estimate scaling relations still remains contentious. Several studies have recommended alternatives to OLS regression, such as reduced major axis regression (RMA) (*10*), because both population density and body mass are subject to measurement error. Schmid and colleagues go one step further and apply a new method, the OLS-bisector: This method calculates the line that bisects the OLS (X versus Y) and OLS (Y versus X) best-fit lines. With this approach they demonstrate that the scaling exponent of population density is different from that of metabolic rate, and that the former varies across functional and taxonomic groups, implying that the pattern cannot be explained solely by the energy equivalence rule.

The OLS-bisector method is commonly used by astrophysicists and has been shown to outperform other approaches if both variables are subject to measurement error and it is not clear which variable should be treated as the independent and which as the dependent (*11*). The usual convention in allometric studies is to use body size as the independent variable (*12*). However, there is no way to logically prove the independent nature or causal role of body size (*13*) because body size and physiological or ecological traits do not evolve in isolation, but affect each other. In fact, plant ecologists have traditionally treated the sizes of individual plants as though they were determined by population density (the Thinning Law) (*14*).

The study of whole communities containing a wide array of taxonomic groups is a daunting task. Such an approach is badly needed, however, if we are to glean insights into the structure of ecological systems. The identification of invariant scaling relations as reported by Schmid *et al.* (*3, 6*) and, more generally, the existence of simple scaling laws (*15*) suggest that general principles underlie the complex organization of ecological systems.

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PERSPECTIVES: GENOMICS

Genomics Happens

Victor J. DiRita

Modern epidemiology is rooted in the work of John Snow, an English physician whose careful study of cholera victims led him to discover the waterborne nature of this disease. Cholera also played a part in the foundation of modern bacteriology—40 years after Snow's seminal discovery, Robert Koch developed the germ theory of disease following his identification of the comma-shaped bacterium *Vibrio cholerae* as the agent that causes cholera (see the figure). Koch's theory was not without its detractors, one of whom was so convinced that *V. cholerae* was not the cause of cholera that he drank a glass of it to prove that it was harmless. For unexplained reasons he remained symptom-free, but nevertheless incorrect.

A notable addition to *V. cholerae* research comes from Heidelberg *et al.* (*1*) who report in a recent issue of *Nature* the complete genome sequence of its two circular chromosomes (*2*). The sequencing team discovered a total of 3885 open reading frames (lengths of DNA that encode proteins): 2770 on the larger chromosome 1 (2.9 million base pairs) and 1115 on the smaller chromosome 2 (1.1 million base pairs). Slightly more than 50% of these open reading frames encode proteins homologous to proteins of known function; the remainder encode proteins without ascribed functions or that are not homologous to any known protein.

Cholera continues to be a scourge throughout much of the world with seven global epidemics (pandemics) recorded since 1817. In 1991, for the first time in 100 years, cholera arrived in the Western Hemisphere from its focal center in Asia. Cases were first reported in Peru, and epidemics throughout South and Central America rapidly followed. The sequenced *V. cholerae* isolate, El Tor strain N16961, is representative of the strains causing the current pandemic. Between epidemics, *V. cholerae* lives in aquatic environments, often in association with marine invertebrates. Although most isolates of *V. cholerae* do not cause disease, some carry genes that have enabled the microbe to adapt to humans and to produce virulence factors. Upon infection of humans, these strains colonize the gut mucosa and produce cholera toxin (CT), which stimulates secretion of water and electrolytes by gut epithelial cells leading to severe diarrhea that can be fatal within hours (see the figure).

Even before completion of the *V. cholerae* genome sequence, it was well established that virulence genes directing the interaction between this pathogen and its human host were acquired by pathogenic *V. cholerae* from bacteriophage and from large gene clusters called pathogenicity islands by horizontal gene transfer. Two major virulence factors, CT and a colonization factor called toxin-coregulated pilus (TCP), are each encoded on genetic elements in chromosome 1 that are not universally found in *V. cholerae* strains; yet they are regulated by genes that appear to predate entry of these elements into the genome (*3*). The genes encoding CT are acquired from the genome of bacteriophage CTXϕ (*4*); those encoding TCP are carried on an element called the *Vibrio* pathogenicity island (VPI), also thought to be of bacteriophage origin (*5, 6*). The genome sequence data challenges the notion that the VPI is of bacteriophage origin because none of the VPI genes encode phage structural or morphogenetic proteins. An integron island (a system for gene capture and dissemination) on chromosome 2 is also likely to be im-

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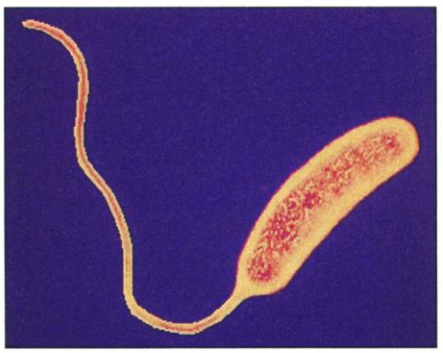
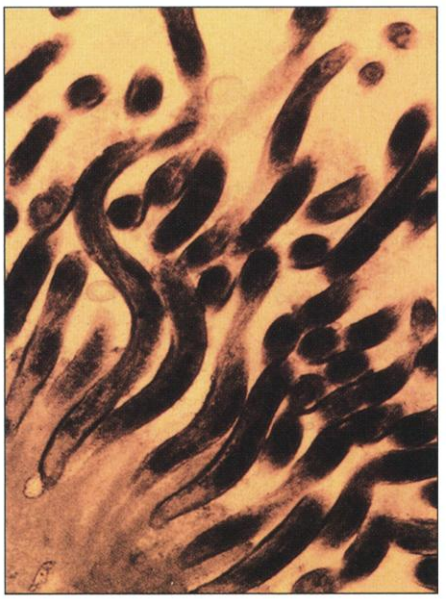
portant for survival of *V. cholerae* in human populations (*7*) as it encodes several genes for antibiotic resistance as well as potential pathogenicity determinants.

Despite extensive knowledge of the major *V. cholerae* virulence factors, development of live vaccine strains for disease control in endemic areas has been vexingly difficult. Horizontal gene transfer and other genetic alterations bode ill for efforts to develop a "one size fits all" vaccine. For example, a change in a cell surface protein of *V. cholerae*, which is important for developing a protective host immune response after infection, resulted from acquisition of a new gene cluster in the O139 serogroup strains that emerged in 1992 (*8*). Another factor confounding vaccine development is that some vaccine strains, although engineered not to express CT, continue to cause unacceptably high levels of residual symptoms such as cramping, diarrhea, and nausea. Investigators have labored hard to identify and remove genes that might contribute to these symptoms, a task that should be made much easier now that the complete genome sequence is at hand.





Alternative life-styles. The comma-shaped bacterium *Vibrio cholerae* (**left**) normally inhabits aquatic environments. However, acquisition of virulence factors enables this microbe to colonize the gut mucosa of human hosts (**right**). Here it releases cholera toxin, which causes rapid release of water and electrolytes from gut epithelial cells resulting in severe, often fatal, diarrhea.

CREDIT: (LEFT) MOREDUN ANIMAL HEALTH LTD/SCIENCE PHOTO LIBRARY; (RIGHT) V. BURMEISTER/VISUALS UNLIMITED

In addition to CT, the genome sequence reveals several unknown toxins that may contribute to the side effects of current vaccine strains. Among these is a toxin called RTX, which is encoded, along with proteins required for its synthesis and export, by a genetic element next to the site of CTXϕ insertion on chromosome 1 in some strains of *V. cholerae* (*9*). RTX cross-links actin, a cellular cytoskeletal protein, and therefore has dramatic effects on cell architecture (*10*). This activity is unexpected for a bacterial toxin, but how RTX is involved in pathogenesis has yet to be determined.

Another gene product mined from the *V. cholerae* genome sequence is PilD (also termed VcpD), which is important in both the pathogenic and nonpathogenic phases of the *V. cholerae* life cycle (*11, 12*). PilD is a protease required for secretion of CT and for assembly of an extracellular organelle called the mannose-sensitive hemagglutinin (MSHA). MSHA is not a virulence factor but rather is involved in biofilm formation, a recently characterized feature of *V. cholerae* that most likely contributes to its survival in aquatic environments (*13*). That PilD is required for both virulence and environmental adaptation is noteworthy because it provides a mechanistic link between the two life-styles of the organism.

In addition to factors of potential importance for pathogenicity, the *V. cholerae* genome contains genes encoding metabolic proteins, transporters, and regulatory proteins befitting a free-living microbe adapted to niches outside of the human gut. For example, *V. cholerae* has a staggering number of methyl-accepting chemotaxis proteins (MCPs) that regulate its swimming behavior in response to amino acids, sugars, and even oxygen. *Escherichia coli* strain K12 has 5 MCPs (*14*), and the pathogen *Campylobacter jejuni*, whose genome sequence was recently completed, has 10 (*15*). By contrast, the *V. cholerae* genome encodes 43 MCPs, whose genes are distributed about equally between the two chromosomes. The MCP genes most probably arose through gene duplication, and so there is likely to be some redundancy in function. However, it is possible that each MCP regulates motility in response to a different chemotactic molecule.

Although much is known about the pathogenicity of *V. cholerae*, the complete genome sequence is sure to keep surprising us as it has already with the revelation of RTX and PilD. Particularly exciting for understanding host-pathogen interactions is the potential for combining genome information with functional genomic approaches to identify essential genes and characterize patterns of gene expression during infection (*16, 17*). Conversely, information about how *V. cholerae* behaves in natural environments outside of its human host is scarce. Interactions of the free-living microbe within biofilms and with marine invertebrates and zooplankton have been described. The factors required for these associations have not been fully defined, although enzymes that digest chitin (a component of the exoskeleton of zooplankton), genes for which have now been identified in the *V. cholerae* genome, are probably important. Furthermore, the "viable but nonculturable" phenotype, a quasi-dormant state that *V. cholerae* is proposed to adopt in nature, is not at all understood at the molecular level (*18*). Recent appreciation of the ecological forces influencing the epidemic behavior of *V. cholerae* (*19*) is likely to promote more research into the adaptation and survival of this bacterium in its natural aquatic environment.

The postgenomic era of *V. cholerae* research has begun, and the challenge for investigators will be to use the genomic sequence information to probe more deeply into all aspects of the life-style of this fascinating, frightening, and often frustrating microbe.

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