Supplementary information

On the Origin of Biological Construction, with a Focus on Multicellularity

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Text S1. Definitions for the unit of biological organization

The plurality of terms used to define new units of biological organization emerging from hierarchical evolutionary transitions (HET) has complicated the field: sometimes, different terms have been used to denote similar types of biological organization (e.g., plants are referred to as both individuals and organisms); other times, the same terms have been used to denote different types of biological organization (e.g., some studies only refer to animals and plants when using the term multicellularity, while others are more inclusive, counting for instance colony-forming bacteria). Without claiming to be complete, here we categorize the most commonly-used definitions for the unit of biological organization in the field of evolutionary biology (for a more general overview, see also (1, 2)). We will also include definitions for multicellularity, since this is the primary focus of our Perspective. Even though a categorization cannot do full justice to the diverse ways in which definitions can be interpreted, by categorizing we acquire a general understanding of the relationship between the different definitions and the criteria they apply.

In general, definitions differ in the minimal criteria that need to be satisfied before a group is considered a unit of biological organization. Figure S1 categorizes the definitions according to six commonly-applied criteria (see also Table S1):

(i) Potential to be a unit of selection. Definitions that employ this criterion consider any group with the potential to evolve by natural selection to be a unit of biological organization. Such groups must have three properties: multiplication, variation and heredity. These three properties form a subset of the properties used by John Maynard Smith and others to define the unit of selection (see next criterion and (3–6)), the only difference being that a potential unit of selection does not have to express heritable fitness differences, whereas an actual unit of selection does (7). This criterion represents the least strict criterion that studies apply for defining a unit of biological organization.

(ii) Unit of selection. According to this criterion, groups are only considered a unit of biological organization when undergoing evolutionary change by natural selection. This is typically expressed using Lewontin’s principles of evolution (8). The important distinction with the previous criterion is that groups, in addition to the three properties described above, should also express heritable fitness differences (see also (6, 7, 9)). Only in the presence of fitness differences, selection can favor some groups over others, and groups form units of selection.

(iii) Cooperation. The previous criteria do not account for the interactions among group members. Yet, as is known from some paradigm examples of biological organization, members of the group often cooperate to bring about group-level adaptations (e.g., cells cooperate in multicellular organism and bees work together in the beehive). Therefore, in addition to the ‘unit of selection’ criterion, some definitions rely on cooperation as the minimal criterion that qualifies a group as a biological unit. For example, in the case of multicellularity, Bonner stated (10): “cells will either compete with one another or cooperate, and it is only as they shift from competition to cooperation that they can rise to the higher multicellular level of selection” (see also (11, 12)).

(iv) No conflict. Since cooperation can occur in many group settings, including in those that still have considerable conflict (e.g., bacterial communities), some researchers prefer a stricter criterion: they not only require a unit of biological organization to be characterized by cooperation, but also by a near lack of
conflict. For example, according to Queller and Strassman (13), "the organism is simply a unit with high cooperation and very low conflict among its parts".

(v) **Mutual dependence.** Instead of ‘no conflict’, some studies prefer the criterion of mutual dependence, in addition to the criterion of cooperation. The most popular formulation of mutual dependence, with regard to HET, is given by Maynard Smith and Szathmáry (14): “entities that were capable of independent replication before the transition can replicate only as part of a large whole after it”. In this definition, mutual dependence is explicitly formulated with respect to replication. Consequently, groups that satisfy this definition automatically form a potential unit of selection and are also implicated to have some type of cooperation, as their members depend on each other for replication.

(vi) **Integration/indivisibility.** This final criterion is formulated to account for a large set of definitions that require any form of functional integration and indivisibility (often expressed in different ways). Although it is rarely specified how these properties can be quantified, it is typically invoked when characterizing the paradigm examples of biological organization: e.g., the multicellular organism, the eusocial bee hive.

A number of general insights can be derived from evaluating the definitions in Figure S1. First, many definitions are inspired by the paradigmatic examples of biological organization. Studies first identify the properties that these paradigmatic examples have in common and subsequently use these properties to formulate their criteria (e.g., cooperation, mutual dependence, integration/indivisibility). Second, most definitions have a nested relationship with respect to each other: groups that satisfy the criteria of the stricter definitions are often implicitly assumed to satisfy those of the less strict definitions as well. Third, the nested layering of definitions gives the false impression that – during a HET – groups undergo a teleological progression towards a certain end-point; the point at which the group resembles one of the paradigmatic examples of biological organization. Groups that deviate from these examples (e.g., facultatively eusocial organisms, aggregative multicellularity, facultative symbionts) are often regarded as incomplete transitions when viewed along the trajectory towards strict cooperation, mutual dependence and integration (see also (15)). Not only is this view false, since many of these deviating examples are the product of alternative evolutionary trajectories, it is also problematic, since it takes the focus away from studying these alternative trajectories, even though they are critical for our understanding of biological construction: only by comparing different evolutionary trajectories towards biological construction can we discriminate between the causal factors that lead to one type of biological organization and not to the other. Fourth, despite considerable disagreement on what is a unit of biological organization, all studies agree that groups can only evolve group-level properties if they are a unit of selection (i.e. if they undergo evolution by natural selection). Studies that apply the least strict criteria—‘potential to be unit of selection’ and ‘unit of selection’—therefore focus on groups that (can) evolve group-level properties, regardless of what these properties might be; studies that apply stricter criteria focus on the evolution of specific group properties (e.g., cooperation), under the assumption that these properties are critical for the evolution of new levels of biological organization.
**Figure S1. Criteria and definitions for the unit of biological organization.** Venn diagram that categorizes definitions for the unit of biological organization based on six criteria: potential to be unit of selection (black); unit of selection (grey); cooperation (green); no conflict (red); mutual dependence (blue) and integration / indivisibility (purple). The six criteria give rise to seven sets of definitions. For each set of definitions, some examples (including references and terminology) are listed below the Venn diagram (see also Table S1).
### Table S1. Overview of definitions

#### 1. Potential to be unit of selection

<table>
<thead>
<tr>
<th>Reference</th>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Griesemer J (2000) The units of evolutionary transition. <em>Selection</em> 1(1-3): 67-80.</td>
<td>Reproducer</td>
<td>“Reproducers are entities that multiply by material overlap of propagules conferring the capacity to develop.” (see also Text S1)</td>
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<td>Libby E, Rainey PB (2013) A conceptual framework for the evolutionary origins of multicellularity. <em>Physical biology</em> 10(3): 035001.</td>
<td>Multicellularity</td>
<td>“(1) Existence. There must be a stage during the life cycle of the organism where a group state is clearly recognizable. (2) Evolution. Groups must be able to multiply and share heritable information with newly created groups.”</td>
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<td>de Monte S, Rainey PB (2014) Nascent multicellular life and the emergence of individuality. <em>Journal of Biosciences</em> 39(2): 237-248.</td>
<td>Multicellularity</td>
<td>“This renders our formulation particularly suited to the earliest manifestations of multicellular life. 1. Identity: a criterion for delimiting collectives ... 2. Recurrence: a relationship between collectives at time t and time t'&gt;t such that at both times the collectives are characterized by the same identity criterion ... 3. Genealogy: the possibility of identifying the precursor(s) of a recurrence, based on the sharing of particle lineages among collectives across successive recurrences.”</td>
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<tr>
<td>Herron MD, Nedelcu AM (2015) Volvocine algae: from simple to complex Multicellularity. <em>Evolutionary Transitions to Multicellular Life</em>, eds Ruiz-Trillo I, Nedelcu AM (Springer), pp. 129-153.</td>
<td>Multicellularity</td>
<td>“Here, we define multicellularity as a category of phenotypes that are based on more than one cell. Such phenotypes can be stable and represent the longest part of a life-cycle or be transient (induced in response to external stimuli) and represent a small (or facultative) portion of a life cycle”</td>
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#### 2. Unit of selection

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<thead>
<tr>
<th>Reference</th>
<th>Term</th>
<th>Definition</th>
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<tr>
<td>Buss LW (1987) <em>Evolution of individuality</em> (Princeton University Press)</td>
<td>Evolutionary transition</td>
<td>“At the heart of my arguments is the simple observation that the history of life is a history of the elaboration of new self-replicating entities by the self-replicating entities contained within them. Self-replicating molecules created self-replicating complexes, such complexes created cells, cells obtained organelles, and cellular complexes gave rise to multicellular individuals ... The history of life is a history of different units of selection. Novel selective scenarios dominate at times of transition between units of selection. Whereas the lower self-replicating unit was previously selected ...”</td>
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by the external environment alone, following the transition it became selected by traits expressed by the higher unit.”

Reference

Term
Evolutionary transition

Definition
“In this article I follow Buss in adopting a selective definition – according to which a major transition consists in a transformation of the hierarchical level at which selection operates on a population. This implies that a mere aggregation of entities into groups is insufficient. The entities need to be compounded in such a way that higher level selection takes place.”

Reference

Term
Evolutionary individuality

Definition
“Evolutionary individuals are those biological entities that satisfy Lewontin’s three conditions for natural selection: they vary, that variation results in differentiation fitness among them, and that variation is heritable.”

3. Cooperation

Reference

Term
Multicellularity

Definition
“The appearance of multicellularity during the course of early evolution is one of the major transitions during the whole span of biological evolution, as Maynard Smith and Szathmáry (1995) and others have pointed out. These transitions are especially important in their implications for natural selection because with each transition one moves from one level of selection to another. This is the case with the invention of multicellularity, where one shifts from the cell as a unit of selection to a multicellular group of cells as a unit. Cells will either compete with one another or cooperate, and it is only as they shift from competition to cooperation that they can rise to the higher multicellular level of selection.”

4. Mutual dependence

Reference

Term
Major transition

Definition
“Entities that were capable of independent replication before the transition can replicate only as part of a large whole after it”

Reference

Term
Multicellularity

Definition
“By a multicellular organism, we understand one in which the activities of the individual cells are coordinated and the cells themselves are either in contact or close enough to interact strongly.”

Reference

Term
Multicellularity

Definition
“What is multicellularity? We agree with Kaiser’s (2001) view, that an overall coordination of function is a necessary and sufficient condition for a colony of cells to qualify as multicellular ... The two basic aspects of any living being are metabolism and informational operations. We can thus say that if at least some parts of the metabolism or the information processing of the cells (confined to
a single cell in unicellular organisms) are shared in a coordinated manner by all cells of the colony, we are dealing with a multicellular organism. Sharing must have an evolved genetic basis not found in unicellular organisms.”

5. No conflict

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<tr>
<td>Term</td>
<td>Organismality</td>
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<tr>
<td>Definition</td>
<td>“The organism is simply a unit with high cooperation and very low conflict among its parts”</td>
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<tr>
<td>Term</td>
<td>Individuality (This definitions falls in multiple categories simultaneously)</td>
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<tr>
<td>Definition</td>
<td>“We describe three nested views of individuality, each of which builds on the previous ... The first view defines an individual organism as a living entity in which the fitness interests of its components are aligned such that little or no actual conflict is expressed ... The second view defines an individual organism as a living entity in which the components are interdependent on one another for reproduction, such that fitness is exported from the lower to the higher level, and the whole organism reproduces itself to create a similar entity with heritable fitness ... The third view defines an individual organism as an integrated functional agent, whose components work together in coordinated action analogous to the pieces of a machine, thus demonstrating adaptation at the level of the whole organism.”</td>
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6. Mutual dependence + no conflict

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<tr>
<td>Term</td>
<td>Major transition</td>
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<tr>
<td>Definition</td>
<td>“First, entities that were capable of independent replication before the transition can replicate only as a part of a larger unit after it ... 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) on its own right.”</td>
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<tr>
<td>Term</td>
<td>Individuality</td>
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<tr>
<td>Definition</td>
<td>“By ‘individual’ in this book I mean some stable, physically discrete entity that is composed of interdependent parts acting in a coordinated manner to achieve common goals and is typified by the very property of lacking a high degree of within/individual conflict (e.g. Dawkins 1982, 1990; Queller 1997, 2000). ‘Physically discrete’ here means that the parts of the individual are either physically joined to one another or tend to remain in close proximity”</td>
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7. Integration / Indivisibility

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<td>Term</td>
<td>Superorganism</td>
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<tr>
<td>Definition</td>
<td>“We define a superorganism as a collection of single creatures that together possess the functional organization implicit in the formal definition of organism. Just as genes and organs do not qualify as organisms, the single creatures that make up a superorganism also may not qualify as organisms in the formal sense of the word.”</td>
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<tr>
<td>Term</td>
<td>Evolutionary individuals</td>
</tr>
<tr>
<td>Definition</td>
<td>“Evolutionary individuals are integrated and indivisible wholes with the property of heritable variation in fitness so that they may evolve adaptations at their level of organization.”</td>
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<tbody>
<tr>
<td>Term</td>
<td>Organismality + individuality (combination of both organismality and individuality)</td>
</tr>
<tr>
<td>Definition</td>
<td>“A distinction can be made between organisms and Darwinian individuals. Organisms, in this sense, are metabolic units, which may or may not reproduce. Darwinian individuals are reproducing entities, which may or may not have the metabolic features of organisms. Both are important kinds of “individuals” from a biological point of view. Within mainstream views of reproduction and metabolism, entities such as people and pigeons are examples of both. Viruses, in contrast, are Darwinian individuals without the metabolic features of organisms, and some symbiotic collectives might be organisms without being Darwinian individuals.”</td>
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Text S2. A glimpse of what is known.
Studies spanning the diversity of research fields in biology have greatly advanced our knowledge and improved our ability to approach the above questions for diverse HET. Here, we briefly review some of these key advances before outlining our bottom-up approach.

*Phylogenetic studies* have revealed a wealth of information about HET. They have identified the multiple independent transitions to new levels of biological organization and have revealed the order of organizational changes that characterize some of these transitions (e.g. (16–18)). They have demonstrated that the genetic changes underlying HET are typified by both conservation and innovation (19–23). For example, many genes that regulate multicellular development were already present in the solitary ancestor (i.e. conservation) (20, 22, 24); but multicellular organisms also show a relative enrichment of genes involved in transcriptional regulation, cell adhesion and cell-to-cell communication, often as a consequence of gene or whole-genome duplication (i.e. innovation) (19, 25, 26). Phylogenetic studies have further documented the prevalence of complementary gene loss in symbiotic partnerships, where one partner typically undergoes strong genome reduction (e.g. (27)). Finally, they have also been used to infer the potential ecological factors important for evolutionary changes in organizing complexity (28, 29).

*Empirical studies* have provided key insights into the ecological factors (both biotic and abiotic) important for group formation, the evolution of groups, and the organizing principles underlying group formation. For example, experimental studies have examined both the initiation of groups in response to ecological cues, such as predation (30), and the evolution of groups in lab settings, as a result of ecological selective pressures, such as the selection for more efficient resource consumption, for bigger size, or for better dispersal (31–33). Bonner observed that the abiotic environment also seems to constrain grouping mechanisms (34): “all the aquatic organisms began their multicellularity by the products of cell division failing to separate, while most terrestrial microorganisms involve some form of motile aggregation of cells or nuclei in a multinucleate syncytium” (34). Experimental studies have further uncovered organizing principles in groups from many species (35, 36), such as the division of labor between heterocysts and photosynthetic cells in filamentous cyanobacteria (37), or the folding of cellular bundles underlying colony spread in the bacterium *Bacillus subtilis* (38).

*Theoretical studies* have focused on the evolution and self-organization of groups. Evolutionary models have examined the evolution of within-group cooperation and the shift in the level of selection, from selection within groups to selection between groups. The study of cooperation has identified important assortment mechanisms (39) that facilitate the assortative interaction between cooperative individuals, thereby promoting cooperation and preventing conflict: kin recognition, spatial structure, limited dispersal, reciprocity, vertical transmission, bottlenecks, monogamy, etc. (12, 13, 40, 41). Multilevel selection theory (42–44) has inspired the formulation of individuating mechanisms (45, 46) that inhibit selection within groups and/or promote selection between groups (e.g., single-cell bottleneck, sexual recombination, policing; (47, 48)). Self-organization models have examined the group properties that emerge from the interaction between group members, thereby also uncovering organizing principles of existing groups (35). Studies have shown how differential adhesion results in cell sorting and morphogenesis (49, 50); how reaction-diffusion systems can give rise to pattern formation (51); or how cell differentiation could spontaneously arise in groups of interacting cells (52). Theoretical studies have furthermore structured the discussion of HET by categorizing transitions based on who forms a group and how (53–56): group members can either be similar (fraternal transitions; e.g., multicellularity, animal
sociality) or different (egalitarian transitions; e.g., endosymbiosis, obligatory mutualisms) and they can form a group by either failing to separate after reproduction (also referred to as staying together; e.g., clonal development, subsociality) or by aggregating (also referred to as coming together; e.g., aggregative multicellularity, parasociality).
Text S3. Life cycles: reproduction, development and evolution

In this Perspective the life cycle plays an important role in the evaluation of hierarchical evolutionary transitions (HET) towards new units of biological organization, as we define a group to have the potential to undergo a HET only when it is part of a life cycle. This life cycle perspective is inspired by the seminal work of John Tyler Bonner, one of the first to emphasize the importance of the life cycle in biology (57):

“The view taken here is that the life cycle is the central unit in biology. The notion of the organism is used in this sense, rather than that of an individual at a moment in time, such as the adult at maturity. Evolution then becomes the alternation of life cycles through time; genetics the inheritance mechanism between cycles, and development all the changes in structure that take place during the life cycle” [p.3]. By emphasizing the role of the life cycle, Bonner attempted to (re)unite the fields of evolutionary and developmental biology. Yet, in the midst of remarkable genetic discoveries – such as the DNA (58, 59), the isolation of the first bacterial gene (60) and the first gene sequence (61) – Bonner’s conceptual insights did not resonate in the scientific literature. In the 90s, the importance of the life cycle was revived as part of Developmental Systems Theory (62–64). Paul Griffiths and Russell Gray (63) described the life cycle as follows: “The developmental process is a series of events which initiates new cycles of itself. We conceive of an evolving lineage as a series of cycles of a developmental process, where tokens of the cycle are connected by the fact that one cycle is initiated as a causal consequence of one or more previous cycles, and where small changes are introduced into the characteristic cycle as ancestral cycles initiate descendant cycles” [p. 291]. They continued by saying, "we claim that the individual, from a developmental systems perspective, is a process – the life cycle. It is a series of developmental events which forms an atomic unit of repetition in a lineage. Each life cycle is initiated by a period in which the functional structures characteristic of the lineage must be reconstructed from relatively simple resources” [p. 296].

The description of the life cycle as the ‘atomic unit of repetition in a lineage’ was later criticized by James Griesemer (6), who convincingly argued that this description lacked specificity. As no two instantiations of a life cycle are exactly the same, one has to specify what traits should be repeated and to which extent traits should be similar between different instantiations of the life cycle. Griesemer approached this problem by determining the minimal set of recurrent traits (6): “The evolutionary minimum concept of development is the acquisition of the capacity to reproduce. Being of the same relevant kind means being of the reproducing kind, i.e., having the capacity to reproduce. No particular degree of re-semblance in any particular trait is required in general for reproduction to operate. Of course, realization in offspring of the capacity to reproduce will undoubtedly entail many particular trait resemblances” [p. S360]. Hence, according to Griesemer, “progenogenesis is multiplication with material overlap of mechanisms conferring the capacity to develop” [p. S361]. In other words, across successive instantiations of the life cycle (i.e. generations), at least those components of development should be inherited that are required for the capacity to reproduce. This view of development is largely in agreement with that of Bonner, who stated that “in a very literal sense our concern with development is a concern with reproduction: development is the copy-making process” [p. 14] (65). Since the life cycle goes hand in hand with its developmental underpinnings1, Bonner concluded (65): “It is impossible to have reproduction, in the sense in which we

1 Bonner and Griesemer both adopt a broad definition of development, in which developmental processes are assumed to underlie the entire life cycle and are not confined to any particular life stage. A similar view is adopted in this Perspective (see Table 1), Bonner stated (10): “Since for simple organisms their life cycle is their development, the two stand in close relation to each other” [p. 15]. Griesemer stated (66): “On the account of reproduction I favor, development is not a phase of a life cycle.
have defined it here, without life cycles”; he then continued, “It is equally impossible to have inherited variation without life cycles [...]. Since reproduction and inherited variation are the prerequisites of natural selection, it follows that life cycles are required for selection” [p. 15]. Thus, according to Bonner, the life cycle forms the basic premise for evolution by natural selection. It encapsulates the properties needed for an organism’s potential to evolve. In contrast to Bonner’s notion of the life cycle, Griesemer summarizes his arguments in the concept of the reproducer: “Reproducers are entities that multiply by material overlap of propagules conferring the capacity to develop”. Like Bonner’s notion of the life cycle, Griesemer’s reproducer forms a unit with the potential to undergo evolutionary change by natural selection (inspired by the subset of criteria – multiplication, inheritance and variation – used by John Maynard Smith to define the unit of selection (4, 5, 7); see also Text S1). By focusing on a unit’s potential to evolve, as opposed to its selective advantage, Bonner and Griesemer explore the evolutionary origin of the unit without considering its fitness consequences. In other words, one can examine how a unit gains the capacity to evolve, before studying the selective pressures that favor or oppose its evolution.

Griesemer’s concept of the reproducer emphasizes that two successive instantiations of a life cycle can express considerable differences, since only the capacity to reproduce should be propagated across generations. This variability can make it difficult to demarcate successive instantiations of a life cycle: where does one instantiation of the life cycle end and where does the next one begin? Griesemer acknowledged that the abstract notion of the reproducer is problematic when it comes to demarcating generations (6). Recently, Silvia de Monte and Paul Rainey (9) proposed an alternative approach, in which a unit’s potential to evolve can be studied without demarcating successive generations: “we suggest that evolution by natural selection may occur provided: 1, there are identifiable collectives; 2, they recur; and; 3, there is a genealogical connection between recurrences” [p. 242]. De Monte and Rainey particularly focused on the evolutionary origin of multicellularity (i.e. identifiable collectives), but their arguments can also be applied to the evolution of other phenotypic traits. They argue that by examining trait recurrences along a genealogy, instead of those across generations, there is no need to identify successive instantiations of the life cycle (i.e. parent-offspring relationships). Yet, in a way, recurrent traits already entail a form of parent-offspring relationship and, therefore, give rise to similar questions about reproducibility as those encountered when studying successive instantiations of the life cycle along a genealogy, as done by Griffiths and Gray (63): at which time intervals should a trait recur along the

Note that Griesemer’s concept of the reproducer is distinct from Dawkin’s concept of the replicator (67): whereas replication solely concerns gene copying (i.e. replicators are the genes inside an organism), reproduction entails all developmental processes necessary for acquiring the capacity to reproduce. Although Maynard Smith and Szathmáry did not include the concept of the reproducer in their original publication on the major transitions in evolution (14), they did discuss the role of reproducers in a later publication (68): “(i) it is reproducers, rather than replicators, of a higher level that arose during the transitions; (ii) when a higher level reproducer appears, a novel type of development is worked out; and (iii) rather old-fashioned replicators are packaged into novel reproducers” [p. 569] (see also ‘replicators versus reproducers’ in supporting information of (56)).

*By making fitness secondary to the other properties in his units analysis, Maynard Smith draws attention to the evolutionary problem of the origin of levels of the hierarchy itself: under what conditions will entities evolve that are capable of being units of evolution and/or selection at that level?” [p. 70] (7). Ellen Clarke also emphasizes the importance of studying the origins of HET (69): “This tendency to blackbox questions about the origins of transitions is problematic, because these questions are interesting in their own right, and not as easy to answer as is perhaps assumed. Furthermore, the details of how the origin questions can be and have been solved, during each of the numerous transitions that have taken place in the history of life, surely have implications for the maintenance problem [i.e. the question on how the higher-order organization is maintained, in the light of potential conflict] too.” [p. 306]
genealogy compared to that of other traits and how similar should those traits be at each reoccurrence? Only traits that reoccur sufficiently often with respect to an organism’s life cycle have the potential to evolve by natural selection (i.e. if a trait only reoccurs once in a thousand generations, there is no or little potential for selection to act on this trait).

Thus, the life cycle forms a basic premise for evolution by natural selection. It is defined by the repetitive cycles of recurrent phenotypic properties along the genealogy of an organism. At the minimum, those properties necessary for the capacity to reproduce need to reoccur across successive instantiations of the life cycle (i.e. generations), although in most cases many other properties will reoccur as well. The recurrent properties along the genealogy of an organism can be used to demarcate successive generations, which is necessary for determining the potential of new recurrent properties (e.g. recurrent group formation) to evolve by natural selection (see also (66, 70, 71)).

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4 De Monte and Rainey stated (9): “the appropriate choice for the time of observation of recurrence may be self-evident, and defined by the life cycle. In those instances where there is no obvious cycle, the observation time will be set by the dynamic of particles within and among collectives.” [p. 243]

5 This has also been emphasized recently by Eörs Szathmáry (56): “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 or the accuracy of reproduction rather than replication sensu stricto” [p.10109]
Text S4. Animal sociality

In this section, we illustrate the wider applicability of our framework by briefly examining the origin of animal groups. The evolutionary origin of animal sociality differs from that of multicellularity in multiple aspects. First, animal groups are not characterized by physical attachment. Second, animals have multiple life stages: in the juvenile life stage individuals undergo maturation and in the adult life stage individuals become reproductively active. In many cases, the parents provide a form of parental care to the juveniles during maturation, thus forming a temporary grouping. To account for these differences, we have to specify what constitutes an animal group. We define an animal group to be a collection of closely-interacting adults, which may or may not be sexually-active in the newly-emerged group. A single breeding pair is not considered a group, an assumption implicitly made for multicellular groups as well, where we did not consider the adhesion of two cells prior to syngamy as a type of multicellularity. Finally, juveniles may or may not be present within such groups. This definition excludes social groups made entirely of juveniles, as is the case in subsocial spiders where siblings capture and share prey cooperatively until they reach adulthood, at which point they revert to solitary life (72). This choice is motivated by simplicity of presentation but studying such subsocial groupings is both necessary and an easy extension of our integrative, bottom-up framework.

Although differences exist, the parallels between multicellularity and animal sociality are striking (13, 14, 55, 73, 74). The same two scenarios identified for multicellularity are also likely responsible for triggering the appearance of the first animal group stage within a life cycle: (i) ecology first scenario, in which an ecological change results in the origination of the first group and (ii) mutation first scenario, in which a genetic change results in the origination of the first group. The ecology first scenario could, for example, act via a decline in available nesting space that might lead to an imposed overlap in generations (i.e. adult offspring could stay at the parental nest while scouting for nest locations or while waiting to inherit the nest from their parents, as is the case in some cooperatively breeding birds; see (75)). The mutation first scenario has been proposed to explain the evolution of eusociality in ants: some mutant daughters might have reduced flying ability and therefore be forced to stay at the parental nest after they reach sexual maturity (76). This scenario, however, is harder to evaluate in animal groups since manipulative lab experiments (e.g., knocking off genes to observe behavior) are less feasible. Nevertheless, there is increasing support for the existence of social genes (77) and recent work has opened the possibility of creating mutagenic insects that might allow direct testing for such genes (78). Whether the ecology triggers the first groups or it simply permits their persistence, it is undeniable that it plays a crucial role in the origin of animal groups. A compelling example comes from the Halictidae, which can be solitary, intermediately social or eusocial depending on elevation (79). The Halictidae also reveal important interactions between ecology and development (80).

Figure S3 shows the potential life cycles that could emerge at the origin of the very first animal groups. As for the origin of multicellularity, Figure S3 separates life cycles according to (i) the presence/absence of the solitary life stage, (ii) the mechanisms underlying group formation, and (iii) the life stage at which reproduction occurs (necessary to support the propagation of the life cycle). In addition to these criteria, for animal groups we also need to specify (iv) the existence of overlapping generations, as juveniles can stay with the group or leave before maturity. This additional criterion leads to two additional life cycles, not present among the multicellular motifs: individuals could come together and reproduce inside the group; subsequently, the juvenile offspring could leave the group before maturity and form a new group upon maturation. These life cycles were not present in the case of multicellularity since there is no distinction between juvenile and adult cells and, therefore, reproduction inside the group automatically
implies a form of staying together. The same two grouping mechanisms can be found in animals as well: individuals can either stay together (ST) when offspring fail to leave the parental nest after maturation (e.g., ants, termites), or they can come together (CT) by means of aggregation (e.g., bark beetles, starling flocks). Much like in the case of multicellularity, the ecology can constrain the grouping mechanism: for example, when large groups need to form relatively quickly – e.g., to escape predators, fight competitors, or overcome prey defenses – CT is the only viable option (81).

In Other HET in the main text we highlight another striking parallel between the HET to multicellularity and the HET to animal sociality. Much as in the case of multicellularity, the life cycle of the solitary ancestor is also of critical importance for the HET to animal sociality. We illustrate this by focusing on the Polistes wasps (Fig. 2), in which the bivoltine life cycle of the solitary ancestor forms a stepping-stone to cast differentiation in eusociality. In the case of eusociality, ancestral properties are often referred to as preadaptations (82–85), which emphasizes their role in facilitating the transition to eusociality (e.g. (85)). Preadaptations are also discussed in the literature on the HET to multicellularity (86–90), but seem to play a less central role in the overall approach. This might in part be explained by our more incomplete understanding of how unicellular organisms function in nature (e.g., what are the ecological conditions they face and how do they respond to these conditions?), compared to the analogous understanding for solitary animals, which is more readily available (e.g., studies on life history traits, behavior, physiology, habitat usage, ecology, etc.). In general, the comparisons above between the HET to animal sociality and the HET to multicellularity show the importance of comparing different types of transitions. By necessity, studies on different HET often have to employ different methods, which can lead to non-overlapping insights; by combining these insights we can improve our general understanding of HET.
Figure S2. The origination of life cycles with a group life phase and emergent group properties that could be selected for. Left: two alternative scenarios that lead to the origination of the first group life cycles: the ecology first scenario and the mutation first scenario. Right: possible properties that can be selected after the formation of the first life cycles with a group life phase. Group properties are divided between those involved in group formation, group features and propagation.
**Figure S3.** Potential animal life cycles that could emerge upon the origin of the first animal groups (figure shows possible life cycles, irrespective of their likelihood of emerging). The life cycle motifs give a simple and schematic representation of the transitions that could occur within the first life cycles. S = solitary life stage (black); CT = group life stage formed by individuals coming together (red); ST = group life stage formed by individuals staying together (blue); CT/ST = group life stage in which individuals both come together and stay together (e.g. animal group with overlapping generations that allows for immigrants). Arrows show reproduction of the (i) solitary life stage, (ii) transitions from solitary life stage to the group life stage and vice versa and (iii) potential fragmentation of group (dotted line). Right-hand column provides examples of species that have a life cycle comparable to the schematic life cycle motifs (75, 91–96).
References


