Kin Discrimination and Cooperation in Microbes

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Abstract

Recognition of relatives is important in microbes because they perform many behaviors that have costs to the actor while benefiting neighbors. Microbes cooperate for nourishment, movement, virulence, iron acquisition, protection, quorum sensing, and production of multicellular biofilms or fruiting bodies. Helping others is evolutionarily favored if it benefits others who share genes for helping, as specified by kin selection theory. If microbes generally find themselves in clonal patches, then no special means of discrimination is necessary. Much real discrimination is actually of kinds, not kin, as in poison-antidote systems, such as bacteriocins, in which cells benefit their own kind by poisoning others, and in adhesion systems, in which cells of the same kind bind together. These behaviors can elevate kinship generally and make cooperation easier to evolve and maintain.

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INTRODUCTION

Kin discrimination is the differential treatment of related individuals compared with unrelated individuals of the same species. Traits that cause one individual to aid another at a cost to itself cannot evolve unless it benefits others who have genes for those same traits. With kin recognition or discrimination, individuals can assist relatives, harm or ignore nonrelatives, and coordinate group activity (27, 106).

Recognition seems likely to be particularly important to microbes because they undertake many processes extracellularly in the public sphere that larger organisms privatize inside. Microbes secrete substances for nourishment, movement, virulence, iron acquisition, protection, and biofilm production (11, 19, 31, 33, 48, 63, 111, 118, 119). Other kinds of microbial cooperation involve specialization among individuals of a population, with some taking on potentially fatal altruistic roles, such as forming a stalk, that benefit others (108). These kinds of cooperation have clear analogs with cooperation in macroorganisms such as social insects.

There is a large literature documenting the importance of kin recognition to social interactions in animals (21, 27, 35, 36, 73, 82, 106), and this area has fueled a more recent upsurge of research in microbes (11, 77, 115, 118). We proceed by presenting the theoretical background, reviewing the microbial research, and discussing how microbes change our thinking about recognition.

Kin selection: selection that results from effects on relatives, such as altruism; usually described by Hamilton's Rule

THEORETICAL BACKGROUND

Kin Selection and Hamilton's Rule

It is not easy to evolve cooperative or altruistic traits, but it is possible under certain conditions. One of those conditions concerns genetic structure. A genetic allele that causes help to others can be favored by selection if the help goes to others who share the allele, a process known as kin selection (49, 50). Kin selection has been instrumental in understanding sociality in groups such as insects (107) and vertebrates (18). In microorganisms, it can explain, or promises to explain, many kinds of social interactions.

How kin selection works is summarized by Hamilton's rule and the associated concept of inclusive fitness (8, 34, 49, 50, 88, 118). Hamilton's rule sums up all the fitness effects of a behavior, each with a relatedness correction. Hamilton's rule shows how altruism is favored. Suppose actor individuals (X) experience an average fitness cost c_x but give average fitness benefit b_y to others (Y). This trait is favored if $c_x + r_{yx}b_y > 0$, where r_{yx} is the relatedness of the actor to beneficiaries and measures the probability above random chance that the beneficiaries carry the actor's gene. The concept explains the evolution of worker sterility in social insects, whose colonies consist of relatives (107). It also

explains why social amoeba cells (*Dictyostelium*) sacrifice their lives to build a stalk (40).

The equation also accounts for other types of behavior. If the behavior benefits the actor as well as the recipient, as in secretion of invertase by yeast (45), then Hamilton's rule is $b_x + r_{yx}b_y > 0$. Cooperation is selected more strongly if it benefits kin, but it can even be favored if $r_{yx} = 0$ because of benefits to self. A selfish behavior that benefits the actor but harms others is favored when $b_x + r_{yx}c_y > 0$. Relatedness can moderate selfishness, but even in the most cooperative of social insects, the honey bee, sister queens fight to the death over who gets to head the colony (41). Spiteful behavior, in which the actor gives up fitness to harm others (y), can be favored only if it also aids other relatives (z): $-c_x - r_{yx}c_y + r_{zx}b_z > 0$. For example, suicidally releasing bacteriocins to kill unrelated competitors can be favored if they eliminate competitors of relatives (38).

Genetic Relatedness

Relatedness does not measure absolute gene sharing, but the degree to which alleles are shared above random expectation, based on the population of the allele's competitors. Affecting others at random allele frequencies does not change frequencies (**Figure 1*a***).

Genetic relatedness: the fraction of genes shared between two individuals above the fraction held in common by the population of potential interactants

What matters is similarity above random levels at the locus under social selection, but

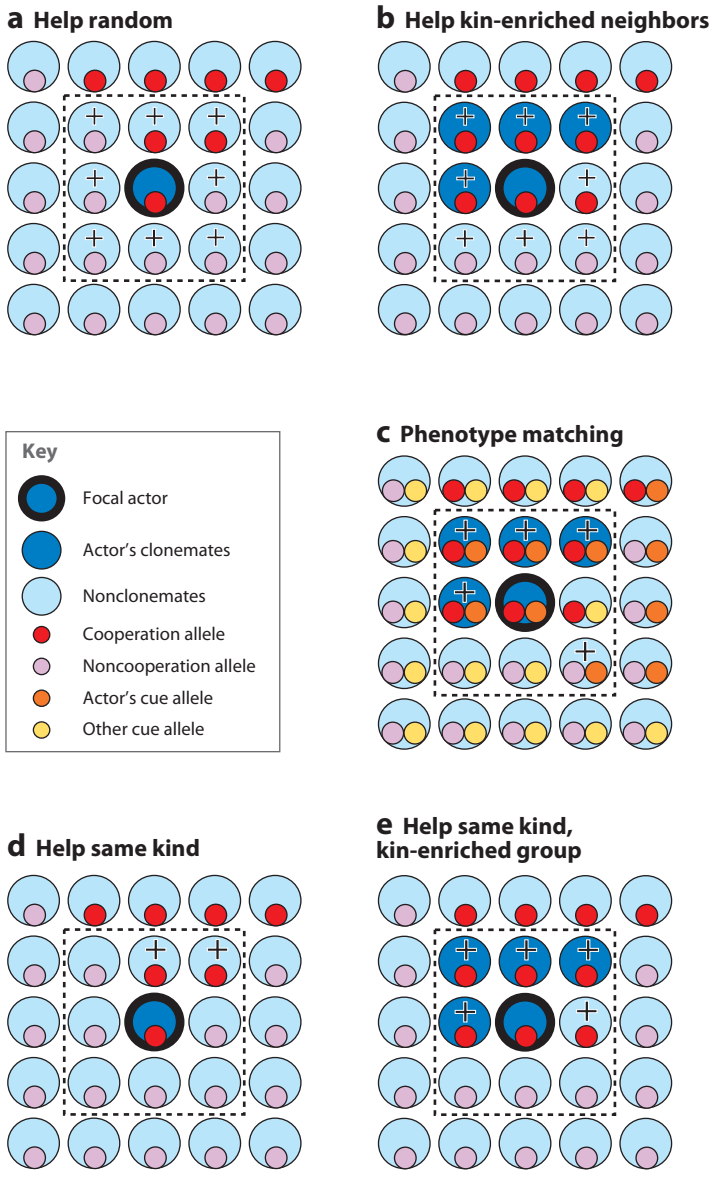

Figure 1

Each plot of 25 individuals represents a population, with the focal actor (*heavy border*) performing an action that benefits (*plus sign*) everyone within a local neighborhood (*inside the dashed line*). Individuals outside the dashed line represent the rest of the population (which is usually much larger in number). (*a*) The cooperator benefits neighbors who are genetically like the whole population, so these benefits do not favor either allele. (*b*) The cooperator's neighborhood includes four clonemates who must have the cooperator red allele; the rest of the beneficiaries are a random sample of the population. Here, benefits go preferentially to the cooperative allele so cooperation can be favored. Relatedness to beneficiaries is one-half because half of them bear the cooperator allele, above the random level. (*c*) Phenotype matching using genetic cues at a different locus (*orange versus yellow*) in a population that has the same configuration as panel *b*. The actor uses identity at its cue locus (*orange*) to target its beneficiaries, which has the effect of increasing the proportion of clonemates among the beneficiaries from four-eighths in panel *b* to four-fifths in panel *c*. (*d*) Greenbeard or kind discrimination, in which the individual helps others of its kind, and cooperation can be favored even with no clonemates in the neighborhood. (*e*) Kind discrimination results when clonemates are present.

Broad-sense kin discrimination: all mechanisms that lead to differential treatment of neighbors according to kinship, including kind discrimination

Cue: a genetic trait that is used to recognize relatives

Phenotype matching: using matches at other loci to identify relatives

this is often the same across loci because similarity is determined by pedigree connections. Thus, in diploids, full siblings are related by one-half, half siblings by one-fourth, first cousins by one-eighth, and so on. These relatedness values can be determined by tracing gene-passing probabilities on pedigrees. Microbes differ in two ways. First, these particular relationships may not matter (in bacteria without meiosis-based sex) or matter only rarely (in eukaryotes with occasional sex). Second, the most important distinction is often whether partners are clonemates, descended mitotically from a recent common ancestor. Moreover, we argue that microbes more often show discrimination based on relatedness at a social locus, which may not be the same as relatedness at other loci. This works for all genes if the social locus is used primarily to differentiate clonemates from nonclonemates.

Although we focus here on a kin selection theoretical approach, there is an alternative group selection approach. We simply note that group selection also requires genetic variation between groups, which is equivalent to relatedness within groups (89). Therefore, the issues addressed by this paper are just as relevant to the group selection perspective.

The importance of relatedness in microbial interactions is well documented. For example, in *Pseudomonas aeruginosa* both cooperative iron uptake (48) and quorum sensing (25) are favored more at high relatedness, effects seen not only in the laboratory but also in infections in mice (51). Further microbial examples of the importance of relatedness include altruistic stalk formation in social amoebas (40), fast versus efficient feeding in yeast (72), toxin production in *Escherichia coli* (69), and invertase secretion in yeast (47).

What is Recognition and Discrimination?

There is no universal agreement on what constitutes kin recognition. In the narrow sense of recognition that requires a cognitive process, likely involving neurons, microbes have no kin recognition. Yet they do often manage to direct benefits of cooperation preferentially to kin, and we need a vocabulary to address this question. To begin, we define a broad category of broad-sense kin discrimination to describe the whole range of ways that kin are preferentially affected, compared with the rest of the population, regardless of mechanism.

Discrimination can be based on either environmental or genetic cues. For example, if neighbors tend to be relatives, the environmental cue of proximity can suffice: Simply help all neighbors within a certain distance. This is the situation diagrammed in **Figure 1*b***. This is often augmented by other environmental cues, giving environmental kin discrimination. An example for a nesting bird might be its natal nest—any other nestling in that location will be a sibling. For a bacterium, cues that it is growing in liquid would likely indicate a more random structure unsuitable for cooperation, as in **Figure 1*a***, whereas cues that they are on a substrate may indicate a structured environment with kin, as in **Figure 1*b***.

Genetic cues, or labels, can improve discrimination through a process called phenotype matching (54, 55, 73, 103, 116). In animals this usually involves learning. For example, an animal learns its own smell and later compares that to the smell of unfamiliar individuals. **Figure 1*c*** shows how a cue can improve the identification of relatives, who are more likely to share a cue allele, or any allele, with the actor. In animals, there is considerable evidence for such matching via learning (4, 68, 75). The process of phenotype matching involves three components (4). First, there is the production or acquisition of cues (110). Second, these cues must be detected and matched (74). Finally, some action is performed for sufficiently good matches (71). Microbes do not have the cue-learning mechanisms of animals, but we consider whether they have systems with similar effect.

Kin and Kind

Animals are thought to act on the basis of cues giving information about identity by descent

Table 1 Kin and kind discrimination

	Narrow-sense kin discrimination	Kind discrimination
Beneficiaries	Genealogical kin	Same trait or kind
Genetic identity	By descent only	All identities
Mechanism	Proximity or differential action	Usually differential effects
Genes	Multigenic	Often one, or linked complex
Relatedness	Same across genome	Higher at kind locus
Complex cooperation	Possible	Unlikely

due to genealogy, whether the cues are based on proximity, environment, or gene products. Because all genes derive from a common pedigree, this creates unanimity among the genes and can therefore support complex cooperation involving many genes. We call this narrow-sense kin discrimination to distinguish it from the broad sense that includes all mechanisms, including those mechanisms we think are more appropriately called kind discrimination because they specifically identify others with the same trait (**Table 1**).

Kind discrimination is usually discussed under the heading of greenbeard alleles, an idea that goes back to Hamilton (50) but is named after a thought experiment of Richard Dawkins, who imagined a gene that simultaneously produces a green beard, recognizes green beards in others, and directs aid toward them (22, 37, 50). Thus, a greenbeard gene effectively causes all three components that are carried out by different genes under phenotype matching, but it has some different consequences. **Figure 1*d*** shows that greenbeard discrimination can lead to cooperation even if there are no true relatives in the neighborhood, because the greenbeard gene helps copies of itself even in nonrelatives. When relatives are present, the greenbeard (**Figure 1*d***) more effectively targets bearers of its allele than phenotype matching based on other cue genes (**Figure 1*c***).

This kind of nepotism can still be thought of as kin selection and treated via Hamilton's rule, with the relatedness in a perfect greenbeard being 1 (37). But this is no longer genome-wide pedigree relatedness. A greenbeard is not identifying genealogical classes of *kin*, although kin may be included in the beneficiaries. It is identifying those of the same *kind*—whether the similarity be random or due to recent genealogical connections. Other alleles at this and other loci do not share this same high relatedness of 1 to the beneficiaries. Therefore, they will not be selected to behave as if relatedness is 1, but instead according to whatever pedigree relatedness applies to them.

Greenbeards were generally thought to be rare, though a few examples have been reported (37). Producing a trait, recognizing it in others, and acting appropriately seem to be a tall order for a single gene. But this way of framing it exaggerates the difficulty. It is not required for the individual to pick out kinds and perform differential actions to them. All that is really needed for kind discrimination is the expression of a trait that, for whatever reason, has differential effects on bearers and nonbearers of the trait. Frequency-dependent effects, which are common in microbes (70, 99), fulfill this condition (86, 87, 90). Here the costs and benefits of a behavior change with its frequency, because actors have different effects on their kind versus other kinds, albeit often indirectly. For example, nestlings that beg loudly can benefit all loud chicks purely at the expense of quiet nestmates when parent birds divide a fixed food amount according to begging intensity. Brighter warning coloration makes an individual more likely to be seen and eaten by naïve predators, but if it is eaten, it teaches the predator to avoid others with that coloration. Loud chicks and bright insects each benefit others of their kind, without ever taking differential actions toward them (86).

Narrow-sense kin discrimination: mechanisms that specifically target kin; includes proximity and phenotype matching, but not kind discrimination

Kind discrimination: mechanisms, such as greenbeard traits, that specifically target others who possess the same trait (including but not restricted to kin)

Table 1 summarizes the broad differences between kin and kind effects. We suggest that kind discrimination is common in microbes. For example, the *FLO1* in yeast gene codes for an adhesion protein that allows cells to clump and physically protect the interior cells against chemically harsh conditions such as treatment with solutions of peroxide, ethanol, or antibiotics (105). The benefits go to others with the gene, kind but not specifically kin, because those lacking the gene form weaker bonds and clump less.

SURVEY OF MECHANISMS

Microbes have multiple ways of differentially impacting others according to kinship or kind, ranging from simple proximity to much more complex forms of discrimination of kin or kind. We treat them roughly in order of sophistication, starting with proximity and moving toward more complex mechanisms.

Population Structure and Proximity

Cooperation evolves in the context of genetic population structure. The structure of interest is at the microscale—what an individual microbe will experience and specifically who it will affect by its behavior. Sadly, the most studied population structure is the least structured, and highly unnatural: the shaking Erlenmeyer flask containing a single clone. Natural aquatic habitats can have some physical structure at the scale of a bacterium. Indeed, water can be experienced more as a gel than as mixed liquids, in part because of the polymer gels that are so common in water (112), though the extent to which this elevates relatedness among neighbors is unknown.

Solid substrates afford much better opportunities for long-term high relatedness. The simplest natural way to generate high relatedness is through dispersal of propagules to unoccupied patches suitable for growth. When microbes grow out in clonal patches from a single originating cell or spore, secreted public goods will naturally reach only clonemates.

High relatedness can be generated not only by dispersal to empty habitats, but also by more conventional slow migration or growth. A remarkable series of simulations has shown that under low-nutrient conditions, cell proliferation alone can result in sorting into clonal populations (80). As a mixture of clones grows outward, sufficiently low nutrients mean that only a few cells succeed in moving out in any given direction, eventually causing one clone to dominate by random genetic drift (selection would tend to increase this effect). This sorting is sufficient to favor the invasion of a new altruist mutant that secretes a cooperative good that benefits neighbors. Thus, where natural conditions involve low nutrients on substrates, clonemates are often the main recipients of secreted products, even in the absence of any mechanism of recognition.

These mechanisms of broad-sense kin discrimination can be augmented by environmental cues, such as nutrient level, that provide information about how high local relatedness is likely to be. Such cues allow recognition and facultative cooperation in situations favoring cooperation, without any active distinction between kin and nonkin among interactants.

Unfortunately, there is remarkably little work on genetic population structure of free-living microbes in nature, particularly at the appropriate scale for microbial interactions (6, 30, 52, 113). In the free-living soil bacterium *Myxococcus xanthus*, 78 isolates were found in 100 samples taken from a 16 × 16 cm patch of soil, and made up at least 21 genotypes (113). *M. xanthus* is a predatory species with cooperating motility and predation that, when starved, cooperatively forms multicellular fruiting bodies. The isolated clones differed in social behaviors such as social motility and showed competition during fruiting body formation, with some clones cheating others (66, 114).

Similarly, in the eukaryote *Dictyostelium discoideum*, there is a great deal of genetic diversity among clones (30). *Dictyostelium* is a genus of eukaryotic haploid amoebae that live in the soil and prey on bacteria. When they starve, cells aggregate into a multicellular fruiting body

with fertile spores at the top of a stalk made up of dead cells. Cheaters, which produce more than their fair share of spores, are known from both natural isolates (14, 29, 108) and lab mutations (26, 101). It is in the interests of the altruistic stalk-forming cells to die only to benefit relatives. From a 25-m transect near Mountain Lake Biological Station in Virginia, 102 *D. discoideum* isolates were collected from 26 of 50 collected 0.2 g samples (30). Of these, 46 were genetically distinct, and 63% of the soil samples yielding isolates had more than one haplotype. Genetic relatedness within samples was 0.519 ± SE 0.014 (30). Thus, genetically distinct clones co-occur in nature at proximities likely to be close enough for coaggregation. These clones readily formed chimeras in the laboratory. Wild-collected clones from a similar location in North Carolina exhibited cheating, with some clones contributing more than their fair share of spore cells (29).

However, high rates of clonality were found in actual fruiting bodies, collected at Mountain Lake, mostly from isolated white-tail deer scat (40). Average relatedness within fruiting bodies was 0.86 to 0.975, depending on the sample and method used. The deer scat often contained multiple genotypes. Two qualifications should be mentioned about this rare study of relatedness among actual interactants. First, this method does not detect the 20% of cells that form a stalk. Second, as we note below, some of the high relatedness may be due not to pure proximity, but to different clones recognizing and segregating.

Population structure can also be revealed though bacteriocins, which are poisons that kill foreign clones (see below). In a natural population of *Xenorhabdus bovienii*, a bacterium carried by nematodes that kills insect hosts, bacteriocins of *X. bovienii* from the same soil sample did not harm other bacteria from that soil sample but did attack bacteria from samples only a few meters away (52).

Microbes that colonize larger organisms are of particular interest, both because colonization may be limited, resulting in high relatedness, and because the host organism provides a way for the microbes to help (or harm) each other over long distances. Most microbial social interactions are limited to short spatial scales, but when a microbe helps or harms its host, it may help or harm all the other microbes in its host, however far away they may be. Pathogen infections provide a common example. There is a large literature on multiplicity of infection, the number of different strains infecting a host (confusingly the term is also used for the number of infectious particles per cell). Here we simply note that there can be great variation even within the same species (94). Low multiplicity of infection should have higher cooperation, other things equal, although higher cooperation could lead to either greater or lower virulence (12).

Symbiotic microbes are sometimes sequestered in specific organs. For example, the Hawaiian sepiolid squid *Euprymna scolopes* has a light organ that has six separate crypts, each of which is colonized by a single light-producing *Vibrio fischeri* bacterium (121). Even more clonal are the many endosymbionts that are vertically transmitted as their host reproduces, as is the case with *Buchnera* (117).

Nonspecific Contact Prevention

In addition to clonal population structure, there is a possible mechanism for generating high relatedness that does not require overt detection of foreigners. As clonal colonies grow outward, they can encounter other colonies causing mixtures that will reduce relatedness, but such dilution could be reduced or eliminated by any mechanism that prevents contact. Such mechanisms often depend on specific genetic differences (see below) but they need not do so.

For example, two genetically identical colonies of *Paenibacillus dendritiformis* grow outward, but growth slows and stops where they approach each other, while proceeding in other directions (3). The mechanism involves subtilisin, a secreted serine protease (2). At low concentrations, subtilisin increases colony growth, perhaps by making proteins more available. Higher concentrations, for example, occurring

Poison-antidote system: a type of discrimination in which secreted poisons kill others lacking the antidote; the poisons are generally coded for by adjacent genes in gene complexes

from the summed contributions of two converging colonies, stimulate the release of Slf (sibling lethal factor), a 12-kDa protein that lyses *P. dendritiformis* cells. The system thus forms a boundary, keeping approaching colonies separate.

These studies do not report what happens between nonidentical colonies, but they would presumably behave similarly, provided they also produce subtilisin and Slf. Thus, one possibility is that the killing system evolved to keep nonclonemates at bay, and that preventing fusion of identical colonies is a side effect. Regardless of its evolved function, such a system would serve to keep colonies clonal, making cooperation easier to evolve and maintain.

The frequency of such contact inhibition is unknown. A similar system has been reported in aflagellate *hag* mutants of *Bacillus subtilis* (58). Under low-nutrient conditions, but not at high nutrients, colonies grow out in radiating tendrils, which avoid each other, as do tendrils from adjacent colonies. Perhaps a common mechanism underlies avoidance of tendrils and colonies, in which case dendritiform growth patterns might be an indicator of such colony contact inhibition.

As a cautionary note, these examples show that overt recognition cannot necessarily be inferred from rejection of foreign colonies alone. Fortunately, the standard Dienes test for recognition includes a control of two colonies of the same type. We now proceed to discuss cases of more overt recognition.

Specific Contact Prevention

Specific systems that block mixing with other clones, but not with self, are much better known. This requires some kind of specific marker that distinguishes clones, a role typically fulfilled by poison-antidote bacteriocins, discussed in the following section.

Perhaps the first indication of kin discrimination in bacteria came from the observation that two strains of the gram-negative bacterium *Proteus mirabilis* did not fuse but instead formed a visible boundary, now known as a Dienes line (13, 24). Production of a Dienes line is an indication of genetic difference, and this is often used in clinical typing of *P. mirabilis* infections (79). This segregation occurs during swarming, a cooperative movement involving secretion of surfactants (45, 62). Segregation may prevent these public goods from being exploited by cheaters.

Exactly how *P. mirabilis* cells recognize relatives is unclear and is likely to be multifactorial, depending on genes (39), bacteriocin production (67), and unidentified products of a special round cell form (13). Disruption of a single open reading frame with six genes caused discrimination from the parent clone (39).

Velicer & Vos (111) investigated interactions during the active feeding stage of the *M. xanthus* clonal isolates from the 16 mm^2 of soil discussed above. There were clear colony boundaries between all clones that had different genotypes and no such boundaries between control spots of the same clone. The mechanism of this response is unknown, though it may involve bacteriocins (76), which have been identified in this and other species of *Myxococcus* (53). Exclusion of others allows the benefits of secreted lytic enzymes and antibiotics to go to clonemates.

Secretion: Poison-Antidote Systems

Poison-antidote systems involve releasing a bacteriocin protein that can be rendered ineffectual by another protein, the antidote, but will kill strains lacking it. They are therefore a type of kind discrimination, though these spiteful systems function best in structured populations with relatives, typically clonemates, nearby (38, 52, 56, 64). Bacteriocin systems are extremely widespread in bacteria and also occur in archaea (98, 109).

The bacteriocins from *E. coli*, called colicins, are probably the best known (15). Colicins are made up of three genes encoded on plasmids: the toxin, the antidote, and the gene whose product causes a subset of cells to lyse and release the toxin (98). The bacteriocin genes are not activated until quorum-sensing

mechanisms indicate the bacteria are sufficiently dense, a time when competition is likely to be intense (98). Colicins are large proteins and act as nucleases or, more commonly, form pores in the cell walls of their targets (15). *E. coli* also produces another kind of bacteriocin called a microcin that is smaller, does not depend on cell lysis, and is generally less well understood (10, 43). Low environmental iron levels apparently stimulate its production.

Bacteriocins are a form of kind discrimination; each type targets individuals that lack the same kind of bacteriocin. They benefit their own kind, including kin (clonemates), so they are included under broad-sense kin recognition.

They are sometimes diverse and some individuals carry multiple colicins (42–44, 97). Eight different colicins and all of the seven known microcins were detected in a screen of 266 fecal isolates of *E. coli*, each from a different human host in Canberra, Australia (43). Thirty-eight percent of the clones they picked up produced either a colicin (24%) or a microcin (20%), and some had multiple bacteriocins. In a study of mouse colicins in Australia, nearly half of all isolates produced colicins (15, 44).

SECRETION: GIFT-PASSWORD BENEFITS

Just as poison-antidote systems target harm to individuals lacking the system, it is possible that only those who have the appropriate password can receive benefits, or gifts. In some sense, all benefits may have a gift and password nature because the recipients must have the appropriate molecular pathways to utilize them, but here we are concerned with polymorphic systems that could target other cooperators, a relatively unexplored possibility (118).

A simple gift-password system occurs in *Agrobacterium tumefaciens*, which can induce its plant host to produce opines that feed the bacteria (83, 120). Although inducing opine production is a public good that could benefit nonproducers, the ability to induce opines and the ability to catabolize them are encoded on the Ti plasmid, so that the benefits go only to other producers. Similarly, certain *Rhizobium* strains in legume nodules produce a private nutritional source that is catabolized only by others outside the nodule who share the same plasmid that bears genes for production and catabolism (7).

Gift-password system: a type of discrimination in which beneficial products cannot be used by nonproducers, who lack the password

Pseudomonas aeruginosa provides a possible example with more diversity (118). This species secretes an iron-binding siderophore, pyoverdine. Strains vary both in the form of pyoverdine produced and in their ability to take up iron from the different forms (78). Interestingly, strains are able to take up iron from more forms of pyoverdines than they are able to produce, suggesting some ability to exploit other's pyoverdine-sequestered iron (85, 104). Moreover, evidence suggests that pyoverdine production genes are under diversifying selection, as would be expected if selection favored making pyoverdines that could benefit only self and relatives (104).

Staphylococcus aureus shows similar diversity in its *agr* quorum-sensing system, which upregulates a set of secreted proteins. Three types use three different autoinducing peptides that each activates its own kind but often inhibits other kinds (60). Although many quorum-sensing systems are general, this one appears to work only when it counts enough of its own type and not too many of the other types.

Gift-password benefit polymorphisms seem to be less common than the widespread poison-antidote harms of bacteriocin systems. It appears that in bacteria it is easier to evolve to kill your enemies than to favor your friends. Why this should be so is not obvious, but there is one significant consequence. We noted above that bacteriocins, by changing local relatedness, pave the way for greater cooperation in general. Gift-password systems do not generally do this.

Secretion: Attraction and Repulsion

None of the systems discussed so far seems to involve actively seeking out one's own type.

Greenbeard recognition: cue, recognition of the cue, and cooperative action bundled into the same gene or linked gene complex

The ciliate *Tetrahymena thermophila* is common in dead vegetation in ponds and other water bodies where it preys on bacteria. Under low-nutrient conditions, cells often clump together and release factors called *Tetrahymena* cell survival factors that may increase survival under harsh conditions (16, 17). Different strains differ in both their propensity to aggregate and their propensity to disperse (102). Aggregators seem to grow more slowly, release more cell survival factors, and disperse less well.

Lower levels of aggregation are observed in mixtures of two clones than in one clone alone, indicating some form of kin recognition and discrimination (16). The study also tested whether clones moved preferentially to medium that previously housed clonemates or to medium that had housed a different clone. Interestingly, greater aggregators moved toward the clonemate medium, whereas low aggregators did the opposite (16). One interpretation is that aggregators cooperate more and seek out clonemates and that uncooperative dispersers move to minimize contact with kin. The mechanism is not yet known, except that it must involve detection of something in the medium.

Contact: Poison-Antidote

There are also systems that function rather like bacteriocins but require cell contact (1). These systems are deployed during logarithmic growth to inhibit strains lacking the same system. There are at least two types in *E. coli*, each with a nonhomologous region on the *CdiA* gene that confers both inhibition and specificity, and with nonhomologous *CdiI* genes that encode immunity to their own *CdiA*. These systems occur widely in gram-negative bacteria, but not in all strains of a species. Originally thought to be involved in differentiation processes, the discovery of the mechanism and the specificity shifted the focus to competition (1). Cell lysis is not required for release of the toxin; instead the toxin resides in the cell membrane. The target cells cease growth but appear to be viable, on the basis of propidium iodide assays.

Contact: Adhesion

Another way to target benefits to one's own type is adhesion. When like binds to like, kind discrimination occurs. Adhesion is important in biofilms, though it is still unclear how often biofilms are cooperative versus competitive structures (122). In one single-species biofilm, the wrinkly spreader mutants of *Pseudomonas flourescens* cooperate by adhering in a mat at the broth-air interface (93). However, new nonsticky mutants that occur within the mat spread because they enjoy the benefits of being in the mat without paying the costs (92).

The previously mentioned *FLO1* adhesion system of *Saccharomyces cerevisiae* allows formation of protective clumps or flocculates under certain kinds of harsh conditions, though this trait has been bred out of commonly used laboratory strains (105). Production of this adhesion protein is costly; producers grow much less quickly than nonproducers. Perhaps this contributes to variability and even loss of the trait. The *FLO1* gene has also been called a greenbeard, and it is one in the sense of kind discrimination in which benefits go to one's own kind. However, it operates by differential effects rather than by differential action. The adhesion protein binds with mannan oligosaccharide chains that make up the outermost layer cell wall. Thus, the protein itself recognizes all other cells, and favoring its own kind comes more indirectly because two-way bonding between *FLO1* cells is stronger than one-way bonding between a *FLO1* and a *FLO1* knockout (105). The *FLO1* gene is variable across strains, particularly in numbers of a DNA repeat unit, and increasing numbers of repeats increase flocculation (105). But it is unknown whether these different variants generally bind preferentially with each other.

More specific greenbeard recognition is shown by a *D. discoideum* cell adhesion gene, *csaA*, and its knockout mutant (91). The wild type does the three things required of a greenbeard: It possesses a trait, identifies the trait in others, and acts altruistically on the basis of the trait. Its cells adhere homotypically and

then go through the social cycle of aggregation, slug formation, and sterile stalk formation. *CsaA* knockouts without the gene tend to be excluded from these aggregations (84), particularly on soil. If they do get into aggregations they tend to cheat, presumably because less adhesion puts them in the rear of the slug, where spores develop (91). Thus, by adhering preferentially with its own kind, the wild type avoids being cheated. This was an important proof-of-principle for greenbeards, but because it is nearly invariant across natural clones, it is not a gene that regularly discriminates among clones in nature (91).

Highly variable adhesion molecules appear to mediate segregation in *D. discoideum*. Amoebas of different clones, and even different species, coaggregate by responding to common signals (57, 108). Clonal segregation, which prevents exploitation by foreign clones, was first studied in *D. purpureum* and *D. giganteum* by labeling one clone in a mixture (5, 40, 57, 61, 77, 81).

In *D. discoideum*, one study showed that segregation increased with overall genetic divergence (81) and another showed that this was not the case (28). However, there is a specific genetic component, a mediated pair of adhesion genes (5). These genes have several transmembrane immunoglobulin-like repeats. They are adjacent in the genome and coexpressed, peaking at 8 to 12 h after starvation, a time when the formerly independent amoebae are in physical contact with each other (5). Knockouts of either gene separate from wild type, confirming their active role in kin discrimination. As would be expected for sequences coding for recognition cues, they are extremely variable, and the variation in sorting that is seen in the geographic study (81) is well explained by parts of these sequences (5).

The big question is how *D. discoideum* uses kin discrimination in natural circumstances. The diversity of genetically different clones in tiny soil samples (30) argues that the role for discrimination is to avoid cheaters (14, 29, 108). But actual sorting in *D. discoideum* is usually rather weak (28, 81). Sorting could have a cost, because not segregating would produce larger slugs, which move farther (32). It could be that sorting is dependent on the balance of costs and benefits, and that this balance varies in different habitats.

M. xanthus also produces a multicellular fruiting body when starved, with most cells dying, possibly to provide an advantage such as protection, nutrition, or dispersal to surviving spores (111). However, clones do not segregate at the fruiting body stage (115) possibly because recognition in the growth stage may keep groups highly related; further recognition and sorting during fruiting may be redundant.

Kind Discrimination Enhancing Relatedness

Complex cooperation, involving many genes, probably requires that many or all genes in the genome share high relatedness with partners. Population structure and environmental cues alone can sometimes provide this. Alternatively, certain types of kind discrimination could provide the basis for increasing relatedness at all genes. Forming borders between colonies, killing or inhibiting of unlike strains, and adhesion are all examples of kind discrimination, but they can also increase overall relatedness among interactants and therefore lead to complex interactions. In the extreme case, a rare bacteriocin may kill off everyone except clonemates, leaving the local group with a relatedness of unity at all loci.

If a bacteriocin exists at higher frequency in the population, the effect is not as large, but still present. In **Figure 2*a***, the actor's clonemates make up one-half of its neighbors, who will all survive its bacteriocin, as will one-fourth of the rest of its neighbors, who have the bacteriocin allele at its population frequency. After killing, the local neighborhood has higher relatedness at all loci, perhaps allowing for more complex cooperation (**Figure 2*b***). These systems approach animal phenotype matching, where action genes use cues at other loci. Here the cues are provided by kind discrimination. But when

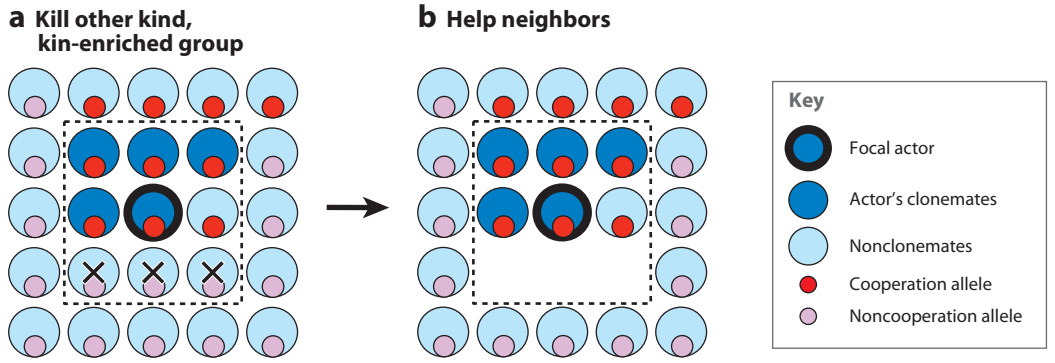

Figure 2

(*a*) Bacteriocins kill cells lacking the bacteriocin genes. (*b*) This results in an increased percentage of clonemates in the neighborhood making cooperation more favorable at all loci.

animals use memory, this system uses a sort of population memory, in which past decisions are stored via killing or adhesion. And in cases in which animals can pick out kin at each subsequent interaction (differential action), this system relies on altered population structure and proximity.

Assessment of Relatedness in Mixtures

Although the evidence for broad-sense kin discrimination that we have discussed in this paper involves either kind discrimination, or proximity-based environmental discrimination, this does not mean that fine-grained kin discrimination can be ruled out. It may be that cases in which clones consistently act differently simply because they are in the presence of other clones point to such kin discrimination, though they do not require it. If there are just two or three kinds that treat each other differently, then kind discrimination seems a likely explanation. If there are many types that recognize foreigners, then they are coming close to assessing actual relatedness; a rare cue or combination of cues will pick out clonemates effectively. Examples of behavioral responses to overall relatedness can be found in both bacteria and eukaryotes.

The rodent malarial parasite *Plasmodium chabaudi* adjusts sex ratio in an adaptive manner according to the number of strains infecting the host (95). This implies that it can detect the presence of foreign clones, although the mechanism is unknown.

Similarly, *D. discoideum* adjusts some behaviors in mixtures in apparently adaptive ways. First, mixtures travel less far in the slug stage than do the same number of cells of a single clone (32), consistent with decreasing altruism by staying out of the anterior portion of the slug, which is the region that produces stalk. Second, mixtures of two clones typically produce more spores than the corresponding single clones do (14). The highly variable adhesion genes (84) could mediate these traits, but so far they have been implicated only in clonal segregation.

Chimeras in the bacterium *M. xanthus* show the opposite effect. In all mixes, at least one clone produced fewer spores than it would have made alone, and in 11 of 36 mixes, both partners produced fewer spores than they would have made alone (115).

These *Dictyostelium* and *Myxococcus* results are consistent with recognition and competition in fruiting bodies. Alternatively, they might result from diminished coordination of distinct genotypes in fruiting body development. Further study of mechanisms are needed to distinguish these two hypotheses.

DISCUSSION

Microbes are surprisingly social, having myriad ways to affect their neighbors for good or ill.

Selection rewards genes that give fitness benefits to others of the same kin or kind. Microbes offer many advantages for the study of social evolution compared with animals. Those that are easy to grow in the laboratory allow easy experimental manipulations that might be impossible with animals. Their short life spans make true multigeneration evolutionary experiments possible. The genetic toolkits available for many microbes make it comparatively easy to find and manipulate the genes involved in sociality.

The major disadvantage of studying microbes may be the difficulty of studying them in the field. First, how social behaviors are expressed may be altered in laboratory environments. For example, movement of quorum-sensing signals can be altered by both the physical and the biological environment (23). Similarly, cell adhesion in the amoeba *D. discoideum* is different on soil than on agar (84), and this difference alters the direction of selection (91). In fact, laboratory-adapted organisms often lose crucial social traits, such as swarm motility in some bacteria (62), the floccing behavior in yeast (105), and social sporulation in *B. subtilis* (9). It would not be surprising if this were often true for recognition traits, because the uniclonal populations often maintained in the laboratory remove selection for recognition. Similarly, studying one or few laboratory strains will not suffice for understanding highly polymorphic discrimination cues. Just as important, we know little about the relatedness structure in natural environments. Given the importance of kinship, we cannot claim much understanding of natural social behavior until we learn more.

Although the same broad selective processes, as embodied in Hamilton's rule, govern microbial and animal social interactions, they are executed in partially different manners. We see no evidence that microbial recognition is mediated by learning or social experience. Instead, it seems to be mediated largely by pure population structure, kind recognition, or both.

The importance of greenbeards and kind discrimination seems to be much greater in microbes than in animals. Greenbeards, generally thought to be rare, are difficult to distinguish from other processes in which individuals preferentially help (or hinder) others of their own kind. In microbes these include many frequency-dependent processes, but poison-antidote systems and adhesion are particularly important.

Indeed there is little evidence in microbes for narrow-sense kin discrimination except through proximity cues. But something similar to phenotype matching occurs when poisoning or adhesion results in higher relatedness across the genome, allowing greater cooperation. Because complex cooperation, such as fruiting body formation, requires action of many genes, we suggest that the stage for it could often be set by elevated relatedness due to poisoning of others or adhesion. We can say that a sort of recognition has occurred, not through history encoded in memory, but through history encoded in the results of past behavior.

An important unresolved question is how antidotes and passwords are kept private. Why does a *P. aeruginosa* strain not evolve to use multiple pyoverdines, whether it produces them or not? And what prevents *E. coli* strains from accumulating antidotes to all colicins? Undoubtedly, the clonality of bacteria, with relatively infrequent recombination, helps by keeping traits together, but gene transfer can put initially separate genes together. Perhaps costs of antidotes or passwords are significant, especially if frequencies of the corresponding poisons or benefits are low.

A related conundrum concerns how diversity is maintained in systems that distinguish kin or kind. If benefits are given to the same kind, or harms to the other kind, there is positive frequency-dependent selection that favors common kinds over rare ones. A common kind receives more benefits, or fewer harms, and therefore is selectively favored. This selection therefore tends to remove the genetic variation that is necessary to allow discrimination in the first place, a puzzle known as Crozier's paradox (20, 110). The solution is tied up with the privacy problem. If a particular poison-antidote

system becomes common, it might pay to lose the poison gene but not the antidote. The resulting free rider or cheater genotype would benefit when others poison competitors, but it would avoid the costs of producing and releasing poison (46). The success of this genotype could prevent the poison-antidote system from becoming fixed. Experimental work on colicins shows that in structured populations there can be a dynamic balance among poisoning, cheaters, and susceptible strains (64, 65), even though these studies did not address the fate of multiple poisoning strains. Theoretical models suggest that the range of conditions that preserve variation may be limited, requiring intermediate levels of recombination (59, 100). Perhaps this condition is met more often in microbes than in eukaryotes that recombine every generation.

Riley (96) proposes that colicins evolve through a process called diversifying recombination. This process has two steps. The first step is a mutation in the immunity gene, which broadens immunity. The second mutation is in the colicin gene and results in the production of a new colicin that the carrier of the first mutation can withstand but others in the ancestral population cannot. Such a process seems consistent with the observed patterns of diversity in immunity binding and killing functions (98).

Kin discrimination is a field that originated with animal studies. Its extension to brainless microbes has generated many fascinating advances in our understanding. Although unsolved puzzles like Crozier's paradox remain, they may well be solved first in a natural microbial system.

SUMMARY POINTS

1. Cooperative traits in microbes are to be favored most likely by selection when they disproportionately benefit others with the cooperation genes.
2. In many microbes the most important type of kin is a clonemate.
3. When neighbors are often clonemates, proximity can be a sufficient cue.
4. Much discrimination in microbes is based on kind rather than kin.
5. Poison-antidote systems and adhesion are common types of kind discrimination.
6. Kind selection that leads to death of other kinds or segregation away from them can increases kinship and provide the basis for true kin discrimination.

FUTURE ISSUES

1. How high is relatedness in natural situations?
2. How much cue variation is there and how is it maintained by selection?
3. How are passwords and antidotes kept private?
4. Do pathogens assess multiplicity of infection and cooperate more when it is low?
5. How important are kin and kind discrimination in biofilms?
6. How many microbes seek out kin?

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LITERATURE CITED

1. Aoki SK, Pamma R, Hernday AD, Bickham JE, Braaten BA, Low DA. 2005. Contact-dependent inhibition of growth in *Escherichia coli*. *Science* 309:1245–48
2. Be'er A, Ariel G, Kalisman O, Helman Y, Sirota-Madi A, et al. 2010. Lethal protein produced in response to competition between sibling bacterial colonies. *Proc. Natl. Acad. Sci. USA* 107:6258–63
3. Be'er A, Zhang HP, Florin EL, Payne SM, Ben-Jacob E, Swinney HL. 2009. Deadly competition between sibling bacterial colonies. *Proc. Natl. Acad. Sci. USA* 106:428–33
4. Beecher MD. 1982. Signature systems and kin recognition. *Am. Zool.* 22:477–90
5. Benabentos R, Hirose S, Sucgang R, Curk T, Katoh M, et al. 2009. Polymorphic members of the *lag* gene family mediate kin discrimination in *Dictyostelium*. *Curr. Biol.* 19:567–72
6. Bent SJ, Gucker CL, Oda Y, Forney LJ. 2003. Spatial distribution of *Rhodopseudomonas palustris* ecotypes on a local scale. *Appl. Environ. Microbiol.* 69:5192–97
7. Bever JD, Simms EL. 2000. Evolution of nitrogen fixation in spatially structured populations of *Rhizobium*. *Heredity* 85:366–72
8. Boyd R, Richerson PJ. 1980. Effect of phenotypic variation on kin selection. *Proc. Natl. Acad. Sci. USA* 77:7506–7
9. Branda SS, González-Pastor JE, Ben-Yehuda S, Losick R, Kolter R. 2001. Fruiting body formation by *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* 98:11621–26
10. Braun V, Patzer SI, Hantke K. 2002. Ton-dependent colicins and microcins: modular design and evolution. *Biochimie* 84:365–80
11. Brown SP, Buckling A. 2008. A social life for discerning microbes. *Cell* 135:600–3
12. Brown SP, Hochberg ME, Grenfell BT. 2002. Does multiple infection select for raised virulence? *Trends Microbiol.* 10:401–5
13. Budding AE, Ingham CJ, Bitter W, Vandenbroucke-Grauls CM, Schneeberger PM. 2009. The dienes phenomenon: competition and territoriality in swarming *Proteus mirabilis*. *J. Bacteriol.* 191:3892–900
14. Buttery NJ, Rozen DE, Wolf JB, Thompson CR. 2009. Quantification of social behavior in D. discoideum reveals complex fixed and facultative strategies. *Curr. Biol.* 19:1373–77
15. Cascales E, Buchanan SK, Duche D, Kleanthous C, Lloubes R, et al. 2007. Colicin biology. *Microbiol. Mol. Biol. Rev.* 71:158–229
16. Chaine AS, Schtickzelle N, Polard T, Huet M, Clobert J. 2010. Kin-based recognition and social aggregation in a ciliate. *Evolution* 64:1290–300
17. Christensen ST, Sorensen H, Beyer NH, Kristiansen K, Rasmussen L, Rasmussen MI. 2001. Cell death in *Tetrahymena thermophila*: new observations on culture conditions. *Cell Biol. Int.* 25:509–19
18. Cornwallis CK, West SA, Davis KE, Griffin AS. 2010. Promiscuity and the evolutionary transition to complex societies. *Nature* 466:969–72
19. Crespi BJ. 2001. The evolution of social behavior in microorganisms. *Trends Ecol. Evol.* 16:178–83
20. Crozier RH. 1986. Genetic clonal recognition abilities in marine invertebrates must be maintained by selection for something else. *Evolution* 40:1100–1
21. Crozier RH. 1987. Genetic aspects of kin recognition: concepts, models, and synthesis. See Ref. 27, pp. 55–73
22. Dawkins R. 1976. *The Selfish Gene*. Oxford: Oxford Univ. Press
23. Decho AW, Norman RS, Visscher PT. 2010. Quorum sensing in natural environments: emerging views from microbial mats. *Trends Microbiol.* 18:73–80
24. Dienes L. 1946. Reproductive processes in *Proteus* cultures. *Proc. Soc. Exp. Biol. Med.* 63:265–70
25. Diggle SP, Griffin AS, Campbell GS, West SA. 2007. Cooperation and conflict in quorum-sensing bacterial populations. *Nature* 450:411–14

26. Ennis HL, Dao DN, Pukatzki SU, Kessin RH. 2000. *Dictyostelium* amoebae lacking an F-box protein form spores rather than stalk in chimeras with wild type. *Proc. Natl. Acad. Sci. USA* 97:3292–97
27. Fletcher DC, Michener CD. 1987. *Kin Recognition in Animals*. Chichester, UK: John Wiley
28. Flowers JM, Li SI, Stathos A, Saxer G, Ostrowski EA, et al. 2010. Variation, sex, and social cooperation: molecular population genetics of the social amoeba *Dictyostelium discoideum*. *PLoS Genet.* 6:e1001013
29. Fortunato A, Queller DC, Strassmann JE. 2003. A linear dominance hierarchy among clones in chimeras of the social amoeba, *Dictyostelium discoideum*. *J. Evol. Biol.* 16:438–45
30. Fortunato A, Strassmann JE, Santorelli LA, Queller DC. 2003. Co-occurrence in nature of different clones of the social amoeba, *Dictyostelium discoideum*. *Mol. Ecol.* 12:1031–38
31. Foster K. 2011. Social behavior in microorganisms. In *Social Behaviour: Genes, Ecology and Evolution*, ed. T Szekely, A Moore, J Komdeur, pp. 331–56. Cambridge, UK: Cambridge Univ. Press
32. Foster KR, Fortunato A, Strassmann JE, Queller DC. 2002. The costs and benefits of being a chimera. *Proc. R. Soc. Lond. B* 269:2357–62
33. Foster KR, Parkinson K, Thompson CRL. 2007. What can microbial genetics teach sociobiology? *Trends Genet.* 23:74–80
34. Frank SA. 1998. *Foundations of Social Evolution*. Princeton, NJ: Princeton Univ. Press
35. Gadagkar R. 1985. Kin recognition in social insects and other animals—a review of recent findings and a consideration of their relevance for the theory of kin selection. *Proc. Indian Acad. Sci.* 94:587–621
36. Gamboa GJ, Reeve HK, Pfennig DW. 1986. The evolution and ontogeny of nestmate recognition in social wasps. *Annu. Rev. Entomol.* 31:431–54
37. Gardner A, West SA. 2010. Greenbeards. *Evolution* 64:25–38
38. Gardner A, West SA, Buckling A. 2004. Bacteriocins, spite and virulence. *Proc. R. Soc. Lond. B* 271:1529–35
39. Gibbs KA, Urbanowski ML, Greenberg EP. 2008. Genetic determinants of self identity and social recognition in bacteria. *Science* 321:256–59
40. Gilbert OM, Foster KR, Mehdiabadi NJ, Strassmann JE, Queller DC. 2007. High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proc. Natl. Acad. Sci. USA* 104:8913–17
41. Gilley DC. 2001. The behavior of honey bees (*Apis mellifera ligustica*) during queen duels. *Ethology* 107:601–22
42. Gillor O, Giladi I, Riley MA. 2009. Persistence of colicinogenic *Escherichia coli* in the mouse gastrointestinal tract. *BMC Microbiol.* 9:165
43. Gordon DM, O'Brien CL. 2006. Bacteriocin diversity and the frequency of multiple bacterlocin production in *Escherichia coli*. *Microbiology* 152:3239–44
44. Gordon DM, Riley MA, Pinou T. 1998. Temporal changes in the frequency of colicinogeny in *Escherichia coli* from house mice. *Microbiology* 144:2233–40
45. Gore J, Hyuk H, van Oudenaarden A. 2009. Snowdrift game dynamics and facultative cheating in yeast. *Nature* 459:253–56
46. Grafen A. 1990. Do animals really recognize kin? *Anim. Behav.* 39:42–54
47. Greig D, Travisano M. 2004. The prisoner's dilemma and polymorphism in yeast SUC genes. *Proc. R. Soc. Lond. B* 271:S25–S26
48. Griffin AS, West SA, Buckling A. 2004. Cooperation and competition in pathogenic bacteria. *Nature* 430:1024–27
49. Hamilton WD. 1964. The genetical evolution of social behaviour. I. *J. Theor. Biol.* 7:1–16
50. Hamilton WD. 1964. The genetical evolution of social behaviour. II. *J. Theor. Biol.* 7:17–52
51. Harrison F, Browning L, Vos M, Buckling A. 2006. Cooperation and virulence in acute *Pseudomonas aeruginosa* infections. *BMC Biol.* 4:21
52. Hawlena H, Bashey F, Mendes-Soares H, Lively CM. 2010. Spiteful interactions in a natural population of the bacterium *Xenorhabdus bovienii*. *Am. Nat.* 175:374–81
53. Hirsch HJ. 1977. Bacteriocins from *Myxococcus fulvus* (Myxobacterales). *Arch. Microbiol.* 115:45–49
54. Holmes WG. 2004. The early history of Hamiltonian-based research on kin recognition. *Ann. Zool. Fenn.* 41:691–711

55. Holmes WG, Sherman PW. 1982. The ontogeny of kin recognition in two species of ground squirrels. *Am. Zool.* 22:491–517
56. Inglis RF, Gardner A, Cornelis P, Buckling A. 2009. Spite and virulence in the bacterium *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 106:5703–7
57. Jack CN, Ridgeway JG, Mehdiabadi NJ, Jones EI, Edwards TA, et al. 2008. Segregate or cooperate—a study of the interaction between two species of *Dictyostelium*. *BMC Evol. Biol.* 8:293
58. James BL, Kret J, Patrick JE, Kearns DB, Fall R. 2009. Growing *Bacillus subtilis* tendrils sense and avoid each other. *FEMS Microbiol. Lett.* 298:12–19
59. Jansen VAA, van Baalen M. 2006. Altruism through beard chromodynamics. *Nature* 440:663–66
60. Ji GY, Beavis R, Novick RP. 1997. Bacterial interference caused by autoinducing peptide variants. *Science* 276:2027–30
61. Kaushik S, Katoch B, Nanjundiah V. 2006. Social behaviour in genetically heterogeneous groups of *Dictyostelium giganteum*. *Behav. Ecol. Sociobiol.* 59:521–30
62. Kearns DB. 2010. A field guide to bacterial swarming motility. *Nat. Rev. Microbiol.* 8:634–44
63. Keller L, Surette MG. 2006. Communication in bacteria: an ecological and evolutionary perspective. *Nat. Rev. Microbiol.* 4:249–58
64. Kerr B, Riley MA, Feldman MW, Bohannan BJM. 2002. Local dispersal promotes biodiversity in a real-life game of rock-scissors-paper. *Nature* 418:171–74
65. Kirkup BC, Riley MA. 2004. Antibiotic-mediated antagonism leads to a bacterial game of rock-paper-scissors in vivo. *Nature* 428:412–14
66. Kraemer SA, Toups MA, Velicer GJ. 2010. Natural variation in developmental life-history traits of the bacterium *Myxococcus xanthus*. *FEMS Microbiol. Ecol.* 73:226–33
67. Kusek JW, Herman LG. 1980. Typing of *Proteus mirabilius* by bacteriocin production and sensitivity as a possible epidemiological marker. *J. Clin. Microbiol.* 12:112–20
68. Lacy RC, Sherman PW. 1983. Kin recognition by phenotype matching. *Am. Nat.* 121:489–512
69. Le Gac M, Doebeli M. 2010. Environmental viscosity does not affect the evolution of cooperation during experimental evolution of colicigenic bacteria. *Evolution* 64:522–33
70. Levin BR, Antonovics J, Sharma H. 1988. Frequency-dependent selection in bacterial populations. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 319:459–72
71. Liebert AE, Starks PT. 2004. The action component of recognition systems: a focus on the response. *Ann. Zool. Fenn.* 41:747–64
72. MacLean RC, Gudelj I. 2006. Resource competition and social conflict in experimental populations of yeast. *Nature* 441:498–501
73. Mateo J. 2002. Kin-recognition abilities and nepotism as a function of sociality. *Proc. R. Soc. Lond. Biol. Sci.* 269:721–27
74. Mateo JM. 2004. Recognition systems and biological organization: The perception component of social recognition. *Ann. Zool. Fenn.* 41:729–45
75. Mateo JM. 2010. Self-referent phenotype matching and long-term maintenance of kin recognition. *Anim. Behav.* 80:929–35
76. McCurdy HD, Macrae TH. 1974. Xanthacin—bacteriocin of *Myxococcus xanthus* FB. *Can. J. Microbiol.* 20:131–35
77. Mehdiabadi NJ, Talley-Farnum T, Jack C, Platt TG, Shaulsky G, et al. 2006. Kin preference in a social microorganism. *Nature* 442:881–82
78. Meyer JM, Stintzi A, DeVos D, Cornelis P, Tappe R, et al. 1997. Use of siderophores to type pseudomonads: the three *Pseudomonas aeruginosa* pyoverdine systems. *Microbiology* 143:35–43
79. Munson EL, Pfaller MA, Doern GV. 2002. Modification of dienes mutual inhibition test for epidemiological characterization of *Pseudomonas aeruginosa* isolates. *J. Clin. Microbiol.* 40:4285–88
80. Nadell CD, Foster KR, Xavier JB. 2010. Emergence of spatial structure in cell groups and the evolution of cooperation. *PLoS Comput. Biol.* 6:e1000716
81. Ostrowski EA, Katoh M, Shaulsky G, Queller DC, Strassmann JE. 2008. Kin discrimination increases with genetic distance in a social amoeba. *PLoS Biol.* 6:e287
82. Pfennig DW. 1990. "Kin recognition" among spadefoot toad tadpoles: a side-effect of habitat selection? *Evolution* 44:785–98

75. Good source for recent animal kin discrimination studies.

83. Platt TG, Bever JD. 2009. Kin competition and the evolution of cooperation. *Trends Ecol. Evol.* 24:370–77
84. Ponte E, Bracco E, Faix J, Bozzaro S. 1998. Detection of subtle phenotypes: the case of the cell adhesion molecule csA in *Dictyostelium*. *Proc. Natl. Acad. Sci. USA* 95:9360–65
85. Poole K, McKay GA. 2003. Iron acquisition and its control in *Pseudomonas aeruginosa*: Many roads lead to Rome. *Front. Biosci.* 8:D661–86
86. Queller DC. 1984. Kin selection and frequency dependence: a game theoretic approach. *Biol. J. Linn. Soc.* 23:133–43
87. Queller DC. 1985. Kinship, reciprocity and synergism in the evolution of social behaviour. *Nature* 318:366–67

88. Demonstrates the robustness of kin selection.

88. Queller DC. 1992. A general model for kin selection. *Evolution* 46:376–80
89. Queller DC. 1992. Quantitative genetics, inclusive fitness, and group selection. *Am. Nat.* 139:540–58

90. Development of the theory of kind discrimination.

90. Queller DC. 2011. Expanded social fitness and Hamilton's rule for kin kith, and kind. *Proc. Natl. Acad. Sci. USA* 108:10792–99
91. Queller DC, Ponte E, Bozzaro S, Strassmann JE. 2003. Single-gene greenbeard effects in the social amoeba, *Dictyostelium discoideum*. *Science* 299:105–6
92. Rainey PB, Rainey K. 2003. Evolution of cooperation and conflict in experimental bacterial populations. *Nature* 72–74
93. Rainey PB, Travisano M. 1998. Adaptive radiation in a heterogeneous environment. *Nature* 394:69–72
94. Read AF, Mackinnon MJ, Anwra A, Taylor LH. 2002. Kin-selection models as evolutionary explanations of malaria. In *Adaptive Dynamics of Infectious Diseases: In Pursuit of Virulence Management*, ed. U Dieckmann, JAJ Metz, MW Sabelis, K Sigmund, pp. 165–78. Cambridge, UK: Cambridge Univ. Press
95. Reece SE, Drew DR, Gardner A. 2008. Sex ratio adjustment and kin discrimination in malaria parasites. *Nature* 453:609–14
96. Riley MA. 1998. Molecular mechanisms of bacteriocin evolution. *Annu. Rev. Genet.* 32:255–78
97. Riley MA, Gordon DM. 1999. The ecological role of bacteriocins in bacterial competition. *Trends Microbiol.* 7:129–33

98. Best overview of bacteriocins.

98. Riley MA, Wertz JE. 2002. Bacteriocins: evolution, ecology, and application. *Annu. Rev. Microbiol.* 56:117–37
99. Ross-Gillespie A, Gardner A, West SA, Griffin AS. 2007. Frequency dependence and cooperation: theory and a test with bacteria. *Am. Nat.* 170:331–42
100. Rousset F, Roze D. 2007. Constraints on the origin and maintenance of genetic kin recognition. *Evolution* 61:2320–30

101. A selection for cheater mutants that demonstrates their commonness.

101. Santorelli L, Thompson C, Villegas E, Svetz J, Dinh C, et al. 2008. Facultative cheater mutants reveal the genetic complexity of cooperation in social amoebae. *Nature* 451:1107–10
102. Schtickzelle N, Fjerdingstad EJ, Chaine A, Clobert J. 2009. Cooperative social clusters are not destroyed by dispersal in a ciliate. *BMC Evol. Biol.* 9:251
103. Sherman PW, Holmes WG. 1983. Kin recognition in animals. *Am. Sci.* 71:46–55
104. Smith EE, Sims EH, Spencer DH, Kaul R, Olson MV. 2005. Evidence for diversifying selection at the pyoverdine locus of *Pseudomonas aeruginosa*. *J. Bacteriol.* 187:2138–47
105. Smukalla S, Caldara M, Pochet N, Beauvais A, Guadagnini S, et al. 2008. *FLO1* is a variable green beard gene that drives biofilm-like cooperation in budding yeast. *Cell* 135:726–37
106. Starks PT. 2004. Recognition systems: from components to conservation. *Ann. Zool. Fenn.* 41:689–90
107. Strassmann JE, Queller D. 2007. Insect societies as divided organisms: the complexities of purpose and cross-purpose. *Proc. Natl. Acad. Sci. USA* 104:8619–26
108. Strassmann JE, Zhu Y, Queller DC. 2000. Altruism and social cheating in the social amoeba, *Dictyostelium discoideum*. *Nature* 408:965–67

111. Good overview of a social bacterium that includes field-collected clones.

109. Torreblanca M, Meseguer I, Ventosa A. 1994. Production of halocin is a practically universal feature of archaeal halophilic rods. *Lett. Appl. Microbiol.* 19:201–5
110. Tsutsui ND. 2004. Scents of self: the expression component of self/nonself recognition systems. *Ann. Zool. Fenn.* 41:713–27
111. Velicer GJ, Vos M. 2009. Sociobiology of the myxobacteria. *Annu. Rev. Microbiol.* 63:599–623

112. Verdugo P, Alldredge AL, Azam F, Kirchman DL, Passow U, Santschi PH. 2004. The oceanic gel phase: a bridge in the DOM-POM continuum. *Mar. Chem.* 92:67–85
113. Vos M, Velicer GJ. 2006. Genetic population structure of the soil bacterium *Myxococcus xanthus* at the centimeter scale. *Appl. Environ. Microbiol.* 72:3615–25
114. Vos M, Velicer GJ. 2008. Natural variation of gliding motility in a centimetre-scale population of *Myxococcus xanthus*. *FEMS Microbiol. Ecol.* 64:343–50
115. Vos M, Velicer GJ. 2009. Social conflict in centimeter and global-scale populations of the bacterium *Myxococcus xanthus*. *Curr. Biol.* 19:1763–67
116. Waldman B, Frumhoff PC, Sherman PW. 1988. Problems of kin recognition. *Trends Ecol. Evol.* 3:8–13
117. Wernegreen J, Moran N. 2001. Vertical transmission of biosynthetic plasmids in aphid endosymbionts (*Buchnera*). *J. Bacteriol.* 183:785–90
118. West SA, Diggle SP, Buckling A, Gardner A, Griffin AS. 2007. The social lives of microbes. *Annu. Rev. Ecol. Evol. Syst.* 38:53–77
119. West SA, Griffin AS, Gardner A, Diggle SP. 2006. Social evolution theory for microorganisms. *Nat. Rev. Microbiol.* 4:597–609
120. White CE, Winans SC. 2007. Cell-cell communication in the plant pathogen *Agrobacterium tumefaciens*. *Philos. Trans. R. Soc. B Biol. Sci.* 362:1135–48
121. Wollenberg MS, Ruby EG. 2009. Population structure of *Vibrio fischeri* within the light organs of *Euprymna scolopes* squid from two Oahu (Hawaii) populations. *Appl. Environ. Microbiol.* 75:193–202
122. Xavier J, Foster K. 2007. Cooperation and conflict in microbial biofilms. *Proc. Natl. Acad. Sci. USA* 104:876–81

118. A good general review of microbial sociobiology.

122. Demonstrates that biofilm production can be competitive not cooperative.