune system, the avidity with which they adhere to the host consequently varies, too. Rather, genomic context determines its fate. In the context of the *M. gallisepticum* genome, the gene and trait remain stable. And because sialidase activity is necessarily coordinated with avidity of adherence, direct selection on V1hA indirectly drives diversity in the evolutionary passenger gene.

Genomic Context Can Determine Evolvability of Traits

When diversity in sialidase activity is favored in *M. synoviae* why is the same trait encoded by the same gene so stable in *M. gallisepticum*? It comes down to pressure to perform. Selective pressures can be either direct or indirect, and the affected traits can thus be thought of as either drivers or passengers of evolution. In nature, the *M. synoviae* V1hA proteins per-

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elle, it lacks a driver of diversification and, thus, remains stable.

Mycoplasmas are parasitic bacteria with minimal, streamlined genomes. By their very nature, these organisms avoid introducing potentially confounding variables in evolutionary studies such as co-dominance, inheritance, redundant functions, alternative gene splicing, and environmental survival. Thus, for the first time, we can see markedly different selective forces acting on homologous genes in two distinct species occupying the same niche in a shared habitat. These forces can be measured and phenotypically verified, tying together informatics, mathematical, and biological data.

In short, this system demonstrates that evolvability is not necessarily inherent to a particular trait, but is heavily influenced by the genomic context in which that trait is found. Determining the evolutionary pressures acting on disease-associated traits, along with the evolvability in context of the genes encoding those traits, creates the exciting potential for forecasting infectious disease. In other words, by thinking about infectious diseases in the same manner as evolutionary biologists consider this subject more broadly, we can come a bit closer to answering that critical question: •what is coming next?Ž

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Suggested Reading

Bloom, J. D., L. I. Gong, and D. Baltimore. 2010. Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. *Science* **328**:1272...1275.

Bozic, I., T. Antal, H. Ohtsuki, et al. 2010. Accumulation of driver and passenger mutations during tumor progression. *Proc. Natl. Acad. Sci. USA* **107**:18545...18550.

Conrad M. The brain-machine disanalogy. 1989. *Biosystems* **22**:197...213.

Gatenby, R. A., J. J. Cunningham, and J. S. Brown. 2014. Evolutionary triage governs fitness in driver and passenger mutations and suggests targeting never mutations. *Nature Commun* **5**:5499.

Graves, C. J., V. I. Ros, B. Stevenson, P. D. Sniegowski, and D. Brisson. 2013. Natural selection promotes antigenic evolvability. *PLoS Pathog.*

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