Review

The cooperative amoeba: *Dictyostelium* as a model for social evolution

Si. I. Li and Michael D. Purugganan

Department of Biology and Center for Genomics and Systems Biology, 1009 Silver, 100 Washington Square East, New York University, New York, NY 10003, USA

Social interactions, including cooperation and altruism, are characteristic of numerous species, but many aspects of the evolution, ecology and genetics of social behavior remain unclear. The microbial soil amoeba *Dictyostelium discoideum* is a model system for the study of social evolution and provides insights into the nature of social cooperation and its genetic basis. This species exhibits altruism during both asexual and sexual cycles of its life history, and recent studies have uncovered several possible genetic mechanisms associated with kin discrimination and cheating behavior during asexual fruiting-body formation. By contrast, the molecular and evolutionary mechanisms that underlie sexual macrocyst formation remain largely enigmatic. *D. discoideum*, given its utility in molecular genetic studies, should continue to help us address these and other relevant questions in sociobiology, and thereby contribute to a coherent theoretical framework for the nature of social cooperation.

The evolution of social cooperation

Cooperation is common in nature. In social insects such as ants and bees, sterile or semi-sterile workers collectively rear eggs laid by the queen, and in mammals wolves hunt in packs, cooperatively trapping prey to secure their meal [1]. In these and other similar cases, social cooperation between individuals can be mutually beneficial and result in gains in fitness. The origins and maintenance of sociality, however, has long been recognized as presenting some difficulties for evolutionary theory because several aspects of social behavior appear to lead to reduced direct fitness. Altruism, for example, can lead to a decrease in direct fitness of the altruistic individual, and should thus be eliminated by selection. Moreover, although social cooperation could enhance the fitness of group members, it is also vulnerable to the invasion of cheaters who benefit from a social trait without paying the fair cost.

Several theories have been advanced to explain how social cooperation arises, most prominently kin selection theory; this posits the importance of inclusive fitness in determining altruism and cooperative behaviors [2] (Box 1). There have also been a large number of empirical studies dissecting the evolution and mechanisms of social cooperation in diverse species, and attention has recently focused on sociality in microorganisms. In microbial species, research is facilitated by their relatively facile genetic systems, short generation times, simple life histories and the ability to readily manipulate their environments. The great range of social cooperative behaviors observed in microbes, including the production of public goods such as siderophores [3], quorum sensing (QS) [4,5], biofilm formation [6], cooperative motility [7,8] and the formation of fruiting bodies, is now increasingly appreciated.

Dictyostelium as a model system for the study of social evolution

One of the best-studied examples of microbial sociality is in *Dictyostelium discoideum*, a soil amoeba principally distributed in eastern North America, Japan, and the east coast of China [9]. This species has been a key model system for understanding the genetic basis of social cooperation as well as development and cell–cell signaling [10]. The 34 Mb genome of *D. discoideum* has been completely sequenced and carefully annotated. In addition, several molecular approaches are available to dissect molecular and cellular functions, thereby facilitating the genetic analysis of cooperative behavior [11].

D. discoideum lives largely as single-celled individuals in the soil, but enters a social phase of its life cycle when starvation conditions lead to a transition from solitary lives to swarming cooperative aggregates that eventually

Glossary

Altruism: a behavior that benefits the recipient(s) at the cost of the direct fitness of the actor. A clear example of altruism in *D. discoideum* is provided by the cells which sacrifice themselves in the stalk of fruiting bodies to increase the reproductive success of other coaggregate cells.
Cheater: an individual who gains benefit from social cooperation but without contributing proportionally to the collective production of the social trait. During fruiting-body formation, cheaters in *D. discoideum* occupy a disproportionately higher cell proportion in spores compared other coaggregate cells.
Coercion: a behavior that forces the recipient to behave in an involuntary way, usually aimed at preventing selfish action of the recipient and enforcing cooperation.
Cooperation: a social interaction, such as fruiting-body formation in *D. discoideum*, that increases the direct fitness of the recipient(s).
Direct fitness: a measure of an individual's ability to produce viable offspring.
Inclusive fitness: a measure of fitness that takes into account both the direct fitness of the actor and the effect of the behavior on the fitness of the recipient(s), weighted by genetic relatedness (Box 1 for more details).

Corresponding author: Purugganan, M.D. (mp132@nyu.edu).

0168-9525/$ – see front matter doi:10.1016/j.tig.2010.11.003 Trends in Genetics, February 2011, Vol. 27, No. 2

Box 1. Kin selection theory

First proposed by R.A. Fisher and J.B.S. Haldane, the theory of kin selection was formally established by W.D. Hamilton in the 1960s and has exerted a profound influence on the development of social evolution theory [2,63,64]. To apply this theory to fruiting-body formation one imagines a prestalk cell *A*, which at maturity becomes part of the dead stalk, and *n* prespore cells R_1, ..., R_n, which are related to *A* and eventually develop into viable spores sitting on top of the stalk. With social cooperation, each R_i gains the potential for reproducing at the cost of the survival of *A*.

Kin selection theory states that the fitness of *A* is best measured as inclusive fitness – this reflects the genetic effects of survival and reproduction both on actor *A* itself, and on the recipients, R_1 to R_n, multiplied by the genetic relatedness between actor and recipients. In general, inclusive fitness of actor *A* can be expressed as

$$w + \sum_i r_i \Delta w_i \quad [1]$$

where w is the effect of the social behavior on the fitness of actor *A*, Δw_i is the effect on fitness of the *i*th recipient R_i, and r_i is the relatedness between the *i*th recipient R_i and actor *A*.

In a simplified case, there is only one recipient in a social interaction. When the actor behaves altruistically to its own detriment, w can be replaced by the cost c to the actor, and Δw_i can be shown as the benefit b of the recipient. Under positive selection, the fitness must increase, which leads to the inequality.

$$-c + rb > 0 \quad [2]$$

A behavior is thus favored if inclusive fitness has a value greater than zero, a formulation known as Hamilton's rule. When the cost to the actor is smaller than the benefit to the recipient multiplied by genetic relatedness, altruism would be favored. This requires a high degree of relatedness between interacting individuals, and altruism is thus theoretically more likely to occur between close relatives.

Hamilton proposed two mechanisms to ensure high relatedness [2]. First, relatives tend to remain in close proximity because of limited dispersal and/or physical barriers hindering efficient dispersal. Thus, even indiscriminate altruism can be favorable because neighbors are more likely to be relatives. A second mechanism is kin discrimination, in which an individual discriminates against distant relatives and preferentially cooperates with closer relatives. 'Greenbeard' genes take kin discrimination to an extreme [35], where cooperation is specifically directed towards individuals carrying the same allele at a particular gene, regardless of genetic similarity in the rest of genome.

develop into multicellular fruiting bodies [12] (Figure 1). Aggregation of individual amoebae is mediated by the release of cAMP, which leads to the chemotactic movement of cells, the polarized secretion of more cAMP, and the initiation of changes in developmental gene expression [12]. As many as 10^5 individual cells aggregate in the fruiting body, which is comprised of the stalk surmounted by the terminal sorus. The stalk cells perish during fruiting-body maturation, whereas the sorus contains haploid cells encapsulated as spores that await dispersal and germination when conditions are favorable for single-cell growth [10,12].

How does this sophisticated cooperative behavior benefit *D. discoideum* under conditions of resource limitation? Ecologically, there are three major benefits to the formation of fruiting bodies. First, migrating slugs – an early phase of fruiting-body formation – display higher mobility compared to solitary amoebae. They can cross soil barriers that single cells cannot, providing an advantage in terms of local dispersal to new food sources [13]. Second, social cooperation protects cells against nematodes and other soil predators [14]. It is reported, for example, that *Caenorhabditis elegans* feeds on solitary amoebae, but fails to do so once the extracellular matrix (or slime sheath) of multicellular aggregates forms. Finally, the holding of the spores aloft by the stalk appears to facilitate their long-range dispersal by water or passing animals.

One interesting feature of *D. discoideum* is that different strains are found to co-occur in North America [15], and chimeric fruiting bodies composed of different genotypes can thus form. There are both benefits and costs to being a chimera. On the one hand, larger slugs travel further, and slugs tend to be larger when composed of strains of different genotypes [16]. Chimeric slugs, on the other hand, travel less efficiently compared to clonal peers of the same size, and this could be due to internal competition between cells of distinct genetic backgrounds [16].

Molecular mechanisms of social cooperation

The onset of the social fruiting-body phase leads to a major shift in developmental gene expression, which is followed by morphological change and cell fate determination (Figure 1). During the early mound stage, prestalk and prespore cells initially differentiate and sort into different positions; the former move more effectively in response to the cAMP signal, and exhibit differential cell adhesion, thereby progressing to the top of the mound [17]. Continued elongation of the mound leads to the formation of a migrating slug in which prestalk and prespore cells are arranged in an anterior–posterior axis and their ratio stabilizes at ~1:4 [10].

Extracellular signal molecules play an important role in the establishment of cell fates, among the best characterized are differentiation-inducing factor-1 (DIF-1) and cAMP [12,18]. DIF-1 is a chlorinated hexaphenone that represses prespore fate and induces one prestalk cell type, the pstO cells [19,20]. DIF-1 is generated by the prespore cells, and production is regulated by a negative-feedback loop where extracellular DIF-1 is inactivated by a dechlorinase (DIFase) produced by prestalk cells [18,21]. Notably, DIF-1 is not required for the differentiation of the other major prestalk cell type, the pstA cells, which indicates that extra signal molecules must also be involved [22,23]. cAMP is also known to regulate cell differentiation, and its action in the prespore pathway is dependent on distinct membrane receptors – its action is activated via receptor cAR3 whereas it is repressed via cAR4 [24]. cAMP thus exerts opposite effects based on relative expression or activation levels of alternative receptors, the expression of which is selectively enriched in different cell types – cAR3 is preferentially expressed in prespore cells whereas cAR4 expression is localized to prestalk cells [25,26]. Cross-talk between the DIF-1, cAMP and other unknown signaling pathways controls the distribution of cells between stalk and spore, and the relative proportions in each compartment can be very plastic as a result of complex hierarchical regulation. The fruiting body of *Dictyostelium* is a classic example of social cooperation, and these molecular mechanisms – which control the formation and development of the fruiting body – represent the underlying basis that governs the cooperative interactions among cells.

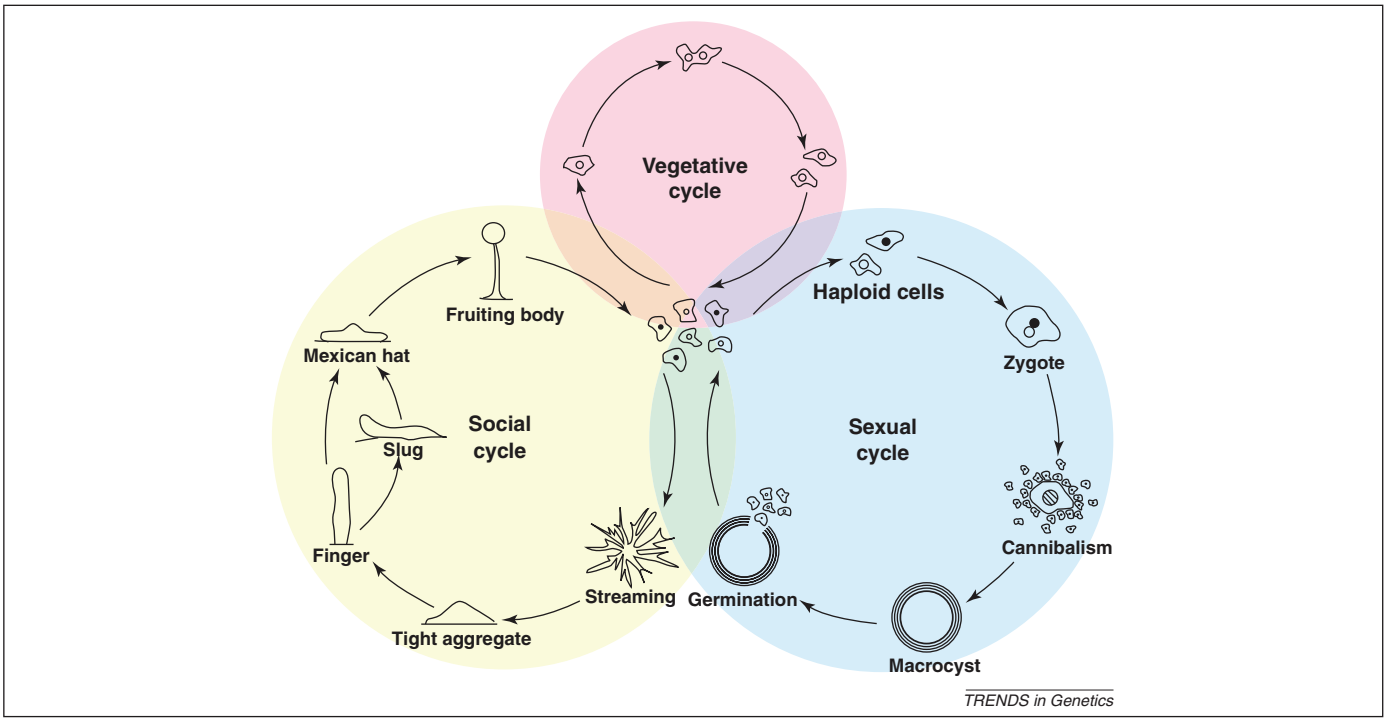

Figure 1. The life cycle of *Dictyostelium discoideum*. When food supply is sufficient, *D. discoideum* lead solitary lives as single haploid cells and reproduce by mitotic cell division (vegetative cycle). When food is scarce, however, these haploid cells enter a multicellular phase of their life cycle, which can be either asexual (the social cycle) or sexual. At the onset of the social cycle, individual cells aggregate by streaming, and the resulting tight multicellular aggregates elongate into fingers and then migrating slugs. After a series of morphological and physiological changes, these multicellular structures eventually develop into fruiting bodies, and spores from atop the fruiting bodies can disperse and germinate into solitary haploid cells. In the sexual cycle, two haploid cells of opposite mating types (indicated by open and closed circles in the cells to denote nuclei of different mating types) fuse to form a diploid zygote (indicated by the hatched circle). The zygote attracts surrounding solitary cells and cannibalizes them as nutrients, developing into a mature macrocyst. Meiosis occurs and recombinant progeny are generated and released during germination.

Altruism and kin discrimination in *Dictyostelium*

The development of the multicellular fruiting body in *D. discoideum* is accompanied by the appearance of altruism as a key component of social cooperation. Approximately 20% of the cells that aggregate to form the fruiting body form the stalk cells, and these altruistically die to ensure the survival of the 80% of the cells that make up the spore-bearing sorus. In general, programmed cell death in multicellular systems is commonplace, but the case of *Dictyostelium* has a unique feature – the stalk cells had a previous existence as solitary individuals, and so their altruistic death is the ultimate expression of subsuming individual fitness for the cooperative group.

Chimeric fruiting bodies are likely to occur in nature, and the presence of different genotypes in fruiting bodies could thus lead to conflicts of interest between cells. According to kin selection theory, the altruistic behavior of stalk cells can be explained by sufficiently high genetic relatedness among individuals in a fruiting body. When the costs and benefits involved in a social interaction are fixed, individuals are expected to cooperate altruistically more frequently with close relatives because this allows for an increase in inclusive fitness (Box 1). In general, genetic relatedness within *D. discoideum* fruiting bodies in nature has been found to be high [27], and two mechanisms have been proposed to explain this observation. First, limited dispersal of individuals is a passive mechanism that leads to spatial structuring in which close relatives are found in closer physically proximity to each other, and thus are more likely to cooperate with each other. Moreover, although diverse strains can co-occur, large clonal patches (one as large as 12 m in diameter discovered in Texas, USA) are also observed, which suggests that it is possible for *D. discoideum* to maintain high relatedness at local spatial scales [28].

Another mechanism for ensuring high genetic relatedness in fruiting bodies is some form of kin discrimination, where *D. discoideum* cells preferentially associate with kin and discriminate against unrelated non-kin. The process of kin recognition usually involves an ability to measure and react to genetic relatedness between actor and recipient in a social interaction, and several studies have been performed to identify levels of kin discrimination in different *Dictyostelium* species. In *D. purpureum*, a species with extensive genetic variation and clear population structure, fruiting-body formation is affected by genetic distance between strains as measured by rDNA sequences [29], with strong kin discrimination observed between distinct genotypes [30]. In addition, the degree of kin discrimination appears to be positively correlated to the genetic divergence between strains that contribute to the chimeric fruiting body.

An investigation of *D. discoideum* fruiting-body formation between one fixed laboratory axenic strain (which can grow in artificial media independent of live bacteria) and a series of natural strains of variable genetic distance, as measured using microsatellite loci, also found a positive correlation between kin discrimination and genetic divergence [31]. However, another study using strain pairs that show different levels of divergence based on >100 single nucleotide polymorphisms (SNPs) across the genome did not reveal any such correlation [32]. Interestingly,

D. discoideum strains from the same geographical location (i.e. sympatric strains) showed greater levels of kin discrimination compared to allopatric strain pairs, regardless of their actual genetic distances as measured by overall SNP divergence. This suggests that strains that have an increased probability of interaction tend to recognize each other better and preferentially cooperate only with cells of the same genotype.

Although it is unclear why these different studies gave different results, this raises the question of whether *D. discoideum* strains actually discriminate based on genome-wide genetic relatedness. For this single-celled organism it is possible that discrimination depends on specific set of recognition genes rather than general genetic similarity due to genealogical relationships. If this is true, then the question arises – which genes are associated with kin/non-kin recognition and do levels of kin discrimination correlate with divergence at these specific loci? In *D. discoideum*, kin discrimination does appear to be mediated in part by two polymorphic genes, *tgrB1* and *tgrC1*, which encode transmembrane glycoproteins and play a role in cell–cell recognition/adhesion. As expected, the degree of kin discrimination in this species increases with the level of sequence dissimilarity at these two genes [33].

Sociobiological theory posits that gene-specific recognition and behavior between social interactors is taken to the extreme by so-called 'greenbeard' genes – these possess three characteristics: (i) they encode hypothetical green beards (i.e. a recognizable trait or signal), (ii) they allow individuals to recognize green beards in others and (iii) to cooperate only with individuals displaying green beards [34,35]. The first greenbeard effect was found in the red fire ant, possibly involving multiple tightly-linked genes [36,37], but the first single-gene greenbeard effect was observed in *D. discoideum* [38]. In this species, cells carrying a wild-type *csaA* allele aggregate predominantly with other wild-type cells when mixed with *csaA* null cells in soil. By contrast, on non-natural laboratory substrates, such as agar, an anti-greenbeard effect was found, where *csaA*-knockout cells were able to adhere to aggregation streams and thereby preferentially become spores. Thus, on more natural soil plates the early-onset greenbeard effect prevents the anti-greenbeard effect from occurring by excluding null mutant cells from aggregates. Interestingly, the *csaA* gene is not polymorphic in natural populations, and the existence of such polymorphisms in the laboratory might not be fully representative of the situation in the wild. This genetic study, however, does indicate that greenbeard genes can exist and it remains to be seen whether they are an important component of the regulation of the social dynamics in this social amoeba.

The invasion of cheaters

One aspect of social cooperation is that it sets the stage for the evolution of cheaters – individuals that take advantage of the system to increase their own fitness but without contributing to the maintenance of cooperative behavior. In fruiting-body formation in *D. discoideum*, cheating genotypes are those that preferentially form spore cells at the expense of becoming the altruistic stalk cells that perish during fruiting-body development.

Cheating behavior could have diverse mechanisms and involve different portions of the *D. discoideum* life cycle. Cheating behaviors, for example, could be established early in fruiting-body formation. It is reported that cells that initiate fruiting-body development tend to form spores rather than stalk cells, although the energy reserve in these cells is lower than in latecomers [39,40]. Intrinsic properties of vegetative growth and other factors could also result in cheating behavior. Nutrition history, for example, leads to a bias in cell-fate determination [41,42]; cells grown in the presence of glucose are biased to spore fates, although it must be noted that cells which coexist in the wild should tend to share similar nutrition histories. Cells early in the cell cycle also preferentially go to the stalk, and cheaters can exploit this by initiating development late in the cell cycle [42–44]. Finally, the relative allocation of cells between stalk and spore fates is known to differ between genotypes and can affect chimeric fruiting-body formation [45]. During chimeric development, strains with higher sporulation efficiency are more likely to be socially dominant, in other words they become preferentially enriched in spores rather than stalks in chimeric fruiting bodies.

There has been concerted interest in dissecting the genetics of cheating in *D. discoideum*. The first cheater mutant found in this species was *fbxA*; this gene encodes an F-box protein associated with ubiquitination and protein degradation [46]. The proteolytic apparatus containing FbxA protein degrades RegA, a phosphodiesterase that controls the intracellular level of cAMP [47]. Mutant strains of *fbxA* appear normal at aggregation and early differentiation, but exploit wild-type cells later in development by outcompeting them in chimeric mixtures. Although precisely how FbxA protein controls cell-type proportioning and cheating is still unclear, it has been suggested that the cheating mechanism of the *fbxA* mutant could involve manipulating or ignoring extracellular signals [48].

The *fbxA* mutant fails to produce mature spores when they are clonal, and their cheating behavior is therefore only evident in chimeric fruiting bodies. Other laboratory mutants, however, facultatively cheat and are more likely to survive in nature [49]. A genetic screen identified 100 genes associated with cheating, and half of these showed equal or better sporulation efficiency compared to the wild type. These findings revealed a wide range of genes associated with cheating behaviors, and imply that cheaters can evolve spontaneously in nature. The best-described mutant strain had a defect in *chtC*, which encodes a putative transmembrane protein. The *chtC* mutant cells fail to maintain a prestalk fate and instead transdifferentiate into prespore cells [50]; this indicates that cheating can arise by simply exploiting existing developmental pathways.

Given that cheaters, by definition, can outcompete other cells and thus have potentially elevated fitness, how is cooperation maintained in their presence? The fitness of cheaters is likely to be negatively frequency-dependent – at low frequency cheater genotypes spread readily, but they threaten the entire group with greater severity as their frequency increases. As a result, both wild-type and cheater genotypes should coexist as a balanced polymorphism,

limiting the prevalence of the latter genotype – such negative frequency-dependency has indeed been observed in *D. discoideum* [27]. Other mechanisms to subvert cheating behaviors have also been observed in *D. discoideum*. Kin discrimination, for example, works to maintain high relatedness within cooperative structures and prevent the spread of cheaters. As an illustration, wild-type cells in soil do not cooperate with individuals lacking the functional greenbeard gene *csaA*, although this mutant would otherwise act as a potential cheater on less natural (i.e. laboratory) substrates [38]. Cells can also exclude cheaters by cooperating only with individuals expressing an identical set of recognition signals, such as the *tgrB1* and *trgC1* genes [33].

Pleiotropy could also provide a mechanism to mitigate the deleterious effect of potential cheater genes. For example, *dimA* is required to receive the DIF-1 signal that leads to the formation of approximately one half of prestalk cells, which are designated as subtype pstO. Null *dimA* mutant cells cannot be induced by DIF-1 and are consequently enriched in the posterior of the slug where the prespore region is located [51]. Despite this, these cells are excluded from spores because of the pleiotropic effect of the *dimA* gene; later in chimeric development, *dimA* null cells become enriched in the stalk. The two contrasting effects of *dimA* suggest that pleiotropy can limit the spread of selfish individuals.

Finally, the presence of cheaters in the populations could select for cheater-resisters. In *D. discoideum*, several cheater-resisters successfully emerged in a selection experiment using the cheater *chtC* as the selector [52]. Notably, the cheater-resister *rccA* not only resisted exploitation by *chtC* but also cooperated fairly in the same manner as the wild-type genotype.

The enigmatic sexual phase

Compared to the intensively studied asexual fruiting-body phase, much less is known about sex in *D. discoideum*. The sexual cycle in this species, which also has a social dimension, involves (i) sexual maturation of solitary amoebae, (ii) cell and nuclear fusion between cells of distinct mating types, (iii) aggregation of other solitary cells and cannibalization by the developing zygote, (iv) maturation of the macrocyst (a cellulose-coated zygotic structure [10]), and (v) germination of progeny from the macrocyst [53]. As with fruiting-body initiation, macrocyst formation involves cAMP signaling to other single cells by the developing zygote, but these attracted cells are consumed by the zygote, a cannibalistic phenomenon that also represents another example of altruistic behavior in *D. discoideum*. The importance of sex in the *D. discoideum* life cycle, and the implied altruistic behavior displayed by cannibalized cells, was not appreciated for a long time. Germination of macrocysts has proved difficult in the laboratory, and it was also unclear to what extent the sexual phase is important in this species. A recent study based on genome-wide SNP data using several North American strains, however, showed a rapid decay of linkage disequilibrium with distance and the presence of recombinant genotypes among wild strains [32]. These two observations indicate that meiotic recombination is not uncommon in this species [32], suggesting that the sexual cycle occurs readily in the wild. The sexual cycle could thus represent another important type of social cooperation in *D. discoideum*.

Several genes are known to be involved in sexual cycle, some of which also play a role in the asexual phase [53,54]. This could suggest that similar mechanisms underlie at least some aspects of social interaction in both phases, possibly through evolutionary cooption. The dynamics of the altruistic behavior associated with macrocyst formation has not been studied, and some pressing issues require investigation. For example, one can imagine that the sexual cycle could also be vulnerable to cheaters who engage in cell fusion more actively than normal, and thereby escape the fate of cannibalization, but whether this occurs in nature is not known. It is also very possible that the newly-formed zygote can selectively cannibalize surrounding solitary cells that are not closely related to either of the two mating cells, leading to the question of how this altruistic behavior on the part of the cannibalized cells has evolved and is maintained.

Macrocyst formation could represent an example of coercion to enforce altruistic cooperation. This might not be surprising given that recent observations indicate that coercion can be an important mechanism enforcing altruism in insect societies with intermediate level of relatedness [55]. There are several features of macrocyst formation that suggests a coercive mechanism to ensure altruistic behavior of the surrounding cells. It has been shown, for example, that once the zygote is formed it secretes a low molecular weight autoinhibitor which prevents cell fusion in surrounding cells by inhibiting calmodulin [56], thus ensuring that these cells remain solitary and available for the zygote to cannibalize. Moreover, chemoattraction of solitary cells to the developing zygote utilizes cAMP, the same signal used to initiate aggregation for fruiting-body formation. In this context, however, the fate of the attracted cells is not to participate in the formation of the cooperative fruiting-body but to be consumed by the zygote. Several other signaling molecules associated with macrocyst development have been found, suggesting that pathways of cell fate determination are complicit in this social interaction [53,57]. The key question here is whether cannibalism of cells during macrocyst formation also relies on genetic relatedness between zygotes and consumed cells, as predicted by kin selection theory, and if so whether mechanisms ensuring genetic relatedness arise passively (e.g. limited dispersal of cells ensures relatedness of near neighbors) or through an active mechanism of kin discrimination.

Concluding remarks: the genomics era and beyond

In the past decades *D. discoideum* has emerged as a pre-eminent genetic model system for social evolution (Table 1). Using several different approaches it has been possible to study in this microbial species the genetic basis of social cooperation and altruism, and to begin to identify mechanisms associated with the origin and maintenance of sociality. The use of *D. discoideum* as a model system has been reinforced by the development of several genomic resources that will facilitate future research. The completion of the whole genome sequencing project in 2005, as

Table 1. A list of selected genes affecting social cooperation in *D. discoideum*

Gene	Description	Ref.
fbxA	Part of a complex that targets proteins for ubiquination; null mutants are obligate cheaters	[46]
csaA	Adhesion molecule that mediates single-gene greenbeard effects	[38]
dimA	bZIP/bRLZ transcription factor required for the response to DIF-1; null mutants show a pleiotropic effect in chimera development	[51,65]
tgrB1, tgrC1	Highly polymorphic transmembrane glycoproteins that mediate kin discrimination	[33]
rccA	Insertion mutant resists the exploitation by *chtC* and does not cheat on the wild-type	[52]
chtC	Putative transmembrane protein; null mutants are facultative cheaters	[50]

well as of expressed sequence tag (EST) sequencing projects, have led to the discovery of genes involved in different developmental stages and biological processes using microarray (and now RNA-Seq) analyses [58,59]. A comparative transcriptomic study, for example, found that ~69% of the genome (around 8435 genes) is expressed across all developmental stages in the *D. discoideum* isolates analyzed [60]. Population-based resequencing projects also provide insights into the evolutionary genetics of this species [32], and these set the stage for sequence-based scans of selection and possibly association-based genetic analysis of phenotypic variation.

The availability of these genomic resources, coupled with the ease of laboratory and molecular manipulation of this social amoeba, suggests that the time is right for more focused sociogenomic analyses of *D. discoideum*. This microbial species provides an excellent system in which to explore and test alternative models surrounding the origin and maintenance of social cooperation and the evolutionary forces and ecological factors associated with sociality. One could explore, for instance, whether coercion is a significant mechanism in promoting altruism in the sexual cycle of this social amoeba, and to what extent kin discrimination mediated by specific genes underlies social cooperation. Integrating molecular, evolutionary and ecological viewpoints and making full use of the genetic and genomic resources of this model species, coupled with a clear theoretical framework, allows these and other questions to be addressed in greater depth, promising to advance our understanding of sociobiology [61,62].

Acknowledgments

We thank Joan Strassmann, Ulises Rosas and Jeanmaire Molina for constructive comments on the manuscript. Gareth Bloomfield is also thanked for insights into cannibalism during macrocyst formation.

References

1 Edward, O.W. (2000) *Sociobiology: The New Synthesis* (25th Anniversary Edn), Harvard University Press
2 Hamilton, W.D. (1964) The genetical evolution of social behavior, I & II. *J. Theor. Biol.* 7, 1–52
3 Griffin, A.S. *et al.* (2004) Cooperation and competition in pathogenic bacteria. *Nature* 430, 1024–1027
4 Parsek, M.R. and Greenberg, E.P. (2005) Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends Microbiol.* 13, 27–33
5 Keller, L. and Surette, M.G. (2006) Communication in bacteria: an ecological and evolutionary perspective. *Nat. Rev. Microbiol.* 4, 249–258
6 Nadell, C.D. (2009) The sociobiology of biofilms. *FEMS Microbiol. Rev.* 33, 206–224
7 Velicer, G.J. and Yu, Y.T. (2003) Evolution of novel cooperative swarming in the bacterium *Myxococcus xanthus*. *Nature* 425, 75–78
8 Velicer, G.J. and Vos, M. (2009) Sociobiology of the myxobacteria. *Annu. Rev. Microbiol.* 63, 599–623
9 Swanson, A.R. *et al.* (1999) Global distribution of forest soil dictyostelids. *J. Biogeogr.* 26, 133–148
10 Kessin, R.H. (2001) *Dictyostelium – Evolution, Cell Biology, and The Development of Multicellularity*, Cambridge University Press
11 Eichinger, L. *et al.* (2005) The genome of the social amoeba *Dictyostelium discoideum*. *Nature* 435, 43–57
12 Chisholm, R.L. and Firtel, R.A. (2004) Insights into morphogenesis from a simple developmental system. *Nat. Rev. Mol. Cell Biol.* 5, 531–541
13 Kuzdzal-Fick, J.J. *et al.* (2007) Exploiting new terrain: an advantage to sociality in the slime mold *Dictyostelium discoideum*. *Behav. Ecol.* 18, 433–437
14 Kessin, R.H. *et al.* (1996) How cellular slime molds evade nematodes. *Proc. Natl. Acad. Sci. U. S. A.* 93, 4857–4861
15 Fortunato, A. *et al.* (2003) Co-occurrence in nature of different clones of the social amoeba, *Dictyostelium discoideum*. *Mol. Ecol.* 12, 1031–1038
16 Foster, K.R. *et al.* (2002) The costs and benefits of being a chimera. *Proc. Biol. Sci.* 269, 2357–2362
17 Clow, P.A. *et al.* (2000) Three-dimensional *in vivo* analysis of *Dictyostelium* mounds reveals directional sorting of prestalk cells and defines a role for the myosin II regulatory light chain in prestalk cell sorting and tip protrusion. *Development* 127, 2715–2728
18 Williams, J.G. (2006) Transcriptional regulation of *Dictyostelium* pattern formation. *EMBO Rep.* 7, 694–698
19 Kay, R.R. and Jermyn, K.A. (1983) A possible morphogen controlling differentiation in *Dictyostelium*. *Nature* 303, 242–244
20 Kopachik, W. *et al.* (1983) *Dictyostelium* mutants lacking DIF, a putative morphogen. *Cell* 33, 397–403
21 Kay, R.R. and Thompson, C.R. (2001) Cross-induction of cell types in *Dictyostelium*: evidence that DIF-1 is made by prespore cells. *Development* 128, 4959–4966
22 Shaulsky, G. and Loomis, W.F. (1996) Initial cell type divergence in *Dictyostelium* is independent of DIF-1. *Dev. Biol.* 174, 214–220
23 Thompson, C.R. and Kay, R.R. (2000) The role of DIF-1 signaling in *Dictyostelium* development. *Mol. Cell* 6, 1509–1514
24 Kim, L. *et al.* (2002) Receptor-dependent and tyrosine phosphatase-mediated inhibition of GSK3 regulates cell fate choice. *Dev. Cell* 3, 523–532
25 Louis, J.M. *et al.* (1994) The cAMP receptor CAR4 regulates axial patterning and cellular differentiation during late development of *Dictyostelium*. *Genes Dev.* 8, 2086–2096
26 Yu, Y. and Saxe, C.L., III (1996) Differential distribution of cAMP receptors cAR2 and cAR3 during *Dictyostelium* development. *Dev. Biol.* 173, 353–356
27 Gilbert, O.M. *et al.* (2007) High relatedness maintains multicellular cooperation in a social amoebae by controlling cheater mutants. *Proc. Natl. Acad. Sci. U. S. A.* 104, 8913–8917
28 Gilbert, O.M. *et al.* (2009) Discovery of a large clonal patch of a social amoeba: implications for social evolution. *Mol. Ecol.* 18, 1273–1281
29 Mehdiabadi, N.J. *et al.* (2008) Phylogeny, reproductive isolation and kin recognition in the social amoeba *Dictyostelium purpureum*. *Evolution* 63, 542–548
30 Mehdiabadi, N.J. *et al.* (2006) Social evolution: kin preference in a social microbe. *Nature* 442, 881–882
31 Ostrowski, E.A. *et al.* (2008) Kin discrimination increases with genetic distance in a social amoeba. *PLoS Biol.* 6, e287
32 Flowers, J.M. *et al.* (2010) Variation, sex and social cooperation: molecular population genetics of the social amoeba *Dictyostelium discoideum*. *PLoS Genet.* 6, e1001013

33 Benabentos, R. *et al.* (2009) Polymorphic members of the lag gene family mediate kin discrimination in *Dictyostelium*. *Curr. Biol.* 19, 567–572
34 Gardner, A. and West, S.A. (2009) Greenbeards. *Evolution* 64, 25–38
35 Dawkins, R. (1976) *The Selfish Gene*, Oxford University Press
36 Keller, L. and Ross, K.G. (1998) Selfish genes: a green beard in the red fire ant. *Nature* 394, 573–575
37 Krieger, M.J.B. and Ross, K.G. (2002) Identification of a major gene regulating complex social behavior. *Science* 295, 328–332
38 Queller, D.C. *et al.* (2003) Single-gene greenbeard effects in the social amoeba *Dictyostelium discoideum*. *Science* 299, 105–106
39 Huang, H.J. *et al.* (1997) Cells at the center of *Dictyostelium* aggregates become spores. *Dev. Biol.* 192, 564–571
40 Kuzdzal-Fick, J.J. *et al.* (2010) An invitation to die: initiators of sociality in a social amoeba become selfish spores. *Biol. Lett.* 6, 800–802
41 Blaschke, A. *et al.* (1986) *Dictyostelium discoideum*: cell-type proportioning, cell-differentiation preference, cell fate, and the behavior of anterior-like cells in Hs1/Hs2 and G+/G- mixtures. *Differentiation* 32, 1–9
42 Thompson, C.R. and Kay, R.R. (2000) Cell-fate choice in *Dictyostelium*: intrinsic biases modulate sensitivity to DIF signaling. *Dev. Biol.* 227, 56–64
43 Gomer, R.H. and Firtel, R.A. (1987) Cell-autonomous determination of cell-type choice in *Dictyostelium* development by cell-cycle phase. *Science* 237, 758–762
44 Ohmori, R. and Maeda, Y. (1987) The developmental fate of *Dictyostelium discoideum* cells depends greatly on the cell-cycle position at the onset of starvation. *Cell Differ.* 22, 11–18
45 Buttery, N.J. *et al.* (2009) Quantification of social behavior in *D. discoideum* reveals complex fixed and facultative strategies. *Curr. Biol.* 19, 1373–1377
46 Ennis, H.L. *et al.* (2000) *Dictyostelium* amoebae lacking an F-box protein form spores rather than stalk in chimeras with wild type. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3292–3297
47 Mohanty, S. *et al.* (2001) Regulated protein degradation controls PKA function and cell-type differentiation in *Dictyostelium*. *Genes Dev.* 15, 1435–1448
48 Shaulsky, G. and Kessin, R.H. (2007) The cold war of the social amoebae. *Curr. Biol.* 17, R684–R692
49 Santorelli, L.A. *et al.* (2008) Facultative cheater mutants reveal the genetic complexity of cooperation in social amoebae. *Nature* 451, 1107–1110
50 Khare, A. and Shaulsky, G. (2010) Cheating by exploitation of developmental prestalk patterning in *Dictyostelium discoideum*. *PLoS Genet.* 6, e1000854
51 Foster, K.R. (2004) Pleiotropy as a mechanism to stabilize cooperation. *Nature* 431, 693–696
52 Khare, A. *et al.* (2009) Cheater-resistance is not futile. *Nature* 461, 980–982
53 Urushihara, H. and Muramoto, T. (2006) Genes involved in *Dictyostelium discoideum* sexual reproduction. *Eur. J. Cell. Biol.* 85, 961–968
54 Shimizu, H. *et al.* (1997) A mutation in the cAMP signaling pathway affects sexual development of *Dictyostelium discoideum*. *Dev. Growth Differ.* 39, 227–234
55 Ratnieks, F.L. and Wenseleers, T. (2008) Altruism in insect societies and beyond: voluntary or enforced? *Trends Ecol. Evol.* 23, 45–52
56 Lydan, M.A. and O'Day, D.H. (1989) The autoinhibitor of cell fusion in *Dictyostelium* inhibits calmodulin. *Biochem. Biophys. Res. Commun.* 164, 1176–1181
57 O'Day, D.H. and Lydan, M.A. (1989) The regulation of membrane fusion during sexual development in *Dictyostelium discoideum*. *Biochem. Cell Biol.* 67, 321–326
58 Morio, T. *et al.* (1998) The *Dictyostelium* developmental cDNA project: generation and analysis of expressed sequence tags from the first-finger stage of development. *DNA Res.* 5, 335–340
59 Urushihara, H. *et al.* (2004) Analysis of cDNAs from growth and slug stages of *Dictyostelium discoideum*. *Nucleic Acids Res.* 32, 1647–1653
60 Parikh, A. *et al.* (2010) Conserved developmental transcriptomes in evolutionarily divergent species. *Genome Biol.* 11, R35
61 West, S.A. *et al.* (2006) Social evolution theory for microorganisms. *Nat. Rev. Microbiol.* 4, 597–607
62 Robinson, G.E. *et al.* (2005) Sociogenomics: social life in molecular terms. *Nat. Rev. Genet.* 6, 257–270
63 Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*, Clarendon Press
64 Haldane, J.B.S. (1955) Population genetics. *New Biol.* 18, 34–51
65 Thompson, C.R. *et al.* (2004) A bZIP/bRLZ transcription factor required for DIF signaling in *Dictyostelium*. *Development* 131, 513–523