Toward major evolutionary transitions theory 2.0

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The impressive body of work on the major evolutionary transitions in the last 20 y calls for a reconstruction of the theory although a 2D account (evolution of informational systems and transitions in individuality) remains. Significant advances include the concept of fraternal and egalitarian transitions (lower-level units like and unlike, respectively). Multilevel selection, first without, then with, the collectives in focus is an important explanatory mechanism. Transitions are decomposed into phases of origin, maintenance, and transformation (i.e., further evolution) of the higher level units, which helps reduce the number of transitions in the revised list by two so that it is less top-heavy. After the transition, units show strong cooperation and very limited realized conflict. The origins of cells, the emergence of the genetic code and translation, the evolution of the eukaryotic cell, multicellularity, and the origin of human groups with language are reconsidered in some detail in the light of new data and considerations. Arguments are given why sex is not in the revised list as a separate transition. Some of the transitions can be recursive (e.g., plastids, multicellularity) or limited (transitions that share the usual features of major transitions without a massive phylogenetic impact, such as the micro- and macronuclei in ciliates). During transitions, new units of reproduction emerge, and establishment of such units requires high fidelity of reproduction (as opposed to mere replication).

egalitarian transitions | fraternal transitions | multilevel selection | aggregative unit formation | cohesive unit formation | cooperation | recursive transitions

The Major Transitions in Evolution was published 20 y ago (1) and popularized 16 y ago (2). The impressive work accomplished by the interested community has made time ripe for a resynthesis of the field. In this paper, I outline the revised theory while noting that the full account can be taken only in a new book. First, I present the key points of the theory, followed by an impressionist overview of some of the transitions, highlighting (without being all-inclusive) some of the most exciting findings pertinent to the major transitions in a revised list. In doing so, I rebuild some of the foundations of the theory. A scholarly account of all relevant contributions is beyond the scope of the present paper. For lack of space, I deliberately omit discussion on the origin of animal societies (3), except humans.

Brief Survey of the Conceptual Landscape of the Major Transitions

Bonner (4), Buss (5), Maynard Smith (6, 7), Leigh (8), Jablonka (9), and Szathmáry (10–13) have significantly helped open this field of inquiry. A succinct exposition of the original theory is to be found in ref. 14. In this section, I highlight some general considerations; others will be discussed for didactic reasons in association with some example transitions later.

Increase in Complexity. By any sensible measure of complexity, one is likely to conclude that biological units of evolution in certain lineages got more complex through the 3.5 billion years of evolution (1). This ■■■ does not contradict the fact that the earth can still be regarded as a habitat dominated by prokaryotes. We are not focusing on ecosystem complexity, but the complexity of the players (organisms, etc.) belonging to certain lineages, acting in the ecological theater. One can ask the question then: Why and how has complexity increased? A diffusion model (15) could be regarded as a null hypothesis: If there is a "wall" on the left, indicating the minimal complexity of living systems, then a random walk in complexity would drag the mean away from the wall with time. This increase in complexity may have been achieved as a result of a series of major evolutionary transitions. These ■■■ involved changes in the way information is stored and transmitted" (ref. 14, p. 227). Maynard Smith and Szathmáry presented a table of such transitions (I present a revised Table 1). A list by itself can be defined in any arbitrary way; the crucial question is how the listed items belong together. "There are common features that recur in many of the transitions" (14). It has never been claimed that all transitions would possess all common features or that the possessed features would have uniform weights across all of the transitions.

From Lower to Higher Level Evolutionary Units. The first common feature is the transition from independent replicators to form higher level units: for example, genes ganged up in protocells, prokaryotes joined to constitute the eukaryotic cell, protist cells stacked together to form multicellular organisms, and so on. In order for such a transition to be successful, evolution at the lower level must be somehow constrained by the higher level. I adopt the view of Bourke (3), who suggested that major transitions should typically be cut into three phases: the formation, maintenance, and transformation of "social groups." I suggest replacing the somewhat too broad term "social group" with that of a higher evolutionary level, traditionally understood as populations of higher level units. It should be noted, however, that the fluid nature of the state of the art does not allow yet a systematic delineation of these phases for all transitions.

Division of Labor and Selection. The recurrent emergence of the division of labor or the combination of functions allows the higher level units to be more efficient under certain conditions, which has to translate into a fitness advantage. Synergistic fitness interactions are regarded as one of the crucial driving forces behind the major transitions (14, 16). "If cooperation is to evolve, non-additive, or synergistic, fitness interactions are needed. If two or more cooperating individuals achieve something that a similar number of isolated individuals cannot, the preconditions exist. … But the dangers of intragenomic conflict remain: both relatedness and synergistic fitness interactions are likely to be needed" (ref. 14, p. 229). Local interactions in some sorts of groups have played a role in all transitions (17): models based on the assumption of spatial homogeneity are notoriously unable to account for the necessary dynamics.

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Table 1. Revised major transitions

Origin of:	Formation, maintenance, transformation phases	Transition in individuality	New type of information storage, use, and transmission	Limited transitions
Protocells	1. Autocatalytic networks on the rocks cooperate 2. Naked genes escape into compartments 3. Chromosomes form	MLS1 on the rocks MLS2 in compartments Chromosomes as conflict mediators	Catalysts based on informational replication arise Genetic information encapsulated in cells	
Genetic code and translation: prokaryotic cells	1. Limited coding before translation (coenzyme amino acids and peptides) 2. Early ribosomes and primitive translation 3. Vocabulary extension by bacterial sex	Establishment of symbiotic autocatalytic molecular networks, including complementary subcodes	Symbolic as opposed to earlier iconic hereditary system (code) Coded sexuality	21st and 22nd amino acids (selenocystein and pyrrolisine) Highly polyploid bacteria
Eukaryotic cells	1. Fusion–fission cycle (early sex) 2. Mitochondrial symbiont (before or after phagocytosis) 3. Nucleus, meiosis, and mitosis	Different cells come and stay together as a higher level whole	Genome composed of functionally synergistic compartments Separation of transcription from translation	Within-cell soma and germ (ciliates)
Plastids	1. Engulfment of plastids 2. Transfer of plastid genes to nucleus 3. Posttranslational import and regulation of division	Different cells come and stay together as a higher level whole	Genome composed of functionally synergistic compartments	Tertiary plastids *Paulinella*
Multicellularity (plants, animals, fungi)	1. Size advantage from cohesion 2. Programmed regulation of cell division 3. Soma and early-sequestered germ line	Associative multicellularity allows for differentiation and division of labor	Epigenetic inheritance systems with high hereditary potential	Multicellularity in other lineages Multi-multi symbioses (e.g., lichens)
Eusocial animal societies	1. Origin of societies 2. Control of conflict (dominance, punishment, policing) 3. Dimorphic reproductive and nonreproductive castes	Formation of (super)organisms	Animal signaling and social learning	Unicolonial ant supercolonies
Societies with natural language	1. Confrontational scavenging, first words 2. Eusociality (grandmothers) and protolanguage 3. Cultural group selection and syntax	Non-kin, large-sized cooperation based on negotiated division of labor Food sharing and reproductive leveling Cultural group selection	Symbolic communication with complex syntax	Animal cultures

Limited transitions are cases in which the formation and the maintenance of the units did not lead to vast adaptive radiations as seen in phylogeny. For example, ciliates with micro- and macronuclei are important, but they do not match the impact of segregated soma and germ in the eukaryotic multicells, and the same holds for other examples in this table. It is fair to say that these ■■■ have been potentially major transitions that remained in bud so far. Some of these buds may flower, however, in the (hopefully) billions of years to come.

Novel Inheritance Systems. There are hereditary mechanisms below and before, as well as above and after, DNA that emerged in evolution: the RNA world, epigenetic inheritance, and language are important examples. This ■■■ is a feature that is arguably present in some form in all of the transitions listed in Table 1. It was noted that new inheritance systems arise first in a rudimentary form, offering so-called limited heredity, where a few types, typically vastly below the number of individuals, can be propagated (1). Further evolution generalizes the system so that a hyperastronomically vast combinatorial space can be sampled by evolutionary search: for all practical reasons, we are dealing with unlimited heredity when the number of possible types vastly exceeds the number of individuals, even across the history of the entire biota. Evolution progressed from unlimited to limited heredity in the genetic, epigenetic, and linguistic domains.

Two Dimensions of Major Evolutionary Transitions. Far from being an arbitrary collection of merely interesting anecdotes about evolution, transition theory has been presented as exploring the topic in two dimensions. As Queller (18) aptly noted, the major transitions might be regarded as a combination of two books: "The Acquisition of Inheritance Characteristics" and "Cooperators since Life Began," with overlapping and complementary features. Buss (5) before, and Michod (19) after, 1995 were concerned with the second problem whereas Jablonka and Lamb (20, 21) were concentrating on the first. I think that this dual approach is a feature rather than a bug. It would be somewhat surprising if major achievements of evolution could be satisfactorily coerced into a Procrustean bed of either dimension. More importantly, this view is linked to the notion of units of evolution that multiply, show inheritance, and have variability (22–24). Uniting the last two criteria in hereditary variability, one has two major features: the nature of multiplication and the nature of inheritance, the major evolutionary transitions that we are interested in.

Egalitarian and Fraternal Transitions. Queller (18) has identified two types of major transition: fraternal and egalitarian. In the first, like units join or remain joined, reaping the first benefits from the economy of scale, and then evolving division of labor

by differentiation. In the second, unlike units come together, complementing their functions in a higher unit. The origins of complex multicellularity and that of the eukaryotic cell serve as respective examples. The main control of conflicts is ensured by kinship and fairness in reproduction for the fraternal and egalitarian transitions, respectively.

Origins of Life: Three Early Phases of Transitions to Cells

Progress about the origins of life has been considerable although the nut is still hard to crack. New experiments and theoretical insights have been generated, but, equally important, we now have a much better understanding of what we do not understand (moving from "unknown unknowns" to "known unknowns"). I expand on this topic in some detail because several general points can be clearly illustrated by relatively simple examples that serve as a kind of introduction to related issues tackled later.

The Origin of the First Hereditary Replicators. This ■■■ is still an unsolved problem. By itself, this transition is not an evolutionary one because, without hereditary replicators, no Darwinian evolution is possible. However, we have to consider the gray zone where chemistry and evolution had the first overlap. As Orgel noted: "All replicating systems are, by definition, autocatalytic and all autocatalytic systems result, in some sense, in replication" (ref. 25, p. 203). This ■■■ is the view that transition theory has adopted throughout the years, which also led to a new way of classifying replicators (26). [As Okasha (27) notes, this approach rests on a broader conceptualization than that by Dawkins.] Autocatalysis is at the heart of template replication as well as that of metabolic growth (1).

There is a possibility that autocatalytic macromolecular networks without template replication could exist, a view advocated by Kauffman (28, 29) since 1971. Imagine a network of peptides in which some peptides can catalyze the formation of other peptides from amino acids and simpler peptides. Recent calculations show that the probability of formation is higher than previously thought (30) and that there is limited evolvability, provided that reflexively autocatalytic networks are compartmentalized (31). This option is also compatible with the view that the RNA world may have never been clean and that amino acids and peptides played some important role in the beginning: for example, in the handling of membrane permeability (32).

There is ample evidence supporting the view that the RNA world in fact existed (33), but many agree that it may not have been the earliest genetic system, because of difficulties with its origin. Despite recent progress, we still have no general RNA-based replicase that could replicate a great variety of sequences, including copies of its own. I briefly consider novel issues in turn. A potential way out of the missing RNA replicase problem could be a network in which two types of ribozymes act together: replicases replicate short strands that would be linked by ligases (34). Both ligases and replicases would form in this way. Template effects are important, and the system as a whole is collectively autocatalytic. We have nice examples of a ligase-based anabolic autocatalytic system (35) and a collectively autocatalytic set of minimalist nucleic acid replicators (36).

The Error Threshold of Molecular Replication and the Maintenance of Integrated Information. Once RNA genes could be mechanistically replicated one way or another, a first appearance of intragenomic conflict arises due to Eigen's error threshold (37). Limited replication accuracy in early systems would have allowed the maintenance by selection of single genes only that in turn would have competed with each other. Eigen suggested the hypercycle (37) as a solution (Fig. S1*A*). The hypercycle is a system of molecular cooperators. Each member grows due to a combination of autocatalytic effect and heterocatalytic aid provided by the other member: thus, kinetically, we are dealing with at least second-order growth. Such a system is ecologically stable, but evolutionarily unstable because of the parasite problem (38). Parasites replicate faster than cooperators but do not return aid to the system. Many do not realize the importance of this definition: there is a notoriously recurring error in the literature equating any collectively autocatalytic network with hypercycles, which leads to dramatic confusion by implying that the dynamical theory of hypercycles is applicable whereas it is not (39). Cross-catalytic peptides or anabolic ligases are collective autocatalysts but their members are not cooperators in the evolutionary sense.

Cases of Multilevel Selection. Because the hypercycle was conceived in the pre–RNA-world era of this field, Michod considered the effect of population structure on the evolutionary stability of the system. Imagine one replicating gene that somehow also catalyzes the formation of a protein replicase and that in turn replicates the gene and its parasitic mutants (Fig. S1*B*). Michod (40) applied the trait-group model of Wilson (41) to show that, in a spatially inhomogeneous setting, parasites cannot take over. The reason for this ■■■ is that genes are weak altruists in this case: they help parasites better but they also help themselves to a lesser degree. In other words, these altruists can "scratch their own back" (they pay a relative cost). This form of population structure is regarded recently by many as the first to ensure genomic coexistence in the early days of evolution; localization of the genes could have happened either on mineral surfaces (42, 43) or the holes in porous rocks (44). It is known that weak altruists do not require kin selection to spread whereas strong altruists need assortative grouping (45): imagine, in contrast to Michod's case, a self-replicating RNA replicase challenged by its own parasitic copies. Here, a single replicase is a strong altruist because it pays an absolute cost in fitness. Indeed, a cellular automaton model (42) shows that limited diffusion causing interaction of relatives is necessary for the spread of efficient replicases in coexistence with a parasite population: a trait-group model is not sufficient. A cellular-automaton model also shows that, once there is population structure, a hypercyclic interaction among the replicators is not necessary (43). Because here ribozymes act not on themselves but on metabolites, they again can scratch their own back: a trait-group model is thus as good as a cellular automaton model. All passive models of compartmentation are examples of multilevel selection models of type 1 (MLS1) where the focal units are still the individual replicators rather than the groups (46).

However, passive localization of replicators to mineral surfaces or a trait-group type lifecycle is a poor man's form of compartmentation. Information integration is more efficient by reproducing compartments (11), as in the nearly 30-y-old stochastic corrector model (Fig. S1*C*). This ■■■ is a clear example of multilevel selection of the second type (MLS2) where the focal units are groups (or collectives), despite the fact that replicators (particles) are also reproducing. Variation on which selection among the cells can act is provided by demographic stochasticity within compartments and chance assortment of genes into offspring compartments. Due to the metabolic coupling, protocells with a balanced fitness enjoy a fitness advantage. The construction can be followed to yield group selection–mutation balance. Group selection is effective because group size is much smaller than population size at the group level; there is no migration between groups and each group has only one parent (47). In contrast to traditional models of altruism, there is an optimal frequency of different types of cooperator. Multilevel selection is integral to account for the dynamics of the major transitions (5, 17, 19, 27, 48). The formation of protocells is a major transition in individuality (MTI).

Protocell Transformation: Chromosomes and Efficient Metabolism. The stochastic-corrector model was used to account for the spread of chromosomes within protocells (49): even with replicative disadvantage to longer chromosomes relative to unlinked genes, suppression of internal competition and reduction in assortment load are potent selective forces. The chromosome is a conflict-mediating institution whereby different particle fitnesses and that of the protocell are aligned and particle and cell

reproduction become fully synchronized. In this sense, the internal gene population is under tight control although, of course, transposons also can break this rule (1). As recently shown, evolution of efficient and specific enzymes in general requires this step because, without chromosomes, generalist but inefficient enzymes are better because their presence reduces the considerable assortment load (protocells do not lose an essential gene upon random cell fission) (50).

The Genetic Code, the Prokaryotic Cell, and Bacterial Sexuality

The genetic code allowed for the full division of labor between genes and enzymes; the genetic and catalytic alphabets thus became distinct. The presence of a genetic code is an enabling constraint (51, 52): because protein enzymes do not have to reproduce, they can explore a larger functionality space. This exploration in an RNA world is limited because ribozymes had to replicate and also do work in the protocell. Under such circumstances, the optimal size of the genetic alphabet is modest: more base-pair types increase the catalytic potential but reduce copying fidelity. If fitness is a product of the two, an optimum is ensured (53). Only inventing a separate catalytic macromolecular set can help the system leave this trap.

Origin of the Genetic Code. Remarkably, there is recent indication that a group of amino acids could be stereochemically recognized by, and possibly charged to, simple RNA molecules, as experiments on artificial selection for RNA aptamers show (54). Stereochemical match is aided by codonic or anticodonic triplets in the corresponding binding sites although an open question is the accuracy when all amino acids and aptamers are present in the same milieu. Should this mechanism turn out to be robust, it offers a convenient road toward initial establishment of the code. The question "what for" remains, however. Still, before the advent of ritualized translation, amino acids and peptides could have boosted RNA protocells by enhancing catalytic potential (55, 56) or regulating membrane permeability and transport (32). When speculating on the origin of translation, one should consider that a pentanucleotide (!) ribozyme is capable of catalyzing peptide bond formation (57).

All this ■■■ has led to a major change in how inheritance was executed. The origin of the code is an important example of the division of labor (1). In the RNA-world phase, we have only RNA replicators, even if possibly aided by amino acids and peptides. Then there came a phase when ribozymes still existed and replicated and some encoded peptides were already operational: such a transitional form is inevitable to maintain functionality (58). As soon as proteinaceous aminoacyl-tRNA synthetases appeared on the scene, a new kind of autocatalysis (replication) emerged. Whereas, previously, nucleic acids were autonomously autocatalytic, in the DNA–RNA–protein world, autonomous autocatalysis is shown by the collective network only, even if informational replication is ensured by nucleic acids. Modern metabolism is likely to be a palimpsest of the RNA world (59).

Horizontal Gene Transfer. Woese and coworkers (60, 61) have recently argued that (*i*) early evolution relied on massive horizontal gene transfer, (*ii*) early cells were not Darwinian because they have acquired many genes by horizontal, therefore Lamarckian, mechanism, and, (*iii*) most important for the present topic, no universal code could have emerged and been optimized without horizontal gene transfer (HGT).

Let us dissect the above claims because there are valid and invalid statements. First, as Poole noted (62), there is nothing Lamarckian here but only multilevel selection. Second, it is a big mistake to ignore, as those authors did, the parasitic genetic elements as a menace to the integrity of the genome. For example, in the case of the stochastic corrector model, HGT is far from universally optimal because of the spread of selfish replicators (63); in other words, group selection is rendered ineffective and sex is selected against. Therefore, the phase of massive HGT is unlikely to predate the origin of chromosomes. Another precondition is the evolution of the sexual apparatuses of prokaryotic cells. It seems impossible to realize controlled bacterial sex without proteins. This ■■■ is complemented with the valid point that the extension and optimization of the genetic code (in reasonable time) needed HGT (61). To this ■■■, we add that HGT then was aided by evolving translation. HGT and translation were thus evolutionarily synergistic. This ■■■ has important consequences. Imagine two cell lineages with partly overlapping codes. The interesting parts are the nonoverlapping sets A and B. As things are, A and B are not yet mutually needed for function. If they come together in the same cell, however, respective coded amino acids will invade the proteins, including the synthetases associated with A and B (network symbiosis). Now, the two sets cannot replicate independently any longer. Aided by symbioses in the same cell, the two translation systems merged into one. There is practically no way back: the expanded code is now locked in by contingent irreversibility (1). It thus seems that the origin of the genetic code qualifies as a bona fide egalitarian transition (taken in several smaller steps, but this ■■■ is true for all transitions).

Maintenance and Transformation of the Fluid Bacterial Genome. The recent view is that sex seems indispensable for the maintenance of bacteria, in at least two related ways. First, there is strong selection for a fast cell cycle, which selects for the loss of dispensable genes in any particular environment. However, environments and bacteria are not stationary in time and space either. Therefore, bacteria having transformation competence can be stably maintained due to the advantage of HGT, resulting in gene reloading (64). It also seems that, on the whole, bacteria could not avoid Muller's ratchet either without some form of recombination (65) because, despite occasionally very high population numbers, starvation and bottlenecks are also common. So, whereas, in the very early days, recombination was more likely to be harmful (because of parasitic elements, combined with a lack of linkage), neither the subsequent origin of the genetic code/translation nor the maintenance of the bacterial genome was feasible without bacterial sex. This ■■■ necessarily implies massive HGT for present-day prokaryotes also, in contrast to views (60) to the contrary.

The Origin of the Eukaryotic Cell

Although bacteria can sometimes be as large as a typical eukaryotic cell and can harbor as many as10,000 genes (66), spectacular individual complexity is a feature of the eukaryotes. Indeed, the divide between prokaryotes and eukaryotes is the biggest known evolutionary discontinuity. What allows this increase in complexity? A consensus seems to emerge that the answer lies in energy. It was the acquisition of mitochondria that allowed more energy per gene available for cells (67–69), which, in turn, allowed experimentation with a higher number of genes. This ■■■ was accompanied by a more K-selected lifestyle relative to the prokaryotes (70) and optimization for lower death rates (71).

Order of Appearance of Phagocytosis and Mitochondria. There is no space here to enter the whole maze of the recent debate about the origin of the eukaryotic cells; suffice it to say that the picture seems more obscure than 20 y ago. I illustrate the situation by two strong competing views: phagocytosis (and associated cellular traits) followed by acquisition of mitochondria (72) and the opposite, the acquisition of mitochondria, followed by the evolution of phagocytosis (68, 69). Phylogeny could in principle tell this difference in order, but the analyses are inconclusive (73). The major argument against the phagocytosis-early scenario is once again energetic. According to this view, the boost provided by mitochondria not only was necessary for the evolution of very complex eukaryotic genomes but also was essential for the origin of the eukaryotic condition (69). It is important to realize that these ■■■ are two different claims, and that the first is often portrayed to imply the latter, which is wrong. The snag is that "archezoan" protists lack mitochondria. Archezoa were once a

high taxonomic rank (1) until it became clear that all known examples have or had mitochondria. This ■■■ has dethroned Archezoa and at the same time has weakened the position of the phagocytosis-early hypothesis although the latter step is not a logical necessity (73). The "archezoan niche" admittedly exists (69). So why cannot one imagine an archezoan-like intermediate? An attempted answer is again related to the energy. The genome sizes of prokaryotes and eukaryotes overlap around 10 Mb and around 10,000 genes (66). This ■■■ is exactly why frequent reference to average genome sizes is irrelevant for the discussion of origins. The overlap suggests that a lineage of prokaryotes could have evolved a small but sufficient pre-eukaryotic genome without mitochondria. If not, why not? Here it is: "the energetic cost for the de novo 'invention' of complex traits like phagocytosis must far exceed the costs of simply inheriting a functional system" (ref. 69, p. 8) and "it must take many more than the total number of genes that are required in the end. Ten times as many?" (ref. 69, p. 35). If the argument holds, then it should hold in principle for any complex eukaryotic trait (mitosis and meiosis, nucleus, cilia, etc.), and indeed for any complex prokaryotic trait (photosynthesis, multicellularity with fruiting bodies, ribosomes, flagella) as well because both empires experimented with novel gene families and folds relative to what had been there before. There is no theoretical or comparative evidence to support the imagination of such "exuberant evolutionary scaffolding" that would require a transient appearance of a huge number of genes exceeding the final count by up to an order of magnitude. If it is not phagocytosis, then it can only be syntrophy or bacteriovory that allowed the entry of the ancestor of mitochondria. There are comparative concerns with these ideas (73). Archaea are not known to harbor prokaryotic symbionts; only eubacteria harbor (rarely) other eubacteria so the appropriate cross-domain analogy is missing. The same holds for known cases of syntrophy. Moreover, there is no example of a relevant cross-domain syntrophic endosymbiosis. However, it is logically true that it is not necessary for a prokaryote to get into another prokaryote without phagocytosis, but it is equally true that one does not need mitochondria for phagocytosis. Archaea have a cytoskeleton and can even fuse their cells (see below), and there is the undeniable ecological advantage of the phagotrophic niche. Theoretical (72, 74) and phylogenetic (75) considerations are consistent with the idea of a primitively phagotrophic, but otherwise archaeal, host cell [see *SI Text S1* for a discussion of possible early advantages of not digesting the mitochondrial ancestor, through either benefiting from its photosynthesis (76) or farming (77) by the host cell].

The Nucleocytoplasm and Meiotic Sex. The origin of the nucleocytoplasm cannot be considered in detail here, but there are two novel, important points to mention. One is that the breaking up of the tight prokaryotic genome organization was presumably due to the invasion of self-splicing introns from mitochondria (68, 78), followed by the evolution of the spliceosome. This ■■■ would have been impossible unless the protoeukaryote evolved sexual recombination rather early: asexual genomes are a challenge to the spread of selfish genetic symbionts. Meiosis is a shared ancestral character state in eukaryotes (79). As testified by halobacteria, a form of fusion–recombination–fission cycle may have been strictly speaking the first (80, 81). Rather than a separate major transition, meiosis and syngamy seem to be better regarded as a coevolving form of maintenance or transformation of an emerging higher-level evolutionary unit. The other component of the genetic revolution is the emergence of the nucleus itself, from which the name eukaryote is derived. The evolution of introns and eukaryotic gene regulation would have been impossible without the spatial separation of transcription and translation (82). Without the nucleus the genome expansion allowed by the mitochondrial extra energy could not have been realized. The division of labor between cytoplasm in eukaryotes is as important as that between nucleic acids and proteins in prokaryotes: both are enabling constrains.

Several people have questioned the validity of eukaryotic sex as a separate major transition. Although it is true that, during sex, two individuals are needed instead of one (1) and that they share the benefits equally (83), giving it an egalitarian flavor (18), there are two heavy counterarguments: mating pairs do not become parts in the further hierarchy (like cells, for example) and they do not give rise to mating pairs as propagating units (83). The equal sharing of benefits can be realized through haploid or diploid offspring. Enduring diploidy is an optional consequence of sex that arose in certain lineages independently. Now, it seems that the origin of sex is coincident with the origin of the eukaryotic cells, and, in a loose form, it may have preceded it as an archaeal legacy. Whether demoting sex from the major transitions remains justified or not time will tell: we need an updated, detailed scenario for the very origin of the eukaryotic cell. It could be that some stages of the origin of meiosis preceded, others were coincident, and the remaining once followed the acquisition of mitochondria—we do not know. However, just as the prokaryotic stage as we know it may not have been established and maintained without horizontal gene transfer, the eukaryotic condition may never have arisen and been maintained without evolving meiosis.

Dynamics and Levels of Selection. Curiously little modeling has been done on eukaryotic origins. The stochastic corrector model (Fig. S1*C*) was published first as applied to a eukaryotic host with two types of asynchronously dividing, complementarily essential organelles, such as mitochondria and plastids (10), and the relation to the origin of protocells by creating shared interests was noted (13, 84). However, mitochondria are much older than plastids so a stage like this ■■■ may have never existed. However, the stochastic-corrector principle works also with one host and one unsynchronized symbiont just as well. Viewed carefully, the origin of the eukaryotic cell is a prime example of repeated, and sometimes recursive, egalitarian transitions: the origins of mitochondria, meiosis and syngamy, and plastids are variations on this theme.

The Second Eukaryotic Transition: Plastids

Repeated and Recursive Transitions. The origin of plastids is less controversial than the earlier case of the mitochondrion. It now seems that, although in many ways the transition to plastids is analogous to that of mitochondria, the former came much later in an already well-established eukaryotic cell (there are several eukaryotic lineages that do not seem to have had plastids ever). These considerations justify the promotion of plastids to major transition rank in Table 1. There is a further important difference: In contrast to plastids, there are no secondary and tertiary mitochondria. Although it seems that all plastids go back to the same stock of endosymbiotic cyanobacteria, it happened recursively that a eukaryotic cell enslaved another eukaryotic cell because of its photosynthetic potential (76, 85). It is puzzling why we have not seen the analogous case of a protist with archezoan features acquire a second mitochondrion of either pro- or eukaryotic origin (such a discovery would be fascinating). The membrane structure, inheritance, and import mechanisms of nonprimary plastids are complex (76). Recent data indicate that *Paulinella* might represent a repeated, independent origin of a primary plasmid by the engulfment of a cyanobacterium by an amoeboid cell. This new primary endosymbiosis happened ~60 million years ago and resulted in a novel way of protein retargeting into the plastid through the Golgi (86).

The Origin of Multicellularity: Fraternal and Egalitarian

Although multicellularity arose more than 20 times, the "spectacular" forms arose only in plants, animals, and fungi. I focus on the basic classification of multicellularity, the role of the levels of selection and the apparent recursion in the evolution of multicellularity.

Aggregative and Cohesive Forms. A particularly appealing recent account is given by Bonner (87) about forms and the selective rationale of multicellularity. In the lifecycle, the multicellular

condition arises either by cells (or nuclei) coming together or by cell division, followed by sticking together. The first type is terrestrial and the latter is of aquatic origins. Aggregation of cells evolved four times independently (some eubacteria, two kinds of cellular slime molds, and some ciliates). Multicellularity in any one lineage always meant an increase in size—which could have been a neural trait, especially in the aquatic forms. Then, the economy of scale kicked in, offering advantages in dispersal or feeding or both (18, 87, 88).

Transitional Forms and Levels of Selection. Okasha (27, 89) newly recognized clearly that major transitions are intimately linked with the shift from MLS1 to MLS2 in relation to particles (lower-level units) and collectives (higher-level units). He distinguishes three phases in this regard: "(Stage 1) Collective fitness defined as average particle fitness (cooperation spreads among particles). (Stage 2) Collective fitness not defined as average particle fitness, but still proportional to average particle fitness (collectives start to emerge as entities in their own right). (Stage 3.) Collective fitness neither defined as nor proportional to average particle fitness (collectives have fully emerged; fitnesses are decoupled)" (ref. 27, p. 1023).

This idea is important because it realizes that one needs a diachronic rather than synchronic approach to the problem of levels in hierarchical selection. We have already seen the fruitfulness of this approach in relation to the origin of cells. Shelton and Michod (90) observe that it is a proper research program, supported by theory (1) to map this list to real cases; they offer a tentative analysis in the case of multicellularity in the Volvocales, where all multicellular forms are cohesive. Michod and Nedelcu describe by writing: "as the evolutionary transition proceeds, group fitness becomes decoupled from the fitness of its lower-level components" (ref. 92, p. 66). People have noted that, although lower-level units are progressively de-Darwinized (93), in the majority of multicells, several individual cells remain reproductive.

Egalité and the Accuracy of Reproduction. There is confusion here that should be cleared up. The first observation is that, if the number of particles per collective is constant, the fittest will be the same by using either MLS1 or MLS2 criteria (27). The second, related problem is that these phases have not been mapped onto the fraternal–egalitarian dimension. In the case of symbiosis, the increase in complexity is accompanied by the emergence of synchronized replication (1). In egalitarian transitions, particle fitness values cannot go down to zero, but they need to be nearly controlled through the mediation of conflicts (reproductive leveling), sometimes up to the point of near equalization (genes in the same chromosome). There is no stage 3 for egalitarian transitions because no reproductive division of labor can exist. This conclusion is valid for the egalitarian forms of multicellulary (see below) as well. Fig. S2 shows the combination of (egalitarian and fraternal) × (aggregative and cohesive) forms of transitions. What matters is the frequency of different particles across the generation of collectives. A common feature I argue is the repeatability of the life cycle (94) or the accuracy of reproduction (ref. 95) rather than replication sensu stricto (see *SI Text S2* for discussion). Faithfulness can be achieved either by controlled reproduction of particles (egalitarian) or controlled development (evolved fraternal) across the generations. In simple forms, reproduction is compositional (only numbers of different particle types matter) whereas, in more complex forms, it is positional, resting on positional information in development, recreating also morphological rather than merely compositional patterns of particles. Note that recursive multicellularity has apparently happened in the cnidarian siphonophores (2). Their most integrated development is associated with cormidia that look like segments of repeated units of the same set of different zooids. Each cormidium forms by the subdivision of a bud (96, 97). Growing from a zygote ensures maximal possible kinship. Integration in the latter case is remarkable, granting these creatures a high degree of "organismality" (98). Another case of recursive multicellularity is in the anglerfish, which can also be regarded as the ultimate integration of the sexes, where even the circulatory systems of the female and the much smaller male(s) become one (99).

Egalitarian Multicellularity. Certain cases of symbioses sit rather comfortably in the organism category (98), despite the fact that their egalitarian nature precludes reproductive division of labor: There is no way for the fungal cell to give rise to an algal cell in case of lichens, for example. I think the original accounts (1, 2, 14) on the major transitions are outdated on this issue: Although they discuss symbiosis, they do not assign the right importance to it beyond the formation of protocells and the eukaryotic cell (3). Lichens, the *Buchnera*–aphid symbiosis, and some plant-pollinator pairs qualify as important examples (98). Ultimately, what allows organism formation from lower level units is a high level of cooperation and a low level of realized conflicts (98).

The Origin of Human "Eusociality," Cooperation, and Language

Human society with language has been, and it still is, the last item on the list (Table 1). For many, the burning question is: Can this part of evolution be regarded as an MTI? The answer is not, if one thinks in the context of multicellular organisms or termite mounds and beehives, but in another sense the answer is, as I shall argue below, affirmative. This transition is one where fraternal and egalitarian features are intermingled. I shall consider recent support to four key components: (*i*) language, (*ii*) human cooperation, (*iii*) human eusociality, and (*iv*) cultural group selection.

Communication and Cooperation Hand in Hand. The confrontational scavenging scenario (101, 102) argues that the rudiments of human language coevolved in *Homo erectus* with the beginning of general cooperation (where individuals were not necessarily closely related; see *SI Text S3* for further details). It was language, with its unlimited hereditary potential, that opened up the possibility of open-ended cumulative cultural evolution, also specific to humans. Cooperation among relatives does exist in humans, but it significantly goes beyond. Shared interest can elicit extensive cooperation among unrelated individuals. A feature of confrontational scavenging is that it links the origins of two human-specific traits closely together in a synergistic fashion (16) where none works without the other, and, if they do not, the cost in fitness is substantial. The dynamics of cooperation here is that of a teamwork dilemma (103), where the collective benefit increases with the number of cooperators in a sigmoid fashion. This ■■■ has the important consequence that it is not an *n*-person Prisoners' Dilemma game that assumes a linear benefit function. In contrast, with a sigmoid benefit function, there is an internal cooperative equilibrium in the system without punishment or repeated interaction among the same individuals (104). Language allows for something unprecedented: negotiated division of labor (2). Just as the evolution of powerful epigenetic inheritance systems allowed the evolution of complex multicellularity, natural language allowed the emergence of complex human societies (9).

Human Eusociality? It was noted that grandmothers represent a temporal nonreproductive caste (105), and, in this sense, humans can be regarded as weakly eusocial (note that grandmothers care for descendant kin). This trait was suggested to originate with *erectus* also (106). In a comparative context, it is noteworthy that a similar condition is found in dolphins with complex cognition, vocal imitation, and cultural differences (107). Grandmothers carry not only related genes but also relevant cultural information. With the gradual complexification of protolanguage, this trait was reinforced. Ultimately, it may have been critical for the origin of efficient teaching (as opposed to learning, which is common), which, in turn, was necessary for cumulative cultural adaptation. According to a recent model (108), fertile females could transfer resources to grandmothers, enabling the latter to redirect their efforts from inefficient foraging to grandchildren care. During this time, fertile females would have been free from

caring, and they could have gone to forage with higher efficiency than grandmothers. This ■■■ is a synergistic situation through intergenerational division of labor whereby everyone does the task she is the most efficient in.

Cultural Group Selection. Human families or local groups are not like beehives or termite mounds. Group structure is too transitory to allow for a major transition in evolution in a purely biological sense. However, it seems compelling that multilevel selection is somehow relevant to this problem and that, in some sense, certain human groups are more advanced than beehives or termite mounds (48). How and why? As recognized by Boyd and Richerson (109), language and cooperation within groups allows for group selection of coherent cultural content, and mechanisms like imitation and in-group bias can maintain cultural diversity among groups. Groups can flourish or decline depending on such cultural content. Intergroup competition and prestige-biased imitation of more successful groups offer the mechanism (110). The dynamics of group cultural content is somewhat similar to the phase of bacterial evolution with frequent horizontal gene transfer. This process has helped build complex societies where genetic relatedness did matter even less than before.

So Is It a Major Transition? We see key elements that are highlighted in other transitions: cooperation (including reproductive leveling and food sharing), a form of eusociality, a powerful novel inheritance system, and living in groups. "Although a cultural group behaves like a well-integrated individual, some of the 'parts' of this individual, such as some behaviors or products of behavior, are potentially independent and 'mobile'… it is the cultural traditions, language, rules and laws that are the cohesiveness-maintaining mechanisms that integrate the 'cultural individual' " (ref. 9, p. 308). It sounds just right: biology gives room to technological and communal cultural evolution. Due to social care (including medicine) and agriculture, the biology of humans has become gradually de-Darwinized. It is culture where the main action is going on.

Conclusion and Outlook

At the list level (Table 1), there are four major novelties: the revision of the first half, the promotion of plastid origin and the demotion of eukaryotic sex, and the inclusion of limited transitions. The transition to cells now includes the origin of chromosomes, and the origin of meiosis and syngamy is included in the transition to eukaryotic cells. The downgrading of two transitions, previously ranked as major, shrinks the top half of the table. Accepting the view of Bourke (3) about origin, maintenance, and transformation phases, we can look at the flow in a more balanced manner.

I have paid considerable attention to the multilevel selection perspective. There is no space here to survey the recent debate on individual, kin, and group selection (cf. ref. 16), but a few remarks are in order. Maynard Smith has thought that the gene's eye view is "a heuristic perspective, not an empirical hypothesis about the course of evolution" (ref. 111, p. 997), and missing this perspective can lead to shaky conclusions: e.g., about aspects of the origin of multicellularity (ref. 1, pp. 244–245). However, to conclude from this ■■■ that there is kin selection and nothing else is a non sequitur. The egalitarian transitions are notoriously resistant to a kin-selectionist approach: Recall the working of the stochastic corrector model. It is a continuous-time, fully dynamic model with reproducing and dying-out groups. Simon (112) has shown that kin-selection versions of such group-selection models are dynamically insufficient. Once you solve the group-selection model, you can always post hoc make up one using inclusive fitness, but this ■■■ yields no additional information, and it is impossible to go the other way round.

The categories of associated recursive and limited transitions have been identified. A major outstanding issue is what I call filial transitions: origin and evolution of new Darwinian systems within the hierarchy, such as the nervous system (20, 113) and the adaptive immune system in vertebrates (113). Previous books Q:23 (1, 3), as well as the present review, have dealt with some common principles of major transitions. The question can justifiably be raised whether we have a theory or not. I think we do, but with qualifications. Theories do not have to be predictive but still can have considerable explanatory power. After all, the predictive aspect of evolutionary biology as such is limited as well; and this ■■■ especially applies to the quantitative aspects. There are two questions that one can raise: (*i*) Is it possible to have a "transitometer" that would tell us whether a lineage or a small set of lineages have transited to 20% or 90% (J. Peck, personal communication)? I think this ■■■ can be answered in the fu- Q:24 ture if one can show that the evolutionary dynamics of transitions has something in common with phase transitions in physics. (*ii*) Related to this ■■■, can we predict, by looking at an evolving population, that a major transition is "imminent"? It is surely impossible to predict whether it is a really major transition or a limited transition—this ■■■ only phylogenetic time can tell. However, transition theory strongly suggests that, if we see, even in rudimentary form, that originally independently reproducing units join, somehow use functional synergies among the units, and that there is some novelty in the inheritance system as well, then the population is definitely on its way to a "major transition."

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Q: 5_Please verify that all supporting information (SI) citations are correct. Note, however, that the hyperlinks for SI citations will not work until the article is published online. In addition, SI that is not composed in the main SI PDF (appendices, datasets, movies, and "Other Supporting Information Files") have not been changed from your originally submitted file and so are not included in this set of proofs. The proofs for any composed portion of your SI are included in this proof as subsequent pages following the last page of the main text. If you did not receive the proofs for your SI, please contact PNAS_Specialist.djs@sheridan.com.

Q: 6_PNAS allows up to five keywords. Please delete as many keywords as necessary to meet the five-term limit.

Q: 7_In the affiliations line, please confirm spell-out of "MTA-ELTE". Also, please confirm whether all units, divisions, departments, laboratories, or sections have been included in the affiliations line for each footnote symbol or add if missing. PNAS requires smallest institutional unit(s) to be listed for each author in each affiliation. The order is from smallest unit to largest.

Q: 8_If appropriate, please clarify what exactly are "the field" and "the revised theory".

Q: 9_PNAS style does not allow italics for emphasis (e.g., "*in certain lineages*).

Q: 10_PNAS mandates unambiguous pronoun antecedents. Please provide the appropriate noun here and throughout the article where indicated by ■■■.

AUTHOR QUERIES

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Q: 11_Please note that the reference list has been renumbered to address numbering problems present in the original manuscript (specifically, refs. 15 and 16 were out of order).

Q: 12_To show where the quotation begins, please insert an opening set of quotation marks to match the ending quotation marks after “transitions”.

Q: 13_For clarity throughout, please consider introducing each quotation with the author of the source. Please also indicate where the quotation ending "is stored and transmitted" begins.

Q: 14_The phrase “the Maintenance of Integrated Of Information” was changed to “the Maintenance of Integrated Information”. Please check for correctness.

Q: 15_Per PNAS style, all citations of figure panels in the text use capital letters, regardless of whether the actual figures use lowercase letters.

Q: 16_Please confirm the following for correctness as edited: “Imagine one replicating gene that somehow also catalyzes the formation of a protein replicase and that in turn replicates the gene and its parasitic mutants”

Q: 17_The word “phagocytocis” was changed to “phagocytosis”. Please check for correctness.

Q: 18_Rather than saying “see below”, please identify the specific section by name.

Q: 19_Please cite ref. 91 in order in the main text

Q: 20_Please confirm “multicellulary” in the following: “egalitarian forms of multicellulary”.

Q: 21_Rather than saying “see below”, please identify the specific section by name.

Q: 22_Please cite ref. 100 in order in the main text.

Q: 23_PNAS does not allow statements that work is planned or in progress. Therefore, the following statement has been deleted: “to be discussed soon in forthcoming publications”.

Q: 24_PNAS no longer allows references to unsupported data (e.g., personal communications). For the statement based on “J. Peck, personal communication”, please either (*i*) provide the data as Supporting Information, (*ii*) provide an at least “in press” reference for an article that has been accepted for publication, or (*iii*) remove the statement in the text.

Q: 25_In the Acknowledgments, please confirm spell-outs of ERC, EU, and COST.

Q: 26_Please confirm that all journal references include issue numbers if the journal provides them.

Q: 27_Main text refs. 26, 31, 39, 50, 69, 74, 102, and 107 and SI ref. 13 include only a first page number. Please provide the full page range for multi-page articles.

Q: 28_If available, please update ref. 33 with volume, issue, and page range.

AUTHOR QUERIES

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Q: 29_Reference 91 "Michod, 1997" is not cited in the text. Please add an in-text citation or delete the reference.

Q: 30_Reference 100 "Maynard Smith, Harper, 2003" is not cited in the text. Please add an in-text citation or delete the reference.

Q: 31_In Table 1, “prokarytotic” was changed to “prokaryotic”. Please check for correctness.

Q: 32_In Table 1, please confirm “Multi-multi symbioses”

Supporting Information

Szathmáry 10.1073/pnas.1421398112

SI Text S1

Possible Advantages of Indigestion. Because the crucial argument against early phagocytosis is questionable, we are free to return to the idea of early phagocytosis. In this context, it must be stressed that, in the recent scenario, no full-blown archezoan-type cell is imagined. The critical transitional form is the very origin of rudimentary phagocytosis (figure 2b in ref. 1), still without many of the known cell organelles, such as the nucleus. This transitional form could well have been sustained by a genome in the higher prokaryotic range, while already providing the ecological benefits of internal digestion. The question we still have to discuss is the initial advantage. The final advantage is of course ATP production, but the ADP/ATP antiporter is a eukaryotic invention, the emergence of which must have taken some time. So unless some benefit from the promitochondrion to the urkaryote (mutualist transitional forms) could have been provided, the initial symbiosis was endoparasitic, which would have meant another burden for the fragile transitional prekaryote. In other words, cells without the protomitochondrial indigestion would have been better off, especially because, in this picture, the protomitochondrion would have been obligatorily endosymbiotic, so evolution could not have been driven by parasitic selfishness with frequent horizontal transfer between hosts. There are two possible ways out. Because mitochondria seem to descend from α-proteobacteria, protomitochondria could have still been photosynthetic. Some of these bacteria are known to eject photosynthate into their environment, which could have benefited the prekaryote (2). Another (not exclusive) way out is farming/prudent predation of protomitochondria (3). Interestingly, there exists today a similar phenomenon in the social amoeba *Dictyostelium* (4) although there the bacteria are carried extracellularly by the multicellular slug and fruiting body. In the case of the protoeukaryote, the symbiont would have been intracellular private property, and it would have been inherited through division.

SI Text S2

Replicators Versus Reproducers. Griesemer (5) insightfully analyzes the problem of transitions in the light of the problem of reproduction. Compare replication by photocopying of a sheet of paper with, say, bacterial reproduction. As we have seen, a cell is a collectively autocatalytic system. DNA is an autocatalyst in need of obligatory heterocatalytic aid by proteins. The photocopier is not part of such a collectively autocatalytic system. This ■■■ relates to the problem of whether viruses are alive or not. My resolution is that there are units of evolution and units of life, and, between these sets, the overlap is large but not complete (6). Viruses are, in this light, only units of evolution. Or, as others would say, autonomous living systems (7) can propagate only through reproduction: replication is necessary but not sufficient. In Gánti's minimal life model (8), there are three autocatalytic subsystems: (*i*) a metabolic network, (*ii*) template replication, and (*iii*) a growing boundary. This chemoton model shows perhaps what Griesemer's reproducer is. "Special or developmental progeneration is multiplication with material overlap of mechanisms conferring the capacity to develop. Development is acquisition of the capacity to reproduce. Reproduction therefore, is progeneration of entities that develop. Because development is analyzed in terms of the capacity to reproduce and progeneration transfers the capacity to develop, reproduction can be understood as the recursive realization of the capacity to reproduce. The capacity to reproduce is the capacity to progenerate entities with the capacity to acquire the capacity to reproduce. Reproduction requires both progeneration and development" (ref. 9, p. S361). It is in this sense that Szathmáry and Maynard Smith (10) adopted the view that evolutionary transitions can create new levels of reproduction.

SI Text S3

The Confrontational Scavenging Scenario. Although animal communication systems do exist (11), they mostly include self-regarding signals about things here and now (12). Natural language is very different: There is a lot of displacement (referring to items that are not present now or are purely imaginary), and it is full of symbolic (arbitrarily conventional rather than indexical or iconic) reference, aided by complex syntax. No other species comes nearly close to such a synergistic package, the origin of which we need not explain. This transition happened in early *Homo erectus*, who faced the problem of starvation due to the disappearance of fruits in that period. There was, however, plenty of meat around, including carcasses of the megafauna. Whereas weapons of the time were not good for hunting elephants or rhinos, they were sufficient to butcher carcasses that rival predators were unable to access before the carcasses exploded. To use this resource, three crucial actions are needed: First, members of the group who cannot know about the carcass must be informed about its nature, location, and distance; second, they need to be recruited; and, third, execution of the task requires intense cooperation with limited opportunity to cheat. The work consists of fighting off the predators around, butchering, and transporting home the carcass. It was this niche that allowed a wedge to penetrate the previous animal communication system by signals for displaced items. Given the fact that by then *erectus* had already had a large brain and was very likely equipped with Machiavellian social intelligence (13, 14), the process did not stop there, and protolanguage with increasing richness of symbolic reference started to evolve, to be followed by syntax that presumably emerged with the speciation of *Homo sapiens* (12).

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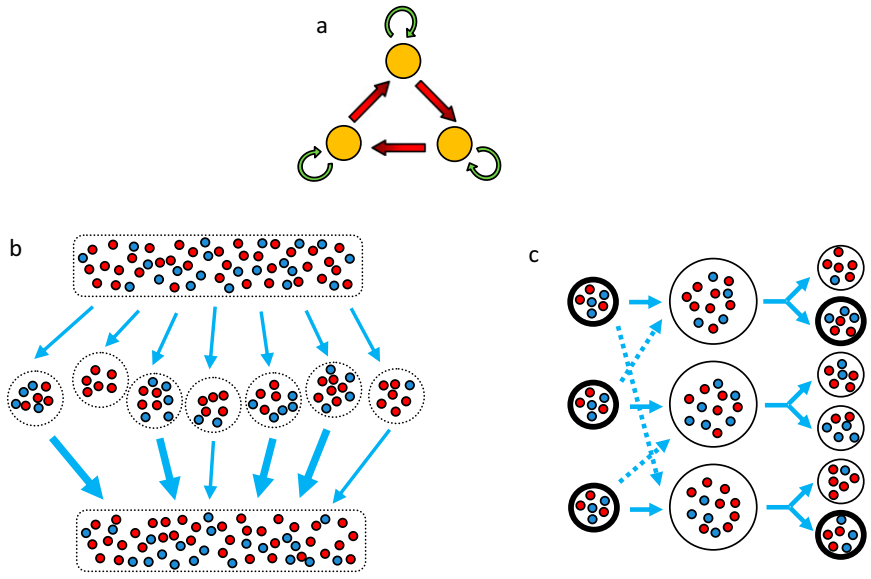

Fig. S1. Replicator topologies and forms of multilevel selection (MLS). (*A*) There are three different information carriers. Green arrows stand for autocatalysis, and red arrows depict heterocatalytic aid. Without the green arrows, we have a collectively autocatalytic system whose members are not replicators (1–5). With all of the arrows, we see a hypercycle of replicators (6). Both systems are ecologically stable, but, without some multilevel selection, the hypercycle is ecologically unstable. (*B*) Multilevel selection of the first type (MLS1), where the focal units are the different (red and blue) replicators, and transient groups provide the context of selection (7–11). Suppose that red replicators are faster than blue ones, but groups with more blue replicators produce more particles. There is an equilibrium frequency in the global population of altruists and selfish replicators, even with random group formation, if the altruists pay a relative cost (they help the reds more than themselves). [A difference between many of the cited models and the original trait group model of Wilson (10) is that, in the former, more than one round of reproduction within a group is possible.] (*C*) In multilevel selection of type 2 (MLS2), the groups are bona fide evolutionary units that multiplay and hereditary variation with fitness effects at the collective level. In the stochastic corrector model (12, 13), the two different types of replicator complement each other synergistically, but there is also intragenomic conflict: Reds replicate faster than blues. Nevertheless, group selection among protocells can maintain a stable population. An Eigen equation at the compartment level can thus be derived, where the mutation terms correspond to the change in gene composition between parent and offspring, due to internal competition and stochasticity. The construction is, unlike many others, fully analytic. Variation is generated by demographic stochasticity in protocells and the chance assortment of replicators into offspring cells. This model is a prototype of how MLS2 can treat egalitarian transitions, including the origin of simple and, later, eukaryotic cells. Note that there was some inconsistency in treating such transitions in the original publications about major transitions. Whereas generally a kin selectionist view was endorsed (12, 14), in some cases, it was complemented by true multilevel selection (*Bottom,* MLS2). Replication of genes and reproduction of protocells are not synchronized, time is continuous, and generations overlap. The model is set up in such a way that protocells reproduce when the total number of genes reaches a threshold. Thus, upon division, each protocell contributes the same number of particles (genes) to the population. However, because of the effect of genes on metabolism, collectives (protocells) with more balanced gene content reproduce faster. Such cells contribute more particles, as well as protocells, per unit time to the population.

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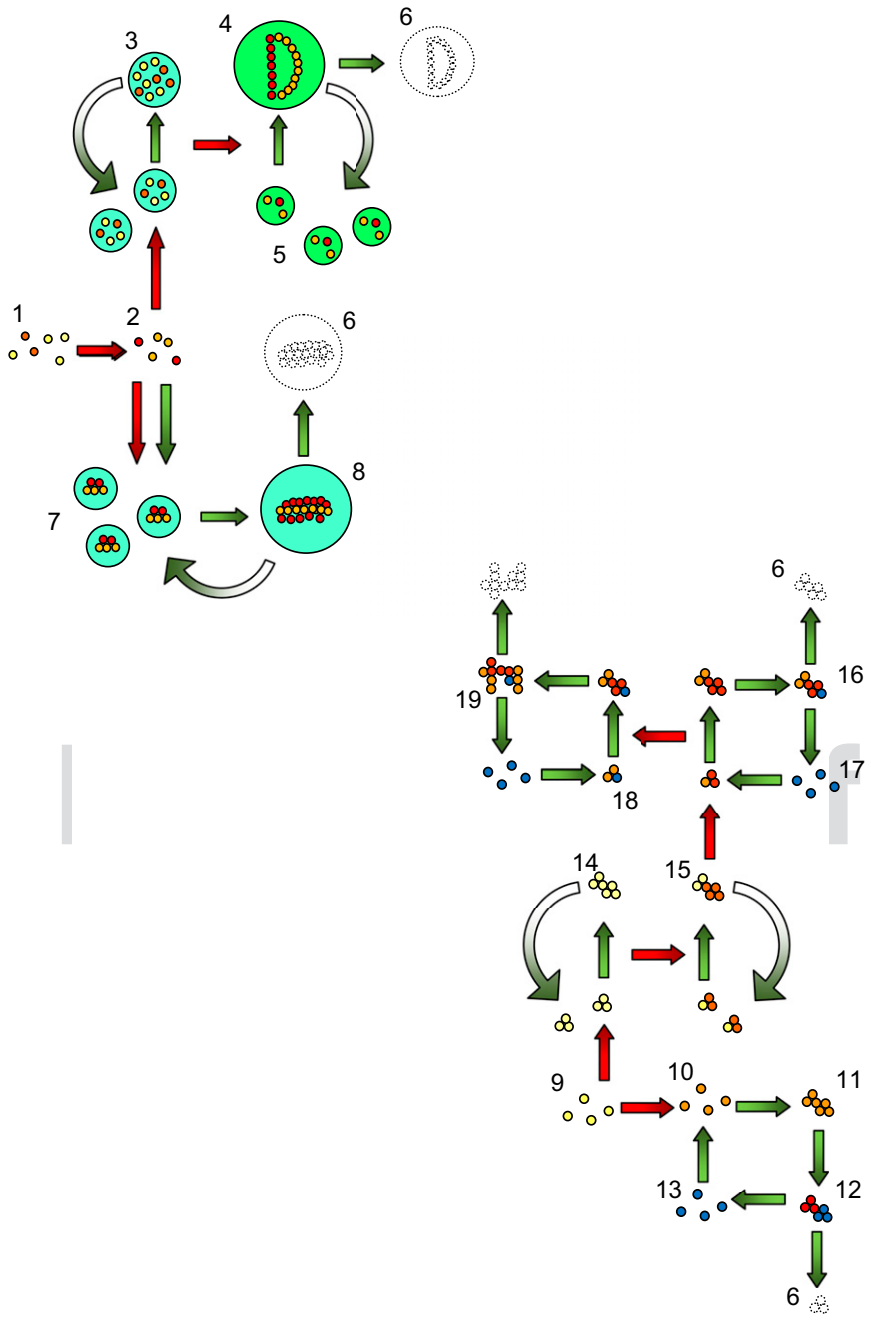

Fig. S2. (Egalitarian and fraternal) × (cohesive and associative) origins of higher-level evolutionary units, exemplified by the origin of multicellularity. Green arrows stand for lifecycle transitions, and red arrows indicate evolution. (*Top*) Egalitarian transitions. The cohesive route: different replicators interact (transition 1) in an MLS1 manner, which results in a coevolved set (transition 2), which then can be encapsulated in a common reproducing unit (transition 3) that may evolve into a morphophysiologically complex organism (transition 4), possibly producing propagules of different size (transition 5) and dead even bodies (transition 6). The aggregative route: different units may optionally reestablish the higher-level unit repeatedly (transition 8) that may produce propagules of different size (transition 7). (*Bottom*) Fraternal transitions. Units of the same type (transition 9) may follow different evolutionary routes. The aggregative route: units can evolve into populations of interacting (transition 10) and aggregating (transition 11) cells that can differentiate to establish reproductive division of labor (transition 12) and produce unicellular propagules (transition 13). The cohesive route: a blob of cells may stay together (transition 14) and reproduce by fragmentation. This form may evolve some cell differentiation, maybe based on location in the clump (transition 15). Further evolution can produce larger, differentiated bodies (transition 16), with late sequestration of germ cells that produce unicellular propagules (transition 17). However, further evolution yields early sequestration of germ cells (transition 18), which allows the evolution of even more complex organisms (transition 19).

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Q: 1_PNAS outline style requires at least two subheadings beneath each main heading. Therefore, concerning SI Text S1, SI Text S2, and SI Text S3: please either (i) combine headings, (ii) add a second subheading beneath each, or (iii) delete each single subheading.

Q: 2_Please confirm "urkaryote".

Q: 3_PNAS does not allow statements that work is planned or in progress. Therefore, the following statement has been deleted: "Modeling is under way to see how this idea works in a quantitative fashion.".

Q: 4_A closing set of quotation marks was inserted after "development". Please check for correctness.

Q: 5_Per PNAS style, if an SI reference is cited only in a figure or table legend and not in the SI running text, only the legend will carry the reference number. Please check the citations carefully (refers to SI refs. 15–27, now renumbered per style beneath the Fig. S1 legend). Note: the citation of ref. 3 was interpreted as original SI ref. 3. Please confirm.

Q: 6_In the Fig. S1 legend, please revise the following for clarity: "In multilevel selection of type 2 (MLS2), the groups are bona fide evolutionary units that multiplay and hereditary variation with fitness effects at the collective level."

Q: 7_In ref. 3, if available, please insert volume, issue, and page range.

Q: 8_In the Fig. S2 legend, the word "transition" was introduced to avoid confusion with reference citation style. Please check for correctness.