Major evolutionary transitions in individuality

Stuart A. West[a,b,1], Roberta M. Fisher[a], Andy Gardner[c], and E. Toby Kiers[d]

[a]Department of Zoology, University of Oxford, Oxford OX1 3PS, United Kingdom; [b]Magdalen College, Oxford OX1 4AU, United Kingdom; [c]School of Biology, University of St. Andrews, Dyers Brae, St. Andrews KY16 9TH, United Kingdom; and [d]Institute of Ecological Sciences, Faculty of Earth and Life Sciences, Vrije Universiteit, 1081 HV, Amsterdam, The Netherlands

The evolution of life on earth has been driven by a small number of major evolutionary transitions. These transitions have been characterized by individuals that could previously replicate independently, cooperating to form a new, more complex life form. For example, archaea and eubacteria formed eukaryotic cells, and cells formed multicellular organisms. However, not all cooperative groups are en route to major transitions. How can we explain why major evolutionary transitions have or haven't taken place on different branches of the tree of life? We break down major transitions into two steps: the formation of a cooperative group and the transformation of that group into an integrated entity. We show how these steps require cooperation, division of labor, communication, mutual dependence, and negligible within-group conflict. We find that certain ecological conditions and the ways in which groups form have played recurrent roles in driving multiple transitions. In contrast, we find that other factors have played relatively minor roles at many key points, such as within-group kin discrimination and mechanisms to actively repress competition. More generally, by identifying the small number of factors that have driven major transitions, we provide a simpler and more unified description of how life on earth has evolved.

cooperation | altruism | signaling | division of labor | conflict

The evolution of life, from simple organic compounds in a primordial soup to the amazing diversity of contemporary organisms, has taken roughly 3.5 billion years. How can we explain the evolution of increasingly complex organisms over this period? A traditional approach has been to consider the succession of taxonomic groups, such as the age of fishes giving rise to the age of amphibians, which gave way to the age of reptiles, and so on. Although this approach has some uses, it is biased toward relatively large plants and animals and lacks a conceptual or predictive framework, in that it suggests we look for different explanations for each succession (1).

Twenty years ago, Maynard Smith and Szathmáry (2) revolutionized our understanding of life on earth by showing how the key steps in the evolution of life on earth had been driven by a small number of "major evolutionary transitions." In each transition, a group of individuals that could previously replicate independently cooperate to form a new, more complex life form. For example, genes cooperated to form genomes, archaea and eubacteria formed eukaryotic cells, and cells cooperated to form multicellular organisms (Table 1).

The major transitions approach provides a conceptual framework that facilitates comparison across pivotal moments in the history of life (2, 3). It suggests that the same problem arises at each transition: How are the potentially selfish interests of individuals overcome to form mutually dependent cooperative groups? We can then ask whether there are any similarities across transitions in the answers to this problem. Consequently, rather than looking for different explanations for the succession of different taxonomic groups, we could potentially identify a few key factors that have been important again and again at driving increases in organismal complexity. This approach would both unify and simplify our understanding of the evolution of life on earth.

We define the steps and processes in major transitions and show that the problem of explaining major transitions can be broken down into six questions. We explore what is already known about the factors facilitating transitions, examining the extent to which we can generalize across the different transitions. Ultimately, we are interested in the underlying evolutionary and ecological factors that drive major transitions.

Defining Major Transitions

A major evolutionary transition has been most broadly defined as a change in the way that heritable information is stored and transmitted (2). We focus on the major transitions that lead to a new form of individual (Table 1), where the same problems arise, in a way that facilitates comparison, and so exclude the evolution of the genetic code, sex and language (1, 2, 4, 5).

A major evolutionary transition in individuality is defined by two conditions (1, 2). First, entities that were capable of independent replication before the transition can replicate only as part of a larger unit after it, termed mutual dependence, interdependence, or contingent irreversibility. Second, there is a relative lack of within-group conflict such that the larger unit can be thought of as a fitness-maximizing individual (or organism) in its own right. For example, it is common to think of the nucleus and organelles of a eukaryotic cell, or the group of cells that makes up a multicellular animal, as a single organism (6).

When these two conditions are met, evolution can lead to a new higher level individual (organism). We have a group that can't be broken up (condition 1), and we can conceptualize the group acting with a single purpose, where the interests of the previously independent individuals are now aligned (condition 2). We provide examples of how this definition can be applied in Table 1. A precise definition matters because a blurring of exactly what is being examined can obscure the relative importance of the underlying selective forces (7, 8).

Steps

Major evolutionary transitions can be divided into two steps: (*i*) the formation of a cooperative group and (*ii*) the transformation of the cooperative group into a more cohesive and integrated entity that can be considered a new level of individual (organism) (Fig. 1) (1, 2). The second step typically involves a number of common features, including the following: the individuals in a group evolving to perform different tasks (division of labor); division of labor becoming so specialized that the members of the group become dependent upon each other; and communication to coordinate cooperation at the group level.

Although these two steps may not have clear borders, and can move gradually from one to the next, they can occur in some order (1). For example, in transitions involving members of the same species, we would not expect division of labor to evolve until after cooperative groups have formed. Consequently, the

Table 1. The major evolutionary transitions in individuality

Major transitions			Not major transitions		
				Why not?	
Transition			Examples	Mutual dependence?	Negligible conflict?
		Examples			
Independent replicators	→	Populations of replicators	Genome	—	—	—
Separate unicells	→	Symbiotic unicell	Eukaryotic cell*, primary plastid endosymbiosis*, *Paulinella** Secondary/tertiary plastid endosymbiosis*	—	—	—
Unicells	→	Multicellular organism	Animals, plants, fungi, red algae, brown algae	*Dictyostelid* slime molds[†] *Pseudomonas* biofilms	No No	No No
Multicellular organisms	→	Eusocial society	Higher termites, leaf-cutter ants, honeybee	Social aphids[‡] *Melipona* bees Cooperative vertebrates Humans	No Yes No No	Yes No No No
Multicellular organisms	→	Obligate interspecific mutualism	Some obligate endosymbionts (e.g., *Buchnera* in aphids*), leaf-cutter ants and their fungi, Paracatenula flatworms and their chemoautrophic symbionts	Legumes-*Rhizobia*[§] Squid-*Vibrio* Gut microbiota	No No No	No No No

*Highly integrated/obligate symbioses, where the hosts cannot survive without their symbionts, and there seems to be a relative lack of conflict, such that the symbionts can best increase their own fitness by helping their hosts reproduce (45).
[†]Facultatively multicellular species, which can remain in their unicellular state for many generations, without the need to form a multicellular fruiting body, which they do only under certain harsh conditions to increase dispersal success (no mutual dependence) (37). Sufficient conflict to select for noncooperative cheats, and within-group kin discrimination (68, 69).
[‡]Facultatively eusocial species, where queens are still able to breed if the workers/soldiers are removed (no mutual dependence).
[§]Hosts are able to grow and replicate without these symbionts (no mutual dependence), and there is sufficient conflict to select for noncooperative cheats (**69**).

benefits of having different cell types in multicellular organisms may not provide an explanation for why multicellular groups initially formed. In contrast, with transitions between members of different species, individuals can be specialized to perform different tasks before the formation of cooperative groups, and group transformation involves specialization to help each other.

What Are the Big Questions?

We suggest six questions that are key to understanding the evolutionary and ecological drivers of major transitions:

i) What conditions favor the formation of cooperative groups?
ii) What conditions maintain cooperation during group transformation?
iii) What conditions favor division of labor?
iv) What conditions favor communication that coordinates cooperation at the group level?
v) What conditions lead to negligible conflict within groups?
vi) What conditions favor mutual dependence?

Question *i* concerns the first step in a major transition (the formation of a cooperative group) whereas the other five questions concern the second step in a major transition (the transformation of the cooperative group into a new level of individuality) (Fig. 1). We have divided these questions to identify specific research problems. Our division emphasizes that major transitions are not just about cooperation or extreme altruism (sterility); they are about an extreme form of cooperation, involving multiple traits, division of labor, mutual dependence, and a lack of conflict.

What Conditions Favor Cooperation?

The first step in a major transition is that individuals come together and form a cooperative group. Cooperation then needs to be maintained while group transformation takes place. We consider the factors that may favor the formation and maintenance of cooperative groups together because the same selective forces

can be involved. A trait is cooperative if it provides a benefit to another individual and has evolved at least partially because of that benefit (8). The problem of cooperation is that, all else being equal, cooperators could be exploited and outcompeted by non-cooperators (cheats), who gain the benefits from the cooperation of others, but avoid the cost of cooperating (9). The evolution of cooperation requires two conditions (1, 10–12). First, there is some ecological or efficiency benefit to cooperation. Second, there is some mechanism that leads to the benefits of cooperation being directed back to the cooperator and/or their relatives.

Ecological or Efficiency Benefits. The ecological benefits of cooperation have been well studied in many taxa. Cells form cooperative multicellular clumps to evade predators, make more efficient use of factors that are excreted from individual cells, and form fruiting bodies that aid dispersal (1, 13). Cooperation
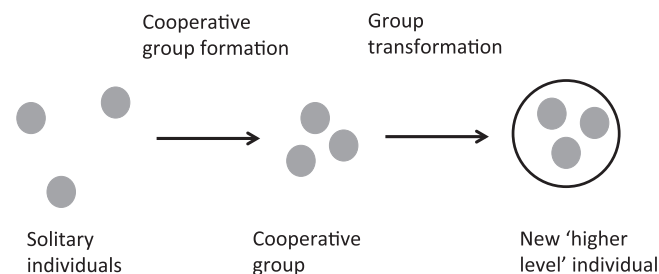

Fig. 1. A major evolutionary transition involves two steps: first, the formation of a cooperative group; second, the transition to a new level of organism, with division of labor, interdependence, and coordination of the parts. Although the first step is well-understood, the second is not. We follow Bourke, except that he divides transitions into three steps, distinguishing between maintenance and transformation (1).
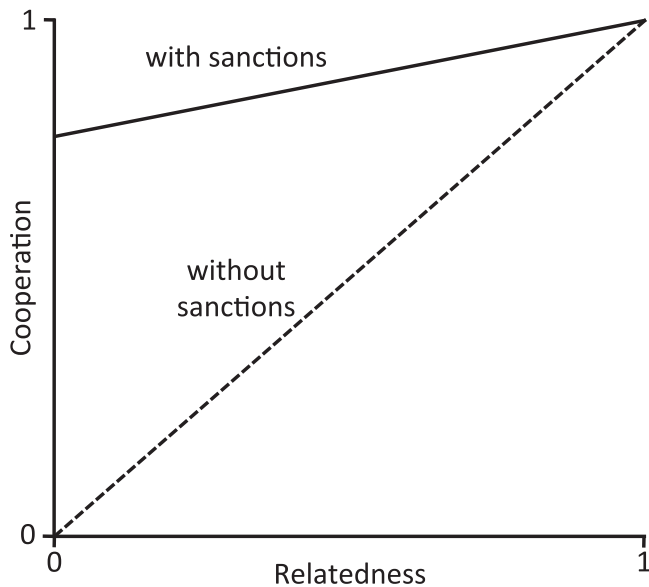
Fig. 2. The hypothetical level of cooperative helping in a symbiont plotted against the relatedness between the symbionts infecting a host. If the hosts sanction uncooperative symbionts, then a high level of cooperation is predicted, relatively independent of relatedness. If the hosts do not carry out sanctions, then the level of cooperation is predicted to depend strongly upon relatedness between symbionts (23).

between different species often involves a symbiont providing a service, such as a resource that allows their host to grow better (14–16). Cooperation is favored in eusocial species to care for young over an extended period where the parent may die, or to defend a valuable resource (17).

Mechanism to Direct Benefits Back (Within Species). Cooperation can be favored if the benefits go to relatives who carry the same genes for cooperation, termed kin selection (18). By helping a close relative reproduce, an individual is still passing on copies of its genes to the next generation, just indirectly. Kin selection is often encapsulated with Hamilton's rule (18), which predicts that altruistic cooperation will be favored when $rB - C > 0$, where C is the fitness cost to the altruist, B is the fitness benefit to the beneficiary, and r is their genetic relatedness. Hamilton's rule shows that altruism can be favored when the indirect benefits of helping relatives (rB) outweigh the direct costs (C). There is a large empirical literature supporting the role of relatedness and showing how a high relatedness can arise either through limited dispersal, which keeps relatives together, or kin discrimination, where cooperation is preferentially directed toward relatives (1, 11).

Cooperation can be favored between nonrelatives in the same species if it provides a direct benefit to the cooperator: for example, when unrelated ants cooperate to increase their chance of founding a new colony (19). It can also occur via mechanisms, such as reciprocity, that reward cooperators or punish noncooperators (20). In these cases, the costs incurred by cooperating are outweighed by the benefits received in return, and so cooperation is mutually beneficial rather than altruistic.

Mechanism to Direct Benefits Back (Between Species). Cooperation between species also requires mechanisms that lead to the benefits of cooperation being directed back to the cooperator and/or their relatives (21). Broadly speaking, two mechanisms are likely to be important for producing this feedback. The first mechanism is if individuals tend to be associated in such a way that their fitness becomes entwined (partner-fidelity feedback) (12). For example, in a vertically transmitted clonal symbiont, then helping the host could increase the number of host offspring that the symbiont would be transmitted to. Relatedness among the symbionts matters because it determines who receives the benefits

of helping the host, and thus whether cheats could exploit the cooperation (22) (Fig. 2). A high relatedness among vertically transmitted symbionts in a host is likely to be important with mitochondria, plastids, and endosymbionts such as *Buchnera*.

The second mechanism is if the host preferentially rewards more cooperative symbionts and/or punishes less cooperative symbionts (23). For example, legumes provide more resources to more cooperative rhizobia, and squid eliminate symbiotic bacteria that do not luminesce (24, 25). Such "sanctions" mechanisms can favor cooperation even when symbiont relatedness is low at the level of the host (Fig. 2).

What Conditions Favor Division of Labor?

Organisms must perform a number of tasks to survive and reproduce. When will natural selection favor a division of labor, with different individuals performing different tasks? To illustrate this problem with a specific example, consider two tasks, A and B. We assume that investment into these two activities must be traded off against each other because time and energy spent on A cannot be spent on B. Division of labor will tend to be favored when the shape of the relationship between the proportion of resources allocated to each task and the fitness return are accelerating (Fig. 3) (2). The fitness return might be accelerating if a task becomes more efficient as more effort is put into it, or if tasks A and B don't mix well. In contrast, if the fitness returns on tasks are decelerating, then it can be more efficient to have all individuals perform some A and some B.

There are numerous examples of division of labor. In some volvocine algae, there is a division between large cells that reproduce and small cells who beat their flagella to keep the colony afloat (5). In some cyanobacteria, there is a division between cells that photosynthesize and cells that fix nitrogen into ammonia (heterocysts)—this division seems to be favored because nitrogenase, the enzyme that converts nitrogen gas to ammonia, is rapidly destroyed in the presence of oxygen (26). Symbioses by definition involve a division of labor, with partners providing different services for each other. In some cases, one partner will provide a service that the other partner cannot perform, such as the intracellular chemoautotrophic bacterial symbionts that provide nutrition for marine flatworms lacking a digestive tract (27). Hosts can sometimes harbor multiple symbionts, with different symbionts performing different tasks (28). Mitochondria

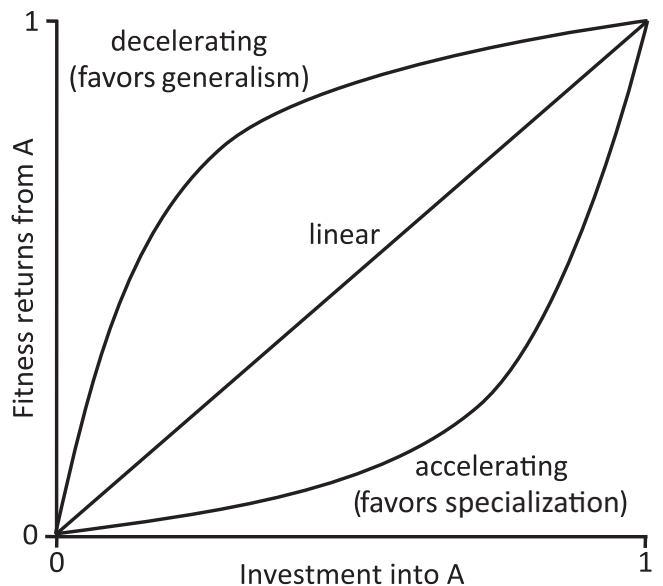**Fig. 3.** The relationship between the proportion of resources invested into a trait (A) and the fitness return from that trait. We assume that a proportion of resources X is put intro trait A, and the remaining proportion $1 − X$ into trait B.

and plastids provide clear examples of extreme specialization in transitions between species.

The general principle is that a division of labor will be favored when there are efficiency benefits to specialization (Fig. 3). There is a lack of research showing why division will be the favored in specific systems. Both theoretical models demonstrating how trade-offs between different traits can lead to benefits of specialization, and empirical studies measuring these trade-offs, are required to address this gap (29). Furthermore, our discussion has ignored many complexities. For example, how would factors such as within-group conflict, variation in relatedness, or different abilities to coordinate at the group level influence selection for division of labor?

What Conditions Favor Communication That Coordinates Cooperation at the Group Level?

Communication can be key to all stages of a major transition, playing multiple roles in the formation and transformation of cooperative groups: for example, coordinating cooperation and the division of labor at the group level. However, communication systems are potentially exploitable by cheats who fail to respond to signals or who signal dishonestly (30). Individuals could ignore signals to cooperate or could signal in a way to make others cooperate more. What maintains the honesty of the communication systems that help drive major transitions?

Theory has shown that honest signaling requires either that signals can't be faked (indices), that signals are costly to fake (handicaps), and/or that the sender and receiver have a common interest (30). A common interest is particularly relevant in major transitions because the same conditions that favor cooperation can also favor honest communication—high relatedness or aligned reproductive interests. The importance of high relatedness in favoring honest signaling within cooperative groups has been demonstrated experimentally with quorum sensing (QS), the process whereby bacteria use small signal molecules to regulate the production of extracellular factors that aid growth, motility, and biofilm formation. QS is favored at high relatedness and disfavored at low relatedness (31). Common interest presumably explains a range of signals in cases where major transitions have been made, from the waggle dance of the honeybee to among cell coordination in animals. Comparative studies could examine how communication systems vary across species that differ in their degree of conflict.

Symbioses involve many forms of communication, including the acquisition of symbionts from the environment, to the coordination of tasks. Specific cases range from partnerships in which effective communication allows both partners to prosper, to cases in which signals produced from one partner are used to manipulate the other for selfish gain. With luminous bacteria and their squid hosts, there seems to be a mutual interest in coordinating rhythms such that bacterial luminescence peaks when hosts most need the camouflage provided by the bacteria. This coordination involves the host genes' being regulated by the light and molecules produced by the bacteria (32). In other cases, conflict among partners is higher, and signals may be used to manipulate others.

What Conditions Lead to Negligible Conflict Within Groups?

Even when there are clear benefits to evolving cooperation, conflicts can still arise in cooperative groups. Evolutionary theory predicts that individuals are adapted to maximize their inclusive fitness, even if this maximization comes at a cost to other individuals or the group (18). Inclusive fitness is the sum of an individual's influence on its own reproductive success and that of related individuals. The question here is, as required for a major transition, what conditions are required for individuals maximizing their own inclusive fitness to also be maximizing the fitness of the group (33) (Fig. 4). We focus on inclusive fitness because it is under the full control of the actor, and so represents a measure of fitness that could be maximized (34). It is useful to consider within- and between-species transitions separately.

Within-Species Transitions. What do the empirical data show? Group formation seems to play a key role in whether a major transition to multicellularity or eusociality can take place (Fig. 5)

(1, 35–37). The major transition to obligate multicellularity has taken place only in species where daughter cells stick together after division and so group formation is clonal, such as the animals, fungi, red algae, green plants, volvocine algae, brown algae, some ciliates, and some cyanobacteria (37, 38). Although nonclonal group formation occurs via aggregation in many species, such as the cellular slime molds, ciliates, and acrasid slime molds, and can lead to very high relatedness (39), it has led only to facultative multicellularity.

Similarly, the transitions to both facultative and obligate eusociality have taken place only in species where offspring stay to help their parents and there is either strict lifetime monogamy or asexual reproduction (35, 36, 40). Multiple mating and multiple queens occur in some eusocial species, but they are derived states that evolved after eusociality was fixed. Although cooperation occurs in many species where females mate multiple times, such as the cooperative breeding birds and mammals, no multiply mating species has made a major transition to obligate eusociality (41).

Group Formation. How groups form is important because of its influence on relatedness and conflict. Consider a focal individual who is choosing either (*i*) to perform a cooperative behavior that helps another individual produce B offspring, to whom the focal actor is related r_h, or (*ii*) to produce C of their own offspring to whom they are related by r_o. In this case, helping is favored if $r_h B - r_o C > 0$, which represents a form of Hamilton's rule. However, we are interested not in whether helping is favored, but whether there is conflict between different individuals over whether to help. Conflict occurs when individuals disagree about the best strategy (6).

What conditions would be required to remove conflict between the helpers (workers) in a social group, such that they could be considered a single maximizing unit? One way to remove this conflict would be if the potential helper is equally related to the offspring that it could help raise and its own offspring ($r_h = r_o$) (33, 35–37). In this case, the different offspring are worth equal value to the potential helper, and they are selected to do whatever leads to
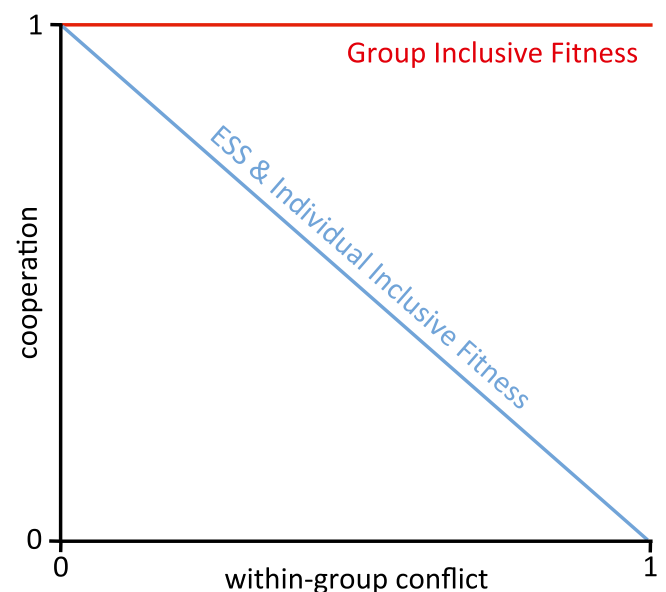

Fig. 4. The individual and the group. The hypothetical level of a cooperative trait, such as the amount of an extracellular factor produced by bacterial cells, plotted against the extent of conflict between interacting individuals. The different lines show the optimal strategy from the perspective of an individual's inclusive fitness (blue line) and group fitness (red line). Natural selection will lead to the evolutionarily stable strategy (ESS), which will be the strategy that maximizes inclusive fitness (i.e., the blue line), irrespective of the consequences at the group level. We would expect natural selection to lead to maximization of group fitness, and thus think of the group as a fitness-maximizing individual, only in extreme cases where there is no within-group conflict.

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Route of social group formation leads to negligible conflict

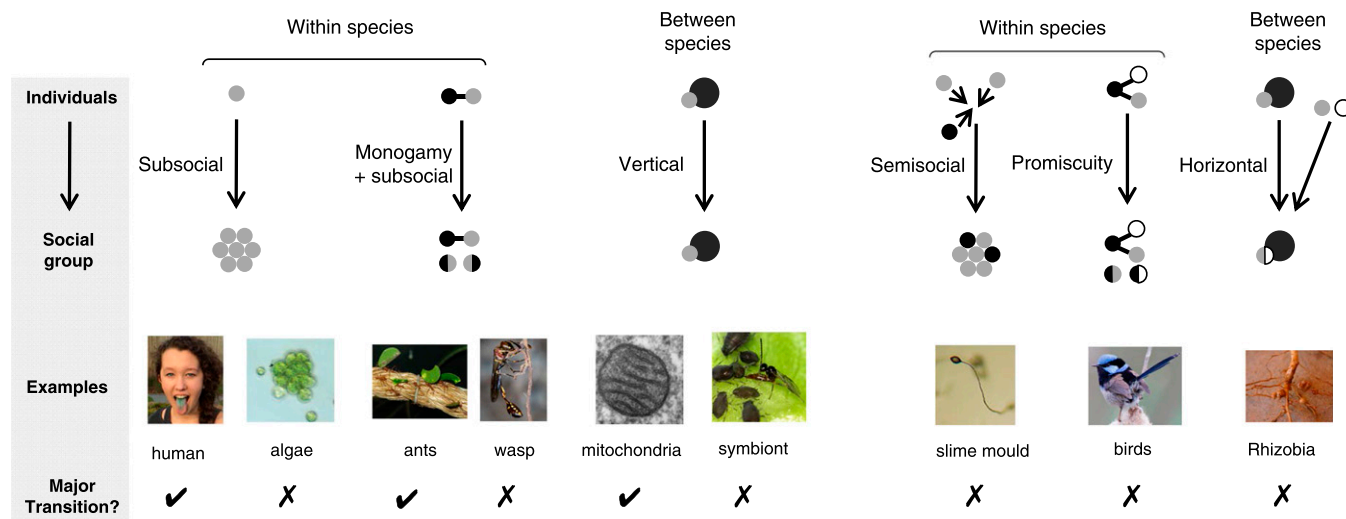**Route of social group formation leads to appreciable conflict**

Fig. 5. The way in which groups form is a major determinant of when major transitions have taken place, because it determines relatedness and the potential for within-group conflict. Within-species transitions have taken place only when offspring stay to help their parents (subsocial), and reproduction is either asexual or sexual with lifetime monogamy. Between-species transitions seem to involve similarly restrictive group formation, such as vertical transmission leading to clonal symbionts whose interests are aligned with their hosts. Different colored circles represent either genetically distinct individuals (within-species) or individuals of different species. Larger circles represent hosts with smaller circles representing their symbionts. The images, from left to right show: obligately multicellular human (image courtesy of Stu West), facultatively multicellular *Chlamydomonas* algae (image courtesy of Will Ratcliff), obligately eusocial *Atta* ants (image courtesy of Wikimedia commons/Arpingstone), facultatively eusocial Stenogastrine hover wasp (image courtesy of Wikimedia commons/David Baracchi), mitochondrion (image courtesy of Wikimedia commons/Louisa Howard), *Hamiltonella defensa* symbiont in black bean aphids (image courtesy of Christoph Vorburger), facultatively multicellular *Dictyostelium* slime mould (image courtesy of Wikimedia commons/Bruno in Columbus), cooperatively breeding superb fairy wren (image courtesy of Wikimedia commons/JJ Harrison), Legume-*Rhizobia* mutualism (image courtesy of Dave Whitinger, All Things Plants). Negligible conflict is not sufficient for a major transition—the algae, wasp, and symbiont examples have not made a major transition because there is not mutual dependence (see also Table 1).

the production of the most offspring, which will depend upon B and C. In a sexual species, this condition is met with strict lifetime monogamy, where potential helpers are aiding their parents, such that they are helping rear full siblings ($r_h = r_o = 1/2$). In an asexual species, these conditions arise in clonal groups ($r_h = r_o = 1$), which also removes conflict between helpers and their parent. Multicellularity and eusociality have evolved only under these conditions.

Our above discussion makes clear that we need to specify whom we are considering potential conflict between, and their behavioral options. We have considered conflict among helpers, and not between helpers and their parents. This assumption is reasonable in large colonies where the workers are effectively in control, but not in smaller colonies or when the reproductives can still wield power over factors such as caste or sex ratio. Consequently, factors such as colony size and caste determination can influence whether a major transition can be made because they will determine the impact of conflict between queens and workers at the colony level.

Conflict can still arise in monogamous species, when the decision a helper faces is not whether to help raise full siblings. In *Melipona* bees, conflict arises as to whether individuals rear their own offspring, or their niblings (nieces and nephews), resulting in a wasteful 5–14% of individuals competing to become queens (42). A general issue here is that the Hamilton's rule approach is relatively heuristic, and explicit theory is required to determine when different individuals within a social group will act as a single maximizing agent (33).

Repression. Another way to eliminate within-group conflict is if there is complete repression of competition within groups, such that individuals cannot increase their reproductive success via any form of cheating (33, 43). As with the relatedness scenario described above, repression of competition unites the interest of the group. Repression of competition has been important in some social hymenoptera, where workers destroy or "police" the eggs laid by other workers (44). For example, in the honeybee,

worker policing is so efficient that workers are effectively selected to not try to reproduce.

Haplodiploidy. Our above discussion of eusociality ignored the complication of haplodiploidy. In diploids, when offspring stay to help their monogamous parents, the helpers are equally related to offspring that they could help raise and their own offspring ($r_o = r_h = 1/2$). This same condition holds in haplodiploids if helpers cannot differentiate between male and female offspring. However, if we allow for the possibility that males and females can be distinguished from each other, then we find that workers are more related to (*i*) their own sons ($r = 0.5$) than their brothers ($r = 0.25$), (*ii*) their sisters ($r = 0.75$) than their daughters ($r = 0.5$), (*iii*) their daughters ($r = 0.5$) than their brothers ($r = 0.25$), and (*iv*) their nephews ($r = 0.375$) than their brothers ($r = 0.25$). These relatedness asymmetries can lead to conflicts between workers in haplodiploid species where diploidy would not. Consequently, haplodiploidy hinders major transitions, such that haplodiploid species can require an extra step. Monogamy and sufficient ecological benefit of cooperation can take species to obligate eusociality (35, 40) and, in the case of diploids, a major transition. However, then, in haplodiploids, an extra step is required to suppress conflict and complete a major transition: for example, the evolution of multiple mating leading to worker policing (44). This point emphasizes that, in haplodiploids, mating frequency can have different roles at different stages, with monogamy required to make the transition to obligate eusociality, but then multiple mating driving from eusociality to a major transition.

Transitions Between Species. Less attention has been paid to what conditions lead to reduced within-group conflict in between-species transitions. Again, the way in which groups form may be key. There will be no conflict between vertically transmitted clonal symbionts, who could transmit to more individuals only by increasing the reproductive success of their host. Examples that

seem to lack appreciable conflict include mitochondria, plasmids, the various secondary and tertiary plastid endosymbioses, and possibly the *Buchnera* bacteria that infect aphids (14, 45). In contrast, both the opportunity for horizontal transmission, and within-host symbiont diversity, could lead to conflicts that select for less cooperative symbionts (22, 46). Consistent with the predicted role of transmission route, the evolutionary transition from a parasitic to a mutualistic lifestyle in a range of bacterial lineages is associated with the loss of horizontal transmission (47).

Repression of competition could play a role in transitions between species. Sanction mechanisms seem to be more common in partnerships when symbionts transmit horizontally, where we predict that there will be more conflict to resolve, such as in partnerships with root symbionts, luminous symbionts, and pollinator mutualisms (24, 25, 48, 49).

Hosts could structure or transmit their symbionts in a way that better aligns their interests and removes conflict. However, mechanisms that repress competition, such as reducing symbiont diversity, will be favored only if they have an immediate fitness benefit, and not because they will select for more cooperative symbionts in the future (46). For example, leaf-cutter ants actively remove foreign mycelial fragments to prevent incompatibility interactions that can reduce fungal-garden productivity; this removal has the byproduct benefit of more closely aligning the interests of the ants and their fungi over evolutionary time (50). A general problem here is that we lack formal theory for the conditions required to eliminate conflict in between-species transitions.

What Conditions Favor Mutual Dependence?

In many cases, if a cooperative group is broken up, then individuals are still able to reproduce. In contrast, cases can arise in which different members of the group have lost the capacity for independent replication they once had. For example, mitochondria cannot reproduce without the rest of the eukaryotic cell, and higher termite queens cannot reproduce without the help of their workers. In most cases, mutual dependence seems to arise as a result of extreme division of labor. Individuals become so specialized that they lose the ability to perform other tasks. The question here is what conditions favor the loss of any potential to perform certain tasks that are necessary to replication? Why did higher termite queens lose the ability to feed/rear their offspring, and their workers lose the ability to produce offspring? There is an almost complete lack of work addressing why certain traits are lost, both generally and for specific cases (51).

Presumably, there is some efficiency benefit from becoming irreversibly specialized, which will depend upon a range of biological factors, such as how groups are formed (relatedness), mortality rates, and how those that reproduce (breeders) are replaced. Symbionts offer excellent opportunities for comparative studies on the evolutionary and ecological correlates of mutual dependence. For example, why does the interaction between photosynthetic symbionts and their hosts vary from the obligate symbiosis typified by plastids in plants to ciliates that can "culture" the chloroplasts found in their algal food (45, 52, 53)? Are partnerships in which symbionts access and deliver new forms of energy for their hosts more likely to lead to major transitions than cases where the symbionts provide a resource that the host can also obtain directly? Repression of competition can also favor mutual dependence, as demonstrated by the influence of worker policing on whether hymenopteran workers develop ovaries (44).

Genetic drift can play a role in the evolution of mutual dependence. When symbionts have small asexual populations, drift can be a significant factor, leading to the inactivation and eventual deletion of mildly beneficial genes (54). As symbionts lose functionality in traits or structures, such as transporters and cell walls, the host may evolve to take over these roles, coadapting in a way that leads to mutual dependence. Similarly, hosts can lose traits that the symbionts perform. Oligochaete worms have lost the ability to excrete their own waste, relying instead on metabolic pathways introduced by endosymbionts to provide and process nutrients (55), whereas coral hosts (e.g., *Acropora* sp.) have lost their cysteine synthesis pathway in favor of symbiont-derived sources (56). When multiple symbiont lineages are within a host, different lineages may lose different traits, such that multiple symbionts and the host can become interdependent (28).

Conflict and Maximizing Agents

A major transition requires that there is a relative lack of within-group conflict such that a larger unit can be thought of as a fitness-maximizing agent (individual or organism) in its own right. We are interested in when a group can be considered a fitness-maximizing agent for at least three reasons (6, 34). First, the maximizing agent analogy informs us when group-level adaptation will occur (33). Almost all interesting traits and behaviors are the construction of multiple entities (genes, cells, etc.), pulling in the same direction cooperatively with minimal conflict. If there is negligible conflict, and we can think of a group as a single maximizing agent, then we can expect adaptation at the group level to maximize the fitness of the group and, thus, substantial increases in organismal complexity. For example, clonal multicellular groups have led to animals and plants whereas nonclonal multicellular groups have led only to things like slime molds.

Second, this analogy facilitates progress at the interface of theory and data in evolutionary biology (34). It allows us to think about a single, individual-level agent, rather than trillions of cell- or gene-level agents (57). By black-boxing these lower layers, we can focus on other aspects of biology, such as ecology and behavior, making it easier to develop models and test the robustness of those models to changes in the underlying biological parameters. Nonetheless, the maximizing agent analogy is a heuristic approach that should be used only when the advantages outweigh the disadvantages, which is best judged empirically (58). Third, a focus on maximizing agents makes it easier to identify potential conflicts and determine how they are resolved, both between and within individuals: for example, how conflict can arise over who produces male offspring in haplodiploid social insects, and how conflict is suppressed by worker policing (44).

More generally, we emphasize that the fundamental question being asked with major transitions is one of individuality, and not other issues such as sterility, altruism, complexity, ecological impact, or whether gene transfer has occurred (33). Although traits such as sterility raise important evolutionary questions and can be correlated with whether a major transition has occurred, they are neither necessary nor sufficient for a major transition. Indeed, other examples of complete altruism can be found, which are clearly not major transitions, including bacteria bursting suicidally to release factors that reduce competition (59).

Pragmatism

Identifying whether a major transition has been made is an empirical problem, where pragmatism can be important. From a theoretical perspective, we can identify the ideal qualities that would unequivocally define individuality and identify when a major transition has been made. For example, we can examine what conditions would lead to no conflict, such as clonality or complete repression of competition (33). In the real world, conflict between genes or cells can still exist. For example, in animals and plants, there is still some opportunity for conflict among genes, and somatic mutation can lead to conflicts among cells (6, 60, 61). Our aim is to consider when there is so little conflict that the consequences for the group can be effectively ignored.

Whether there is negligible conflict and the group acts as a fitness-maximizing agent can be trait-dependent, even within an organism. For example, although we expect plant traits controlled by nuclear genes to maximize plant fitness, we know that cytoplasmic genes can select for male sterility, to their own selfish benefit. Similarly, in ants such as *Formica exsecta*, we can assume that the different ants are behaving as a single maximizing unit for traits such as foraging, refuse disposal, etc., whereas there is also clear conflict between the queen and the workers over sex allocation (62). What is key is not to argue whether a certain species has made a major transition, but to use the approach in a way that helps us understand the processes that lead to major transitions: for examining what conditions

lead to greater mutual dependence or lower conflict. Evolution is a process of continuous change, and so we should expect blurry edges with a mosaic of features (1).

Pragmatism is also required when considering whether the members of a group are mutually dependent. We are not saying that mutual dependence can never be reversed by evolution—mitochondria have been lost in some parasites of humans, and plastids have been lost multiple times (45, 63). Instead, our aim is to consider whether the breaking up of a group would prevent replication. Related to this point, all organisms are dependent upon other organisms to be able to reproduce, and we are considering mutual dependence relative to a defined social group. For example, we can ask whether the cells that make up a male lion are mutually dependent upon each other, even though that male is also dependent upon a female lion to mate with, zebras to eat, grass to feed those zebras and so on.

The World Made Simpler
Maynard Smith and Szathmáry offered a mixture of explanations for the different transitions, suggesting that a diversity of factors were at play (2). We suggest that both theoretical and empirical advances have provided a more unified explanation for the different major transitions. Major transitions require extreme conditions, with certain factors being either consistently important, or consistently unimportant.

First, at a very general level, the same two factors play a key role in answering all six questions that we posed: (*i*) an ecological benefit to cooperation and (*ii*) a mechanism to unite the interests of different individuals. Consequently, the same ecological and evolutionary conditions can provide the answers to all six questions. For example, the combination of clonality and the right ecological benefit can remove within-group conflict (question *v*) and favor cooperation (questions *i* and *ii*), division of labor (question *iii*), honest communication (question *iv*), and mutual dependence (question *vi*).

Second, at a more specific level, both theory and data suggest that how social groups form has played an analogous and fundamental role, across the different major transitions (Fig. 5). The within-species transitions to multicellularity and eusociality have occurred only when (*i*) the social group passes through a single propagule phase (cell or singly mated female) or (*ii*) the social group forms by offspring staying to help their parent (subsociality) (35–37, 40). We suggest that transitions between species may involve similarly restrictive conditions, such as vertical transmission of clonal symbionts.

Third, the restrictive conditions required for a major transition make it easier to understand where and why major transitions have not taken place: for example, why there have been no major transitions in promiscuous cooperative breeders or multicellular groups formed by aggregation. A major transition is not driven simply by satisfying Hamilton's rule (questions *i* and *ii*); it is about conflict being sufficiently eliminated that the group acts as a single maximizing agent (question *v*). Consequently, a lower relatedness (*r*) cannot be made up for just with a greater ecological benefit to cooperation (*B*/*C*). We would not expect major transitions with certain life histories, such as promiscuity or nonclonal aggregation (33, 35).

Fourth, there are striking similarities in the ecological benefits to cooperation that have driven different transitions. The eusocial insects can be divided depending upon whether the evolution of eusociality was driven by either the advantage in forming defensive groups (e.g., termites, aphids) or the efficiency benefit gained from cooperating to rear young (e.g., hymenoptera) (17). The ecological benefits to multicellularity seem to divide along analogous lines due to the benefits of forming defensive groups (e.g., algae) or to make certain traits more efficient (e.g., yeast, slime molds) (13, 64). Can we make similar generalizations about the transitions between species?

Fifth, within-group kin discrimination seems to have played a limited role in helping within species' major transitions. The transitions to obligate multicellularity and obligate eusociality have taken place only in clonal or monogamous populations ($r_o = r_h$), where there is no or limited potential for kin discrimination.

Consequently, although kin discrimination can be important in facultatively multicellular species or cooperative breeders, this discrimination is only favored when there is appreciable variation in within-group relatedness (65), which also prevents major transitions. Furthermore, some cases of kin discrimination, such as haplodiploid females favoring sons over brothers, increases rather than decreases conflict, and so disfavor rather than favor major transitions. An important exception is the involvement of kin discrimination in the worker policing of the haplodiploid social insects (44).

Sixth, repression of competition seems to have played a limited role in helping within-species major transitions. The transitions to obligate multicellularity and obligate eusociality have taken place only in clonal or monogamous populations ($r_o = r_h$), where there is no or limited competition to be suppressed. Furthermore, theory suggests that it is hard to evolve the complete repression of competition that would be required for a major transition because the marginal benefits of repressing competition will often plateau, such that an intermediate level of repression will be favored (66). As with kin discrimination, an important exception is policing in the haplodiploid social insects (44).

Considering transitions between species, the relative role of repression of competition is less clear but may also be relatively unimportant. In many cases, vertical inheritance of clonal symbionts may mean that there is no competition to repress. With horizontal transmission of symbionts, sanction mechanisms that reward cooperators and/or punish noncooperators can be favored (23, 24), but these sanctions may not be able to suppress competition enough for a major transition to occur.

Future Directions
We conclude by emphasizing that many of the ideas in this paper are tentative. Major theoretical issues remain unanswered, and empirical work has only begun to tackle the issues surrounding the major questions. Insights can be obtained from studies of species that haven't made major transitions, as well as those that have, or by comparing across species that have transitioned to a different extent.

First, although we have a relatively good understanding of cooperation (questions *i* and *ii*), we have a much poorer understanding of group transformation (questions *iii–vi*). In cases where we have a good understanding, other questions arise: for example, how groups form is important, but how can we explain variation in the way in which groups form?

Second, we have a much better understanding of the within-species transitions than the between-species transitions. The within-species transitions have been easier to study because they have happened both more often and usually more recently than transitions such as the evolution of the eukaryote cell or the genome. Advances in genomic methodologies are allowing division of labor and mutual dependence to be much better studied in endosymbionts and organelles (15, 28, 45, 67).

Third, the applicability of the fitness-maximizing agent analogy to certain scenarios remains to be explored. For example, what are the consequences of horizontal gene transfer in bacteria, or cultural evolution (social learning) in humans? There is a lack of formal theory examining the conditions required to remove conflict and thus lead to a group acting as a single maximizing unit, especially for between-species transitions (33).

Fourth, we have focused on determining the ultimate selective forces that have favored major transitions. Mechanistic details can matter if they predispose certain species to making transitions: for example, if bacteria are able to acquire resources required by a potential host, gain entry to and proliferate in the tissues and cells of eukaryotes, and exchange symbiotic loci horizontally. Can generalizations be made about the role of predispositions within or between transitions?

Finally, we have focused on the route to making a major transition. Once a major transition has been made, evolution can lead to scenarios that reintroduce conflict into the group. For example, multicellular groups can become so large that mutation becomes an appreciable problem, and eusocial insects can evolve

multiple mating or multiple queens (40, 60). In addition, mutual dependence can break down, allowing major transitions to be reversed, as has occurred with both mitochondria and plastids. Genomic reduction could lead to endosymbionts becoming so ineffective that they are lost or replaced (54). These points raise at least two more questions:

vii) How are new conflicts of interest suppressed in groups that have already made a major transition?
viii) What conditions favor the breakdown of major transitions?

As when considering questions *i–vi*, the beauty of the major-transition approach is that it facilitates the identification of general patterns. How important is repression of conflict mechanisms for keeping down conflicts that arise after transitions have been made, such as suppressors of selfish genes or cancer (1, 61)? Do major transitions tend to break down because of new conflicts arising, or because the ecological benefits change? A new era of research focused on the commonalities in the major transitions across the tree of life is positioned to tackle these questions.

1. Bourke AFG (2011) *Principles of Social Evolution* (Oxford Univ Press, Oxford).
2. Maynard Smith J, Szathmáry E (1995) *The Major Transitions in Evolution* (Oxford Univ Press).
3. Leigh EG, Jr (1991) Genes, bees and ecosystems: The evolution of a common interest among individuals. *Trends Ecol Evol* 6(8):257–262.
4. Queller DC (2000) Relatedness and the fraternal major transitions. *Philos Trans R Soc Lond B Biol Sci* 355(1403):1647–1655.
5. Michod RE (2007) Evolution of individuality during the transition from unicellular to multicellular life. *Proc Natl Acad Sci USA* 104(Suppl 1):8613–8618.
6. Queller DC, Strassmann JE (2009) Beyond society: The evolution of organismality. *Philos Trans R Soc Lond B Biol Sci* 364(1533):3143–3155.
7. Crespi BJ, Yanega D (1995) The definition of eusociality. *Behav Ecol* 6:109–115.
8. West SA, Griffin AS, Gardner A (2007) Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection. *J Evol Biol* 20(2):415–432.
9. Ghoul M, Griffin AS, West SA (2014) Toward an evolutionary definition of cheating. *Evolution* 68(2):318–331.
10. Lehmann L, Keller L (2006) The evolution of cooperation and altruism: A general framework and a classification of models. *J Evol Biol* 19(5):1365–1376.
11. West SA, Griffin AS, Gardner A (2007) Evolutionary explanations for cooperation. *Curr Biol* 17(16):R661–R672.
12. Sachs JL, Mueller UG, Wilcox TP, Bull JJ (2004) The evolution of cooperation. *Q Rev Biol* 79(2):135–160.
13. Koschwanez JH, Foster KR, Murray AW (2013) Improved use of a public good selects for the evolution of undifferentiated multicellularity. *eLife* 2:e00367.
14. Moran NA (2007) Symbiosis as an adaptive process and source of phenotypic complexity. *Proc Natl Acad Sci USA* 104(Suppl 1):8627–8633.
15. Douglas AE (2010) *The Symbiotic Habit* (Princeton Univ Press, Princeton).
16. Henry LM, et al. (2013) Horizontally transmitted symbionts and host colonization of ecological niches. *Curr Biol* 23(17):1713–1717.
17. Queller DC, Strassmann JE (1998) Kin selection and social insects. *Bioscience* 48:165–175.
18. Hamilton WD (1964) The genetical evolution of social behaviour. I. *J Theor Biol* 7(1):1–16.
19. Bernasconi G, Strassmann JE (1999) Cooperation among unrelated individuals: The ant foundress case. *Trends Ecol Evol* 14(12):477–482.
20. Trivers RL (1971) The evolution of reciprocal altruism. *Q Rev Biol* 46:35–57.
21. Foster KR, Wenseleers T (2006) A general model for the evolution of mutualisms. *J Evol Biol* 19(4):1283–1293.
22. Frank SA (1994) Kin selection and virulence in the evolution of protocells and parasites. *Proc Biol Sci* 258(1352):153–161.
23. West SA, Kiers ET, Simms EL, Denison RF (2002) Sanctions and mutualism stability: Why do rhizobia fix nitrogen? *Proc Biol Sci* 269(1492):685–694.
24. Kiers ET, Rousseau RA, West SA, Denison RF (2003) Host sanctions and the legume-rhizobium mutualism. *Nature* 425(6953):78–81.
25. Koch EJ, Miyashiro T, McFall-Ngai MJ, Ruby EG (2014) Features governing symbiont persistence in the squid-vibrio association. *Mol Ecol* 23(6):1624–1634.
26. Rossetti V, Schirrmeister BE, Bernasconi MV, Bagheri HC (2010) The evolutionary path to terminal differentiation and division of labor in cyanobacteria. *J Theor Biol* 262(1):23–34.
27. Gruber-Vodicka HR, et al. (2011) Paracatenula, an ancient symbiosis between thiotrophic Alphaproteobacteria and catenulid flatworms. *Proc Natl Acad Sci USA* 108(29):12078–12083.
28. Van Leuven JT, Meister RC, Simon C, McCutcheon JP (2014) Sympatric speciation in a bacterial endosymbiont results in two genomes with the functionality of one. *Cell* 158(6):1270–1280.
29. Gavrilets S (2010) Rapid transition towards the Division of Labor via evolution of developmental plasticity. *PLOS Comput Biol* 6(6):e1000805.
30. Maynard Smith J, Harper D (2003) *Animal Signals* (Oxford Univ Press, Oxford).
31. Diggle SP, Griffin AS, Campbell GS, West SA (2007) Cooperation and conflict in quorum-sensing bacterial populations. *Nature* 450(7168):411–414.
32. Heath-Heckman EAC, et al. (2013) Bacterial bioluminescence regulates expression of a host cryptochrome gene in the squid-Vibrio symbiosis. *mBio* 4(2):e00167-13.
33. Gardner A, Grafen A (2009) Capturing the superorganism: A formal theory of group adaptation. *J Evol Biol* 22(4):659–671.
34. West SA, Gardner A (2013) Adaptation and inclusive fitness. *Curr Biol* 23(13):R577–R584.
35. Boomsma JJ (2007) Kin selection versus sexual selection: Why the ends do not meet. *Curr Biol* 17(16):R673–R683.
36. Boomsma JJ (2009) Lifetime monogamy and the evolution of eusociality. *Philos Trans R Soc Lond B Biol Sci* 364(1533):3191–3207.
37. Fisher RM, Cornwallis CK, West SA (2013) Group formation, relatedness, and the evolution of multicellularity. *Curr Biol* 23(12):1120–1125.
38. Grosberg RK, Strathmann RR (2007) The evolution of multicellularity: A minor major transition? *Annu Rev Ecol Evol Syst* 38:621–654.
39. 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(21):8913–8917.
40. Hughes WO, Oldroyd BP, Beekman M, Ratnieks FL (2008) Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science* 320(5880):1213–1216.
41. Cornwallis CK, West SA, Davis KE, Griffin AS (2010) Promiscuity and the evolutionary transition to complex societies. *Nature* 466(7309):969–972.
42. Wenseleers T, Ratnieks FLW (2004) Tragedy of the commons in Melipona bees. *Proc Biol Sci* 271(Suppl 5):S310–S312.
43. Frank SA (1995) Mutual policing and repression of competition in the evolution of cooperative groups. *Nature* 377(6549):520–522.
44. Wenseleers T, Ratnieks FL (2006) Enforced altruism in insect societies. *Nature* 444(7115):50.
45. Keeling PJ (2013) The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. *Annu Rev Plant Biol* 64:583–607.
46. Frank SA (1996) Host-symbiont conflict over the mixing of symbiotic lineages. *Proc Biol Sci* 263(1368):339–344.
47. Sachs JL, Skophammer RG, Regus JU (2011) Evolutionary transitions in bacterial symbiosis. 108(Suppl 2):10800–10807.
48. Kiers ET, et al. (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333(6044):880–882.
49. Jandér KC, Herre EA (2010) Host sanctions and pollinator cheating in the fig tree-fig wasp mutualism. *Proc Biol Sci* 277(1687):1481–1488.
50. Poulsen M, Boomsma JJ (2005) Mutualistic fungi control crop diversity in fungus-growing ants. *Science* 307(5710):741–744.
51. Ellers J, Kiers ET, Currie CR, McDonald BR, Visser B (2012) Ecological interactions drive evolutionary loss of traits. *Ecol Lett* 15(10):1071–1082.
52. Nowack ECM, Melkonian M (2010) Endosymbiotic associations within protists. *Philos Trans R Soc Lond B Biol Sci* 365(1541):699–712.
53. McManus GB, Schoener DM, Haberlandt K (2012) Chloroplast symbiosis in a marine ciliate: Ecophysiology and the risks and rewards of hosting foreign organelles. *Front Microbiol* 3:321–329.
54. McCutcheon JP, Moran NA (2012) Extreme genome reduction in symbiotic bacteria. *Nat Rev Microbiol* 10(1):13–26.
55. Dubilier N, Bergin C, Lott C (2008) Symbiotic diversity in marine animals: The art of harnessing chemosynthesis. *Nat Rev Microbiol* 6(10):725–740.
56. Shinzato C, et al. (2011) Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature* 476(7360):320–323.
57. Gardner A, Welch JJ (2011) A formal theory of the selfish gene. *J Evol Biol* 24(8):1801–1813.
58. Gardner A (2009) Adaptation as organism design. *Biol Lett* 5(6):861–864.
59. Ackermann M, et al. (2008) Self-destructive cooperation mediated by phenotypic noise. *Nature* 454(7207):987–990.
60. Roze D, Michod RE (2001) Mutation, multilevel selection, and the evolution of propagule size during the origin of multicellularity. *Am Nat* 158(6):638–654.
61. Trivers RL (2006) *Genes in Conflict* (Harvard Univ Press, Cambridge, MA).
62. Sundström L, Chapuisat M, Keller L (1996) Conditional manipulation of sex ratios by ant workers: A test of kin selection theory. *Science* 274(5289):993–995.
63. van der Giezen M (2009) Hydrogenosomes and mitosomes: Conservation and evolution of functions. *J Eukaryot Microbiol* 56(3):221–231.
64. Claessen D, Rozen DE, Kuipers OP, Søgaard-Andersen L, van Wezel GP (2014) Bacterial solutions to multicellularity: A tale of biofilms, filaments and fruiting bodies. *Nat Rev Microbiol* 12(2):115–124.
65. Cornwallis CK, West SA, Griffin AS (2009) Routes to indirect fitness in cooperatively breeding vertebrates: Kin discrimination and limited dispersal. *J Evol Biol* 22(12):2445–2457.
66. El Mouden C, West SA, Gardner A (2010) The enforcement of cooperation by policing. *Evolution* 64(7):2139–2152.
67. Moran NA, McCutcheon JP, Nakabachi A (2008) Genomics and evolution of heritable bacterial symbionts. *Annu Rev Genet* 42:165–190.
68. Strassmann JE, Zhu Y, Queller DC (2000) Altruism and social cheating in the social amoeba Dictyostelium discoideum. *Nature* 408(6815):965–967.
69. Mehdiabadi NJ, et al. (2006) Social evolution: Kin preference in a social microbe. *Nature* 442(7105):881–882.
70. Sachs JL, Ehinger MO, Simms EL (2010) Origins of cheating and loss of symbiosis in wild Bradyrhizobium. *J Evol Biol* 23(5):1075–1089.

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