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 egalitarian, 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 kin[1]. 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 food[1]. 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 BIOFILMS[2–4]. 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 infections[3,5–7]. Some oceanic microbes inhabit polymeric structures, similar to biofilms, which have been described as comprising the fabric of the oceans[8]. 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 phenotype[3]. 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 signals[3,6].

The extracellular polymer matrix of biofilms provides protection from desiccation, toxins and antibiotics, and it might also serve to bind and hold nutrients and enhance physiological stability[9]. 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 trees[10]. 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 consumed[11,12]. 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

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Box 1. Examples of division of labor and hypothesized division of labor in microorganisms

Colony structure in *Escherichia coli* and other bacteria
Some bacteria exhibit complex spatially organized cellular differentiation in their colonies, which might function to maximize growth rates and enhance survival and reproduction.

Cytoplasmic male sterility-inducing mitochondria
These matrilineally transmitted mitochondrial variants, present in some plants, cause suppression of male function, which results in reallocation of plant resources towards female function and presumed increased transmission rates of the variants. The mitochondria that cause the suppression die in the process.

Dimethyl sulfide (DMS)-mediated dispersal in dinoflagellates
In some dinoflagellates, some individuals in a clone have been hypothesized to specialize altruistically in the production of DMS.

Fruiting body formation in Myxobacteria and *Dictyostelium*
In developing fruiting bodies, some cells lyse (possibly providing nutrients to other cells), some cells develop into the nonreproductive stalk (which elevates the spores and apparently aids their dispersal) and some cells develop into the spores themselves.

Microbial biofilm structure
Biofilms exhibit complex three-dimensional structures, such as channels for influx of fluid, and differential gene expression in different regions. Such variation might indicate cellular division of labor, with cells in different parts of the biofilm specializing in different roles.

Nitrogen fixation in cyanobacterial heterocysts and nodulating bacteria
In both of these taxa, some cells become specialized for nitrogen fixation and temporarily or permanently lose the ability to reproduce.

Programmed cell death in *E. coli* and other bacteria
A small proportion of cells in a colony specialize in the production of antimicrobial compounds (colicins or bacteriocins) that kill competing strains or species but whose production results in the death of the producing cells. Programmed cell death might also occur in the context of *E. coli* colony morphogenesis.

response[13]. Finally, *Pfiesteria* dinoflagellates act as ambush predators, synchronously releasing toxins to kill all fish over many km^2, after which the dinoflagellates feed on the carcasses[14]. Other, less spectacular forms of apparent cooperation in foraging include coaggregation by metabolically complementary species of bacteria such as aerobic and anaerobic species[2–4,7], and complex growth forms in *Escherichia coli* that result in colony structures that could maximize feeding ability[16,17].

Reproduction

The study of cooperation in reproduction has long been a central focus of evolutionary biologists, often because it engenders the paradoxical evolution of ALTRUISM and reduced reproduction by some individuals. Among social vertebrates and invertebrates, cooperative reproduction involves helpers, which normally engage in foraging, building or defense of the colony, all of which increase the fitness of the reproductives (e.g. aphids, termites, ants and naked mole rats)[1].

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[18–21]. 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[18–21]. 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[15].

A similar loss of reproductive ability also occurs at the sub-microorganism level, in the mitochondria of plants suffering from CYTOPLASMIC MALE STERILITY[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[26]. Moreover, some phages produce an antideath 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[27]. 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

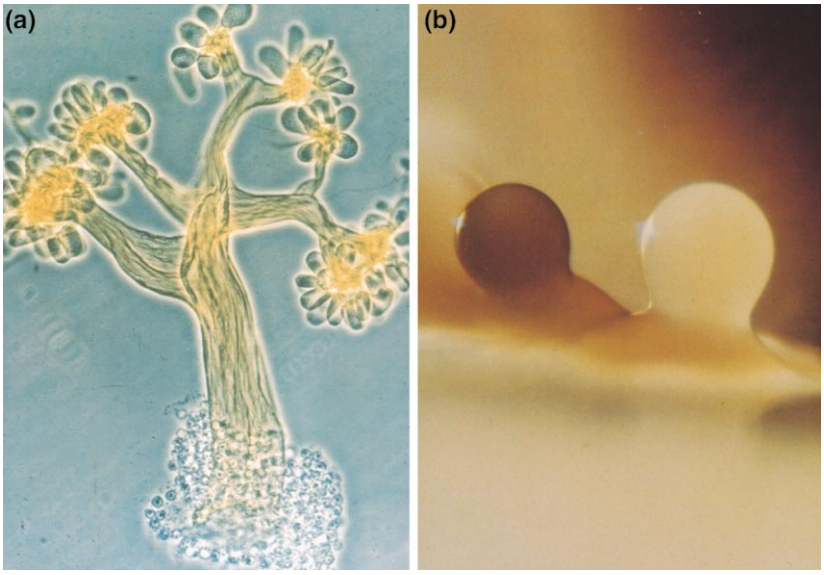

Fig. 1. Fruiting bodies of Myxobacteria species. (a) *Chondromyces crocatus* (~850 μm high) and (b) *Myxococcus fulvus* (~250 μm in diameter). Myxobacteria undergo complex, coordinated development in the formation of fruiting bodies, within which some cells altruistically develop into a nonreproductive stalk, while others develop into reproductive cells (spores).

demonstrated[28]. Further experimental tests utilizing suicide-deficient and suicide-capable strains in competition under various environmental conditions are required to elucidate the adaptive significance of this remarkable trait.

Dispersal

A final context for cooperation is dispersal to new habitats, a problematic process for bacteria given their limited powers of locomotion. Cooperative dispersal has long been known in *Dictyostelium* slime molds, which exhibit chemically coordinated aggregation in response to starvation, followed by formation of fruiting bodies that help to lift cells above the substrate, where they might, as spores, be better dispersed[29,30]. In some species of *Dictyostelium*, cells in the stalk of the fruiting body do not differentiate into spores, whereas in other species, the stalk is either acellular or all the stalk cells develop into spores, so that all aggregants can develop and disperse[29,30]. Myxobacteria exhibit similar dispersal behavior: in response to famine, they pile together into well-structured mounds or other forms (Fig. 1), within which some cells differentiate into spores, others apparently lyse, perhaps providing food for the survivors, whereas other cells differentiate into a population known as peripheral rods, which are hypothesized to protect the developing fruiting body from attacking microbes[31].

Strains of *Dictyostelium* and *Myxococcus* have recently been discovered and characterized that are over-represented among the spores in mixed-strain laboratory cultures[32,33]. These strains have been called cheaters because they exploit the division of labor involved in fruiting-body production. Moreover, Strassmann *et al.*[34] demonstrated that nonidentical genotypes are common in *Dictyostelium discoideum* fruiting bodies in nature, and that cheaters do indeed exist in these social amoebae; cheaters are also found in some colonies of social bees[35].

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[15,17]. 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 dimethyl sulfide (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 clonal 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 crosstalk 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,

Box 2. Quorum sensing: social communication systems in bacteria

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 biosynthesis[a–d]. 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*-acyl-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 initiated[e]. 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 formation[f].

One of the most important consequences of bacterial quorum sensing for humans is its role in infection. In various *Pseudomonas, Staphylococcus*, *Salmonella*, virulent *E. coli* strains and other human pathogens, quorum sensing apparently facilitates bacterial invasion by coordinating the timing of attack, allowing bacteria to escape when they become 'cornered' by the body in an abscess or other localized area, or by allowing the pathogens to reach a critical density before they turn on their virulence genes[a,b,g]. Such a buildup might allow the pathogens to delay initiating a full host immune response until they are much more capable of resisting such a response. Indeed, the regulatory systems of autoinduction differ between pathogenic and nonpathogenic strains of bacteria[h,i] and, possibly, the evolution of a particular form of efficient communication network could be an important factor in the acquisition of virulence status itself.

Quorum-sensing systems have evolved independently in many bacteria, but they have also been lost: some laboratory strains of *E. coli* no longer express their natural, evolved system of social signaling[i], presumably because they have been so long removed from their natural environment. A similar loss of social behavior has been produced experimentally in *M. xanthus*[j].

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and brain worms with a suicidal behavioral morph that invades the brain of the host, exhibit clonal social groups, wherein the benefits of altruistic behavior accrue to genetically identical individuals[1,42]. As such, these groups resemble the cooperating cells of a metazoan body, except that the clones comprise separated rather than attached cells or individuals. This distinction is important because separated clones are much more vulnerable to infiltration by cheater genotypes of the same species[42]. Moreover, all clonal social animals also exhibit sexual reproduction during part of their life cycle, which might aid in defense against parasites[42]. Among nonclonal social animals, within-group genetic relatedness levels are usually substantial (i.e. 0.25–0.75) in eusocial and cooperatively breeding groups, whereas they might be lower in communal forms such as some bees and wasps[1].

What is the genetic structure of bacterial colonies and how might it relate to their social behavior? Until recently, bacteria were presumed to be essentially clonal, with gene exchange between the same or related species occurring only rarely. This was supported by genetic studies for some species, such as *E. coli*, *Haemophilus influenzae*, *Neisseria meningitidus* and some *Salmonella*[43,44]. Social evolution in these species must proceed predominantly under interclonal selection of behavioral traits, because genes for social behavior cannot be transferred between colonies. However, other bacterial species, such as *Neisseria gonorrhoeae* and some *Rhizobium* spp., exhibit considerable levels of gene flow[43,44], although this occurs in *Rhizobium* with a high degree of local population subdivision[21] and in *N. gonorrhoeae* with periodic epidemics, both of which might lead to interpretations of strong clonality without an understanding of the most appropriate spatial or temporal scales[44].

Few links have been made between genetic population structure and social behavior in microorganisms, but what little is known supports the idea that high genetic relatedness facilitates cooperation. Thus, in *Rhizobium*, the nodules are initiated by a single invading cell[19] and 'private', unique food sources produced by the nodule cells might preferentially benefit kin outside the nodule[19,20]. In *M. xanthus*, spores are extremely sticky and might co-disperse with relatives as well as nonrelatives[33] and in *Vibrio fischeri*, bioluminescent organs are colonized by few, probably unrelated individuals that apparently compete fiercely at first

Table 1. Convergent social phenomena in microorganisms and animals

	Microorganisms	Animals	Refs
Domicile creation	Biofilms in many bacteria	Burrows, nests, hives and galls in many social animals	1–7,9
Cooperative hunting or attack	*Myxobacteria* *Pfiesteria* Some pathogens	Wolves, lions and bark beetles	10–14
Specialized food provisioners	*Rhizobium* Cyanobacterial heterocysts	Social insect foragers	1,15,18–21
Specialized defenders	Myxobacteria peripheral rods? Colicin-producing *Escherichia coli*	Aphids, thrips, ants, bees, wasps and sea anemones	1,11,24,25
Specialized dispersal forms	Swarming Fruiting bodies	Naked mole rat dispersers; winged ants and termites	1,15,17,29–34
Altruistic suicide	*Escherichia coli* Cytoplasmic male sterility mitochondria	Sting autotomy in honey bees and wasps; pea aphids	23–26
Communication via chemicals	Quorum sensing in many bacteria	Pheromones in most social animals	13,37–41

via extremely high levels of reproduction[45]. Some bacteria, such as cyanobacteria with sterile heterocyst cells, are connected like beads on a string, and so remain with clonemates throughout development[15]. 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[42,46,47]. Similar reactions have been found among *Myxococcus* spp., which exhibit interspecific territoriality, apparently to defend exclusive rights to their food sources[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*[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[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[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[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[48–50], and development of new theory and models directly applicable to microbes[51–53]. Research areas of particular promise include the study of the roles of cheating and its suppression in the evolution of sociality[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[55]? How common is recognition of clonemates versus nonclonemates, 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.

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Glossary

Altruism: behavior that involves a fitness cost to one individual or cell (the altruist) and a fitness benefit to another individual or cell (the recipient of the altruistic act).
Cheating: engaging in behavior that exploits the cooperative behavior of conspecifics by imposing fitness costs on them, while providing fitness benefits to the cheaters.
Colicins: chemicals released by *Escherichia coli* cells that kill competing strains and species, but whose production results in the death of the colicin-producing cells.
Cooperation: multiple individuals or cells engaging in a common task for mutual benefit.
Cytoplasmic male sterility (CMS): matrilineally transmitted suppression of male function in plants caused by mitochondrial variants, which usually coexist in populations with autosomal genes that repress it. CMS-determining factors increase their reproduction through enhanced production of ovules by the plants that bear them.
Division of labor: two or more classes of individual or cell engaging in specialization involving complementary, synergistic behavior.
Eusociality: a social system that involves two or more discrete and permanent categories of cells or individuals, one of which reproduces less because it helps the other to reproduce.
Genetic relatedness: the probability that an allele (at a polymorphic locus) involved in the expression of social behavior in one individual or cell (the actor) is identical by descent with the allele in the recipient of the social act.
Heterocysts: cyanobacterial cells that are specialized for nitrogen fixation and have lost the ability to reproduce.
Microbial biofilms: thin layers of bacterial colonies on moist or submerged surfaces in nutrient-rich habitats, which are covered by an extracellular polymer matrix that the bacteria have secreted around them.
Quorum sensing: release and uptake of signaling molecules by bacteria, which allows facultative adjustment of behavior (e.g. gene expression, secretion of chemicals, reproduction) in relation to local cell density
Swarming: swimming together, for a prolonged period, of groups of individual bacterial cells that have become specialized for long-distance locomotion; after swarming, the cells dedifferentiate into their usual form.

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