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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 interesting of all, 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.

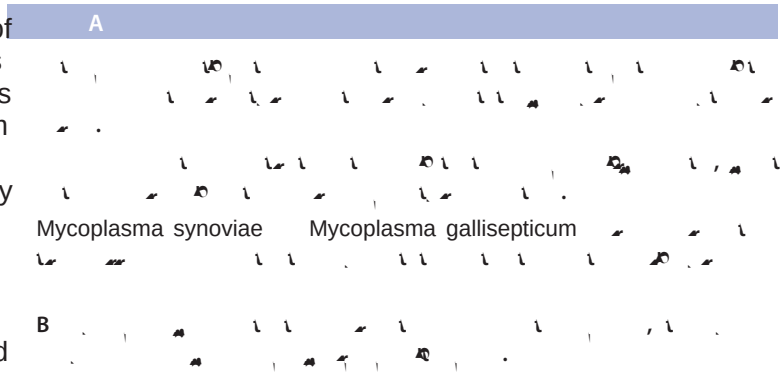
Evolvability was conceived and fšrst studied by examining information processing in the human brain, and was fšrst tested in the fruit fly Drosophila melanogasterThose early studies focused mainly on physiology or developmental biology, and the traits were measured by studying inbred or outcrossed populations. Later, analyses included genetic diversity in the form of specifšc 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.

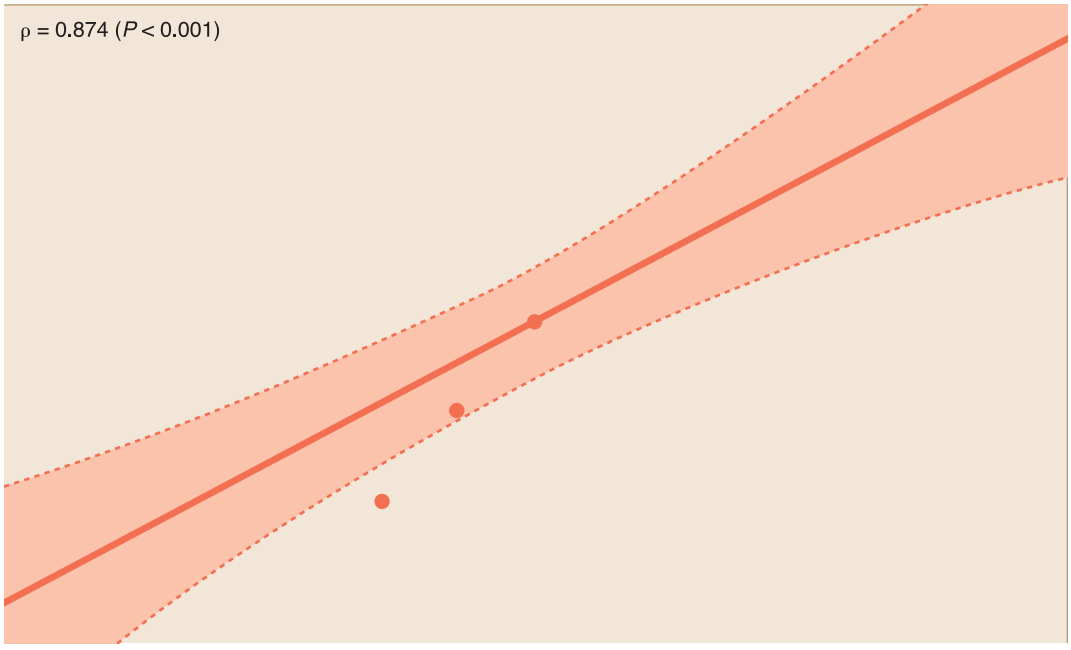
Other factors such as changes in gene expression, dominant and recessive forces, alternative gene splicing, and redundant functions add further complexity to the study of evolvability. However, by using bacterial systems, many of these potentially confounding factors can be more readily controlled.

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, unlike VlsE of B. burgdorferi, this specific adhesive function of VlhA is well known.

Moreover, the strength with which M. synoviae 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 both 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 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 does not make their evolvability universally favorable. To address the broader question of evolvability, we measured selection acting on analogous instead of homologous sialidases of two distantly related bacterial species, Streptococcus pneumoniae and Clostridium perfringens.

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 S. pneumoniae and C. perfringens are largely conserved, and under global purifying selection, suggesting that selection does not always act to diversify bacterial sialidases.

Meanwhile, another question arises. Is there something unique about the nanI gene of M. synoviae 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 the ω value for nanI in M. gallisepticum clearly indicates that it is under purifying rather than diversifying selection.

This critically important finding suggests that no feature of the gene itself makes it evolvable. Rather, genomic context determines its fate. In other words, nanI is evolvable even though, in the context of the *M. gallisepticum* genome, the gene and trait remain stable.

Genomic Context Can Determine Evolvability of Traits

When diversity in nanI and sialidase activity is favored in *M. synoviae*, why is the same trait encoded by the same gene be 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* VlhA proteins perform an indispensible function: host cell attachment. 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. And because sialidase activity is necessarily coordinated with avidity of adherence, direct selection on VlhA indirectly drives diversity in the evolutionary passenger gene, nanI.

However, this relationship is not the case for *M. gallisepticum* because it has a distinctly different primary mechanism of adherence to its host by means of a complex, multimeric attachment organelle (Fig. 2). This structure is stable, constitutive, and completely absent from *M. synoviae*.

elle, it lacks a driver of diversifšcation 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 fšrst 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 verifšed, 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

Acknowledgments

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Suggested Reading

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