PERSPECTIVES: MICROBIOLOGY

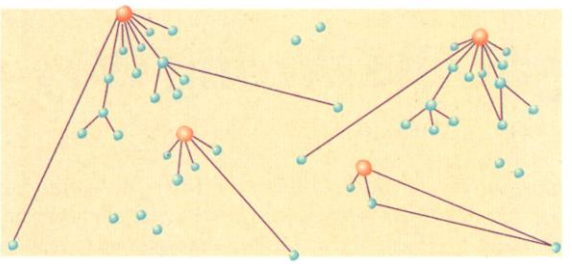
Bacterial Population Genetics and Disease

Marc Lipsitch

What makes a successful pathogen? Genetic studies have addressed this question by elucidating the mechanisms by which bacteria cause disease (pathogenesis)—that is, how they infect hosts, evade the host immune system, secrete toxins, and interrupt and co-opt host signaling pathways (*1*). Evolutionary theory suggests that microbes evolve to a level of virulence that maximizes their transmission from one host to another. This requires that microbes balance the benefit of achieving high numbers with the risk of killing or incapacitating the host, thereby reducing the length of time available for transmission (*2*).

Although compelling for some pathogens, these explanations are harder to reconcile with the life-styles of those infectious agents, particularly commensal bacteria, that appear to "make their living" by colonizing their hosts asymptomatically, yet cause severe disease when they breach the normal barriers of the host and enter the bloodstream. Commensal bacteria include a diverse range of microbes that are carried in the gut, nose, or throat or on the skin of their human hosts. These organisms colonize their hosts without causing symptoms; symptomatic infection is a rare, often accidental, and probably "dead-end" event (*3*). How do the genetically and biochemically complex factors that promote virulence contribute to the evolutionary success of commensal bacteria? On page 114 of this issue, Day *et al.* (*4*) provide intriguing hints about the answer to this question for *Staphylococcus aureus*, an important bacterial pathogen that typically lives as a commensal colonizer of the human nostrils and skin.

If the virulence (invasiveness) of commensal bacteria is truly irrelevant to their transmission and evolutionary success, then one might expect that the organisms that do cause serious disease in humans would be a randomly selected subset of the microbes carried commensally by healthy individuals in any given population. Day and colleagues tested this hypothesis by analyzing 334 isolates of *S. aureus* sampled from the nostrils of healthy individuals and patients with serious *S. aureus* infections (acquired either in or outside of the hospital) in Oxford, England. They characterized all 334 isolates by multilocus sequence typing (MLST), a genetic typing system based on DNA sequences of ~450 base pairs from seven loci, scattered throughout the bacterial chromosome. Consistent with previous studies, they found that disease-causing strains represented a restricted subset of the strains present in the overall *S. aureus* population. This conclusion was strengthened by analyzing *S. aureus* strains isolated from community-acquired as well as hospital-acquired infections. Community-acquired infections are more likely to be representative of invasive *S. aureus* strains circulating in the human population than are infections acquired in a hospital setting.



Defining strain clusters. Isolates of *S. aureus* were obtained from the nostrils of healthy individuals and those with community-acquired or hospital-acquired *S. aureus* infections (*4*). Seven genetic loci in each isolate were sequenced, and pairs of strains that differed from each other at only one or two of the seven loci were identified. These pairs of strains (balls) can be connected by strings: A short string connects strains differing at one locus; a long string connects strains differing at two loci. A cluster can be defined as all of the balls that come along when one picks up a single ball. Within a cluster, the ancestral strain (red ball) is defined as the ball that is connected to the largest number of short strings. These ancestral strains are more likely than nonancestral strains to cause disease (*4*).

The authors then characterized the genetic structure of the *S. aureus* population in greater detail. First, they identified all pairs of strains in their collection that were nearly identical to each other, differing at only one of the seven genetic loci examined. These pairs were then combined into clusters of strains that differed from each other at only one or two loci. Each strain can be envisaged as a ball connected by a string to each of the strains to which it is nearly identical; a cluster, then, is all of the balls that come along when one picks up a single ball (see the figure). Within each cluster, a putative "ancestral strain" is defined as the strain connected to the largest number of other strains that differ from it at only one locus. Day *et al.* found that two other characteristics tended to go along with being an ancestral strain. In most clusters, the ancestral strain was recovered more often than any other strain in the cluster. Furthermore, ancestral strains were overrepresented among disease-causing isolates, relative to their proportion among carriage isolates. Because the most successful strains of *S. aureus* in the human population are more common among invasive isolates than among carriage isolates, the authors infer that whatever factor makes these strains successful in carriage also makes them "hypervirulent," that is, more likely to cause disease.

This interpretation implies that virulence should correlate with improved ability to colonize the host. In an elegantly low-tech confirmation of this prediction, Day *et al.* found that among strains that were recovered from both asymptomatic carriers and infected patients, 95% were present in both nostrils of the carriers. In contrast, only 70% of strains unique to the carriage population were found in both nostrils of carriers.

These observations raise questions about the processes that underlie the population structure of *S. aureus*. Day *et al.* suggest that, from time to time, clones of *S. aureus* arise that are successful colonizers, highly transmissible, and highly virulent. These clones then spread among asymptomatic carriers, causing disease at an elevated rate, and diversifying to produce strains that vary at one or more loci from the ancestral strain. As *S. aureus* ancestral strains diversify by mutation and recombination (the latter, Day *et al.* calculate, being much more frequent), they gradually lose their ability to cause disease.

A formal population genetic model is needed to determine whether this sequence of events could account for the genetic structure of the *S. aureus* population observed by Day and colleagues. If virulence is closely and mechanistically tied to the ability to colonize a host and be transmitted effectively (which should be favored by natural selection), it is difficult to understand why selection would "permit" virulence to decline over time. The authors propose that less-invasive and perhaps less-transmissible strains of *S. aureus* arise by recombination. This makes one wonder which bacteria (perhaps another species?) serve as a reservoir for the DNA that confers less transmission success on *S. aureus* recipients. Also unclear at this stage is the time scale on which the *S. aureus* population structure observed by Day *et al.* developed (a point that could be ad-

The author is in the Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA. E-mail: mlipsitc@hsph.harvard.edu

dressed by further population genetic studies).

Regardless of the exact process that led to the *S. aureus* population structure in the Oxford community, the Day *et al.* work has exciting implications for future research. Their findings suggest an answer to the riddle of why *S. aureus* has evolved mechanisms for causing disease when disease is not a necessary or even a common part of its life cycle—the same features that make this commensal a good colonizer also make it better at causing disease. This hypothesis can be evaluated experimentally by, for example, testing avirulent and hypervirulent mutant strains for features that affect colonization, such as adherence to epithelial cells. Day *et al.* found that recombination at three of the seven loci in particular was associated with a loss of virulence, suggesting that genes for virulence factors may be present near these loci. Identification of particular genes that are associated, at the population level, with virulence and the ability to colonize hosts (*5*) will provide candidate virulence factors whose effects on colonization can be tested.

Epidemiologists and researchers that study how microbes cause disease have been skeptical about the relevance of evolutionary biology and population genetics to their disciplines. This is starting to change as researchers recognize the importance of pathogen population structure for antimicrobial resistance (*6*), interactions between pathogens and the immune system (*7*), identifying candidate antigens for vaccines (*8*), and predicting (*9*, *10*) and assessing (*11*) the effectiveness of vaccination programs. The work of Day *et al.* shows that studies of the population genetics of an infectious agent can generate hypotheses about microbial pathogenesis that are both new and experimentally testable. It also demonstrates that a complete understanding of the epidemiology and transmission of infectious diseases depends on a clear picture of the population genetic structure of the causative organism.

References

1. B. B. Finlay, S. Falkow, *Microbiol. Mol. Biol. Rev.* **61**, 136 (1997).
2. J. J. Bull, *Evolution* **48**, 1423 (1994).
3. B. R. Levin, *Emerg. Infect. Dis.* **2**, 93 (1996).
4. N. P. J. Day, *Science* **292**, 114 (2001).
5. M. C. Booth *et al.*, *Infect. Immun.* **69**, 345 (2001).
6. B. R. Levin, M. Lipsitch, S. Bonhoeffer, *Science* **283**, 806 (1999).
7. S. C. Gilbert *et al.*, *Science* **279**, 1173 (1998).
8. G. R. Bolduc *et al.*, *Infect. Immun.* **68**, 4505 (2000).
9. S. Gupta, K. P. Day, *Parasite Immunol.* **16**, 361 (1994).
10. M. Lipsitch, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 6571 (1997).
11. P. Gilbert *et al.*, *J. Clin. Epidemiol.* **54**, 68 (2001).

PERSPECTIVES: OPTICAL MATERIALS

Bending Light the Wrong Way

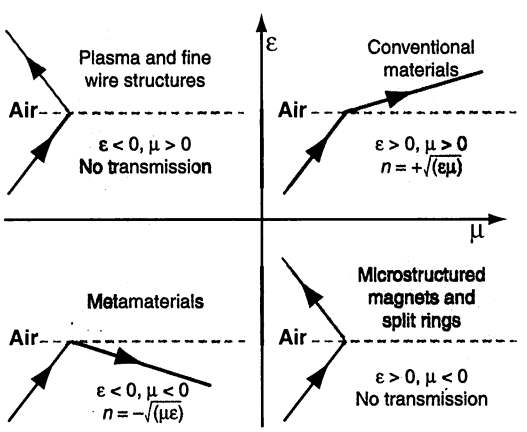
M. C. K. Wiltshire

The refractive index is a basic property of optical materials. It is a measure of how much light slows down when it enters a medium and accounts for the familiar "bent stick in water" phenomenon. This property determines the behavior of lenses and prisms and underpins all optical instrument design. Air and vacuum have a refractive index n of 1, the n for glass is typically between 1.5 and 1.8, and other materials may have n as high as 2.2. This property is not restricted to light waves. Semiconductor materials have a high refractive index in the infrared (for example, $n = 4$ for germanium), and at microwave and radio frequencies, lenses can be made of Teflon. But n is always positive and is normally greater than 1. So what do Shelby *et al.* mean on page 77 of this issue (*1*) when they say that they have verified the existence of negative refractive index materials?

To see how a negative refractive index can arise, we have to go back to Maxwell's equations (*2*) describing the behavior of electromagnetic radiation. These equations relate the behavior of electric and magnetic fields to the dielectric and magnetic properties of the ambient medium, captured respectively by the relative permittivity ε and relative permeability μ. The combined equations predict a wave motion propagating at a speed of $1/\sqrt{(\varepsilon\varepsilon_0\mu\mu_0)}$, where ε_0 and μ_0 are the permittivity and permeability, respectively, of the vacuum. The refractive index of the medium is $n = \sqrt{(\varepsilon\mu)}$.

The author is at Marconi Caswell Ltd., Caswell, Towcester, Northants, UK. E-mail: mike.wiltshire@marconi.com

In the 1960s, the Russian theoretician Veselago (*3*) classified materials according to their ε and μ (see the figure). He realized that it was possible to have negative ε and negative μ and still achieve a propagating wave because $(\varepsilon\mu)$ would be positive. However, to ensure conservation of energy he had to take the negative sign of the square root, which we now interpret as giving a negative refractive index. This meant that light would bend the "wrong" way: Positive lenses would become negative, flat sheets could focus, the Doppler effect would be reversed, and other counterintuitive phenomena would occur. The only problem was that materials with negative n did not exist.



Going beyond convention. Materials can be classified in terms of the sign of their permittivity ε and permeability μ. For each of the possible four cases, the behavior of radiation incident on an air-material interface is shown. In the case of conventional dielectric materials (top right quadrant, with positive ε and μ and thus positive n), the refracted ray lies closer to the normal than the incident ray. Plasmas with negative ε (top left quadrant) reflect radiation. Microstructured magnetic materials with negative μ (bottom right quadrant) also block radiation. The metamaterials (bottom left quadrant, with negative ε and μ and thus negative n) refract to the opposite side of the normal.

The solution lies with artificial optical materials, which provide the scope to tailor an electromagnetic response. If the materials are microstructured and all their constituents are much smaller than the wavelength of the radiation that they interact with, they can be described accurately by their permittivity and permeability. Pendry and co-workers (*4*, *5*) have shown that structures built from fine wires mimic a plasma and have a negative ε in the microwave regime. They have also proposed structures (*6*) that could exhibit negative μ. Shelby *et al.* realized that combining these structures into one "metamaterial" should lead to a material with negative ε and μ. Last year (*7*), they showed proof of principle; now they report the definitive experiment (*1*).

The refractive index of a material is determined by measuring the deviation of light at its surface. Shelby *et al.* did so with their metamaterial at microwave frequencies (10.5 GHz). First, they built a two-dimensional material (*8*) from split rings and fine wires, all based on printed circuit boards. This bulk material was cut into a prism, and microwaves were shone through it and their deflection measured. A Teflon prism was used as a reference. The result is striking: The Teflon prism bends the microwaves in the usual way; the metamaterial bends them the other way (see the figure). It has a negative refractive index.