The evolution of social behavior in microorganisms

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Recent studies of microorganisms have revealed diverse complex social behaviors, including cooperation in foraging, building, reproducing, dispersing and communicating. These microorganisms should provide novel, tractable systems for the analysis of social evolution. The application of evolutionary and ecological theory to understanding their behavior will aid in developing better means to control the many pathogenic bacteria that use social interactions to affect humans.

Ecologists and evolutionary biologists accustomed to working among macroscopic creatures might find the world of microorganisms a very unusual place. However, many recent discoveries in microbial ecology and evolution would be strangely familiar to macrobiologists; for example, cooperation (see Glossary), division of labor, eusociality, cheating, complex communication networks, high genetic relatedness and recognition of kin correlates have all been found in creatures lacking both neurons or nephrons.

Here, I review these remarkable new findings within the context of social phenomena that are already well known in vertebrates and invertebrates, describing how microorganisms are now known to demonstrate all of the hallmarks of a complex and coordinated social life.

Cooperation and division of labor
Cooperation represents the core of sociality. Macroscopic social organisms cooperate to protect themselves from enemies or the elements, and to secure food, reproduction or dispersal to a new locality (Box 1). The cooperation can be egaliitarian, in that all individuals contribute and gain more or less equally, or it can involve division of labor, whereby individuals engage in different tasks from which they might obtain different rewards, directly or via benefits to kin1. Although habitat, morphology and behaviors are profoundly different in microscopic creatures compared with macroorganisms, the selective pressures for sociality often appear to be the same in both groups.

Shelter
One of the most familiar features of vertebrate or invertebrate social cooperation is a burrow, nest, hive or gall (e.g. ant, termite or bird nests, rodent burrows, beehives or aphid galls), usually made by the animals and that provides shelter and sometimes food2. The microbiologist’s conventional view of microorganisms suggested that a similar situation could not exist in such organisms; however, recent work has changed this view dramatically. Many bacterial species have recently been found to create and inhabit structures called microbial biofilms2–4. Biofilms have been found in a variety of habitats, including all nutrient-sufficient aquatic systems, such as on the hulls of ships, in sewage-treatment plants, on our teeth, sometimes in our lungs, and on medical devices and prostheses, where they contribute to chronic infections5–7. Some oceanic microbes inhabit polymeric structures, similar to biofilms, which have been described as comprising the fabric of the oceans8.

Biofilms exhibit organized structures with complex three-dimensional shapes, which might include single bacterial species or specific sets of metabolically complementary species. Within these biofilms, different regions also exhibit differences in gene expression and phenotype3. As with nests, hives, burrows or galls, biofilms can also serve as sites for offspring production: for example, biofilms formed by Pseudomonas bacteria shed planktonic, disperser cells into the water under a diurnal rhythm, and phototrophic Rhodobacter biofilms even release cells in response to intercellular signals9.

The extracellular polymer matrix of biofilms provides protection from desiccation, toxins and antibiotics, and it might also serve to bond and hold nutrients and enhance physiological stability9. However, the cooperative nature of biofilm production and utilization has yet to be firmly established; are some cells in a colony specialized for polymer production, or do all cells contribute? Does the complex structure of biofilms reflect cooperative division of labor, within or between species? Further studies of the adaptive significance of microbial biofilms are needed, especially given their role in human infections and tooth decay.

Foraging
A second form of cooperation found in macroscopic social organisms involves food acquisition. Some vertebrates, such as lions, wolves and wild dogs, cooperate to subdue prey larger than themselves. Others animals, such as bark beetles, engage in temporally coordinated mass attacks on living trees10. Microorganisms are also found to exhibit cooperative foraging, comparable in its sophistication to that seen in macroscopic social organisms. For example, myxobacteria, such as Myxococcus xanthus, engage in mass attacks on microbial prey, which are overwhelmed by force of numbers, broken down with bacterial enzymes and consumed11,12. Many pathogenic bacteria of humans, such as Salmonella and Staphylococcus, only release virulence factors once they have reached high enough numbers to have a better chance of withstanding the human immune
Do microorganisms exhibit cooperation in reproduction? Recent discoveries indicate that they do so in remarkably diverse ways. For example, Rhizobium bacteria form nodules in association with the roots of some plants, wherein they fix nitrogen (N) for the plant and gain carbon (C) in return\(^{16,21}\). In some Rhizobium spp., the bacteria in this association have lost the ability to reproduce, whereas in other species, the bacteria do not reproduce while fixing N for the plant, but retain the capacity to do so after the nodule breaks down\(^{21}\). In both cases, the free-living Rhizobium just outside of the nodule benefit from the C obtained via their encased, altruistic nodule inhabitants\(^{13,21}\). Some cyanobacteria exhibit a similar division of labor related to food acquisition: in response to N limitation, some cells in a linear colony develop into 'heterocysts', which become morphologically and biochemically specialized for N fixation, and, as a result, irreversibly lose the ability to reproduce\(^{23}\).

A similar loss of reproductive ability also occurs at the sub-microorganism level, in the mitochondria of plants suffering from cytoplasmic male sterility (CMS)\(^{22,23}\). The source of cytoplasmic male sterility in Petunia has recently been traced to mitochondria, which deteriorate suicidally in developing male reproductive tissues (which are reproductive dead ends for mitochondria), apparently causing their death while increasing the reproduction of ovular mitochondria\(^{23}\), which might be clonemates.

Programmed suicide might also function in defense: in E. coli, cells attacked by bacteriophage will stop producing a short-lived antidote to a long-lived toxin that they have also been producing, thereby bringing about their own demise with that of the phage\(^{24,25}\). Such suicide could prevent nearby clonemates from also being attacked by the phage, in the same way that parasitized pea aphids in colonies suicidally drop from host plants\(^{16}\). Moreover, some phages produce an anti-death chemical that prevents degradation of the bacteria-produced antidote\(^{24,25}\). In E. coli, cell suicide might also serve as an adaptation to food limitation, in that E. coli starved for amino acids or C will undergo programmed death, thus possibly providing more food for the remaining bacteria\(^{17}\).

Growing colonies of E. coli exhibit a complex structure, with some areas undergoing cell death, and reproduction being limited to a small number of cells at the colony edge\(^{22}\). In some strains of this species, a small proportion of cells in a colony suicidally produce large quantities of chemicals called colicins, which kill bacteria of competing strains and species that lack the ability to detoxify these chemicals\(^{24}\).

Although each of these cases of programmed death involves plausible benefits to surviving adjacent clonemates, such benefits have seldom been
Microorganisms exhibit two other important dispersal mechanisms in addition to fruiting-body formation: swarming and wafting. Swarming, found in species such as Proteus mirabilis, Serratia liquefaciens and Bacillus subtilis, involves differentiation into forms specialized for group swimming\(^{13,17}\). Some species swarm in monospecific groups, whereas others form groups comprising cells of two or more metabolically complementary species\(^{17}\). Wafting into the air using the wind provides an obvious means of long-distance dispersal and, in some marine microorganisms, it might entail cooperation in the production of large amounts of dimethylsulfide (DMS), which facilitates being lofted by wind\(^{36}\). Indeed, Hamilton and Lenton\(^{36}\) suggest that selection could have led to altruistic, specialized DMS-producing morphs of dinoflagellates, especially if these species form colonial patches.

**Communication**

Among social animals, communication can be chemical, visual or physical, and it serves to coordinate activities, delineate group membership or identify individuals or their roles in society\(^1\). Such exchange of behaviorally useful information has also been found to characterize numerous groups of microbes. In some Myxococcus and Dictyostelium, cells aggregating to form fruiting bodies have long been known to engage in chemical communication mediated by secreted chemicals and physical contact, to coordinate their multicellular development\(^{11,12,29,31}\). More recently, communication systems involving continual secretion of specific chemicals into the surrounding environment, and detection of the local concentration of these chemicals to assess and react to local cell density, have been discovered and characterized in a wide range of bacteria; such communication has become known as ‘QUORUM SENSING’ or ‘autoinduction’ (Box 2). In all cases of this phenomenon, quorum sensing appears to represent a mechanism for adaptive cell–cell communication. However, it has yet to be shown experimentally that the traits controlled by quorum sensing are more effective at higher cell densities.

One puzzling feature of quorum-sensing systems is that although some are species-specific, others are shared between species in a genus or even between genera, such that some bacteria might be detecting the signaling molecules of heterospecifics\(^{37–39}\). Could such cross talk be adaptive to both the sender and receiver, or might it involve cheating, deception, or exploitation, as in many animal communication systems\(^{37–40}\)? Future research on the adaptive significance of quorum sensing in complex natural environments and on how such signals can be disrupted\(^{13}\) (as some marine algae are able to do\(^{41}\)) should answer these questions.

**Genetic structure**

Some social animals, including gall aphids, sea anemones, polyembryonic wasps with soldier morphs,
Many species of bacteria have recently been found to demonstrate the capacity to sense and respond to variation in their local density by the release and uptake of signaling molecules. Such ‘quorum sensing’ or ‘autoinduction’ modulates a wide range of microbial activities, such as biofilm differentiation, nodulation, heterocyst differentiation, swarming, plasmid conjugal transfer, interstrain competition, virulence factor production, initiation of chromosome replication and antibiotic biosynthesis4–6. For example, in Vibrio fischeri, a bioluminescent bacterium that inhabits special organs in some fish and squid, a quorum sensing system involving the secretion of N-acetyl-L-homoserine lactones allows the colonizing bacteria to increase in numbers to a density sufficient to meet the light requirements of the host, before bioluminescence activity is initiated6. By contrast, in Myxococcus xanthus, quorum sensing involves secretion of peptides, which allows cells to determine whether there are sufficient starving individuals locally to complete fruiting-body formation7.

One of the most important consequences of bacterial quorum sensing for humans is its role in infection. Quorum-sensing systems have evolved independently in many bacteria, but they have also been lost: some have been so long removed from their natural environment. A similar loss of clonal, with gene exchange between the same or related species occurring only rarely. This was presumably because they have been so long removed from their natural environment. A similar loss of social behavior has been produced experimentally in M. xanthus1.

References
via extremely high levels of reproduction\textsuperscript{45}. Some bacteria, such as cyanobacteria with sterile heterocyst cells, are connected like beads on a string, and so remain with donemes throughout development\textsuperscript{13}. Other more mobile forms, such as E. coli, Proteus mirabilis, and some slime molds, exhibit apparent forms of kin recognition, whereby different colonies exclude one another on contact\textsuperscript{42,46,47}. Similar reactions have been found among Myxococcus spp., which exhibit interspecific territory, apparently to defend exclusive rights to their food sources\textsuperscript{12}. Just as high genetic relatedness or clonality can foster cooperation, mixing of different clones provides opportunities for conflict and cheating, as has been shown for D. discoideum and M. xanthus\textsuperscript{32-34}. Additional studies of kinship in microorganisms will require analysis of genetics and behavior in the natural environments of the rhizosphere, metazoan hosts or biofilms, and they should provide novel tests of social evolution theory as well as increasing our understanding of medically important pathogens\textsuperscript{13}.

**Conclusions and future research**

We are now in an era when molecular technology allows rapid and efficient discovery of the social behavior characterizing bacteria and other microorganisms. The social phenomena uncovered so far allow the first direct comparisons between microorganisms and macroorganisms (Table 1), which reveal convergences in behavior that are clearly suggestive of adaptation. But so far, studies of microorganisms have resulted mainly in a typological understanding of proximate mechanisms, with a focus on species-level cooperation and assumption, rather than demonstration, of adaptation\textsuperscript{2,9}. By contrast, behavioral macrobiologists, with their focus on ultimate causes, intraspecific variation in behavior, and the tension between competition and cooperation, have long been preoccupied with the elucidation of adaptive significance\textsuperscript{1}. Future studies of social behavior in microorganisms would benefit greatly from experimental analyses of the fitness benefits and costs of cooperation, application of social behavior theory developed for macroorganisms\textsuperscript{48-50}, and development of new theory and models directly applicable to microbes\textsuperscript{51-53}. Research areas of particular promise include the study of the roles of cheating and its suppression in the evolution of sociality\textsuperscript{54}, and kin recognition. Are there bacterial strains or species specialized for exploitation of the resources produced by other bacteria, in habitats such as biofilms, human infections and soil\textsuperscript{155}? How common is recognition of donemes versus nondonemes, and does it serve to exclude cheaters? Cooperation and division of labor involving microbiology, ecology, and evolutionary theory should lead to accelerated progress in understanding social worlds both large and small.
References


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