Analyzing Evolvability To Anticipate New Pathogens

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 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

SUMMARY

- **Pathogens adapting to new hosts or constantly shifting to escape the defenses of their natural hosts are subject to measurable evolutionary forces.**
- **The evolvability of a trait is its capacity to change permanently, most notably in response to diversifying natural selection.**
- ***Mycoplasma synoviae* and *Mycoplasma gallisepticum* share a horizontally transferred sialidase that is subject to distinct selective pressures and evolves at different rates in each species.**
- **Because genomic context can drive the evolvability of genes, it should be included when modeling emerging pathogens.**

thought of as exerting either a "purifying" or "diversifying" force. When that force is purifying, the DNA and protein sequences that determine the trait change very little, indicating that the population is at its fittest because the trait in question does not change much. However, when that selective force is diversifying, there is marked variation in the DNA and protein sequences that determine a trait, indicating that the population of organisms is at its fittest when the trait in question varies extensively. A background of random genetic drift, also known as neutral selection, falls between those two extremes.

Examples of proteins under purifying versus diversifying selection are the replication initiation factor DnaA and the variable surface antigen VlsE, respectively. Little variation can be tolerated in DnaA because the function of this enzyme is so critically important to every cell. In other words, DnaA has a low potential for evolvability. However, the situation for VlsE is nearly opposite. Thus, it is advantageous for individual cells within a population to express slightly different antigens along their surfaces, enabling at least some of them to escape when they are all exposed to a host immune surveillance system. Therefore, VlsE is said to have a high potential for evolvability.

The selective force acting on individual amino acid residues can be recognized by aligning homologous DNA codon sequences from multiple isolates within a population of bacteria. Nucleotide changes that do not result in major structural or functional changes in the protein sequence are termed synonymous mutations, whereas changes that do lead to such changes are termed nonsynonymous.

The ratio of nonsynonymous to synonymous mutations (K_a/K_s or d_n/d_s, abbreviated as ω) reveals the type of selective force acting on a particular trait. Neutral selection should result in a ω ratio close to 1. Thus, values less than 1 indicate purifying selection, while ω ratios greater than 1 indicate diversifying selection. Statistical significance can be determined across an entire protein sequence by performing a likelihood ratio test between the native measurements to allow for diversifying selection, and a null model that artificially caps the ω ratio at 1 and thus does not. While inferring selection based on ω ratios allows consideration only of changes in protein sequence rather than changes in gene expression level or timing, in true instances of diversifying selection this limitation would err on the side of false-negative findings rather than false-positive. Any statistically significant diversifying selection is considered remarkable.

Looking more deeply for Evolutionary Drivers

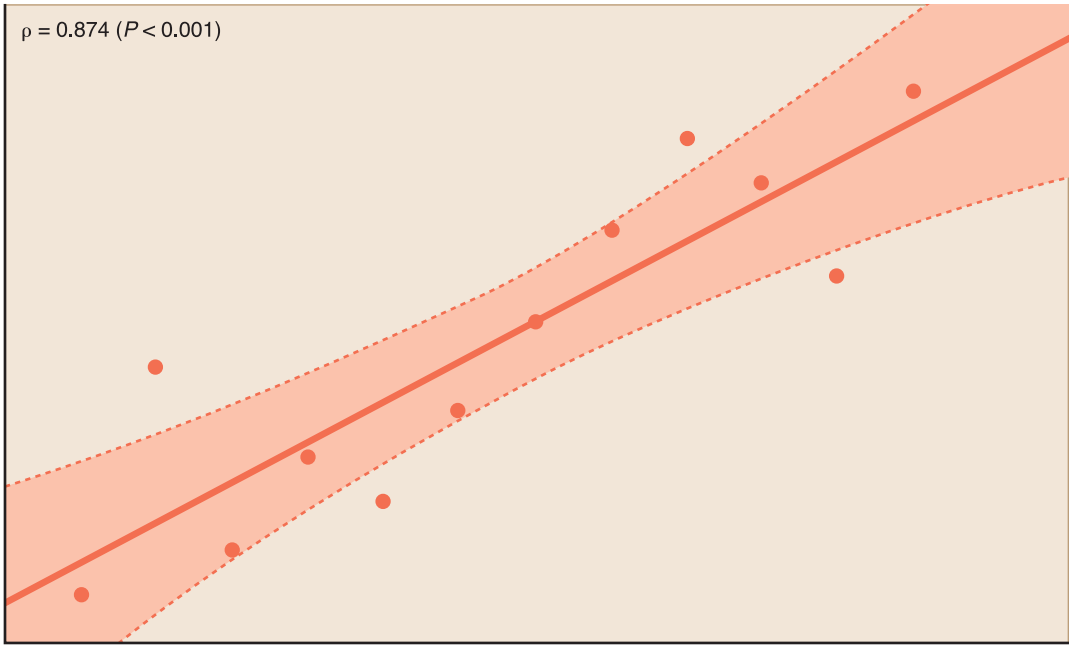
It is possible to observe diversity either by phenotype or by genotype. Fr example, experiments that focus on the VlsE proteins of *Borrelia burgdorferi*, which causes Lyme disease, indicate that these variable antigens are evolvable and diversify in order to escape host immune responses during infection. Further, even unexpressed *vlsE* gene cassettes, which would presumably not be subject to evolutionary pressure from host antibodies, can contribute significant diversity. This unexpected finding provides key evidence that evolvability—in the form elevated mutation rates in unexpressed genes—is itself an evolvable trait.

Further understanding comes from measuring the sialidase enzymes of the avian parasites *Mycoplasma synoviae* and *Mycoplasma gallisepticum* while they adhere to sialic acid residues along the surfaces of host cells. The diversity in enzymatic activity and corresponding genetic diversity for the sialidase (*nanI*) of *M. synoviae* significantly correlates with strain virulence. In other words, the more sialic acid a strain cleaves, the more likely it causes severe disease.

The genetic variation in gene *nanI* arises from significant ($P < 0.001$) diversifying selection. Like the influenza virus, *M. synoviae* adheres to host cells by attaching to sialic acid residues along the host cell surface while retaining the ability to cleave those residues. Indeed, there is a coordinated interplay between the pathogen attaching to those sialic acid residues, and then detaching from them because of sialidase activity. When these two antagonistic phenotypes are not properly balanced, the pathogen becomes less virulent. In other words, extremes of either too strong attachment or detachment lead to less efficient infection or transmission, respectively. For an obligate parasite, "unbalanced" variants would likely be lost from the population.

Knowing that *nanI* sialidase is under diversifying selection in *M. synoviae*, we predicted that the organism's adherence mechanism would also be subject to diversifying selection. *M. synoviae* attaches to host cells is primarily through its immunogenic lipoprotein, called VlhA. Although it,

FIGURE 1



Spearman rank correlation between host cell adherence and sialidase activity in twelve clinical isolates of *Mycoplasma synoviae*. Shading indicates the 90% confidence interval. (Adapted from M. May and D. R. Brown, J. Bacteriol. 193:2116–2121, 2011.)

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).

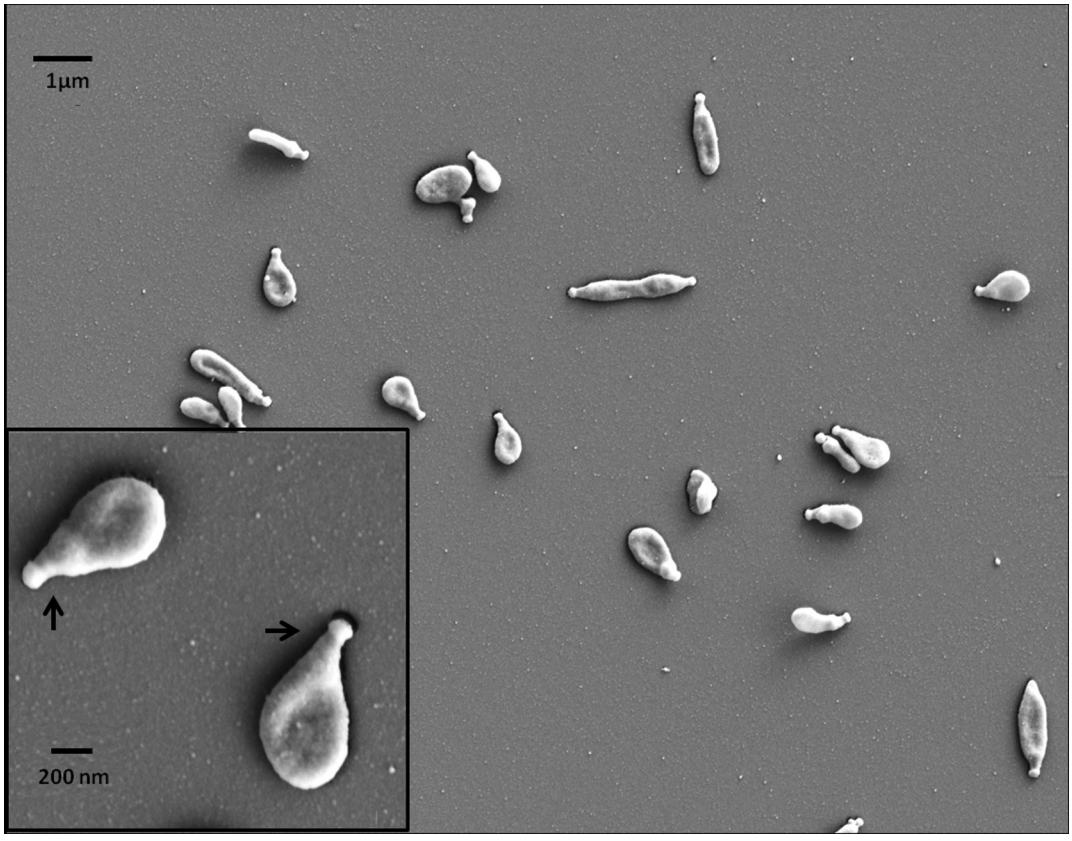
Evolvability Is Not Universally Favored

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 coinfect 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.

FIGURE 2



Visualized here by scanning electron microscopy at magnifications of 20,000 and 100,000 (inset), the attachment organelle (inset, arrows) of *Mycoplasma gallisepticum* is a polar structure that mediates attachment to host cells. In contrast, *Mycoplasma synoviae* lacks an attachment organelle and must rely solely on VlhA adhesins.

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*. Even though *nanI* is an evolvable gene, against the biological backdrop of the attachment organ-

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?"

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