8.1. INTRODUCTION

Most microorganisms live in complex microbial communities. So do their hosts. Eukaryotes have co-evolved with diverse microorganisms on their internal and external surfaces. These microorganisms constitute communities, and the host's fitness may be affected differently by the community than by any single species. Therefore, understanding the structure and function of microbial communities must be integral to the study of host–microbe interactions (see Chapter 5 for further discussion of this point).

Communities are dynamic assemblages whose stability and functions are governed by dependencies and antagonisms among the members. All microbial communities are dynamic and continually responding to their changing physical environments, but those associated with animals must contend with the vicissitudes of their hosts as well. Host tissues may present attractive surfaces and a rich source of nutrients, but they may also expose the microorganisms to extreme conditions due to pH, toxins in food, or precipitous changes in anatomy, such as the shedding of the very tissue that the microorganisms call home.

Spatial and temporal variation presents challenges to those who study communities as well as the microorganisms that comprise them. Although complexity and change typify the natural environment and are thus central to understanding microbial ecology, it is these very features that microbiologists have striven to minimise in ecological models. The emphasis on pure culture over the last century of microbiology has removed microorganisms from their communities and focused on their behaviour in the biologically simple environment of the Petri dish or liquid culture. Although simple model systems have driven the explosion of knowledge in host–microbe interactions
over the last two decades, the reality of natural communities demands that we direct attention to complex assemblages as well. The recent surge of interest in community ecology, coupled with the development of powerful new methods for its study present an unprecedented opportunity to dissect the interactions among microorganisms in communities and elucidate how the function of the entire community determines outcomes for its host. In this context, the reader should refer to Chapter 5 for a discussion of the complexity that can occur in even the simplest monospecies culture system over short time periods.

8.2. GLOBAL ECOLOGICAL QUESTIONS

There are several overarching themes that need to be studied to develop a comprehensive view of the function of any community. Key among these are the influence of physical and chemical factors on community structure and function, succession and community development, mechanisms of mutualism, antagonism, and communication among community members, and the robustness of the community when disturbed or invaded.

An appropriate microbial community can provide a powerful model for studying these principles. Some of the basic tenets of ecology have been well-developed in macroecological systems, and microbial ecology can draw on that existing knowledge, whereas others have been difficult to study at the landscape level and may be better suited for development in microbial models. For example, the keystone species concept has been tested with macroorganisms quite effectively. This is illustrated by the work of Paine in a rocky intertidal zone where he demonstrated that the removal of the top predator, the starfish *Pisaster ochraceus*, resulted in the reduction of species diversity from fifteen species to eight. He showed that the starfish maintains diversity by controlling the population sizes of species that outcompete other members in its absence (Paine 1966). The key findings from this seminal work are broadly applicable to a diverse range of terrestrial and aquatic systems, and a broad range of taxa (Hanke et al. 1992; Naiman et al. 1994; Navarrete and Menge 1996; Crooks and Soulé 1999; Gonzalez-Megias and Gomez 2003). This type of study can provide a model for studying microbial community structure and function, and a framework on which to determine whether similar principles govern micro- and macro-organisms living in communities (Andrews 1991).

In contrast to keystone species, our study of biological invasions lacks opportunity for the same type of experimental approaches, because invasions are not conducive to replicated experiments. Biological invasion theory attempts
to predict invasion patterns, the nature of successful invaders, conditions conducive to invasion, consequences of invasion, and rates and directions of spread (Shigesada and Kawasaki 1997). The nature of invasions makes it challenging to test theory with a replicated experimental design, as most invasions are unexpected, unwanted, and not studied until after the process has begun. Yet the increasing rate of biological invasions necessitates that more general and predictive theories replace the case-by-case, post hoc approach on which we currently rely (Kareiva 1996). The field of biological control, however, provides many examples of replicated studies that document the behaviour of invaders. Unfortunately, many of the planned introductions of insect biocontrol agents rely on the prior establishment of an earlier invader, and so the applicability of biological control experiences to invasion ecology is uncertain.

In an ironic contradiction, invasions by certain insects and microorganisms are responsible for damage to agriculture and forestry, whereas invasions by others, in the form of biological control agents, have been powerful regulators of invasive pests and pathogens. The study of biological control has informed invasion theory. Biocontrol is often dependent on successful invasion by a natural predator or parasitoid of an insect pest. Such invasions have been remarkably effective in some cases. For example, in 1888, the citrus tree pest *Icerya purchasi* was successfully controlled by the introduction of *Rodolia cardinalis* (Shigesada and Kawasaki 1997). More recent successes include control of the cassava mealybug and green mite in Africa using parasitoids from South America (Bellotti et al. 1999). In addition to reducing populations of pests, invasion by biocontrol agents can also affect native communities adversely, as is illustrated by *Compilura concinna*, a parasitoid fly, which was repeatedly introduced to North America to control thirteen pests between 1906 and 1986. This biocontrol agent successfully invaded New England and may now be responsible for the decline in the population of the native, nontarget silk moth (Boettner et al. 2000). Because of this and other examples (Pearson et al. 2000), the potential for further biological control has declined as environmental concerns have risen (Simberloff and Stiling 1996; Daehler and Gordon 1997; Schaffner 2001). Once again, the lack of comprehensive theories that can be empirically validated hinders further advances. Similar problems hinder the field of planned introduction of genetically modified organisms. The enormous potential of this approach is well established, as is our ability to test for potentially direct, immediate effects in closed systems. Unfortunately, most adverse effects that have accompanied other technologies have been delayed, indirect, and only manifested under natural conditions (Kareiva 1996).
Microbiology has provided other systems for exploring invasion biology because some microbiological therapies depend on successful invasions. Biological control of plant disease, for example, involves successful establishment and proliferation in a community by a microorganism, resulting in suppression of plant disease (Cook and Baker 1983; Handelsman and Stabb 1996). Kinkel and Lindow (1993) examined the invasion and exclusion abilities of *Pseudomonas syringae* strains on plant leaves. They found that population size alone did not predict a strain’s ability to invade a community or to exclude others from it, and the characteristics of successful invaders and excluders differed.

Invaders of root-associated communities contend with the highly complex community in the rhizosphere. In general, the invader’s population size is correlated with biological effects, such as disease suppression, but there are some surprises. Gilbert et al. (1993) found that when *Bacillus cereus* was inoculated onto seeds, its population on the root emerging from the seed diminished rapidly, but it continued to have a global impact on the composition of the microbial community long after it was detectable. The complexity of the rhizosphere community makes it difficult to test the hypotheses that are simple enough to be empirically tractable yet robust enough to incorporate community–plant interactions, and thus, simple models would be useful to track bacterial populations and their lasting impacts on communities through which they pass.

A human gut is an ecosystem in which invasion is of particular interest. The success of gut pathogens depends on their invasive ability. Likewise, probiotics, or bacterial inoculants such as *Lactobacillus* and *Bifidobacterium* spp. might be more effective if they survived and colonized the gut (Bengmark 1998; Holzapfel et al. 1998; Goossens et al. 2003; Guarner and Malagelada 2003). Both pathogens and beneficial bacteria must compete with the indigenous community for attachment sites and nutrients. A healthy gut community is highly resistant to invasion, providing the “barrier effect” or “colonisation resistance” that maintains gut function (Bourlioux et al. 2003; Guarner and Malagelada 2003). But little is known about what makes a microbial community robust to or able to recover from invasion.

There is scant knowledge on which to base the design of successful invaders for probiotics or biological control, or to predict how easily a microbial or microbiological community will be penetrated, and perhaps altered, by an invader. Developing the right model system will advance microbial and macroecology by providing a context for testing invasion theory. One of our goals is to develop a model system in which hypotheses can be tested.
rigorously with precisely modulated treatments, controlled variables, and replicated experimental designs.

8.3. MODEL SYSTEMS

The right model system is essential to test ecological principles about microbial community structure, function, succession, and robustness. To be serviceable, a model community must be easily maintained and reproduced and have reproducible composition. There should be means to introduce chemicals or organisms into the community by a process that approximates a natural event, and the community should be sufficiently simple in composition to ensure that all of its members can be studied in culture or by genomics and that the communication networks connecting community members can be mapped.

A number of outstanding model systems have been established for microbial community study over the last two decades (Table 8.1). Each system is suited best to particular investigations. In the following section, we review a few examples of these communities, the advances they have afforded, and their limitations. Many of these model systems involve associations of a single species with a host. Although they may not immediately inform our understanding of complex communities, they have established broad principles of host–microbe interactions and microbe–microbe interactions that are applicable to multispecies communities. The rapid progress made in these simple systems has facilitated the next steps involving complex ones.

8.3.1. Vibrio–Squid

Vibrio fischeri and its host, the squid Euprymna scolopes, have an extraordinary relationship. The bacteria enter and colonise the light organ of the squid in which the bacteria emit light at night, providing counterillumination that enables the host to avoid detection by its prey, who would otherwise see the squid’s shadow in moonlit waters (McFall-Ngai 2000). The partners substantially affect each other’s biology – the bacteria affect development of the squid’s light organ, and the squid provides a non-competitive niche for the bacteria by preventing colonisation by other species (Foster et al. 2000; Visick and McFall-Ngai 2000; Visick et al. 2000). Despite its exotic, perhaps unique, outcome, study of this system has revealed principles in microbial behaviour and host–microbe interactions that appear to be common throughout the microbial world.
Table 8.1. Examples of model systems for studying host–microbe interactions

<table>
<thead>
<tr>
<th>Symbiosis</th>
<th>Approximate number of species</th>
<th>Strengths and contributions of system</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squid–Vibrio</td>
<td>1</td>
<td>Study of signal exchange and colonisation; effect of symbiont on host development</td>
<td>McFall-Ngai 2000; Nyholm et al. 2000; Visick and McFall-Ngai 2000; Visick et al. 2000</td>
</tr>
<tr>
<td>Legume–Rhizobium</td>
<td>1</td>
<td>Study of signal exchange with host; effect on host development; quantitative modelling of interstrain competition</td>
<td>Beattie et al. 1989</td>
</tr>
<tr>
<td>Nematode–Pseudomonas and Enterococcus</td>
<td>1</td>
<td>Identification of virulence genes; comparative virulence in other hosts</td>
<td>Choi et al. 2002; Hendrickson et al. 2001; Jander et al. 2000; Miyata et al. 2003</td>
</tr>
<tr>
<td>Gypsy moth</td>
<td>~11</td>
<td>Relatively simple, multispecies</td>
<td>Broderick et al. 2004</td>
</tr>
<tr>
<td>Cabbage white butterfly</td>
<td>~10</td>
<td>Relatively simple, multispecies</td>
<td>Robinson et al., unpublished</td>
</tr>
<tr>
<td>Termite gut</td>
<td>100–200</td>
<td>Study of carbon, nitrogen, and hydrogen cycling within a community; demonstration of functional redundancy in microbial community</td>
<td>Kane and Breznak 1991; Leadbetter and Breznak 1996; Leadbetter et al. 1998; Leadbetter et al. 1999; Lilburn et al. 1999; Lilburn et al. 2001; Nakashima et al. 2002</td>
</tr>
<tr>
<td>Mouse gut</td>
<td>500–1,000</td>
<td>Effect of bacteria on immune system development</td>
<td>Hooper et al. 2003; Stappenbeck et al. 2002</td>
</tr>
<tr>
<td>Human oral cavity</td>
<td>~500</td>
<td>Bacterial adhesion and succession</td>
<td>Paster et al. 2001</td>
</tr>
</tbody>
</table>
Study of the squid–Vibrio symbiosis led to the discovery of quorum sensing, a mechanism by which many bacteria sense the population density of their species and express genes accordingly. Population density is detected by accumulation of acylated homoserine lactones (AHLLs) whose structures vary, conferring species specificity. Quorum sensing determines expression of the genes responsible for light emission by Vibrio fischeri and regulates genes in other organisms that encode virulence factors, antibiotic production, and other functions that require a high density of cells to be useful to the population (Rice et al. 1999).

The squid–Vibrio system has shaped thinking about microbial ecology by illustrating communication and cooperative behaviours among members of a population of a single species (McFall-Ngai 2000; Nyholm et al. 2000). In fact, some microbiologists have argued that a single species can comprise a community based largely on the principle that there is communication among members of a population (Buckley 2003 – see also the arguments by Rainey in Chapter 5). Community, however, has historically been used in the ecological literature to describe a multiespecies assemblage (Began et al. 1990), and therefore in this chapter we adhere to the traditional definition of community.

### 8.3.2. Rhizobium–Legume Symbiosis

The Rhizobium–legume interaction is another system involving one microbial species and its host that has dramatically shaped thinking about microbial ecology. Members of the Rhizobiaceae family infect the roots of their leguminous hosts and induce the formation of an organ, known as a nodule, in which the bacteria fix nitrogen. Both the bacteria and the plant host produce signals that are interpreted by the other partner, leading to initiation of a new developmental program (Peters et al. 1986; Long 2001; Cullimore and Denarie 2003). Legumes release an array of flavonoids that induce the early nod genes, which results in the synthesis of Nod factors by the bacterial partner. Nod factors are lipooligosaccharides with chitin-like structures that induce morphological changes in the plant. The signals in this symbiosis are highly specific – in general, each species of bacteria infects one or a few species of legumes, and the flavonoids and Nod factors generally define the host range of the rhizobia. This example of signal exchange differs from quorum sensing in that the exchange of signals is between members of different domains of life, the partners are sensitive to minute concentrations (micromolar) of each other’s signals (10^{-6} micromolar), and the events leading to nodulation can be initiated by a single cell (Faucher et al. 1988; Begum et al. 2001).
The *Rhizobium*-legume system has also provided a simple model for studies of bacterial competition. When strains that are able to nodulate a common host are applied together, the proportion of nodules occupied by each strain is often quite different from their representation in the inoculum. The successful competitor establishes a pure culture inside the nodule, making quantitative studies of competition simpler than in a system that remains in an open environment that is bathed in other bacteria. Quantitative modelling and mutant analysis has revealed an ecological picture of interstrain competition.

These relationships are not linear or simple (Fig. 8.1), and mathematical modelling that describes the relationship has offered insights into the biology (Beattie et al. 1989). Extensive mutant analysis indicates that cell surface features (Handelsman et al. 1984; Milner et al. 1992; Lagares et al. 1992; Bittinger et al. 1997; Thomas-Oates et al. 2003), toxin production (Robleto et al. 1998; Oresnik et al. 1999), nutritional competence, and many other physiological factors determine competitiveness (Triplett and Sadowsky 1992). Plants differ in their ability to admit certain strains into their nodules (Rosas et al. 1998; Laguerre et al. 2003), demonstrating that each partner plays an active role in microbial competition. As in the *Vibrio*-squid interaction, interstrain competition in the *Rhizobium*-legume symbiosis has revealed mathematical
and genetic relationships that likely apply to competition in more-complex communities (see Chapter 9 for more details of application of mathematical modelling to such community-based problems).

### 8.3.3. Insect–Microbe Interactions

The interactions between insects and their microbial symbionts have provided the basis for many principles in symbiosis and have revealed unexpected mechanisms that illustrate how organisms cooperate to perform life functions and gain a competitive edge. The *Buchnera*-aphid symbiosis provides an example of an ancient relationship that has evolved into one of mutual dependence. Analysis of the *Buchnera* genome indicates that the bacterium has lost the capacity to synthesize certain amino acids that it acquires from its host. The *Buchnera* genome has been whittled down to a minimal genome, smaller than genomes of most free-living bacteria. Likewise, the aphid cannot synthesize and depends on *Buchnera* for other essential nutrients (Moran and Mira 2001; Ochman and Moran 2001; Moran 2002, 2003; Moran et al. 2003; Wilcox et al. 2003).

Other insects depend on their bacterial associates for production of mating (Brand et al. 1975, 1976) and cohesion pheromones (Dillon et al. 2002). *Wolbachia* alters reproductive behavior in diverse insects (Plantard et al. 1998, 1999), and an unnamed member of the *Cytophaga-Flexibacter-Bacteroides* group induces parthenogenesis in parasitoid wasps (Zchori-Fein et al. 2001). Insect–bacteria cooperation is discussed in detail in Chapter 7, with emphasis on *Wolbachia*, and Chapter 10, on the the intracellular endosymbionts of the Dryophthorididae.

Most of the arthropod symbioses involve highly specific interactions, often between one bacterial species and one insect. There are a few examples of multispecies communities, such as the associates of the hindgut of termites (discussed in Chapter 2). Degradation of complex carbohydrates and fixation of nitrogen form the metabolic foundation for the community’s function (Breznak and Canale-Parola 1972; Breznak et al. 1973; Breznak and Pankratz 1977; Breznak and Kane 1990; Leadbetter and Breznak 1996; Leadbetter et al. 1999; Nakashima et al. 2002; Bakalidou et al. 2002). The intricacies of managing the carbon and nitrogen economy of the hindgut provides evidence for interdependence and co-evolution among the members of the community. A combination of ultrastructural studies, microbiology, and microelectrode measurements indicates that the hindgut is delicately structured to maintain gradients of oxygen and hydrogen, and the microbial community is spatially arranged to take advantage of these and maintain the chemical
gradients (Brune 1998). Perhaps the most ecologically intriguing aspect of the termite hindgut community is the functional redundancy. The community contains numerous and diverse spirochetes, for example, that fix nitrogen (Lilburn et al. 1999, 2001). A future challenge in microbial ecology is to determine the role and significance of functional redundancy in communities, and the termite hindgut provides an ideal venue for such studies.

The termite system offers one of the most powerful existing models for studying nutritional interactions among community members. The exchange of energy and elements is well understood and establishes principles that are likely repeated in other microbial communities. However, its relative complexity prevents a complete genomic analysis of the community members and will make it challenging to construct a comprehensive map of its communication networks.

8.3.4. Microbial Communities in Mammals

The human oral cavity has been the site of intense study of microbial succession, which has revealed a delicately orchestrated sequence of colonisation events that lead to reproducible and consistent three-dimensional community architecture. Cleaned teeth or enamel chips in a normal mouth provide the vehicle for tracking the succession of community membership (Kolenbrander et al. 2002). The early colonisers are predominantly streptococci, which attach directly by adhesion to the salivary receptors on the pellicle that coats the tooth surface in mixed-species biofilms (Palmer et al. 2003). A mixture of species then attaches to the streptococci, forming a complex, multispecies biofilm. A member of the mixed biofilm is *Fusobacterium nucleatum*, which provides the platform to which diverse late colonisers attach (Kolenbrander et al. 2002). In addition to the surface contact, the bacteria communicate with diffusible signals of the type 2 quorum sensing class of inducers (Blehert et al. 2003). The spatial and temporal organisation of the oral community both is shaped by and contributes to community function and impact on the host, thereby providing an impressive model system for relating structure and function.

Gnotobiotic animals have provided the basis for some elegant and informative studies of host–microbe interactions. Such animals are raised from offspring that are delivered by caesarean section and raised in sterile conditions. This approach simplifies experiments and definitively isolates the role of one organism. For example, in a stunning piece of work, Hooper et al. (2003) have shown that a member of the mouse gut microbiota (and a major member of the human gut microbiota), *Bacteroides thetaiotaomicron*, induces
the normal development of the mouse immune system. They implicated angiogenins, which have been associated previously with tumour-associated angiogenesis, in innate immunity. The angiogenin, Ang4, is secreted into the gut lumen and has bactericidal activity against intestinal microbes. Ang4 expression is induced by *B. thetaiotaomicron*, a predominant member of the gut microflora. Moreover, another angiogenin, Ang1, is found in the circulatory system in mice and humans and exhibits microbicidal activity against systemic bacterial and fungal pathogens, suggesting that angiogenins contribute to systemic responses to infection (Stappenbeck et al. 2002; Hooper et al. 2003). The gnotobiotic mouse system has revealed intricacies of bacterial communication with the mammalian immune system that would have been essentially impossible to pinpoint in a more complex microbial environment. The use of this system for studying gut–bacteria interactions is described in detail in Chapter 12.

To address the next level of complexity in host–microbe interactions, we need model systems that offer analytical power comparable to that provided by gnotobiotic plants and animals but have utility for understanding interactions among community members.

### 8.4. APPROACHES TO COMMUNITY STUDY

There are few models that approximate the natural events involved in colonisation by a multispecies community as effectively as the tooth biofilm system. In other systems, far more disruptive approaches have been used. Much of microbial community ecology in host–microbe studies is predicated on the “scorched earth” approach, which involves riddling the environment of all its microorganisms and then adding them back singly or in small groups. Although this method has revealed much about interactions of hosts with one microbial species, the resulting community is too different from a natural community to provide a meaningful basis for analysis.

Many powerful approaches in microbiology involve the removal of one element from an otherwise normal biological system. In genetics, for example, we use mutant analysis to isolate the role of a single gene, and in the study of metabolism, we use inhibitors or mutants to isolate the role of an enzyme in a pathway. There are few tools available for microbial ecology that approximate this degree of rigor or yield arguments with the power of those established through genetics and biochemistry. We propose that a method that selectively removes a single species from a community will provide a powerful and precise strategy to understand community interdependencies. As we cannot reach in and remove members of a species in a microbial
community by hand as can be done with starfish, for example, we must develop alternative strategies.

8.4.1. The Lepidopteran Gut Community

Our goal is to describe the species diversity in a community and then determine the role of each member of the community in the health of the host and in the stability and function of the community. To be tractable for these studies, the community needs to be readily available and reproducible, portable and contained, readily manipulated by addition of chemicals and organisms, and of relatively simple composition. Communities that meet these criteria reside in the midguts of the lepidopteran insects, such as the gypsy moth.

8.4.2. Overall Approach

The overall approach is to remove each species individually from the midgut community and study the resulting effect on the host and the community. We will measure effects on resistance to disease and toxins, development, and fecundity in the host, and nutritional status and robustness of the microbial community following reduction of the population size of one member. Robustness comprises resistance, stability, and resilience. Resistance is the ability of a community to maintain its structure upon challenge by an invader, stability is the ability to return to its original structure, and resilience is the rate of return to original structure (Begon et al. 1990).

8.4.3. Microbial Diversity in Lepidopteran Midguts

We have characterised the microbiota in the larval midguts of the gypsy moth by culture and culture-independent methods. When fed a sterilised diet, the community is comprised of ten members (Table 8.2), seven of which were culturable. Most of the members of the community belong to the γ-Proteobacteria and Firmicute phyla.

The gypsy moth is a generalist that feeds on 300 to 500 plant species that contain a diverse array of allelochemicals. Therefore, we determined the community composition when the larvae were fed foliage from various tree species. The microbial composition of midguts differed substantially among larvae feeding on a sterilised artificial diet, aspen, larch, white oak, or willow. A culturable Enterococcus species, and an Enterobacter species that
Table 8.2. Bacterial phylogotypes identified by culturing and culture-independent analysis of third instar gypsy moth midguts based on 16S rRNA gene sequence analysis

<table>
<thead>
<tr>
<th>Sequence ID</th>
<th>Bacterial division</th>
<th>Genus</th>
<th>Database matches</th>
<th>Population or presence</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(≥ 95% identity)</td>
<td></td>
<td>(≥ 98% identity)</td>
<td>Artificial diet</td>
<td></td>
</tr>
<tr>
<td>Identified by culturing and culture-independent analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAB1</td>
<td>γ-Proteobacteria</td>
<td>Pseudomonas</td>
<td>P. putida</td>
<td>1.8 x 10^5</td>
<td></td>
</tr>
<tr>
<td>NAB3</td>
<td>γ-Proteobacteria</td>
<td>Enterobacter</td>
<td>Uncultured soil</td>
<td>4.8 x 10^6</td>
<td></td>
</tr>
<tr>
<td>NAB4</td>
<td>γ-Proteobacteria</td>
<td>Pantoea</td>
<td>P. agglomerans</td>
<td>2.5 x 10^5</td>
<td></td>
</tr>
<tr>
<td>NAB7</td>
<td>Low G+C</td>
<td>Staphylococcus</td>
<td>S. lentus</td>
<td>8.1 x 10^4</td>
<td></td>
</tr>
<tr>
<td>NAB8</td>
<td>Low G+C</td>
<td>Staphylococcus</td>
<td>S. cohnii</td>
<td>2.9 x 10^6</td>
<td></td>
</tr>
<tr>
<td>NAB9</td>
<td>Low G+C</td>
<td>Staphylococcus</td>
<td>S. xylosus</td>
<td>1.3 x 10^6</td>
<td></td>
</tr>
<tr>
<td>NAB11</td>
<td>Low G+C</td>
<td>Enterococcus</td>
<td>E. faecalis</td>
<td>1.5 x 10^8</td>
<td></td>
</tr>
<tr>
<td>Identified by culture-independent analysis only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAB16</td>
<td>α-Proteobacteria</td>
<td>Agrobacterium</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAB17</td>
<td>γ-Proteobacteria</td>
<td>Enterobacter</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAB20</td>
<td>Low G+C</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Cultured: numbers represent the average population of phylotype based on colony morphology in twenty individual larvae per treatment (cfu/ml gut material). Non-cultured: + indicates presence in guts of insects in diet treatment.

was culturable from some guts but not others were both present in all larvae, regardless of feeding substrate.

8.4.4. Manipulation of Community Composition

Antibiotics

The methods we are using to reduce targeted populations in a community cover a range of specificities. Antibiotics, for example, are not species specific, but by using various drugs of different specificities, the effect on a given species might be isolated. Larvae fed on antibiotics in various combinations
Figure 8.2. Synergy of zwittermicin A and Bt toxin. Zwittermicin A potentiates the activity of the insecticidal toxin produced by *Bacillus thuringiensis* (Broderick et al. 2000).

display numerous signs of reduced health. For example, a cocktail of antibiotics including tetracycline reduces larval survival by fifty percent over the first 20 days after hatching, and the surviving larvae weigh one tenth of the untreated control larvae. Tetracycline alone has a similar effect on development, whereas gentamicin, rifampicin, and penicillin have no effect on survival or growth.

Another antibiotic, zwittermicin A, dramatically increases larval sensitivity to the insecticidal toxin produced by *Bacillus thuringiensis*, although this antibiotic has no measurable effect on the larvae by itself (Fig. 8.2). Zwittermicin A alters the population size of more than one member of the gut community; thus it is not possible to assign the effect on toxin sensitivity to any one member of the community. Gypsy moth larvae fed on aspen leaves have substantially altered gut communities and also show enhanced sensitivity to *B. thuringiensis* and greater susceptibility to virus infection (Hunter and Schultz 1993; Lindroth et al, 1999). In aggregate, these results suggest that a change in the gut microbiota may stunt larval growth, stall development, and make the larvae more sensitive to pathogens and toxin. However, the results do not elucidate the role of any one bacterium or group of bacteria, because all of the treatments affect more than one member of the gut community. To test the hypothesis that a member or members of the gut community contribute to larval development and protect larvae from
pathogens requires methods that isolate the effects of the members of the community. One approach to assigning functions to specific organisms is to feed bacteria to the treated insects to determine whether any of the normal residents of the gut rescues the host. However, methods to selectively remove members from the normal community are also essential because they will generate fewer secondary effects and be less disruptive to overall community function.

**Phage**

Bacteriophage, or phage, are viruses that inject into bacteria a nucleic acid genome that directs synthesis of viral components, leading to the production of more phage particles. Some phage are highly lytic, completing their replication cycle by weakening the bacterial cell wall, which results in cell lysis and release of phage particles. Some phage can release hundreds of infective particles from one infected cell. Their rapid life cycles and high reproductive rates make phage excellent agents to reduce bacterial populations. As agents of bacterial destruction, phage have advantages over antibiotics, such as their high degree of host specificity and amplification over time (Sulakvelidze et al. 2001). The host range of most phage is limited to a single species, and most are selective for certain strains within a species. The host selectivity and explosive killing exhibited by many phage have drawn attention to them as therapeutic agents to cure infectious disease. This idea was introduced by d’Herelle in 1917 (d’Herelle, 1926) and pursued in collaboration with his colleagues in Georgia (formerly USSR), although it did not gain attention until recently in the United States early experiments by d’Herelle and others claimed high survival rates of people treated with phage to control dysentery and cholera, but the experiments were not designed with the rigor of modern experimentation; thus the data, however intriguing, must be interpreted cautiously. A combination of political prejudices and the discovery of antibiotics diverted attention from phage therapeutics in Western medicine until recently (Summers 2001; Sulakvelidze et al. 2001). The last few years have seen renewed interest in phage, as the antibiotic resistance crisis impels microbiologists to find new (or rediscover old, in this case) solutions to infectious disease. Recent reports present rigorous and highly successful in vivo tests of phage to control infectious disease. One study showed that mice infected with *E. faecium* were cured by a single injection of a phage that reproduces in that bacterium. Even mice that were already moribund had a fifty percent survival rate when treated with the phage, compared with zero percent of the untreated ones (Biswas et al. 2002).

Plant pathologists have also used phage to protect plants from infection by bacterial pathogens (Vidaver 1976; Flaherty et al. 2000) and found
that virulent phage interfere with the establishment of a bacterial biocontrol agent (Keel et al. 2002), indicating that phage exert influences on population structures outside the controlled conditions of the laboratory. Of particular interest to us is the fact that d’Herelle first observed phage in cultures of a pathogen that caused an epizootic infection of locusts in Mexico in 1909 (d’Herelle 1926). Thus, observations about the role of phage in bacteria associated with insects date back to the very beginning of phage biology.

The history of phage biology and ecology suggests the potential for their use in ecological studies. Their specificity makes them carefully addressed “letter bombs” that will destroy only one component of a community and remain biologically invisible to the rest. The resistance problem encountered in previous studies can be addressed either by using the phage for short-term studies or by combining more than one phage, in which case the frequency of a doubly resistant mutant is lower than the number of bacteria in the population to be addressed.

Bacteriophage exhibit more specificity than antibiotics, infecting only certain members of a species. The advantage of phage is that they can reduce bacterial populations by four to eight orders of magnitude in vitro. A disadvantage is the difficulty in isolating phage for bacteria that we do not yet know how to culture. A concern with phage was that they might not be able to infect in a gut, especially the gypsy moth gut, which has an average pH greater than 12. Variations and combinations of methods will likely lead to the best tools that incorporate phage into the study of community ecology.

8.5. FUNCTIONAL CONNECTIONS AMONG COMMUNITY MEMBERS: CULTURED AND UNCULTURED

A long-ignored aspect of community ecology is the uncultured majority. Most bacteria in environmental samples are not culturable by standard methods. Therefore, to understand the structure and function of microbial communities, we must include the uncultured bacteria in our analyses. Community structure can be analysed by polymerase chain reaction amplification of 16S rRNA genes from DNA directly extracted from the environmental sample. DNA isolated from environmental samples can also be used for functional genomics by cloning into a suitable vector that replicates in a culturable host. This approach, termed metagenomics, has provided insight into uncultured communities in soil, seawater, sponge tissue, and the human oral cavity (Stein et al. 1996; Schleper et al. 1997; Henne et al. 1999; Beja et al. 2000; Rondon et al. 2000; Courtois et al. 2003; Diaz-Torres et al. 2003; Handelsman et al. 2003).
Metagenomic library construction

Dissect larvae, separate bacteria
\[\downarrow\]
Extract DNA
\[\downarrow\]
Digest
\[\downarrow\]
Ligate into vector
\[\downarrow\]
Transform *E. coli*
\[\downarrow\]
Screen transformants

Figure 8.3. Metagenomics provides a means to access the genomes of as-yet unculturable microorganisms by direct extraction of their DNA from mixed communities (Handelsman 2003; Handelsman et al. 1998; Schloss and Handelsman 2003).

We are characterising the cultured and the as-yet unculturable bacteria in the gypsy moth midgut. We have constructed highly redundant libraries from DNA extracted directly from the gut bacteria that have not been subjected to culturing (Fig. 8.3). Preliminary studies indicate that clones in these libraries express novel functions that have not been found among the cultured bacteria. A major focus of this work is to identify molecules that play a role in communication among bacteria—both culturable and unculturable—in the gut environment. This aspect of host–community interactions will add a new dimension to our understanding of the interactions of animals with their associated microorganisms.

8.6. CONCLUSION

The study of the impact of communities on their hosts is at a new intersection. New tools are available for the dissection of communities, and the knowledge of interactions of single species with their hosts lays a strong foundation for the study of multispecies communities and their hosts. Application of molecular methods that address both the uncultured and cultured members of the communities, computational approaches to model quantitative
events, and diverse biological and chemical approaches to perturb communities will produce an understanding of the complex networks that maintain the structure of the community and govern its influence on the host.

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