Preface

It is a truism among biologists that an organism’s phenotype is the product of both its genotype and its environment. An organism’s genotype contains the total informational potential of the individual, while its environment shapes the expression of the genotype, influences the rate of mutation and occurrence of modifications, and ultimately determines the likelihood that the genotype (or fractions thereof) will survive into the next generation. In the relationship between host and pathogen, therefore, each forms a part of the environment of the other, mutually influencing the biology of both partners on scales ranging from the life history of individuals to the fate of populations or entire species.

Molecular biologists working on problems in pathogenesis generally think of the host organism as the pathogen’s environment and perhaps occasionally consider the pathogen as part of the host’s environment. However, because “environment” can be defined at many scales, so, too, can phenotypes: if a pathogen, as a species, is considered to exist in a host, as a species, then among its phenotypes is the nature of the pandemic disease it can cause within the host community. The contributors to the proceedings of this NATO Advanced Research Workshop have treated the interplay of environment and genotype in the host–pathogen relationship and its relationship to the problem of emerging infectious disease at both the macroscopic and microscopic/molecular levels along this continuum of scale (with some human history thrown in at times for good measure).

Keynote Chapter

The contribution from the meeting’s keynote speaker highlights the importance of understanding the underpinnings of pathogen phenotypes at both scales. The example of *Vibrio cholerae* is considered macroscopically and genetically in an examination of the factors influencing the emergence and spread of new strains of human bacterial pathogens. Citrus greening, caused by the bacterium *Liberibacter asiaticus* and vectored by the Asian citrus psyllid *Diaphorina citri*, is discussed to illustrate the effect of a vector species’ biology on disease emergence and spread. An unfortunate lesson from these examples is that diseases that have already emerged and have spread rapidly may be difficult to control; however, any hope of disease control will be founded on an understanding of the genetic and molecular basis for pathogenesis and the environmental factors (including vectors) that contribute to the transmission of the microorganism.

Section I: Surveillance

The next four chapters treat country-specific approaches, and their results, in one of the most fundamental tasks in combating emerging infectious disease: detecting and
describing the incidence of disease in a geographic region. By its very nature, this effort is labor-intensive in terms of fieldwork (both human and environmental) and in the subsequent laboratory analysis of samples. In the Balkans, the Caucasus, and the Central Asian republics, like elsewhere in the developed and developing worlds, surveillance work ranges from the basic (trapping and culturing from members of a reservoir species) to the complex (use of sensitive laboratory molecular methods, such as PCR) and the application of the resulting data to forestalling and controlling the outbreak of endemic diseases. Akimbayev et al., from Kazakhstan, and Gurbanov and Akhmedova, from Azerbaijan, provide a description of surveillance efforts in recent years that highlight the human and economic factors that influence disease transmission. From the Republic of Georgia, Bakanidze et al. provide a historical perspective that demonstrates the role that militaries have played in the development of public health methods and practices, born of necessity: throughout history, armies over time have lost more soldiers to disease than to violence. Complementing the paper by Bakanidze and colleagues, the chapter by Zhgenti et al. reports on the use of modern molecular biological techniques to differentiate closely related strains of pathogenic bacteria isolated from both environmental and clinical samples in Georgia and throughout the Caucasus. Stikova describes a syndrome-based, nationwide effort deployed in the Republic of Macedonia to report priority communicable diseases that is complementary to the routine surveillance system that reports individual confirmed disease cases. This system, called ALERT, aided in forecasting and detecting the start of the influenza season.

The goal of surveillance always has been actionable information that would allow public health workers to forestall the spread of disease. “Classical” surveillance and epidemiologic reporting as described in these first four chapters, however, now also provides data that are being analyzed by advanced computational and geographic methods known collectively as Geographical Information Systems. Blackburn rounds out Section I by describing new tools that enable the fusion of climatologic, geographic, and epidemiologic data with concepts in ecological niche theory to construct models that may predict the future incidence, prevalence, and transmission of *Bacillus anthracis*, but the methods are generalizable to other diseases.

**Section II: Molecular Analysis and Tools**

At the scale of the bacterium and bacterial genome, the contributors to this section each provide an example of how cutting-edge molecular biological methods are being applied to answer key questions in the study of emerging infectious disease.

*How did the pathogens we observe in the world come to their present state?* Technical challenges abound in the analysis of biological specimens for evidence of ancient infections. Aboudharam et al. describe the development of dental pulp as a target material for isolation of bacterial DNA and the diagnosis of ancient bacteremias, including *Yersinia pestis* infections. Key to their methodology is the development of single-use primer pairs for the detection and amplification of ancient target sequences in a method they term “suicide PCR.”

*What determines the severity of disease a pathogen may cause?* Perry et al. demonstrate the utility of comparative genomics in identifying a putative hemagglutinin gene (“Region E”) that is present in *Brucella melitensis* 16M and absent in *Brucella*
abortus. The data suggest that “Region E” has a host-specific influence on virulence, and the authors speculate that expressing the hemagglutinin in certain Brucella strains may improve their performance as vaccines.

**What genome-wide adaptations predispose a pathogen to cause severe disease?** Rakin examines the contributions of both gain-of-function genetic changes (via lateral gene transfer) and negative selection (favoring what is termed pathoadaptive mutations) in the evolution of pathogenic bacteria. His analysis points out the importance of single-nucleotide polymorphisms that, besides being markers for strain identification, can have significant effects on the functions of virulence and pathogenicity genes. The implication of these results is apparent: in a selective environment or host, mutations can occur that lead to a sudden emergence of a virulent bacterial strain.

**What tools are available for practical studies when containment is not available or practical, but safety must be maintained?** Researchers have long used non-pathogenic surrogates, or “simulants” in place of pathogens and protein toxins for reasons of convenience, safety, reduction of expense, and speed of work. Such simulants have included benign enteric bacterial species, bacteriophage (especially MS2), and proteins such as ovalbumin. Ouellette et al. review here information that suggest that baculoviruses, long used in organic agriculture and widely regarded as having no ill effects on humans, animals or plants, may serve as a new class of simulants for some viral pathogens.

**How do recent advances in sequencing affect the genetic analysis of pathogens?** Molecular biologists are relying on the rapidly decreasing cost per base of DNA sequencing to support the continuing effort to detect and identify the genes (as is discussed by Perry et al.) or gene variants (as in Rakin) that influence bacterial pathogenicity and virulence. Khan briefly reviews the procession from Sanger dideoxy sequencing (and the dye-coupled PCR-driven variant) to so-called next-generation sequencing (NGS) methods. NGS methods have a much higher throughput than the Sanger methods but with generally smaller average read lengths. Concurrent increases in computational power allow the rapid querying of databases for bacterial identification. However, although faster computation also speeds contig formation from unique sequences, short read lengths can result in more contigs that require more effort to assemble into finished whole bacterial genomes. Fortunately, complementary technologies such as whole genome optical restriction mapping are emerging that very rapidly provide the scaffolding data needed to match the increased rate at which NGS produces contigs.

Bacterial genome sequencing that 15–20 years ago required years of effort now takes weeks. The rate at which sequencing technology is accelerating has been compared with Moore’s Law in computing power, except that the rate of improvement for sequencing has proven to be steeper than the drop in the cost of memory and clock cycles over time. The “next” in NGS likely is ready to become dated in use as single molecule sequencing methods are being commercialized by at least two companies and faster methods still are certain to follow.

No sequencing technology currently is employed widely outside of laboratories or core facilities. However, entrepreneurs are fervently seeking the right combinations of technology and business models that will put NGS (and beyond) into the hands of nonlaboratory end users (clinicians, epidemiologists, law enforcement, and first responders). The eventual goal is to provide a user with an encyclopedic understanding
of the DNA sequences present in a sample, breaking the barrier that currently separates sensitivity plus specificity from speed of analysis. The possibility of a technology that will permit fast, accurate, complete data from genuinely unknown samples (unlike PCR) may at last be on the horizon.
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