1

Introduction

ANIMALS IN A MICROBIAL WORLD

1.1. What Is An Animal?

We can answer this question in two ways. One answer is relatively straightforward and is provided in the following paragraph, and a first attempt at the really interesting answer occupies the rest of this book.

The animals are a monophyletic group of eukaryotes with a multicellular common ancestor that fed holozoically (i.e., on particulate food) and comprised cells that lacked a cell wall and included multiple morphologically and functionally different cell types. These traits set animals apart from the only other major group of ancestrally multicellular eukaryotes, the terrestrial plants, and other eukaryotes with multicellular representatives, e.g., the fungi and red algae, which have saprophytic or photosynthetic lifestyles and are composed of cells enclosed within a cell wall. Early in their diversification, two key innovations evolved in the animals: the gut, permitting exploitation of large food items; and the nervous system, laying the foundation for the complex behavior displayed by many animals. A further defining feature of the animals is that, as a group, they lack key metabolic capabilities common to various other organisms, including the capacity to photosynthesize, fix nitrogen, synthesize many amino acids contributing to protein, and produce various cofactors required for the function of enzymes central to metabolism. In other words, many animals are morphologically complex and some are clever, but all are metabolically impoverished.

This description of animals is not incorrect, but it is incomplete. It omits 1–10% of the biomass and half or more of the cells in the animal body. The

missing cells are the microbial communities that live persistently with the animal. Most of these microorganisms are bacteria, but they also include unicellular eukaryotes; and large animals additionally bear multicellular eukaryotes, including mycelial fungi, mites, helminth worms, etc. Traditionally, these inhabitants of animals have been ignored unless they are injurious to the health of their animal host, and because many are difficult to culture, their ubiquity and diversity are grossly underestimated by routine culture-based microbiological methods.

The study of microorganisms associated with animals has been transformed by culture-independent methods to identify and study the function of microorganisms. The key technology has been high throughput DNA sequencing (also known as next generation sequencing), by which all the DNA, expressed genes, or specific genomic regions of interest in a sample can be sequenced simultaneously. It is now possible to determine the taxa in the microbial community and their functional traits, for example from a biopsy taken from the lung or intestine of a human patient, from a single soil microarthropod, or from the gills of a bivalve mollusk brought up from a deep sea hydrothermal vent. These ever-improving technologies have supported a decade or more of research on the microbiological natural history of animal bodies. It is now apparent that animals are the habitat for a previously unsuspected diversity and abundance of microbial residents, and this unfolding discovery has empowered experimental science, revealing that these microorganisms are critical to the health and well-being of their animal hosts.

So, what is an animal? It is a multiorganismal entity, comprising animal cells and microbial cells. The phenotype of an animal is not the product of animal genes, proteins, cells, tissues, and organs alone, but the product of the interactions between all of these animal functions with communities of microorganisms, whose composition and function vary with the age, physiological condition, and genotype of their animal host. Following from the growing appreciation of the significance of the microbiology of animals, many aspects of animal biology are being rewritten (McFall-Ngai et al., 2013). The biomedical sciences are increasingly recognizing the pervasive effects of resident microorganisms on human health. These effects extend beyond local impacts, for example of gut microorganisms on gut health and disease, to microbial effects on cardiovascular health, the integrity of the circadian rhythm, and psychiatric health. Many evolutionary biologists are realizing that the response of animals to selection is influenced by the impact of microbial partners on the trait under selection and the heritability of the microorganisms. Applied biologists are appreciating that microbes can shape the capacity of insects, such as mosquitoes, to vector disease agents (e.g., the malaria parasite, dengue virus) and their susceptibility to certain pest

control agents. Furthermore, reliable predictions of the impacts of climate change on animal distributions will require consideration of the environmental requirements and dispersal capability of the microbial partners as well as the animal.

But, before we go any further, we need to address some issues of terminology. As with so many scientific disciplines, multiple terms are being used, sometimes interchangeably but often with different shades of meaning that can sow confusion and misunderstanding. Section 1.2 provides a guide to how some terms are used in this book, as well as why some terms are eschewed.

1.2. Terminology: Dismantling the Tower of Babel

Terms are important not only because they communicate agreed concepts within a discipline but also because they can encapsulate an entire conceptual framework. In this respect, the term "microbiome," coined to describe the catalog of microorganisms and their genes (Lederberg and McCray, 2001), is of central importance. The microbiome is a global, all-encompassing term for the microbiology of an animal, and is particularly useful where, as in shot-gun sequencing, individual genes cannot readily be assigned to particular microbial taxa. A related term is "microbiota," which refers specifically to the microbial taxa associated with an animal. The terms microbiome and microbiota are sometimes used interchangeably when referring to taxa. Alternatively, the microbiome can be used exclusively to refer to genes and genomes, with the microbiota as a taxonomic descriptor. It is usually obvious from the context how the term microbiome is being used. In this book, I will use both terms, with microbiome to describe inventories of genes, especially in relation to function, and microbiota when referring to the organisms.

The terms microbiome and microbiota have meaning for a science that is founded on molecular biology and genomics. A major driver of microbiome research over the last decade has been large consortium projects that have generated microbial sequences associated with humans, and their potential biomedical importance. However, microbiome research is also founded on many decades of pregenomic research on interactions between healthy animals and their resident microorganisms (Sapp, 1994). Although this research endeavor has been largely independent of biomedical science, the melding of the terminology of the pregenomic science of animal-microbial interactions with the terminology of -omic science has, to a large extent, been successful. But there have been some difficulties, and this is has caused some confusion and miscommunication.

A key pregenomic term is "symbiosis," which—as for microbiome—was invented to fulfil a scientific need. Symbiosis was coined in the 1870s to

encapsulate the new discovery of multipartner organisms. It was initially used by Albert Franck to describe the composite nature of lichens, comprising a fungus and alga, and generalized in 1879 by Anton de Bary to describe the "living together of different species." Over the following century, most research on interactions between healthy animals and microorganisms focused on associations that were readily detected morphologically, including highly specialized interactions involving single microbial taxa housed in specific tissues or organs. These associations were categorized as symbioses (or sometimes endosymbioses), with the animal described as the host and microbial partner as the symbiont. The microbial symbionts in these "one-host-one-symbiont" or occasionally "one-host-two/three-symbionts" associations include the zooxanthellae (dinoflagellate algal cells) in corals, luminescent bacteria in the light organs of some fish and squid, and dense bacterial aggregations in specialized organs (bacteriomes) of certain insects. The text of Buchner (1965), together with two multiauthor volumes (Jennings, 1975; Nutman and Mosse, 1963), provide a superb overview of premolecular research on these associations.

Today, the terms microbiome and microbiota are generally used with reference to multitaxon microbial communities, and the microbial partner(s) tend to be called symbiont(s) where the interactions are limited to one or a few microbial taxa. There is a general presumption that, where the animal host is healthy, the terms microbiota and symbionts do not refer to pathogens. However, the impact of many microorganisms on the animal host can be context-dependent, varying with the developmental age, physiological condition, and genotype of the host, as well as environmental conditions. This was appreciated by Elie Metchnikoff who, in the early twentieth century, coined a further term "dysbiosis" as an antonym of symbiosis, to describe a microbial community that is deleterious to host health (Stecher et al., 2013). In the same vein, individual members of a microbial community that display context-dependent pathogenicity are often known as pathobionts (Hornef, 2015).

In the biomedical literature, the term "commensal" is widely used to describe individual taxa of the microbiota. This term poses some important problems. Strictly speaking, the term commensal refers to "eating at the same table," and has come to describe an organism that derives benefit from an association with no discernible effect on the fitness of its partner, akin to the sparrow feeding on the breadcrumbs dropped from a man's dining-table. The term commensal is not widely used in the symbiosis literature because it has all the standard difficulties of a negative definition: if only one used a more sensitive assay or studied the association under different conditions, perhaps benefit or harm would be detected, and the organism would, no longer, be a commensal. Microorganisms associated with the gut and skin

of humans used to be called commensals, in the erroneous belief that they are of no significance. It is unfortunate that commensal has persisted into the era of microbiome research with the full knowledge that these microorganisms are crucial to the health of humans and other animals. Needless to say, verbal modifiers such as "beneficial commensal" further compound the terminological confusion. In the light of the complexities surrounding the term commensal, it is preferable to avoid this term. Alternative terms, such as microorganisms, microbial communities, etc. are sufficient; where it is important to emphasize that pathogens are specifically excluded from consideration, the term "nonpathogenic microorganisms" can be used to avoid any ambiguity.

There is one further set of terms that needs to be addressed: holobiont and hologenome. Lynn Margulis coined new terms for the partners in a symbiosis as "bionts" and the association as a "holobiont" (Margulis, 1991) to emphasize the evolutionary persistence of the association and how selection may operate at the level of the association (or holobiont). This terminology has been brought into the -omic era with a further new term, the hologenome, which refers explicitly to the host genome plus microbiome as the unit of selection (Rosenberg and Zilber-Rosenberg, 2016). The concept of the hologenome is relevant to a very restricted set of associations. In particular, it does not apply to the complex microbial communities that are the focus of much microbiome research, where individual microbial taxa have different selective interests from each other and variable selective overlap with the host. Strong overlap of selective interest between the partners is predicted in some associations involving individual microbial partners that are vertically transmitted (and so have a selective interest in the fitness of the host offspring), but the residual selective conflict limits the applicability of the hologenome concept. Even the most ancient of symbioses, between the eukaryotic cell and the mitochondrion, is subject to genomic conflict (Perlman et al., 2015) and so cannot be classified as a pure hologenome. For these reasons, which are elaborated further by Douglas and Werren (2016) and Moran and Sloan (2015), the hologenome concept is not developed in this book.

1.3. The Microbiology of Animals

Now that the main terms for the discipline are defined, we can consider why animals support microbial communities. The functional explanations are twofold: microorganisms provide metabolic capabilities that are lacking in animals; and microorganisms modulate the signaling networks that regulate animal functions required for sustained animal health and vigor (figure 1.1).

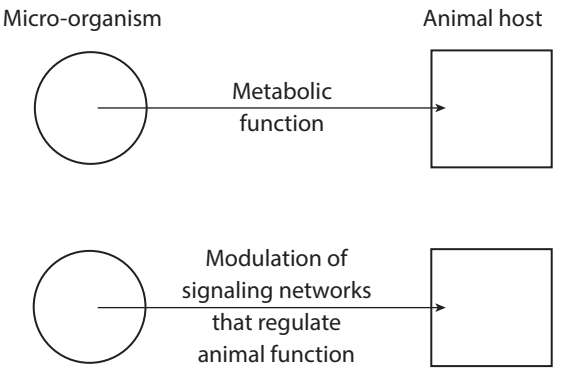

FIG. 1.1. How microorganisms interact with the animal host.

The key metabolic capabilities gained by animals from microbial partners are listed in Table 1.1A. Some of these capabilities were absent from the lineage giving rise to animals, e.g., photosynthesis, nitrogen fixation, and essential amino acid and B vitamin synthesis, and the dietary requirement of various animals for carbon, nitrogen, or specific nutrients has been spared by symbiotic microorganisms. For example, photosynthetic associations have evolved repeatedly in basal animals; some termites possess nitrogen-fixing bacteria in their guts, enabling them to thrive on wood of exceptionally low nitrogen content; and all animals that feed through the life cycle on vertebrate blood, e.g., bedbugs, tsetse flies, leeches, and ticks, are widely believed to derive supplementary B vitamins from microbial partners (Douglas, 2015; Venn et al., 2008).

Symbiotic microorganisms also complement metabolic deficiencies that have evolved in specific animal groups. For example, arthropods cannot synthesize sterols, which are essential constituents of membranes and various hormones, and some insects living on sterol-poor diets derive most of their sterol requirement from yeast symbionts (Douglas, 2015); and, because vertebrates cannot degrade cellulose, most herbivorous vertebrates depend on cellulolytic microorganisms in their guts. Some metabolic traits are mediated either intrinsically (i.e., by the products of animal genes) or via microbial partners, varying among animal taxa. Complex patterns of intrinsic and microbial origins of metabolic traits are evident for cellulose degradation (Calderon-Cortes et al., 2012). Similarly, animal luminescence can be intrinsic or microbial. An inventory of light production in marine fish identified 8 independent origins of intrinsic luminescence and 17 origins of bacterial luminescence (Davis et al., 2016). This includes the deep-sea angler fish that maintain two light organs, one with intrinsic luminescence and the other housing symbiotic bacteria. Secondary metabolism, including the synthesis and degradation of toxins, is also mixed in origin (Table 1.1A)

TABLE 1.1. Services Provided by Microbial and Animal Partners

(A) Microbial services	Examples
CO_2 fixation	Dinoflagellate algae Symbiodinium in shallow-water scleractinian corals
—Photosynthesis —Chemosynthesis	Bacteria in annelid tube worms and various bivalve mollusks at hydrothermal vents and methane seeps
Nitrogen fixation	Various bacteria in some termites
Essential amino acid synthesis	Various bacteria in plant sap–feeding insects
B vitamin synthesis	Various bacteria in insects feeding through the life cycle on blood
Degradation of complex polysaccharides	Bacteria in anoxic gut regions of vertebrate herbivores
Luminescence	*Vibrio* or *Photobacterium* in light organs of some fish and squid
Toxin (e.g., polyketide, antibiotic) synthesis	Various bacteria in benthic marine animals, e.g., sponges, bryozoans, and insects (e.g. *Paederus* rove beetles, attine ants)
Toxin degradation	Various gut bacteria in animals feeding on toxic plant tissues, e.g., detoxification of plant phenolics ingested by desert woodrats, caffeine ingested by the coffee berry borer beetle

(B) Animal services	Examples
Nutrient supply: selective feeding harvests and concentrates nutrient-rich substrates that are utilized by microorganisms	Many gut microorganisms
Protection from adverse abiotic conditions, e.g., high oxygen tensions, desiccation	Obligately anaerobic microorganisms in anoxic fermentation chambers of animal guts
Provide enemy-free space by sequestering microorganisms and by immune system function	Inferred for many intracellular microorganisms
Dispersal, often in nutrient-rich substrate, e.g., mucus, fecal material	Inferred for carriage of some microorganisms through the animal gut

although, as the microbiology of more animals is investigated (e.g., Ceja-Navarro et al., 2015; Florez et al., 2015; Kohl and Dearing, 2016), many more instances of microbial-mediated secondary compound metabolism may be revealed. The evolutionary and ecological factors that determine

why some traits can be either intrinsic or microbial origin have not been investigated systematically.

The many examples of animals that gain access to metabolic capabilities through associations with microorganisms can be explained in terms of an inherited predisposition of animals to associate with microorganisms, especially bacteria. This evolutionary predisposition appears to be common to all eukaryotes. The common ancestor of all modern eukaryotes bore an intracellular *Rickettsia*-like bacterium that evolved into the mitochondrion; unicellular eukaryotes (protists), where investigated, are routinely colonized by bacteria or other microorganisms; the plant microbiome is a very active area of current research (Bai et al., 2015; Lundberg et al., 2012); and discovery of additional fungal or bacterial partners in lichenized and mycorrhizal fungi illustrates how fungi also have a propensity to form associations (Bonfante and Anca, 2009; Spribille et al., 2016). This microbio-philia of animals appears to be matched by advantages to the microorganisms of colonizing the animal, including access to nutrients, protection from natural enemies or harsh abiotic conditions, as well as dispersal (Table 1.1B).

These considerations bring us to the second reason why animals are associated with microorganisms. Because animals originated and diversified in the context of a long evolutionary history of relationships with microorganisms, the key physiological systems of animals, together with the signaling networks that regulate these systems, all evolved in the context of preexisting and ongoing interactions with microorganisms. In other words, the microbiome is expected to play a role, directly or indirectly, in the development and function of the animal nervous system, immune system, endocrinal system, gut physiology, respiratory physiology, and so on. This reasoning takes us beyond the predictions that microorganisms confer various metabolic services, as outlined in Table 1.1A, to the additional prediction that the presence and activities of microorganisms influence many, possibly all, aspects of animal function (figure 1.1B).

Just as the role of microorganisms as a source of metabolic capabilities (figure 1.1A) has been appreciated for many years, the predicted integration of microorganisms into all aspects of animal function also has a strong historical basis. It was Louis Pasteur, the architect of the germ theory of disease, who first addressed the significance of microorganisms for animals, arguing that microorganisms are essential for animal life (Pasteur, 1885). We now know that this is not true, and that some animal species can be maintained under microbiologically sterile conditions; they are known as "axenic" or "germ-free" animals. The viability of germ-free animals was first demonstrated for *Calliphora* blow flies (Wollman, 1911), and the subsequent elucidation of the nutritional requirements of *Drosophila* was

founded on methods devised to eliminate all microorganisms from the fly cultures (Sang, 1956). Today, the production and distribution of germ-free laboratory mice (Smith et al., 2007) is a commercial enterprise. These germ-free mice display many symptoms of ill-health, including stunted growth, depressed fertility, and reduced metabolic rate, commonly accompanied by specific abnormalities of various organs. These multiple deficiencies and abnormalities are the basis for the fundamental role of microorganisms in animal health and well-being. In other words, we can only understand animal function by integrating the microbiology of animals into our explanations of animal biology.

1.4. Scope of This Book

The realization that every animal is colonized by microorganisms that can shape its health and well-being is transforming our understanding of animal biology. The purpose of this book is to provide some initial explanations and hypotheses of the underlying animal-microbial interactions. For this, we need multiple disciplinary perspectives.

We start with evolutionary history in chapter 2. The propensity of animals to associate with microorganisms has ancient roots, derived from both the predisposition of all eukaryotes to participate in associations and, likewise, the tendency of many bacteria to interact with different organisms, often to mutual benefit. Chapter 2 outlines the patterns of these interactions, especially in taxa related to animals and basal animal groups. Interactions are mediated by chemical exchange, enhancing access to energy and nutrients and providing chemical information that enables the interacting organisms to anticipate and respond adaptively to environmental conditions. Many of these core interactions were firmly established in the ancestor of animals. The multicellular condition of animals, sophisticated immunological function of even basal animals, and key animal innovations, including the polarized epithelium and the gut, play important roles in shaping the pattern of animal-microbial interactions.

Although all animals are associated with microorganisms, we know more about the microbiome of humans than any other animal. Chapter 3 addresses current understanding of the role of the microbiome in human health. Studies of the microbiology of humans combined with experimental analyses of model animals are revealing complex problems—and some solutions. The complexity lies in the great diversity of microorganisms within each individual human, as well as considerable among-individual variation; and the importance of the microbiome is reinforced by the increasing evidence for microbial involvement in some diseases, especially metabolic and immunological dysfunctions. Western lifestyles, including diet and antibiotic treatment, have been argued

to contribute to the incidence of microbiome-associated diseases, with opportunities for microbiological restoration by microbial therapies.

Our understanding of interactions between animals and the microbiome is most developed in relation to the immune system, and this is the focus of chapter 4. It is now apparent that animal immune system is a key regulator of the abundance and composition of the microbiota, and that immunological function is strongly regulated by the composition and activities of the microbiome. The immune system cannot be understood fully except in the context of the microbiology of the animal. Furthermore, this highly interactive system is overlain by microbial-mediated protective functions, essentially comprising a second immune system.

Chapter 5 investigates the role of the microbiome in shaping animal, including human, behavior. It has long been known that pathogens can drive animal behavior, and there is now increasing evidence that resident microorganisms can have similar, although often more subtle, effects. Research has focused primarily on three aspects of animal behavior: feeding behavior, chemical communication among animals, especially in relation to social interactions, and the mental well-being of mammals, including humans. As chapter 5 makes clear, this topic has attracted tremendous levels of interest, but fewer definitive data.

The impacts of animal-associated microorganisms on host health and their interactions with the immune system and nervous system of animals (chapters 3–5) have one overriding theme in common: that these interactions are complex, with multiple interacting variables. This complexity can often appear to defy comprehension. Chapter 6 discusses the ecological approaches that have the potential to solve many of these problems of complexity. Treating the animal as an ecosystem, we can ask multiple questions: what are the ecological processes that shape the composition and diversity of microbial communities, and how do these properties of the microbial communities influence overall function of the ecosystem? Research on complex microbiomes, especially in the animal gut, as well as one-host-one-symbiont systems are revealing the role of interactions among microorganisms and interactions between the microorganisms and host in shaping the diversity of the microbiome. Furthermore, the response of individual taxa and interactions can influence the stability of communities to external perturbations, ranging from the bleaching susceptibility of shallow-water corals to the gut microbiota composition of humans administered with antibiotics.

In chapter 7, the evolutionary consequences of animal-microbial associations are considered. There is a general expectation that the fitness of both animal and microbial partners is enhanced by these associations largely through the reciprocal exchange of services. Nevertheless, hosts can exploit their microbial partners, and there are indications that animals can be addicted

to their microbial partners. At a broader scale, this chapter investigates how these associations affect the rate and pattern of evolutionary diversification of the microbial and animal partners. In addition to evidence for coevolutionary interactions and facilitation of horizontal gene transfer, various studies point to a direct role of microbiota in interrupting gene flow and speciation by both prezygotic and postzygotic processes.

Finally, chapter 8 addresses the implications of the microbiology of animals and some key priorities for future research. It is now abundantly clear that the microbiome has pervasive effects on the physiological and developmental systems of animals and the resultant animal phenotype. One of the big biological questions in the life sciences today concerns how the phenotype of an animal maps onto its genotype and the underlying physiological and developmental mechanisms. The answers to this question will require the integration of the microbiome with the traditional animal-only explanations of animal function. As this book illustrates, the technologies and concepts to achieve this intellectual transformation of animal biology are largely in place. Why is this integration of disciplines needed? Beyond the fundamental priority to understand and explain, the microbiome offers important, but currently untapped, routes to promote human health and to mitigate and manage some of the damaging effects of human activities on our environment.