

The major evolutionary transitions in individuality

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The evolution of life on earth has been driven by a small number of major evolutionary transitions. These 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. But not all cooperative groups are on 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 labour, 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 | signalling | division of labour | 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. Whilst this approach has some uses, it is biased towards 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 & Szathmari (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 cooperate to form genomes, archaea and eubacteria formed eukaryotic cells, and cells cooperate 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 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 generalise 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 subset of major transitions which lead to a new form of individual (Table 1), where the same problems arise, in a way that facilitates comparison, and so exclude the transitions to 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 only replicate as part of a larger unit after it. This is 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). This is because we have a group that can't be broken up (condition 1), and we can conceptualise 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: (1) the formation of a cooperative group, and (2) the transformation of the cooperative group into a more cohesive and integrated entity that can be considered a new level of individual (organisms) (Fig. 1) (1, 2). The second step typically involves a number of common features, including: the individuals in a group evolving to perform different tasks (division of labor); division of labour becoming so specialised that the members of the group become dependent upon each other; communication to coordinate cooperation at the group level.

Whilst these two steps may not have clear borders, and can move gradually from one to the next, they can occur in some

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Table 1. The major evolutionary transitions in individuality. ¹Highly integrated / obligate symbioses, where the hosts cannot survive without their symbionts, and there appears to be a relative lack of conflict, such that the symbionts can best increase their own fitness by helping their hosts reproduce (45). ²Facultatively 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 non-cooperative cheats, and within group kin discrimination (67, 68). ³Facultatively eusocial species, where queens are still able to breed if the workers/soldiers are removed (no mutual dependence). ⁴Hosts are able to grow and replicate without these symbionts (no mutual dependence), and there is sufficient conflict to select for non-cooperative cheats(69).

Major Transitions		Not Major transitions			
Transition	Examples	Examples	Why not? Mutual dependence	Negligible conflict	
Independent replicators	Populations of replicators	Genome			
Separate unicells	Symbiotic unicell	Eukaryotic cell ¹ . Primary plastid endosymbiosis ¹ . <i>Paulinella</i> ¹ . Secondary/tertiary plastid endosymbiosis ¹ .			
Unicells	Multicellular organism	Animals. Plants. Fungi. Red algae. Brown algae	<i>Dictyostelid</i> slime moulds ²	No	No
Multicellular organisms	Eusocial society	Higher termites. Leaf-cutter ants. Honey bee.	<i>Pseudomonas</i> biofilms Social aphids ³ <i>Melipona</i> bees Cooperative vertebrates Humans	No	Yes
Multicellular organisms	Obligate interspecific mutualism	Some obligate endosymbionts, e.g. <i>Buchnera</i> in aphids ¹ . Leaf cutter ants and their fungi. Paracatenula flatworms and their chemoautotrophic symbionts	Legumes- <i>Rhizobia</i> ⁴ Squid- <i>Vibrio</i> Gut microbiota	No	No

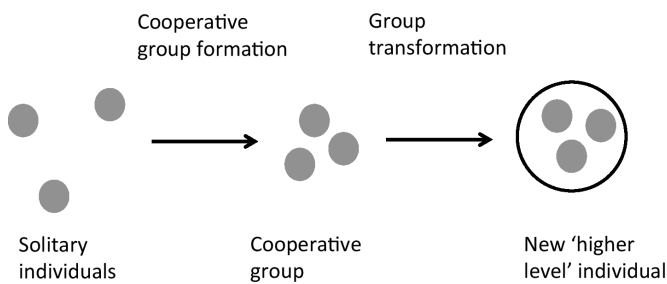


Fig. 1. A major evolutionary transition involves two steps. First, the formation of a cooperative group. Second, the transition to a new level of organism, with division of labour, interdependence and coordination of the parts. Whilst the first step is well understood, the second is not. We follow Bourke, except he divides transitions into three steps, distinguishing between maintenance and transformation (1).

order (1). For example, in transitions involving members of the same species, we would not expect division of labour to evolve until after cooperative groups have formed. Consequently, the 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:

- Q1. What conditions favour the formation of cooperative groups?
- Q2. What conditions maintain cooperation during group transformation?
- Q3. What conditions favour division of labour?

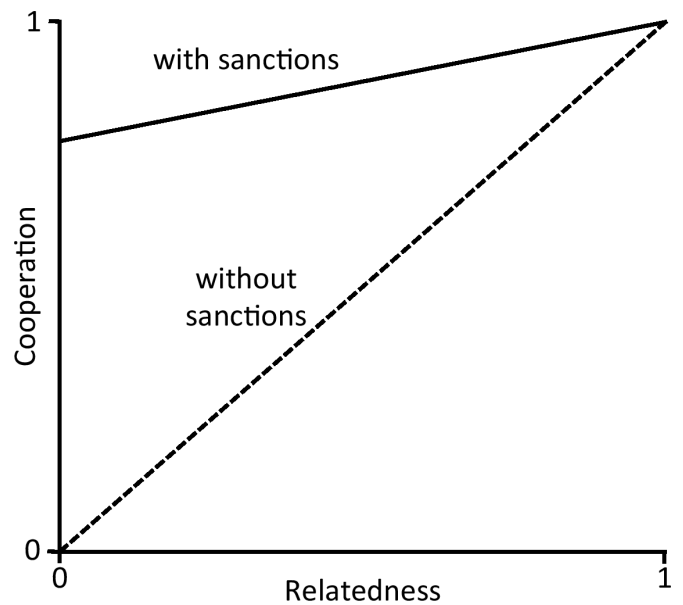


Fig. 2. The hypotheticals level of cooperative helping in a symbiont plotted against the relatedness between the symbionts infecting a host. If the hosts sanction uncooperative symbionts, then a high level of cooperation is predicted, relatively independent of relatedness. If the hosts do not carry out sanctions, then the level of cooperation is predicted to depend strongly upon relatedness (23).

- Q4. What conditions favour communication that coordinates cooperation at the group level?
 - Q5. What conditions lead to negligible conflict within groups?
 - Q6. What conditions favour mutual dependence?
- Question 1 concerns the first step in a major transition (the formation of a cooperative group) while the other five questions

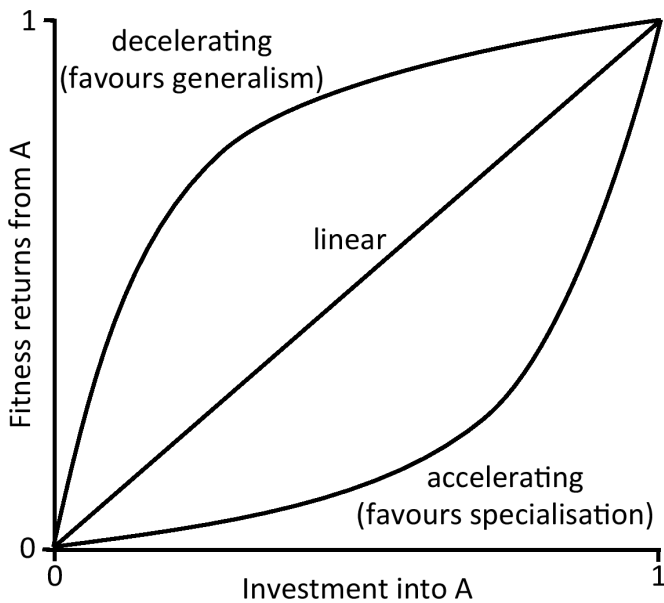


Fig. 3. The relationship between the proportion of resources invested into a trait (A) and the fitness return from that trait. We assume that a proportion of resources X is put into trait A, and the remaining proportion $1-X$ into trait B.

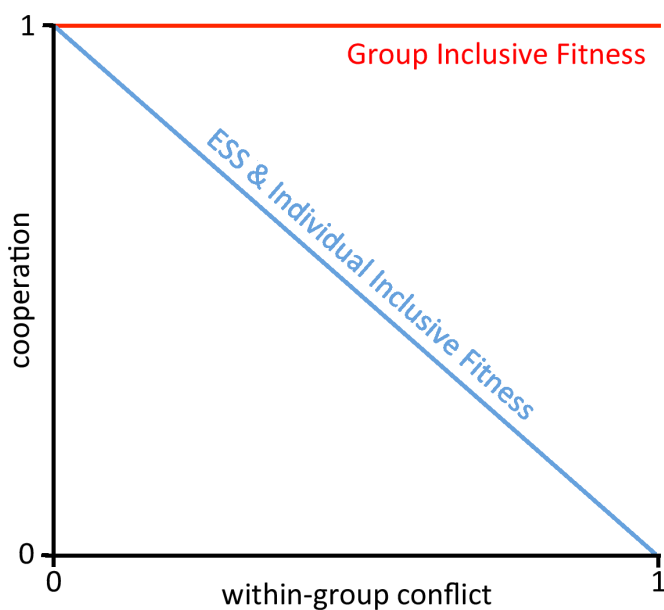


Fig. 4. The individual and the group. The hypothetical level of a cooperative trait, such as the amount of an extracellular factor produced by bacterial cells, plotted against the extent of conflict between interacting individuals. The different lines show the optimal strategy from the perspective of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

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 labour, mutual dependence and a lack of conflict.

Q1 & 2: What conditions favour cooperation?

The first step in a major transition is that individuals come together and form a cooperative group. Cooperation then needs to be maintained whilst group transformation takes place. We consider the factors that may favour 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.

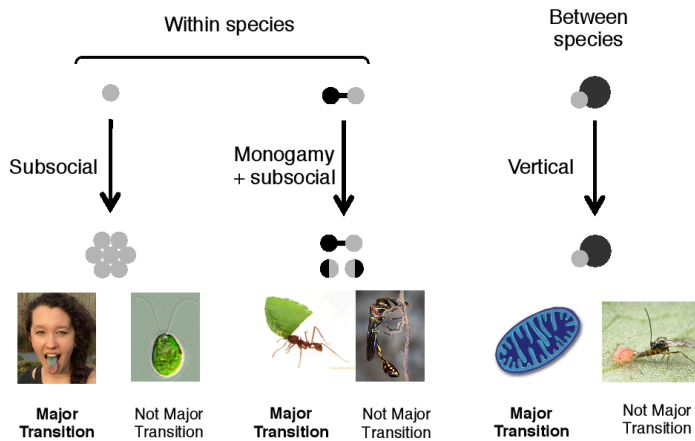
(1) Ecological or efficiency benefits: The ecological benefits of cooperation have been well studied in many taxa. Cells form cooperative multicellular clumps to evade predators, make more efficient use of factors that are excreted from individual cells, and form fruiting bodies that aid dispersal (1, 13). Cooperation between different species often involves a symbiont providing a service, such as resource that allows their host to better grow (14-16). Cooperation is favoured in eusocial species to care for young over an extended period where the parent may die, or to defend a valuable resource (17).

(2) Mechanism to direct benefits back (within species): Cooperation can be favoured 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 favoured 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. This shows that altruism can be favoured 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 towards relatives (1, 11)

Cooperation can be favoured between non-relatives 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 that reward cooperators or punish non-cooperators, such as reciprocity (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.

(3) Mechanism to direct benefits (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 become 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 hence 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*.

a) Route of social group formation leads to negligible conflict



b) Route of social group formation leads to appreciable conflict

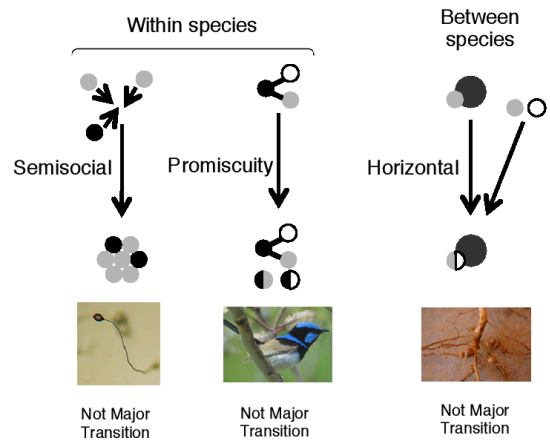


Fig. 5. The way in which groups form is a major determinant of when major transitions have taken place, because it determines the potential for within-group conflict. Within-species transitions have only taken place when offspring stay to help their parents (subsocial) and either asexual reproduction of lifetime monogamy (36, 37, 40). Between-species transitions appear to involve similarly restrictive group formation, such as vertical transmission leading to clonal symbionts whose interests are aligned with their hosts. The images, from left to right show: human, *Chlamydomonas*, *Atta* ants, *Stenogastrine* hover wasp, mitochondrion, *Hamiltonella defensa* (in the aphid), *Dictyostelium*, splendid fairy wren, and *Rhizobia* mutualism.

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 favour cooperation even when symbiont relatedness is low at the level of the host (Fig. 2).

Q3: What conditions favour division of labour?

Organisms must perform a number of tasks to survive and reproduce. When will natural selection favour a division of labour, with different individuals performing different tasks? To illustrate this 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 as time and energy spent on A cannot be spent on B. Division of labour will tend to be favoured when the shape of the relationship between the proportion of resources allocated to each task and the fitness return is 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 labour. In the volvocine algae, there appears to be an advantage to having 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 photosynthesise and cells that fix nitrogen into ammonium (heterocysts) – this division appears to be favoured 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 labour, 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 and plastids provide clear examples of extreme specialization in transitions between species.

The general principle is that a division of labour will be favoured when there are efficiency benefits to specialization (Fig. 3). There is a lack of research showing why this will be the

case 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 labour?

Q4: What conditions favour 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 labour 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), signals are costly to fake (handicaps), and/or the sender and receiver have a common interest (30). A common interest is particularly relevant in major transitions because the same conditions that favour cooperation can also favour honest communication – high relatedness or aligned reproductive interests. The importance of high relatedness in favouring 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 favoured at high relatedness, and disfavoured 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 honey bee 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 appears 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.

Q5: 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 comes at a cost to other individuals or the group (18). Inclusive fitness is the sum of an individual's influence on their own reproductive success and that of related individuals. This leads to the question of what conditions would be required for individuals maximizing their own inclusive fitness, to also be maximizing of 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 does the empirical data show? Group formation appears 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 only taken place 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 non-clonal group formation occurs via aggregation in many species, such as the cellular slime moulds, ciliates, and acrasid slime moulds, and can lead to very high relatedness (39), it has only led to facultative multicellularity.

Similarly, the transitions to both facultative and obligate eusociality have only taken place 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. Whilst cooperation occurs in many species where females mate multiple times, such as the cooperative breeding birds and mammals, this has never led to 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 to either: (i) perform a cooperative behaviour 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 favoured 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 favoured, 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 achieve this 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 the production of the most offspring, which will depend upon B and C . In a sexual species, this arises 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 only evolved under these conditions.

Our above discussion makes clear that we need to specify whom we are considering potential conflict between, and their behavioural options. We have considered conflict among helpers, and not between helpers and their parents. This 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 over other decisions in monogamous species, such as 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 nieces (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: (a) their own sons ($r = 0.5$) than their brothers ($r = 0.25$); (b) their sisters ($r = 0.75$) than their daughters ($r = 0.5$); (c) their daughters ($r = 0.5$) than their brothers ($r = 0.25$); (d) 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. But 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 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 only transmit to more individuals by increasing the reproductive success of their host. Examples which appear to lack conflict include mitochondria, plasmids, the various secondary and tertiary plastid endosymbiosis, 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 conflict that select

681 for less cooperative symbionts (22, 46). Consistent with the pre-
682 dicted role of transmission route, the evolutionary transition from
683 a parasitic to a mutualistic lifestyle in a range of bacterial lineages
684 is associated with the loss of horizontal transmission (47).

685 Repression of competition could play a role in transitions
686 between species. Sanction mechanisms appear to be more com-
687 mon in partnerships when symbionts transmit horizontally, where
688 we predict that there will be more conflict to resolve, such as
689 in partnerships with root symbionts, luminous symbionts and
690 pollinator mutualisms (24, 25, 48, 49).

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

703 **Q6: What conditions favour mutual dependence?**

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

719 Presumably, there is some efficiency benefit from becoming
720 irreversibly specialised, which will depend upon a range of bi-
721 ological factors, such as how groups are formed (relatedness),
722 mortality rates, and how those that reproduce (breeders) are re-
723 placed. Symbionts offer excellent opportunities for comparative
724 studies on the evolutionary and ecological correlates of mutual
725 dependence. For example, why does the interaction between
726 photosynthetic symbionts and their hosts vary from the obligate
727 symbiosis typified by plastids in plants to ciliates that can 'culture'
728 the chloroplasts found in their algal food (45, 52, 53). Are partner-
729 ships in which symbionts access and deliver new forms of energy
730 for their hosts, more likely to lead to major transitions than cases
731 where the symbionts provide a resource that the host can also
732 obtain directly. Repression of competition can also favour mutual
733 dependence, as demonstrated by the influence of worker policing
734 on whether hymenopteran workers develop ovaries (44).

735 Genetic drift can play a role in the evolution of mutual de-
736 pendence. When symbionts have small asexual populations, drift
737 can be a significant factor, leading to the inactivation and even-
738 tual deletion of mildly beneficial genes (54). As symbionts lose
739 functionality in traits or structures, such as transporters and cell
740 walls, the host may evolve to take over these roles, co-adapting in
741 a way that leads to mutual dependence. Similarly, hosts can lose
742 traits that the symbionts perform. Oligochaete worms have lost
743 the ability to excrete their own waste, relying instead on metabolic
744 pathways introduced by endosymbionts to provide and process
745 nutrients (55), while coral hosts (e.g. *Acropora* sp.) have lost their
746 Cysteine synthesis pathway in favor of symbiont derived sources
747 (56). When multiple symbiont lineages are within a host, different
748

749 lineages may lose different traits, such that multiple symbionts
750 and the host can become interdependent (28).

751 **Conflict and Maximising agents**

752 A major transition requires that there is a relative lack of
753 within group conflict such that a larger unit can be thought of as a
754 fitness-maximising agent (individual or organism) in its own right.
755 We are interested in when a group can be considered a fitness-
756 maximizing agent for at least three reasons (6, 34). First, the
757 maximizing agent analogy informs us when group level adaptation
758 will occur (33). Almost all interesting traits and behaviours are
759 the construction of multiple entities (genes, cells etc) pulling
760 in the same direction cooperatively, with minimal conflict. If
761 there is negligible conflict, and we can think of a group as single
762 maximizing agent, then we can expect adaptation at the group
763 level, to maximize the fitness of the group, and hence substantial
764 increases in organismal complexity. For example, clonal multice-
765 llular groups have led to animals and plants, whereas non-clonal
766 multicellular groups have only led to things like slime moulds.

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

782 More generally, we emphasise that the fundamental question
783 being asked with major transitions is one of individuality, and
784 not other issues such as sterility, altruism, complexity, ecological
785 impact or whether gene transfer has occurred (33). Whilst traits
786 such as sterility raise important evolutionary questions, and can
787 be correlated with whether a major transition has occurred, they
788 are neither necessary nor sufficient for a major transition. Indeed,
789 other examples of complete altruism can be found, which are
790 clearly not major transitions, including bacteria bursting suicidally
791 to release factors that reduce competition (58).

792 **Pragmatism**

793 Identifying whether a major transition has been made is an
794 empirical problem, where pragmatism can be important. From
795 a theoretical perspective, we can identify the ideal qualities that
796 would unequivocally define individuality and identify when a ma-
797 jor transition has been made. For example, we can examine what
798 conditions would lead to no conflict, such as clonality or complete
799 repression of competition (33). In the real world, conflict between
800 genes or cells can still exist. For example, in animals and plants
801 there is still some opportunity for conflict among genes, and
802 somatic mutation can lead to conflicts among cells (6, 59, 60).
803 Our aim is to consider when there is so little conflict that the
804 consequences for the group can be effectively ignored.

805 Whether there is negligible conflict and the group acts as a
806 fitness maximizing agent can be trait dependent, even within an
807 organism. For example, whilst we expect plant traits controlled by
808 nuclear genes to maximize plant fitness, we know that cytoplasmic
809 genes can select for male sterility, to their own selfish benefit.
810 Similarly, in ants such as *Formica exsecta*, we can assume that the
811 different ants are behaving as a single maximising unit for traits
812 such as foraging, refuse disposal etc, whilst there is also clear
813 conflict between the queen and the workers over sex allocation (61).
814 What is key is not to argue whether a certain species has made
815 a major transition, but to use the approach in a way that helps
816

817 us understand the processes that lead to major transitions. For
818 examining what conditions lead to greater mutual dependence or
819 lower conflict. Evolution is a process of continuous change and so
820 we should expect blurry edges with a mosaic of features (1).

821 Pragmatism is also required when considering whether the
822 members of a group are mutually dependent. We are not saying
823 that mutual dependence can never be reversed by evolution –
824 mitochondria have been lost in some parasites of humans, and
825 plastids have been lost multiple times (45, 62). Instead, our aim is
826 to consider whether the breaking up of a group would prevent
827 replication. Related to this, all organisms are dependent upon
828 other organisms to be able to reproduce, and we are considering
829 mutual dependence relative to a defined social group. For exam-
830 ple, we can ask whether the cells that make up a male lion are
831 mutually dependent upon each other, even though that male is
832 also dependent upon a female lion to mate with, zebras to eat,
833 grass to feed those zebras and so on.

834 The world made simpler

835 Maynard Smith & Szathmari offered a mixture of explanations
836 for the different transitions, suggesting that a diversity of
837 factors were at play (2). We suggest that both theoretical and
838 empirical advances have provided a more unified explanation for
839 the different major transitions. Major transitions require extreme
840 conditions, with certain factors being either consistently impor-
841 tant, or consistently unimportant.

842 First, at a very general level, the same two factors play a
843 key role in answering all six questions that we posed: (i) an
844 ecological benefit to cooperation and (ii) a mechanism to unite
845 the interests of different individuals. Consequently, the same
846 ecological and evolutionary conditions can provide the answers
847 to all six questions. For example, the combination of clonality
848 and the right ecological benefit can remove within group conflict
849 (Q5), and favour cooperation (Q1&2), division of labour (Q3),
850 honest communication (Q4) and mutual dependence (Q6).

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

861 Third, the restrictive conditions required for a major transi-
862 tion make it easier to understand where and why major transitions
863 have not taken place. For example, why there have been no
864 major transitions in promiscuous cooperative breeders or multi-
865 cellular groups formed by aggregation. A major transition is not
866 simply driven by satisfying Hamilton's rule (Q1&2), it is about
867 conflict being sufficiently eliminated that the group acts as a
868 single maximizing agent (Q5). Consequently, a lower relatedness
869 (r) cannot just be made up for with a greater ecological benefit
870 to cooperation (B/C). This means we would not expect major
871 transitions with certain life histories, such as promiscuity or non-
872 clonal aggregation (33, 35).

873 Fourth, there are striking similarities in the ecological ben-
874 efits to cooperation that have driven different transitions. The
875 eusocial insects can be divided depending upon whether the
876 evolution of eusociality was driven by either the advantage in
877 forming defensive groups (e.g. termites, aphids) or the efficiency
878 benefit gained from cooperating to rear young (e.g. hymenoptera)
879 (17). The ecological benefits to multicellularity seem to divide
880 along analogous lines due to the benefits of forming defensive
881 groups (e.g. algae) or to make certain traits more efficient (e.g.
882 yeast, slime moulds) (13, 63). Can we make similar generalisations
883 about the transitions between species?
884

885 Fifth, within group kin discrimination appears to have played
886 a limited role in helping within species major transitions. The
887 transitions to obligate multicellularity and obligate eusociality
888 have only taken place in clonal or monogamous populations
889 ($r_o=r_h$), where there is no or limited potential for kin discrimina-
890 tion. Consequently, whilst kin discrimination can be important in
891 facultatively multicellular species or cooperative breeders, this is
892 only when they have appreciable variation in within-group relat-
893 edness (64), which also prevents major transitions. Furthermore,
894 some cases of kin discrimination, such as haplodiploid females
895 favouring sons over brothers, increases rather than decreases
896 conflict, and so disfavor rather than favour major transitions. An
897 important exception is the involvement of kin discrimination in
898 the worker policing of the haplodiploid social insects (44).

899 Sixth, repression of competition appears to have played a
900 limited role in helping within species major transitions. The tran-
901 sitions to obligate multicellularity and obligate eusociality have
902 only taken place in clonal or monogamous populations ($r_o=r_h$),
903 where there is no or limited competition to be suppressed. Fur-
904 thermore, theory suggests that it is hard to evolve the complete
905 repression of competition that would be required for a major tran-
906 sition, because the marginal benefits of repressing competition
907 will often plateau, such that an intermediate level of repression
908 will be favoured (65). As with kin discrimination, an important
909 exception is policing in the haplodiploid social insects (44).

910 Considering transitions between species, the relative role of
911 repression of competition is less clear, but may also be rela-
912 tively unimportant. In many cases, vertical inheritance of clonal
913 symbionts may mean that there is no competition to repress.
914 With horizontal transmission of symbionts, sanction mechanisms
915 that reward cooperators and / or punish non-cooperators can
916 be favoured (23, 24), but these may not be able to suppress
917 competition enough for a major transition to occur.

918 Future Directions

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

926 First, whilst we have a relatively good understanding of co-
927 operation (Q1&2), we have a much poorer understanding of
928 group transformation (Q3-6). In cases where we have a good
929 understanding, this often raises other questions – for example,
930 how groups form is important, but how can we explain variation
931 in the way in which groups form?

932 Second, we have a much better understanding of the within-
933 species transitions than the between-species transitions. The
934 within-species transitions have been easier to study because they
935 have happened both more often, and usually more recently than
936 transitions such as the evolution of the eukaryote cell or the
937 genome. Advances in genomic methodologies are allowing divi-
938 sion of labour and mutual dependence to be much better studied
939 in endosymbionts and organelles (15, 28, 45, 66).

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

947 Fourth, we have focused on determining the ultimate selec-
948 tive forces that have favoured major transitions. Mechanistic
949 details can matter if they predispose certain species to making
950 transitions. For example, if bacteria are able to acquire resources
951 required by a potential host, gain entry to and proliferate in
952

the tissues and cells of eukaryotes, and exchange symbiotic loci horizontally. Can generalisations be made about the role of pre-dispositions 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, 59). 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:

Q7. How are new conflicts of interest suppressed in groups that have already made a major transition?

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Q8. What conditions favour the break down of major transitions?

As when considering Q1–6, the beauty of the major transition approach is that it facilitates the identification of general patterns. How important are repression of conflict mechanisms for keeping down conflicts that arise after transitions have been made, such as suppressors of selfish genes or cancer (1, 60)? 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.

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