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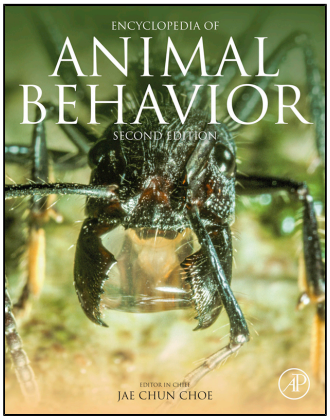


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# Microbes: Social Evolution

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## Glossary

**Altruism** Any trait or behavior that increases the fitness of a recipient while decreasing the fitness of the actor expressing the trait or performing the behavior.

**Autoinducer** In quorum sensing, a low molecular weight secreted molecule that may act as a cue for cell density. Thusly named because production is typically under positive feedback, such that high autoinducer concentrations induce further autoinducer production.

**Inclusive fitness** A fitness measure that incorporates not only the direct fitness consequences of a trait or behavior on an actor but also the indirect fitness consequences on any recipients, weighted by the relatedness between the recipient and the actor.

**Kin discrimination** Differential treatment of related individuals compared with unrelated individuals of the same species.

**Kin selection** Selection on a trait that results from the indirect fitness effects of that trait on relatives.

**Relatedness** The probability above random expectation that an allele present in one individual is present in another.

## Abstract

Microbes engage in diverse interactions to which social evolution theory developed for animals can be usefully applied. In turn, studies on microbes offer insight into social evolution theory as it applies to larger organisms. Here we review key evolutionary concepts as applied to microbial interactions, then describe some prominent examples of how microbes interact to obtain resources, communicate, move, attack and defend themselves from competitors, prey, or predators, and influence multicellular hosts.

## Keywords

Altruism; Antagonism; Bacteria; Conflict; Cooperation; Evolution; Kin selection; Microbe; Multicellularity; Mutualism; Symbiosis

## Introduction

Microbes live rich social lives. They collaborate and compete; they are predators and prey; they are friends and enemies. Microbes live in groups and have for a very long time. Some of the oldest fossils are stromatolites – 3.5 billion year old fossilized biofilms of a sort that can still be found living in modern hypersaline lagoons (Walter, 1977). Microbes often live in large populations and at high densities, often including many species in close proximity. They communicate with kin and non-kin, share and compete, sacrifice for allies, and poison their foes. Their interactions run the gamut from simple to complex, from facultative to obligate, from friendly to lethal.

Many of these interactions find easy comparison to those of more familiar organisms. The soil bacterium *Myxococcus xanthus* is a social predator, swarming across the soil in huge groups searching for bacterial prey (Velicer and Vos, 2009). It does not take much imagination to see why their groups are called wolf packs. *Bacillus subtilis* clones cooperate to build towering biofilm structures that call to mind the mounds of African termites, complete with circulatory systems shaped to draw oxygen inside (Wilking *et al.*, 2013). Other microbes interact in ways that are unique to microbes, like magnetotactic bacteria that align themselves into multicellular magnets (Keim *et al.*, 2004).

Many of the central tools used to understand sociality in microbes are ideas developed by animal behaviorists. Microbes lack conventional nervous or endocrine systems, and yet, as in animals, the evolution and ecology of their behavior is dominated by concepts of cost and benefit, direct and indirect fitness benefits, mutualism and parasitism, and exploitation and kin recognition. Careful application of these concepts can guide our understanding of microbes' evolutionary past and future and their considerable influence on human health, agriculture, and ecosystem function.

In turn, microbes can help us understand non-microbial life. With their uniquely small size, huge populations, metabolic diversity, genetic tractability, and rapid generation times, microbes are useful models that enable experiments testing theories in behavior, evolution, population biology, and ecology that would be impractical with larger organisms. Interactions between microbes even inform our understanding of cell-cell interactions in multicellular organisms. Eukaryotes only came about due to cooperation between microbes, a major evolutionary transition that set the stage for the immense morphological diversity of



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the past half billion years (Hedges *et al.*, 2004). The line between microbial sociality and social (and non-social) function in multicellular eukaryotes is blurrier than one might expect.

In this review we first outline key evolutionary concepts as applied to microbial interactions, then describe some prominent examples of how microbes interact to obtain resources, to communicate, to move and disperse, to attack and defend, and to influence multicellular hosts. Where possible, we will relate microbial examples to conceptually similar examples in macrobes. The term "microbes" here means nothing apart from small size, and encompasses a vast diversity of unicellular and even some multicellular organisms. The examples in this review primarily feature bacteria, but conceptually analogous examples can be found in archaea, viruses, and microbial eukaryotes as well.

## Microbial Organismality – What is a Microbial Individual?

Microbes can straddle the line between single-celled and multicellular life. Many microbes live in densely-packed groups, some with complex morphology that calls to mind the tissues of animals and other large eukaryotes. This complexity has led some researchers to liken some microbial groups not to populations or communities of interacting individuals, but rather to multicellular organisms in their own right.

When is this comparison appropriate? What is a microbial individual? Is it a single cell, or is it a group of cells? At what level of organization is the fitness of a trait expressed? In animals, the answer to these questions is often too obvious to warrant much thought, but the same ambiguities surround some clonal, colonial, or chimeric species. Understanding the evolution of a worker ant's traits is sometimes helped by considering the ant's entire colony, not just the ant, an individual. The same can be true for some microbial groups.

Degree of cooperation and conflict is the key. An individual organism need not be a single body, but its constituent units must have closely aligned fitness interests (Queller and Strassmann, 2009). The balance of cooperation and conflict in a group dictates to what degree it is appropriate to assign a single fitness to it (rather than separate fitnesses for its constituent units). Importantly, 'individual' or 'organism' need not be binary terms – groups with different degrees of cooperation and conflict may be thought of as having different degrees of 'organismality'.

The degree of cooperation and conflict – and thus the organismality – of microbial groups depends upon their ecological and environmental context. Most research on microbes is performed on a single clonal lineage of a single species. Under these conditions, genetic conflicts of interest are minimized, at least until mutation generates new genetic diversity within the experimental population. In nature, however, microbes often live in close association with many competing strains, both conspecific and heterospecific. With genetic differences come potential conflicts, and so highly diverse natural bacterial groups are rarely organismal in the same way the clonal cells in an animal body are. Recent advances in our awareness of microbial sociality have sometimes inspired overzealousness in interpreting microbial traits as adaptive cooperation (Nadell *et al.*, 2008). For most microbes, it is most helpful to think of a clonal lineage as an individual organism (**Fig. 1**). This approach can help explain the evolution of traits and behaviors – like programmed cell death – that are impossible to justify from the perspective of a single cell without overlooking the major role played by conflict between competing lineages.

## Microbial Cooperation

Just as lions band together in prides or wildebeest in herds, most microbes are gregarious and live in groups. Many microbes cooperate within species to gather resources, move, attack, or defend themselves in ways that would be impossible for single cells. As in animals, key to understanding the evolution of intraspecific cooperation in microbes is distinguishing between cooperation that results in a direct fitness benefit for the cooperators and altruistic cooperation, wherein an individual pays a net fitness cost to confer a fitness benefit on a recipient.

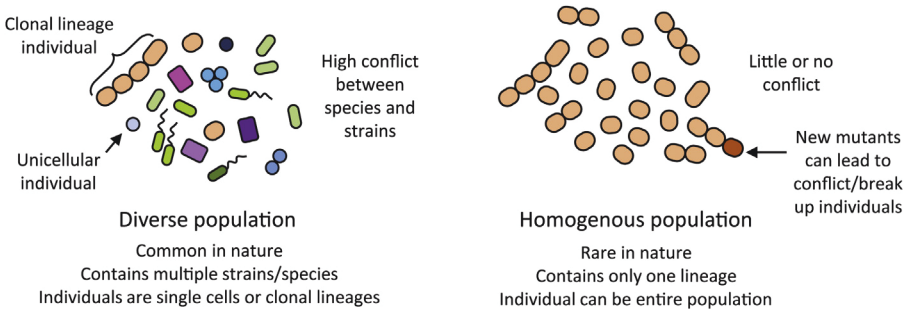


**Fig. 1** Microbial individuals. Microbial individuals are usually single cells or clonal lineages. Genetic differences between strains or species drive conflict between the members of most natural microbial communities. In rare situations when populations are clonal, the entire population may be considered a single individual. Even these, however, break down as mutations occur and introduce new variation.

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Cooperation driven by direct fitness benefits can often be thought of as synergy achieved by performing a task cooperatively that might otherwise be performed alone. Such interactions result in all cooperators gaining fitness from the decision to cooperate, and are widespread in microbes and larger organisms alike. Often more interesting, however, are cases of altruistic cooperation, wherein at least some cooperators incur a net decrease of fitness from cooperating. Altruistic interactions within colonies of ants – wherein most individuals entirely sacrifice their own reproduction to facilitate their queen's – puzzled Charles Darwin, for how could selection result in a trait which reduced fitness? Darwin speculated that selection on family groups may hold the explanation. A century later, this idea was expanded on and formalized as the concept of kin selection on inclusive fitness (Hamilton, 1964). An individual's inclusive fitness incorporates not only the impact of its traits on its own reproduction, but also the impact on relatives who may share the genes underlying those traits. Hamilton's Rule mathematically describes the conditions under which costly – even suicidal – traits can be selected for, and has proven key in understanding altruistic behavior in microbes and macrobes alike.

Kin selection as formalized by Hamilton's Rule emphasizes the importance of three parameters: the cost of a trait to an actor, the benefit of the trait to the recipients, and the relatedness between actors and recipients. Estimating these parameters, especially in natural contexts, is crucial but often non-trivial because costs and benefits are not absolute measures but relative to the selfish alternatives of donor and recipient. Costs and benefits of microbial interactions can vary considerably. The most extreme examples involve – like the ants that puzzled Darwin – some participants sacrificing all fitness, either by specializing in exclusively non-reproductive tasks or even actively destroying themselves. For such costly traits to evolve, the benefits they achieve must be substantial and directed as much as possible to close relatives. Mixed populations of multiple species or even just multiple strains of a single species should not generally evolve altruism.

High relatedness is key for most altruistic traits and there are many ways to achieve it. Mechanisms that direct benefits preferentially to relatives (or harm preferentially to non-relatives) are collectively known as kin discrimination (Strassmann *et al.*, 2011). Simplest and probably most important of these for microbes is limited dispersal. When dispersal is limited, local interactions are most likely to occur between close relatives. Many microbes reproduce clonally and move slowly, resulting in patches of genetically identical cells descended from a single progenitor, especially when a small propagule disperses to a previously-unoccupied patch. At high densities, limited space can drive spontaneous segregation of low-relatedness populations into high-relatedness sectors, such that cooperating strains are likely to interact with cooperating relatives and non-cooperators with non-cooperators (Nadell *et al.*, 2010).

More complex kin discrimination mechanisms increase relatedness via differential effects on relatives and non-relatives. In animals, kin discrimination is often a function of memory or learning, such as when ants and other social insects guard their colonies against conspecifics lacking the correct cues. Without brains, microbes must take different approaches. Some microbes resist mixing with non-relatives – unrelated colonies of the bacterium *Proteus mirabilis* and many other bacteria create distinct boundaries called Dienes lines at points of contact, rather than merging into a larger group (Budding *et al.*, 2009). Other microbes express adhesion molecules which facilitate aggregation with other cells bearing the same adhesion factors (Smukalla *et al.*, 2008). Still other microbes benefit relatives by destroying non-relatives via the secretion of bacteriocins that kill cells lacking the correct immunity gene. Such systems destroy conspecific competitors but leave close kin unharmed, and can increase local relatedness and facilitate the evolution of cooperative traits.

When relatedness is low, exploitation can follow and destabilize altruistic traits. Even an altruistic trait that strongly benefitted a population of cooperators is vulnerable in competition with individuals who benefit from cooperation but do not pay the costs to cooperate themselves. Such individuals gain individual fitness benefits at the expense of the population as a whole. Economic and evolutionary theory calls this phenomenon the tragedy of the commons, and it has been a special focus of evolutionary biology for decades to explain why such conflicts do not preclude the evolution and maintenance of cooperation. Much empirical work suggests that in microbes, at least, they often do.

Microbes perform many biological processes by secreting chemicals into their environment. These chemicals can metabolize resources, defend against attack, facilitate microbial movement, or even communicate information, but they also render many microbes especially vulnerable to the threat of exploitation. Secreted chemicals are energetically expensive and public – their benefits can be enjoyed not just by the producer but by neighboring cells as well – and thus in microbes even many apparently non-social functions gain a social element. Combined with microbes' fast generation times and high mutation rates, this lack of privatization makes exploitation a particular obstacle to cooperation in many microbes, and in fact mutants deficient in cooperative traits are frequently isolated from laboratory, wild, and clinical microbial populations (Dénervaud *et al.*, 2004; Rainey and Rainey, 2003; West and Buckling, 2003).

## How Microbes Use Sociality

### Obtaining Extracellular Resources

Not all resources can be directly drawn from the environment. Often resources exist in an unavailable form, tied up in molecules too large or unwieldy to be imported into the cell. Many microbes secrete exoproducts – often protein enzymes – to liberate these resources. For example, *Myxococcus xanthus* and its relatives are soil bacteria that actively prey on other bacteria by secreting biolytic toxins to kill and enzymes to digest their prey (Daft *et al.*, 1985).

Another family of exoproducts are siderophores, chemically-diverse macromolecules produced by most major bacterial lineages in order to sequester iron from their environments (Hider and Kong, 2010). Though iron is one of the most abundant elements on

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earth, the vast majority of it exists in the biologically-unavailable ferric form ($Fe^{2+}$), and so for many microbes it is a crucial limiting resource. Diverse bacteria solve this limitation by producing and secreting siderophores which bind ferric iron with extremely high avidity. Once bound, siderophores are taken up again into the cells. Cytoplasmic enzymes catalyze the conversion of ferric iron into ferrous iron ($Fe^{3+}$), nondestructively removing it so that the siderophores can be secreted and used again. Siderophores allow microbes to survive in environments with very low iron concentrations, including inside the bodies of macrobe hosts.

Exoenzymes and siderophores are relatively energetically expensive to produce and secrete, and once secreted can diffuse away from the cell that produced them and benefit its neighbors. As such, these are well-studied model systems for the evolution of altruistic cooperation and exploitation. *Pseudomonas aeruginosa* strains that produce siderophores can grow in iron-limited media, but readily evolve non-producing mutants (West and Buckling, 2003). Non-producers lose their ability to grow without exogenous iron, in competition against producers have a higher growth rate due to not incurring the energetic costs of siderophore production. In a well-mixed liquid culture in iron-limited media, non-producers will ultimately outcompete producers to the point of causing a population crash. Such outcomes exemplify the evolutionary instability that can result from exploitable cooperative traits.

Another way microbes cooperate to obtain resources is via cross-feeding mutualisms, typically between species specializing in metabolizing different substrates. This can entail one or more participants actively metabolizing waste products from the others. Interactions like these benefit the participants either by one partner directly providing the other with substrates or by one partner consuming end products such that a metabolic pathway in the other is more energetically favorable. Waste byproduct cross-feeding interactions do not require participants to pay a cost and so generally do not risk the evolution of exploitation (Seth and Taga, 2014).

One very interesting intraspecific cross-feeding interaction takes place between cells in the clonal filaments produced by some species of cyanobacteria (**Fig. 2(a)**). Viewing one of these filaments under magnification makes it immediately obvious that the constituent cells exist in multiple differentiated forms. Strung along the filament of vegetative cells like beads are large, spherical heterocysts – terminally-differentiated, non-photosynthetic cells specializing in nitrogen fixation (Kumar *et al.*, 2010). Heterocysts use nitrogenase to fix biologically unavailable diatomic nitrogen ($N_2$) into ammonia ($NH^{4+}$), which is shared along the filament through channels between adjacent cells. The purpose for this specialization is unusually clear-cut – nitrogenase and its accessory proteins are extremely sensitive to the oxygen produced by photosynthesis, and so a cell can either fix carbon or fix nitrogen, but not both. Other cyanobacterial species address this strong tradeoff temporally rather than spatially, switching back and forth between nitrogen fixing and photosynthesis, but by delegating the tasks to separate cells, filamentous cyanobacteria achieve both processes with greater efficiency. In addition to requiring sugars from their photosynthetic neighbors, heterocysts are incapable of dividing, and so can only have evolved via indirect fitness effects. Like sterile worker ants or the non-reproductive somatic cells that make up most of a multicellular eukaryote's body, heterocysts sacrifice all future fitness to assist their relatives' reproduction.

## Chemical Communication, Cues, and Coercion

Microbes speak a chemical language. Like animals, microbes coerce one another, respond to cues, and even engage in true communication. Explaining the evolution of communication is a difficult problem microbiologists have inherited from animal behaviorists. Why should any organism spend energy to send signals for the benefit of another, particularly between species? Why should a signal's recipient respond honestly? Kin selection can offer an explanation for some intraspecies communication, but between species the risk of exploitation should often preclude true communication.

Complicating the issue is a lack of semantic clarity. In evolutionary biology parlance, true communication is a trait or behavior that influences another organism and that has evolved primarily for this purpose in both sender and recipient (Diggle *et al.*, 2007a). Key here is that in true communication, both parties receive a fitness benefit. Establishing this is often difficult, which has led to the word communication being misapplied to traits that are actually cues (wherein the recipient responds to a trait the sender did not send for that purpose, as when a shark hones in on the electric pulses produced by its prey's nervous system) or coercion (wherein a sender selfishly manipulates a recipient, as when a bola spider mimics moth pheromones to attract prey into its reach). True communication, cue, and coercion are not exclusive categories, and a single trait can function as more than one in different contexts.

The most widely studied such example in microbes is quorum sensing, a potentially social regulatory mechanism that many microbes use to detect and respond to local cell density (**Fig. 2(b)**; Fuqua *et al.*, 1994). Quorum sensing cells constitutively produce autoinducers – small molecules that can readily cross the cell membrane via diffusion or active transporters. As populations increase in density, local autoinducer concentrations increase commensurately. Once autoinducer concentrations rise above a threshold (a 'quorum' of cells), they bind to and activate cytoplasmic transcription factors that in turn upregulate gene expression throughout the genome. With quorum sensing, cells can maintain separate suites of genes for living in low cell density and high cell density environments.

Genes controlled by quorum sensing are many and varied (in the bacterium *Pseudomonas aeruginosa*, more than 10% of the genome is controlled by quorum sensing (Schuster and Greenberg, 2006)), but often are involved in social traits. Most bacterial genes for public goods are controlled by quorum sensing, including production of exoenzymes, antibiotics, biofilm matrix components, and conjugation machinery, as are genes responsible for swarming motility, type VI secretion-mediated killing of competitors, and the formation of biofilms. Typically quorum sensing is believed to primarily benefit participants by allowing them to refrain from producing expensive exoproducts or engaging in social behaviors when there are too few cells present to realize their benefits (Darch *et al.*, 2012). An exoenzyme, for instance, may only be effective enough to recoup the cost of its production if its total concentration is above a threshold. With quorum sensing, cells can suspend production until there are enough cells to reach that

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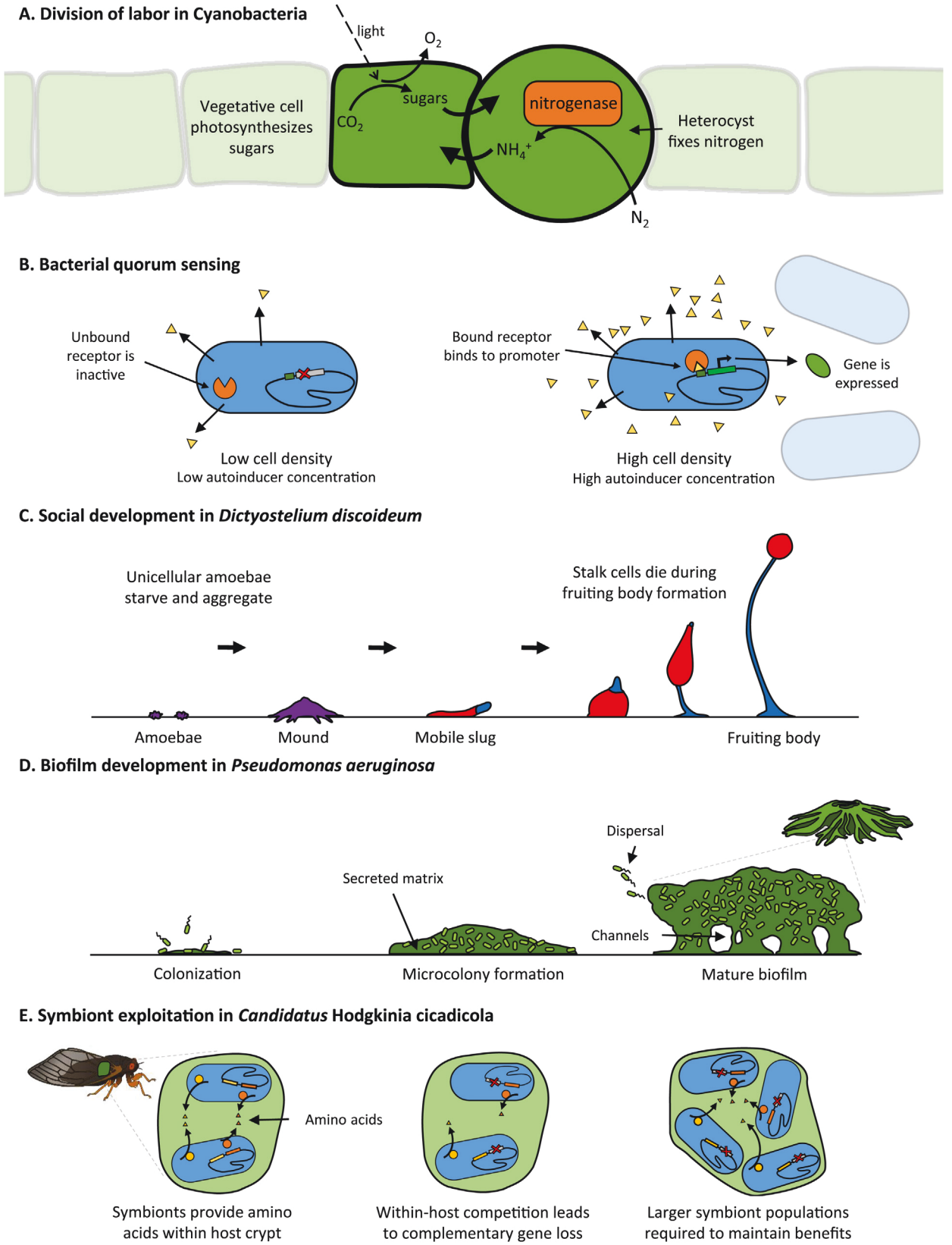


**Fig. 2** Example microbial interactions. (A). Division of labor in Cyanobacteria. Some cells within clonal filaments differentiate into heterocysts (large, round cell, right). Heterocysts abandon oxygen-producing photosynthesis in order to fix nitrogen with the oxygen-sensitive enzyme nitrogenase. Vegetative and heterocyst cells divide labor by exchanging sugars and nitrogen. (B). Bacterial quorum sensing. Quorum sensing cells constitutively secrete autoinducer (yellow triangles). At low cell densities, autoinducer concentration remains low and autoinducer-activated transcription factors (orange circles) are inactive. At high cell densities, autoinducer binds to and activates transcription factors, which in turn bind to and activate gene

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threshold. In pathogens, quorum sensing often controls virulence factor production, and may help cells coordinate 'sneak attack' strategies wherein they remain avirulent and hidden from the host immune system until overwhelming numbers can be mustered (Parsek and Greenberg, 2000).

Quorum sensing is very widespread among bacteria, and similar mechanisms have been found in archaea, fungi, other eukaryotic microbes, viruses, and potentially even multicellular eukaryotes. Many bacteria even maintain multiple quorum sensing circuits in parallel – *P. aeruginosa*, for instance, uses no fewer than seven separate circuits arranged in a hierarchical network. The near-ubiquity of quorum sensing poses interesting questions, as quorum sensing is both a cooperative trait and a communication trait. Given that cells must incur a cost to produce and respond to autoinducer, quorum sensing should be vulnerable to exploitation (Diggle *et al.*, 2007b). Quorum sensing-deficient mutants are common in both experimental populations and in clinical isolates of pathogens (Dénervaud *et al.*, 2004). Of these, 'signal-blind' mutants which produce autoinducer (and so encourage other cells to produce quorum sensing-regulated public goods) but are incapable of responding to it themselves are especially prevalent (Diggle *et al.*, 2007a). Within clonal or near-clonal populations, kin selection on indirect benefits may maintain quorum sensing, but the potential of exploitation to undermine quorum sensing's role for coordinating social traits has led some researchers to propose non-social explanations for its evolution (Redfield, 2002). There is evidence that autoinducers may serve other functions beyond signals of cell density, which may provide sufficient direct benefits to hinder the evolution of quorum sensing-deficient strains in nature. In some bacteria, quorum sensing regulates essential genes in addition to genes for facultative social traits. Quorum sensing signal-blind mutants in *P. aeruginosa* are deficient at synthesizing adenosine, imposing a direct metabolic tradeoff (Dandekar *et al.*, 2012). A similar mechanism in the same species involves the quorum sensing-controlled production of cyanide and a protein conferring immunity to cyanide toxicity (Wang *et al.*, 2015). Cooperating cells produce both cyanide and the immunity protein when in high density conditions. Signal-blind strains cannot produce the immunity protein and are killed by cyanide produced by the cooperators. Cyanide production in *P. aeruginosa* can be thought of as a sanctioning behavior used by cooperators to punish defectors, akin to similar behaviors observed in social insects and macaques.

Kin selection offers a compelling mechanism for maintaining quorum sensing in populations of relatives, but it cannot explain interspecific communication. While quorum sensing systems are widespread across bacteria, most are highly specific, involving autoinducer/receptor pairs discerning enough not to respond to quorum sensing signals produced by other species. A few, however, are more promiscuous. The autoinducer AI-2 is produced by bacteria in many taxa, which has driven speculation that it may function to coordinate actions across multispecies populations like recruitment and development in the complex polymicrobial biofilms that create dental plaques (Kolenbrander *et al.*, 2010). Other studies have shown that *Burkholderia cepacia* growing in the lungs of patients with cystic fibrosis can upregulate virulence factors in response to the autoinducers produced by *P. aeruginosa* (Eberl and Tümmler, 2004). However, without evidence that these interactions have evolved due to fitness benefits in both participants, these are not compelling examples of true communication. A more likely explanation is that *B. cepacia* is using autoinducers produced by *P. aeruginosa* for its own purposes as a cue, or that *P. aeruginosa* is using its autoinducers to manipulate *B. cepacia* into producing virulence factors for its own benefit (Diggle *et al.*, 2007a).

## Cooperative Movement and Dispersal

Microbes use diverse mechanisms to move through their environments, whether as part of their normal lifestyle – like a leopard that must move across its territory in search of prey – or to disperse their offspring – like marine invertebrates releasing gametes to be distributed by ocean currents. Many of these mechanisms require the cooperation of many cells working in concert.

Isolated bacteria move through liquid environments with rotating, turbine-like flagella, but across solid surfaces, surface tension makes this impossible. Many bacteria solve this limitation with a collective motion behavior called swarming motility (Kearns, 2010). Swarming bacteria – like the predatory soil-bacterium *Myxococcus xanthus* – aggregate into large groups called rafts, which produce and secrete surfactants into their environment in order to disrupt surface tension. Cells within rafts link together and develop multiple flagella, which, when rotated, allow the entire raft to pull itself along the surfactant-lubricated terrain. Swarming motility allows *M. xanthus* cells to search for prey in the soil much faster than if they were working alone.

*Myxococcus xanthus* also depends upon cooperation to produce spores and disperse. *M. xanthus* and its relatives undergo a complicated life cycle including both unicellular and multicellular stages (Velicer and Vos, 2009). *M. xanthus* normally inhabit the soil in large, diffuse groups called wolf packs, moving in distinctive rippling patterns via swarming motility and secreting exoenzymes to kill and digest other bacteria. When food resources run low, however, cells must work together to produce spores and disperse to

expression. (C). Social development in *Dictyostelium discoideum*. Upon starving, vegetative amoebae aggregate into a mobile, multicellular slug, which moves through the soil. Fruiting body formation follows, during which a minority of the cells (blue) sacrifice themselves to develop into a stalk to hold the remainder of the aggregate (red) as it develops into durable spores. (D). Biofilm development in *Pseudomonas aeruginosa*. Cells adhere to and colonize a surface through a combination of active migration and division. Secreted extracellular matrix components (dark green) accumulate into a complex structure. Cells detach and disperse from the biofilm's upper layer via autolysis of cells in the lower layer. (E). Symbiont exploitation in *Candidatus* Hodgkinia cicadicola. Hodgkinia cells live within specialized cells in the abdomens of 17-year cicadas (*Magicicada tredecim*) and produce amino acids (yellow and orange triangles) required by their hosts. Competition between strains within a single host favors fast growth rates, selecting for loss of amino acid production. In time, strains fragment into multiple complementary lineages, each producing only a fraction of the necessary amino acids. The host must accommodate increasingly large symbiont populations to maintain sufficient amino acid production.

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greener pastures. Starving cells aggregate into mound-like groups – sometimes containing multiple unrelated strains – and differentiate to produce a fruiting body. Most (more than 90%) of the cells in the aggregate sacrifice themselves, either to produce a stumpy structure around the base of the mound, or autolyzing themselves to liberate resources. Only the final ten percent survive, climbing atop the mound of their dead companions and developing into desiccation- and starvation-resistant spores. Other, less-well-studied myxobacteria produce more elaborate fruiting bodies with tall, branched stalks or densely clustered sori. The formation of the myxobacterial fruiting body and the requisite sacrifice of most of the colony appears to facilitate dispersal by lifting spores out of the soil. The unusual way that these multicellular structures are formed – by aggregation of potentially unrelated cells instead of clonally from the division of a single cell as seen in multicellular eukaryotes – has interesting evolutionary consequences. Myxobacteria aggregates can be chimeric, containing multiple different genotypes, and thus there is potential conflict between genotypes over which cells will make the requisite sacrifices to produce the fruiting body's stalk. Genotypes that do not contribute fairly readily evolve in laboratory studies, and can in some cases outcompete the cooperating wild type cells to the point of rendering the entire aggregate incapable of sporulating. Evidence for the importance of this threat is found in myxobacteria's elaborate kin discrimination behaviors – many genotypes of *Myxococcus* can recognize their own kin and will not aggregate with strains from a different incompatibility type.

Other microbes work together to produce mobile multicellular bodies. The social amoeba *Dictyostelium discoideum* is a eukaryote with a facultatively multicellular life cycle somewhat similar to that of *Myxococcus xanthus*, with starving cells aggregating and many of them sacrificing themselves to produce a tall fruiting body to maximize dispersal of spores (**Fig. 2(c)**; Strassmann and Queller, 2011). Before fruiting, the multicellular aggregate assembles into a large, motile slug, which can crawl through the soil many times faster than the individual amoebae from which it is made and find an optimal spot to fruit. The benefits of moving in a group need not be only mechanical either – some microbes can work together to gain senses. Magnetotactic bacteria found in hyper-saline lagoons form hollow sphere-shaped colonies and align their magnetic-crystal-laden inclusion bodies such that the entire colony has a net magnetic moment. Thusly assembled, the colony can sense and use magnetic fields to navigate through the water column (Keim *et al.*, 2004).

## Defending Against Attack

For many organisms, there is safety in numbers. Defense against attack, especially via predation by larger organisms, is believed to be one of the central selection pressures that has driven the independent evolution of multicellularity in disparate microbial taxa. Algae in the presence of a flagellate predator readily evolve a multicellular lifestyle in the laboratory, growing in clusters that help them resist predation (Boraas *et al.*, 1998).

One very prominent collective defense employed by many bacteria involves growing in dense, sessile colonies called biofilms (Hall-Stoodley *et al.*, 2004; **Fig. 2(d)**). Biofilms place bacteria of a single species or multiple species in close proximity within a secreted extracellular matrix. A biofilm matrix facilitates bacterial adhesion, but also concentrates and retains water or nutrients and forms a physical barrier that can protect the cells within from physical and chemical attack. Cells growing in a biofilm often exhibit markedly increased resistance to desiccation, predation, and toxic chemicals. Biofilm-forming pathogens are of considerable medical significance due to their role in various chronic infections, and their resistance to conventional antibiotic therapies make them difficult and expensive to treat.

We now understand that biofilm growth is the primary lifestyle for most microbes (Hall-Stoodley *et al.*, 2004). Biofilms can be found growing on virtually any surface, including in soil, on the surface of solid particles suspended in water or air, and on the integument and within the bodies of larger organisms. Often a biofilm's structure will be as simple as a layer or pile of cells, but some biofilms grow elaborate morphologies with tower-like structures interspersed with a network of fluid-filled channels that facilitate the movement of water and nutrients into the biofilm's interior. Far from being simple voids, some of these channels even drive active circulation via differential water loss from the biofilm surface (Wilking *et al.*, 2013). Polymicrobial biofilms can feature defined strata of species with different metabolic roles, going from aerobic species on the biofilm exterior to strict anaerobes in the deepest levels (Okabe *et al.*, 1996).

The ubiquity and complexity of some biofilms has led some researchers to liken them to multicellular organisms like plants and animals, with different cells within the biofilm coordinating tasks to altruistically benefit the population as a whole. Social evolution theory gives reason to doubt this interpretation, however, particularly for biofilms composed of multiple species. Many of the collective behaviors of biofilms are more parsimoniously explained by direct benefits to the constituent cells – that is, cooperation in a biofilm is often mutualistic rather than altruistic (Nadell *et al.*, 2008). Much of the structural complexity seen in biofilms need not reflect coordinated signaling between cells as it does in animal or plant tissues, but rather individual cells optimizing their behavior to suit different microniches within the biofilm. Cells growing deep in the interior of the biofilm are exposed to a different environment than that seen by cells on the periphery, with lower resource concentrations and more waste products. Further, conflict between cell lineages within a biofilm can drive structural complexity by incentivizing competitors to grow upwards towards higher nutrient concentrations (Xavier and Foster, 2007) or by specialists competitively excluding cells from microniches (Picioreanu *et al.*, 2004). Often biofilms are more akin to a community of individual cells rather than a single multicellular organism.

Nonetheless, when immobilized within a biofilm, bacterial populations are structured, which can facilitate the evolution of cooperative traits. When cells are embedded in a matrix and unable to move, their neighbors are likely to be disproportionately clonemates. Local relatedness can thus be high, and bacteria more likely to be affected by local biotic and abiotic features of their environment than if they were free-swimming. In laboratories, some single-species bacterial biofilms show robust division of labor

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that may represent altruistic cooperation. In *Bacillis subtilis*, interior and peripheral cells specialize in different tasks (Liu *et al.*, 2015). Peripheral cells have greater access to most resources to support growth, but periodically suspend growth to allow nutrient concentrations in the interior to recover. In return, interior cells supply the peripheral cells with ammonia and act as a protected nest egg from which the biofilm can recover if attacked by antibiotics. *Pseudomonas aeruginosa* biofilms grown under nutrient-limited conditions form distinctive mushroom-like structures, with bulbous crown cell aggregates suspended atop narrow stalks (Klausen *et al.*, 2003; **Fig. 2(d)**). Crown and stalk cells represent distinct subpopulations – stalk cells are non-motile and grow first, after which the motile crown cells climb atop the stalk. Stalk and crown cells have very different gene expression patterns, with stalk cells producing most of the biofilm's necessary public goods and perhaps even autolysing to support the growth and dispersal of the crown cells. In these examples, comparing the biofilm population to a single clonal multicellular organism may be appropriate.

## Chemical Warfare

Microbes engage in constant chemical warfare. Competing strains deploy a diverse arsenal of chemical weapons, either secreted into the environment (Riley and Wertz, 2002) or injected directly into competing cells with syringe-like secretion systems (Coulthurst, 2013). Many of these mechanisms have social elements. Often genes involved in toxin production and deployment are regulated by quorum sensing, only activating when cells grow at high density and their effects are maximized. Sometimes the targets of microbial attacks are other species – predators, prey, or competitors – but some systems specialize on killing conspecifics.

A famous example of the latter are the bacteriocins, a wide category of biocidal peptides produced by many bacterial taxa (Riley and Wertz, 2002). Bacteriocins specifically destroy or inhibit the growth of closely-related strains while leaving untouched any cells carrying the correct immunity protein. The most well-known bacteriocins are the colicins produced by gut bacteria like *Escherichia coli* and its relatives. Colicin systems typically involve three components – the toxin itself, which destroys DNA or tears holes in cell membranes, an immunity protein that confers specific immunity to the toxin, and a lysis protein which causes a fraction of colicin-producing cells to autolyse and deploy their deadly cargoes. The fact that cells must die to release their colicins makes clear that selection for the production of colicins must act on indirect fitness benefits rendered to the producing cells' surviving relatives. By killing conspecifics that do not carry the correct immunity protein, colicin-producing cells reduce competition on the cells that do, which are likely to be kin. While colicins are not a kin recognition system under the strictest definition (they cue onto the presence or absence of the immunity protein rather than kinship per se), by killing competing lineages they can create local patches of high relatedness and may thereby facilitate the evolution of other cooperative traits.

Colicins drive other social dynamics as well. Producing colicins – and the immunity proteins that prevent collateral damage – is energetically expensive. Microbes must leverage the metabolic costs of producing colicins and immunity proteins with the threat posed by competing strains' attacks. These costs likely explain why strains do not accumulate immunity proteins to all colicins simultaneously. Further, the cost of colicin production and resistance can drive a complex nontransitive relationship between colicin-producing cells, non-producing sensitive cells, and non-producing cells resistant to the colicins' effects. Under some conditions, a 'rock-paper-scissors' like dynamic is achieved, where producers outcompete sensitive cells by killing them with colicins, resistant non-producers outcompete producers by not spending energy producing colicins, and sensitive non-producers outcompete resistant non-producers by not spending energy producing immunity proteins (Kerr *et al.*, 2002; Kirkup and Riley, 2004).

## Interacting With Macrobe Hosts

In addition to the microbe–microbe interactions that are the focus of this review, many microbes interact with eukaryotic hosts. All macrobe life evolves and lives against a dense backdrop of diverse microbes, and in recent decades there has been a surge of interest in how host-associated microbes impact host health (McFall-Ngai *et al.*, 2013). Microbes and their hosts can be mutualists or antagonists, their associations can be long-lasting or transient, and they can be horizontally or vertically transmitted. The evolution of conflict and cooperation between a host and a microbial symbiont can be described by many of the same ideas as are applied to microbe-microbe or macrobe-macrobe interactions. Further, often microbes' effects on the hosts they infect are themselves social phenomena that depend upon cooperation between microbes. In such situations interactions occur – and selection can operate – at multiple levels simultaneously.

Some microbes are pathogens that work together to parasitize macrobe hosts like humans. These interactions can be mild and chronic or brief and lethal, and the factors that influence virulence are complex. For many pathogens, virulence is a cooperative trait. Some bacterial pathogens, like *Pseudomonas aeruginosa*, band together to form biofilms which protect them from the host's immune system (as well as antibiotic treatment) (Bjarnsholt *et al.*, 2009). Many virulence-related functions are controlled by quorum sensing (Rutherford and Bassler, 2012), which allows pathogens to coordinate their actions or hide critical antigens from the immune system until sufficient numbers have grown. Some pathogens attack their hosts with secreted toxins which are, from the pathogens' perspective, public goods (and accordingly often controlled by quorum sensing.)

Other pathogens go to more elaborate lengths, sacrificing their own fitness for the cause. One striking example is found in infections of the enteric pathogen *Salmonella enterica* serovar Typhimurium, which infects the guts of humans. Infecting populations of *S.* Typhimurium are made up of distinct subpopulations that play different roles in the infection's overall pathogenesis (Diard *et al.*, 2013). Though transmission to a new host depends upon passing through the intestine, approximately one third of the cells suicidally invade gut tissue rather than remaining in the lumen. In the gut tissue the invading subpopulation secretes a cocktail of inflammatory chemicals, reducing its growth rate and triggering the ire of the host immune system. The host inflammatory response

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modifies the gut lumen, wreaking havoc on non-pathogenic bacteria growing within and benefitting the relatively-resistant two thirds of the *S.* Typhimurium population left behind. Gut-invading *S.* Typhimurium cells sacrifice themselves to trigger the inflammatory state that the non-invasive subpopulation needs to flourish.

Not all microbial symbionts harm their hosts. Some microbes work together to provide benefits to their hosts in exchange for shelter or resources. Some of the best known host-microbe interactions are between sap-feeding insects like aphids and mutualistic bacteria like *Buchnera aphidicola*, which live inside of specialized organs within their hosts and provide amino acids not present in the hosts' nutrient-poor diets (Buchner, 1965). Other hosts depend on microbes to break down molecules in their food – bacteria in the guts of termites, cows, and even humans lend their metabolic flexibility to the task. By producing or digesting molecules not usable by macrobes directly, microbes can enable their hosts to live in niches that would otherwise be impossible.

Other microbes can endow their hosts with new abilities. One famous example is the relationship between the Hawaiian bobtail squid *Euprymna scolopes* and the marine bacterium *Vibrio fischeri* (Nyholm and McFall-Ngai, 2004). *V. fischeri* normally lives in ocean water but can selectively colonize crypts within the squid's mantle. When *V. fischeri* populations inside the crypt reach a sufficient density, a quorum sensing circuit activates the expression of the luciferase gene, producing light. The squid host uses *V. fischeri's* bioluminescence to counter-illuminate itself at night, matching the moonlight coming from above to obscure its silhouette to predators swimming beneath it. Because bioluminescence is energetically expensive, populations of *V. fischeri* growing in squid crypts are theoretically vulnerable to invasion by mutants that do not contribute to light production. How the mutualism is protected from exploitation is an active area of research, but appears to be in part driven by the squid regularly evicting and reacquiring the bacteria from its environment – non-luminescent strains are pleiotropically deficient at colonizing the squid's crypts (Visick *et al.*, 2000).

Other host/microbe interactions are more vulnerable. A striking example can be seen in the symbiosis between the long-lived cicada *Magicicada tredecim* and its intracellular bacterial symbiont *Candidatus* Hodgkinia cicadicola, which produces crucial amino acids for its host (**Fig. 2(e)**; Campbell *et al.*, 2015). The enzymatic pathways that synthesize these amino acids come at a cost to the bacteria, and while collectively the bacteria cannot abandon their duties without killing their host and themselves in the process, there is competition within each host that favors *Ca.* Hodgkinia strains that cut costs and produce fewer benefits. This can lead to within-host divergence of a single *Ca.* Hodgkinia lineage into as many as seventeen separate complementary lineages, each only producing one or a few amino acids and effectively defecting from production of all of the others. With no way to replace underperforming symbionts, the cicada must maintain populations of all of these lineages simultaneously. Here, conflict between bacterial symbionts within a host results in the non-adaptive evolution of reduced benefits for the host.

## Conclusion

Microbes are social for many of the same reasons macrobes are. As in macrobes, interactions between microbes enable them to solve the challenges of life and impose new challenges of their own.

Microbes are worthy of study in their own right. They are ancient, ubiquitous, and of immense ecological significance. They are relevant to human health and agriculture. Most of Earth's history has been spent dominated by microbes, and the argument can be made that they continue to dominate it now.

Microbes also have a lot to teach us about the broader concepts of biology. Many exciting and powerful experimental techniques depend upon the unique properties of microbes, and allow us to test evolutionary and ecological theory at scales that would be impossible for any other organism. The line between microbes and macrobes is fading as we learn more about the origins of eukaryotic life, the prevalence of horizontal gene transfer in our evolutionary history, and the antiquity of physiological complexity. Generalizing our understanding of social evolution in macrobes by incorporating it into a framework that includes microbes is crucial. Rather than regarding microbes as a fringe group deserving of a few special exceptions to the rules developed for macrobes, we should regard macrobes as particularly sophisticated colonies of mostly harmoniously cooperating microbes.

*See also:* **Landmark Studies:** Dictyostelium, the Social Amoeba. **Social Behavior:** Ant, Bee and Wasp Social Evolution; Division of Labor; Kin Selection and Relatedness; Social Evolution in "Other" Insects and Arachnid.

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