# PREFACE

My interest in the propagation of phages within populations of spatially constrained bacteria, as plaques, was first piqued while studying the lysis-inhibition phenotype of phage T4. Display of lysis inhibition by phage T4 is associated with the wild-type genotype whereas *r* mutants are defective in this latent-period extending and burst-size enhancing phenotype. Wild-type T4 plaques are small and turbid, due to lysis inhibition, while *r*-type plaques are large and clear. Despite their smaller size, wild-type plaques contain substantially more phages than do *r*-type plaques. Given the latter's larger size but lower productivity, I reasoned that *r* mutants may succeed in out-competing wild type phages only if plaque numbers were extremely high on plates, e.g., $>10^4$/plate. These *r* mutants with their larger plaque size, in this case, would be able to reach uninfected lawn bacteria before adjacent wild-type competitors. Donivan Sphar, then an undergraduate, and Cameron Thomas-Abedon, my wife, performed the experiments to test this hypothesis. The result? Wild type, contrary to prediction, was competitive, or, at least, nearly so, no matter the plaque density. The reason, as we came to find—though have yet to publish—is that despite the small size of the actual clearing associated with wild-type plaques, their phage-excluding power, an apparent ability to block phages found in other plaques from accessing surrounding bacteria, appears to reach nearly as far into the bacterial lawn as does the bacterial lysis associated with *r*-type plaques. Thus, and despite appearances suggesting otherwise, *r* mutants may reach uninfected lawn bacteria little faster than do wild-type phages. In the course of this research I got into the habit of peering through dissecting scopes at plaques, and it was this latter tendency that led to a deeper interest in the interaction of phages with spatially constrained bacteria.

What I saw when I looked through those dissecting scopes were clearly visible bacterial microcolonies (see, for example, the last figure presented in this book, found on p. 108). Now, I knew very well that bacterial lawns consist of bacterial microcolonies, which are found jammed together to varying degrees. However, there is a profound difference between knowing that something exists and actually seeing it with one's own eyes. Suddenly, in other words, plaques were a complex ecological phenomenon with subtle spatial heterogeneity even within genetically homogeneous lawns. Furthermore, as a consequence of that spatial heterogeneity, during plaque formation phages interacting with bacteria might act a lot more like phages interacting with biofilms—which also are made up of bacterial microcolonies—than I would have previously thought. In addition, consideration of phage interaction with bacterial microcolonies as components of bacterial biofilms, rather than simply phage interaction with individual, planktonic bacteria, may be key to understanding a substantial

portion of phage ecology. This book represents my attempt to explore how phages might interact with naturally occurring, spatially constrained bacteria, that is, with biofilm bacteria.

That I would write a book on this subject came to me as a complete surprise. The manuscript began as a chapter, called simply "Bacteriophages and Biofilms", to be written for a book on biofilms, but for which at first I had only modest ambitions. Indeed, for a time I wasn't sure whether I would ever even write the chapter. However, while sitting in a car as my daughter visited and cared for her horse, I realized that I might be able to recycle some older, as yet unpublished material into that chapter. Three weeks later I had created a working draft of the chapter, but which included only a single paragraph from that older material (see p. 79, "This idea…"). Importantly, my ambition had become excessive, as suddenly I realized that I wanted to take on the entire English-language literature considering phage-biofilm interactions. Once completed, the effort was so long (about 20,000 words in main-text alone) that it made sense to market it also as stand-alone book, one targeted to those individuals with as much of an interest in phages as in biofilms. I realized, however, that it would be helpful to expand the chapter to provide additional material, including more background, an expanded conclusion, various appendices, and some more-speculative, phage-centered prose, e.g., my considerations of why phage plaques grow at a constant rate despite the maturation of the harboring bacterial lawn. The result, I feel, is an effort that starts with the original chapter and then expands that material into quite a bit more. Too, this book can be viewed as an informal second edition to the original chapter.

This work would not have been possible without the help of Paul Hyman, my frequent collaborator, who kindly covered my classes while I was otherwise engaged in writing. I am also grateful to the feedback provided by Dwayne Roach, Catherine Loc-Carrillo, Philip Serwer, Susan Lehman, Hans Ackermann, Ariane Toussaint, Ing-Nang Wang, Sarah Kuhl, Ben Chan, and Cameron Thomas-Abedon on various portions of the manuscript. This work was supported financially by an Ohio State intramural grant awarded to Jeff LeJeune, Brian McSpadden Gardener, and myself. I would also like to thank the Battelle National Biodefense Institute for support which provided me with the time, and motivation, to develop my organizational skills, self-confidence, and critical eye towards phage-therapy experimentation, all of which were essential to the writing of this monograph. Lastly, I would like to dedicate this book to the memory of Professor John Spizizen, a mentor, and friend, recently deceased.