PERSPECTIVE

On the origin of biological construction, with a focus on multicellularity

Jordi van Gestel[a,b,c,d] and Corina E. Tarnita[e,1]

Edited by Gene E. Robinson, University of Illinois at Urbana–Champaign, Urbana, IL, and approved September 1, 2017 (received for review April 9, 2017)

Biology is marked by a hierarchical organization: all life consists of cells; in some cases, these cells assemble into groups, such as endosymbionts or multicellular organisms; in turn, multicellular organisms sometimes assemble into yet other groups, such as primate societies or ant colonies. The construction of new organizational layers results from hierarchical evolutionary transitions, in which biological units (e.g., cells) form groups that evolve into new units of biological organization (e.g., multicellular organisms). Despite considerable advances, there is no bottom-up, dynamical account of how, starting from the solitary ancestor, the first groups originate and subsequently evolve the organizing principles that qualify them as new units. Guided by six central questions, we propose an integrative bottom-up approach for studying the dynamics underlying hierarchical evolutionary transitions, which builds on and synthesizes existing knowledge. This approach highlights the crucial role of the ecology and development of the solitary ancestor in the emergence and subsequent evolution of groups, and it stresses the paramount importance of the life cycle: only by evaluating groups in the context of their life cycle can we unravel the evolutionary trajectory of hierarchical transitions. These insights also provide a starting point for understanding the types of subsequent organizational complexity. The central research questions outlined here naturally link existing research programs on biological construction (e.g., on cooperation, multilevel selection, self-organization, and development) and thereby help integrate knowledge stemming from diverse fields of biology.

major evolutionary transitions | hierarchical evolutionary transitions | bottom-up approach | life cycle | animal sociality

From a primordial soup of elements to the emergence of protocells, from single cells to multicellular organisms, and from multicellular organisms to animal groups, evolution has been punctuated by hierarchical evolutionary transitions (HET), whereby simple units assembled into groups that themselves became new units of biological organization (1–4). The popularization of these HET [also known as transitions in individuality (2, 5)] as part of the "major transitions in evolution" by Maynard Smith and Szathmáry (3), resulted in extensive research efforts—both empirical and theoretical—to understand how new units of biological organization can evolve. However, this endeavor has proved challenging, not least because a unique definition for what constitutes a unit of biological organization has eluded the field; instead, the literature abounds with definitions that differ in the minimal criteria for a group to be considered a unit of biological organization (*SI Appendix, Text S1*, Fig. S1, and Table S1). There seem to be only two points of general agreement: (*i*) a necessary criterion, common to all definitions, for a group to be a unit of biological organization is that the group must be a unit of selection (i.e., it can undergo evolutionary change by natural selection) (*SI Appendix, Text S1*); and (*ii*) there are certain entities that are unambiguously units of biological organization (e.g., animals, plants, eusocial colonies). This has engendered a "top-down" approach for the study of HET that starts with such paradigmatic examples of biological units, identifies their properties (e.g., high level of cooperation, reduced conflict, differentiated types, metabolic specialization) (*SI Appendix, Text S1*), and explores how a group could have evolved each of these properties. While this approach has revealed a wealth of valuable insights, we argue that it is insufficient to understand the origin and evolution of HET.

This type of top-down approach to the study of HET runs into two critical problems. First, by focusing on properties of groups that qualify as paradigmatic

[a]Department of Evolutionary Biology and Environmental Studies, University of Zürich, 8057 Zürich, Switzerland; [b]Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland; [c]Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland; [d]Department of Environmental Microbiology, Swiss Federal Institute of Aquatic Science and Technology (EAWAG), 8600 Dübendorf, Switzerland; and [e]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544

Author contributions: J.v.G. and C.E.T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: ctarnita@princeton.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1704631114/-/DCSupplemental.

examples of biological units, studies largely ignore the ancestor, including its internal organization and properties, the ecological context, and the mechanisms that gave rise to the primitive instantiations of those groups (6–8). As a consequence, it often remains unclear how the organization of the group—including the properties of interest—originated from that of the ancestor, making it impossible to fully unravel the evolutionary trajectory from the solitary ancestor to a new unit of biological organization (9–12): Which organizing principles and properties (e.g., differentiation, conflict suppression, metabolic specialization, cooperation) evolved de novo and which appeared as by-products due to strong interdependencies? What was the order in which organizing principles evolved? How did the organization at one point in time constrain or potentiate the evolution of new organizing principles? What is the relative importance of various factors (e.g., ecological context, conflict avoidance, development/physiology/life history traits) for the evolution of new organizing principles? What types of organizing complexity can emerge from different ancestral properties and evolutionary trajectories?

Second, in addition to ignoring the ancestral properties, by fixating on certain properties common to the known paradigmatic examples of HET, the top-down approach fails to explore the full potential of evolutionary trajectories and transitions, not only the paradigmatic but also the peripheral, and not only the actual (i.e., realized) but also the possible (13). This likely paints an incomplete picture of HET and precludes a valuable comparison across potential evolutionary transitions: only by comparing their full spectrum can we determine the causal factors that explain why certain trajectories did result in new units of biological organization and others did not (14).

Here we identify six questions, Q1–Q6, that, regardless of the definition for what constitutes a new unit of biological organization, need to be addressed in a bottom-up approach to the study of HET:

Q1: When/how does a group originate that has the potential to undergo a HET?

Q2: What emergent properties do these groups have? (For example, in the case of multicellular groups: group size, composition, shape, and the interactions of cells inside the group, including cooperative interactions.)

Q3: How does selection act on these properties?

Q4: How does this affect the ancestral developmental program(s) and change group properties? Selection is only effective when group properties emerge from a heritable developmental program. In the case of newly formed groups, the developmental program is that of the solitary ancestor(s) that make up the group. Selection will therefore exert its effect by affecting the ancestral developmental program(s).

Q5: When/how does this lead to novel organizing/developmental principles within the new unit? (For example, in the case of multicellular groups: differential adhesion, pattern formation and cell signaling.)

Q6: What kinds of organizing complexity can evolve?

These questions separate the origination of the first group and group properties (Q1–Q2) from the selective pressures that underlie the conservation and further evolution of the group (Q3–Q6). This conceptual distinction helps disentangle the causal factors underlying HET; yet, importantly, it does not imply that these processes occur sequentially, since groups can have an instantaneous selective benefit upon their origination. Guided by these six questions, in this Perspective we propose a bottom-up approach to study the dynamics underlying HET, which builds on and integrates knowledge from existing research programs on biological construction: phylogenetic (12, 15–18), empirical (e.g., experimental evolution, developmental biology, sociobiology) (10, 19–22), and theoretical (e.g., on multilevel-selection, cooperation, self-organization) (4, 14, 23–29) (see also *SI Appendix, Text S2*). We illustrate this approach by focusing on the transition to multicellularity, but we showcase its wide applicability by briefly discussing the evolution of animal sociality in *Other HET*, below, and *SI Appendix, Text S4*.

Bottom-Up Approach

Through his work on multicellularity, John T. Bonner was one of the first to study evolutionary transitions in biological organization (1, 30). Bonner focused in particular on the role of the life cycle in the HET to multicellularity (30). He argued that the life cycle encapsulates all properties needed for the potential to evolve by natural selection (1) (i.e., reproduction and heritable variation) and considered the life cycle, and not the organism, to be the unit of biology (30) (*SI Appendix, Text S3*). With this view, biological entities (including groups) have the potential to be a unit of selection if and only if they are part of a life cycle. For example, if a cell acquires a mutation that makes it stick to its daughters after division (e.g., ref. 31), a group life cycle arises, in which cells form clumps that occasionally might break and give rise to new clumps. Over evolutionary time, these clumps could evolve new properties. Groups could also arise as part of the ancestral life cycle. In fact, an increasing number of studies show that groups are often expressed as facultative life stages—triggered by specific environmental conditions—in life cycles of otherwise solitary organisms (32). For example, *Chlamydomonas reinhardtii*, a close relative (i.e., sharing a recent common ancestor) of the multicellular volvocine green algae (33, 34), lives as a unicellular organism, but can induce stickiness and form groups in response to its natural predator *Peranema trichophorum* (35). Similarly, *Capsaspora owczarzaki*, a close relative of the metazoans, can form facultative aggregates in response to environmental stress (36). Even in endosymbioses, facultative associations between the symbiotic partners are hypothesized to have preceded obligate relationships (37).

Following these arguments, henceforth we will define a group as having the potential to undergo a HET (i.e., the potential to be a unit of selection) only when it is part of a life cycle, either as a reproducible life stage in the life cycle of the solitary precursor or as part of a life cycle in which the solitary life stage is effectively absent (i.e., groups that propagate by fragmentation) (see also refs. 38 and 39 and *SI Appendix, Text S1*). The reproducibility requirement pertains strictly to the act of group formation; for all other group properties, such as composition, size, or shape, we allow for potentially low or no reproducibility (for the purpose of this Perspective we distinguish between heritable material and reproducible properties; see Table 1). Therefore, according to this definition, one cannot establish a group's potential to undergo a HET by examining its properties at a given moment in time; instead, one has to trace the group and its descendants over time to determine the reproducibility of group formation. Furthermore, we do not require the group to be formed in every successive instantiation of the life cycle (henceforth generation), only that it is formed sufficiently frequently for selection to potentially act on the group stage. For example, a group could be expressed as a facultative life stage only in response to certain recurrent environmental conditions, as in the examples above.

To determine if groups are part of a life cycle, one needs to determine what constitutes the life cycle (*SI Appendix, Text S3*).

This might seem a trivial task when thinking of the paradigmatic examples of biological organization (animals and plants), but it can be surprisingly difficult in general. Soil-dwelling unicellular organisms are a case in point: in the absence of information about their environment, the life cycle of single cells could be described by their division cycle; but many soil organisms are exposed to fluctuating environmental conditions, such as feast–famine cycles, where short periods of food availability are alternated with long periods of starvation. One could therefore argue that the feast-and-famine cycle, not the division cycle, determines the life cycle of these unicellular organisms. Thus, the feedback between the ecological context (biotic and abiotic interactions; also referred to below as the ecology) and development gives rise to the recurrent trait appearances that characterize the life cycle. Consequently, one can only evaluate life cycles accurately in the appropriate ecological context.

(Q1) Origination of a Group with the Potential to Undergo a HET. Starting from the above definition of what constitutes a group with the potential to undergo a HET, we can examine the conditions necessary for its origination: first, something should trigger group formation; second, group formation should be reproducible across generations, either as an obligatory or as a facultative life stage. We discriminate between two scenarios that could trigger the appearance of the first group stage within a life cycle (*SI Appendix*, Fig. S2): (*i*) the ecology-first scenario, in which an ecological change results in the origination of the first group; and (*ii*) the mutation-first scenario, in which a genetic change results in the origination of the first group. Both scenarios pertain only to the mechanism that underlies the origination of the first groups, not to the selection pressures that might favor or oppose such groups.

In the ecology-first scenario, an ecological change (either biotic or abiotic) acts on preexisting cellular properties to lead to the formation of a group (19, 40). This can happen in many ways. For example, cells might be exposed to an atypical ecological condition that results in the overexpression (via regulatory induction) of a set of proteins. Many proteins carry promiscuous functions (41), such as weak adhesive properties [e.g., proteins involved in phagocytosis (16, 42)]; the overexpression of such proteins could lead to enhanced adhesion that would enable cell-to-cell attachment resulting in group formation. Thus, in this scenario, an ecological change is responsible for triggering group formation by acting on the preexisting plastic response of the solitary ancestor. Crucially, the ecological change should persist or reoccur sufficiently often to support the reproducibility of group formation across generations. It is important to note that here the role of ecology is distinct from the one typically considered in studies on HET: while most studies only consider the ecology when it comes to the selection pressures that favor group formation (e.g., ecological benefits) (see ref. 43), we emphasize that the ecology can also play a critical role in triggering and supporting the origination of the first group life cycles. We also consider the selective (dis)advantages of group formation, but we do so later, in Q3. As noted above, this conceptual separation is not meant to imply that the selective benefits only arise after the origination of the group, since groups can carry instantaneous benefits upon their origination; rather, it is done with the explicit purpose of highlighting the largely ignored, nonselective role that the ecology can play in group origination.

In the mutation-first scenario, a genetic change triggers group formation in a preexisting ecological context. This can also occur in many ways. For example, a genetic mutation could block the expression of an enzyme necessary for hydrolyzing the cell wall at the end of cytokinesis [e.g., a mutation in *CTS1*, a gene encoding for a chitinase that mediates cell separation in *Saccharomyces cerevisiae* (44)]. Then cells would remain attached after cell division and give rise to cell clumps. These clumps could grow and fragment under mechanical stress, thereby giving rise to a group life cycle (31). If the mutation is conditional on the environmental context, in that it only blocks the expression of the hydrolyzing enzyme under certain conditions [e.g., the conditional repression of autolysins in *Bacillus subtilis* (45)], environmental fluctuations might support a life cycle that alternates between a solitary life stage and a group life stage. Thus, although in this scenario ecological changes are not the primary cause for the origination of group formation, they can still play an important role in the emergent group life cycle and the reproducibility of the group stage.

Fig. 1 gives an overview of the life-cycle motifs that could emerge upon origination of a group life stage (triggered by either ecological or genetic changes). These motifs represent the simplest possible life cycles (which could be part of more complex ones; see ref. 46) and they are categorized based on a few criteria (compare with figure S2 in ref. 4): (*i*) the presence/absence of the solitary life stage, (*ii*) the mechanism by which groups are formed, and (*iii*) the life stage at which cell division occurs (necessary to support the propagation of the life cycle). These criteria can be further extended to specify, for example, whether the group life stage is obligatory or facultatively expressed; how transitions between life stages take place (e.g., dispersal, sexual reproduction); or whether the solitary and group life stages coexist in time and space [e.g., when grouping is triggered by a change in ecological

Table 1. Definitions as used in this Perspective

Term	Definition
Unit of biological organization	Multiple definitions (see *SI Appendix, Text S1*, Fig. S1 and Table S1).
Life cycle	The cycle of phenotypic properties that reoccurs every generation [not all properties need to reoccur (see *SI Appendix, Text S3*)].
Group with potential to undergo a HET	Group that is part of a life cycle, such that the act of group formation is reproducible across subsequent instantiations of the life cycle.
Development	The intrinsic processes underlying an organism´s temporal and spatial organization. (Not confined to a particular life stage; encapsulates all processes underlying an organism's life cycle, including solitary and potential group life stages).
Ecology	Biotic (e.g., competitors, predators) and abiotic environment (e.g., temperature, nutrient availability).
Emergent properties	Higher-level (e.g., group) properties that result from interactions between lower-level components (e.g., group members).
Heritable material	Material transmitted from parent to offspring as a direct continuation (e.g., DNA, developmental program).
Reproducible properties	Properties reconstructed in subsequent generations, as the product of the inherited material and the ecology.
Cooperation	Expression of a costly phenotype that is beneficial to others (e.g., public-good production).
Conflict	Expression of a beneficial phenotype that is costly to others (e.g., toxin production, social free-riding, parasitism, competition).

conditions, some cells might remain solitary; see *Dictyostelium discoideum* (47)].

Consistent with Bonner (48), we discriminate between two grouping mechanisms (see also *SI Appendix, Text S2*): cells can either stay together (ST) due to incomplete cell separation after cell division (i.e., clonal development), or they can come together (CT) by means of aggregation (i.e., aggregative development) (4, 26, 49, 50). ST can take many forms: for example, cells could have incomplete cytokinesis, in which the cell walls at the division plane remain fused (44); a daughter cell could be engulfed by the mother cell during cell division (51); coenocytic filamentous cells could cellularize through septa formation (52); cells could undergo complete cell division, but remain attached due to adhesive molecules (53); and so forth. Similarly, CT can also take many forms: for example, cells can aggregate via chemotaxis (54), by binding a common surface (55), or by binding each other (35). Most forms of aggregation are mediated by soluble or membrane-bound adhesive molecules, such as extracellular polysaccharides, protein fibers, and adhesion receptors. ST and CT mechanisms can also be combined: for example, cells (clonal or mixed) could aggregate on a surface to form a group and subsequently undergo cell division without cell separation (22, 55).

Cells in groups formed via ST are necessarily "similar" (since they are clonal), while those in groups formed via CT can be similar (e.g., same or related genotypes) or "different" (e.g., different species). HET, in which group members are similar, are referred to as fraternal transitions, while those in which group members are different are referred to as egalitarian transitions (26). The bottom-up approach we outline can be employed to study both cases but, for simplicity of exposition, below we will focus on the fraternal case; thus, in the case of CT, the aggregating cells will be either clonal or at most of different genotypes of the same species. Bonner (48) pointed out that all aquatic origins of multicellularity arose via ST, while most terrestrial origins arose via CT. This shows that the physics of the environment—for example, a relative lack of surfaces that could support aggregative multicellularity in aquatic systems—can constrain the possible grouping mechanisms, reemphasizing the diverse and critical roles of ecology in the origination of groups.

(Q2) Emergent Group Properties. The origination of a group with the potential to undergo a HET leads to the spontaneous emergence of group properties that fall into three categories (*SI Appendix*, Fig. S2).

Group formation. Multiple properties characterize group formation, such as the rate at which a group forms, its timing relative to other events (e.g., the environmental fluctuations involved in triggering group formation), its location in physical space, or its efficiency. For example, a group that is triggered in response to starvation could form more or less quickly depending on the plastic response of individual cells to starvation (which could be different due to both phenotypic and genotypic variability); it could form in the same place where the cells starved, or elsewhere if cells first migrate to more appropriate conditions; and it could form more or less efficiently in terms of its inherent cohesion, depending on the level of adhesiveness of each cell.

Group features. Group features emerge from the interactions between member cells and depend on cell properties. There can be many emergent group features, but here we briefly focus on group size, group composition, within-group interactions, and group shape. Group size is determined by the strength with which cells adhere to each other: stronger adhesion results in less fragmentation and hence bigger groups (56). Group composition is determined by members that make up the group, which could

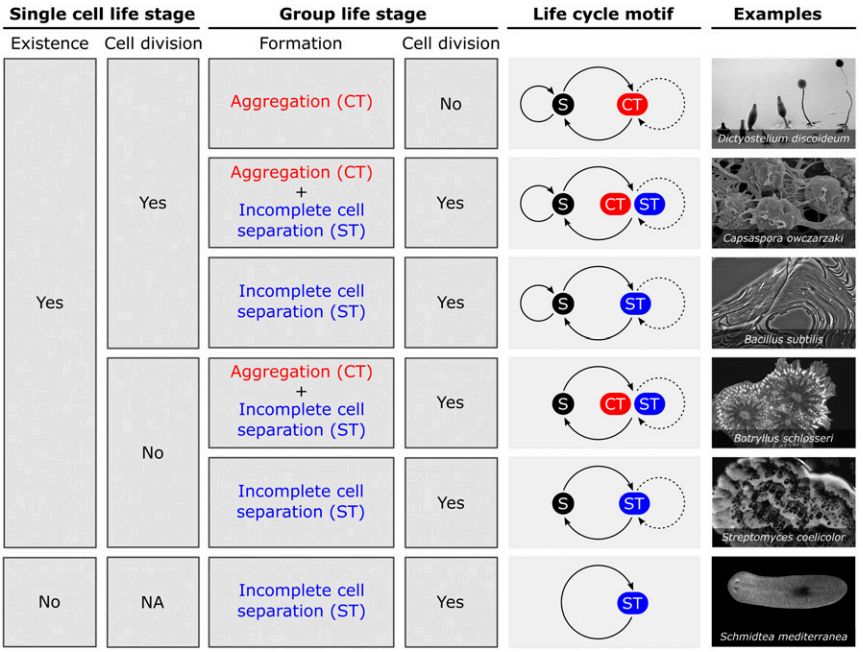

Fig. 1. Potential multicellular life cycles that could emerge upon the formation of the first multicellular groups. Categorization based on (*i*) existence of single cell (S), (*ii*) mechanism of group formation (CT/ST), and (*iii*) life stage where cell division occurs. Two life cycles have a group life stage formed by both CT and ST; here, aggregated cells divide inside the group. Arrows indicate cell division in solitary life stage, transition between solitary and group life stages, and potential fragmentation of the group (dotted line). Images show examples of species with a life cycle comparable to each life cycle motif. (*Top* to *Bottom*) *D. discoideum*, image courtesy of MJ Grimson and RL Blanton (17, 90), *C. owczarzaki*, image adapted from ref. 36, *B. subtilis* (107, 108), *Botryllus schlosseri*, reprinted from ref. 109 with permission from Elsevier, *Streptomyces coelicolor*, image courtesy of VM Zacharia and MF Traxler (52, 107), *Schmidtea mediterranea* asexual biotype CIW4, image adapted from ref. 110.

be clonal or nonclonal (different genotypes). Within the group, spontaneous interactions could emerge between cells. For example, in groups consisting of multiple genotypes, cells might spontaneously engage in antagonistic interactions via the production of toxins, but they might also engage in metabolic interactions, such as cross-feeding, whereby they exchange metabolites that improve growth (57). Such mutualistic interactions could further influence the organization of the group by promoting genotypic intermixing (58). In clonal groups (i.e., consisting of a single genotype), cells could spontaneously engage in a variety of interactions as well (25), some of which could be cooperative (31). Clonal groups could also spontaneously express phenotypic heterogeneity [e.g., via cell responses to local environmental gradients (see ref. 59)]. This capacity of cells to express phenotypic differences inside the group is in most cases already latently present in the ancestor (60). Solitary cells face a multitude of ecological challenges, which they overcome by adjusting their phenotype: for example, cells can express different metabolic pathways in response to the available resources, become motile in search for food, or induce dormancy to survive stress. The phenotypic states that the ancestor expresses in time can become expressed in space when cells form a group (61). Thus, the plasticity of the ancestor in response to its environment will likely influence the propensity of cells to vary inside the group. This phenotypic variability could even result in pattern formation if cells respond to each other through extracellular signals (62). Finally, group shape can also be affected by member cells. Models and experiments have shown that when cells differ in their adhesive properties, simple morphogenic processes could emerge (e.g., cell sorting, engulfment, folding) that can influence group shape (27, 63, 64). Differential adhesion is relevant to both clonal and nonclonal groups.

Propagation. As part of a life cycle, groups need to propagate (*SI Appendix, Text S3*) to prevent the life cycle from ending with the group stage. Propagation can take many forms: groups might release single cells, they might shed fragments or fission, or they might dissolve altogether. The mode and rate of propagule production depend on the viscoelastic properties of the group as well as on the environmental conditions (65). For example, when groups are exposed to stronger shear stresses, they are expected to shed more propagules. The processes of propagule production and group formation are antagonistic (21, 66, 67): whereas the latter requires the attachment of cells, the former relies on their separation. This was experimentally illustrated in *Vibrio cholerae* (68): constitutive production of extracellular matrix enhanced group formation and growth due to cells firmly sticking together, but dramatically reduced propagule production. The trade-off between group formation and propagule production is just one of the many possible interdependencies that might characterize the first groups.

(Q3 and Q4) Selection and New Emergent Properties. Selection could act on any of the emergent group properties and, due to interdependencies, indirectly affect others. For example, when there is selection for bigger group sizes, cells that produce more adhesive molecules might be favored, which strengthens their cohesion (56). This increased adhesiveness is likely to affect the group composition as well: for example, cells might start to sort based on their adhesive properties (69) or they might bind to nonadhesive cells in the environment. Increased adhesiveness can also change the group shape: for instance, adhesive molecules might alter the growth dynamics of the group (70) or change its viscoelastic properties (71), thereby changing the group response to external mechanical forces (e.g., shear stress). Finally, as mentioned above, increased adhesiveness can also influence propagule production: for example, adhesive molecules might decrease the rate of propagule production (68) and increase propagule size (65). Thus, selection for one property—group size—is likely to have consequences for many other group properties as well, some of which could be deleterious (e.g., reduced propagule production). Such interdependencies make it difficult to discriminate a posteriori between properties that were favored by selection and those that emerged as side-effects. For instance, in the study of HET, it is often claimed that the single-cell bottleneck evolved because it results in strict genetic homogeneity and, thereby, prevents conflict. However, the single-cell bottleneck might just as well be conserved because it can promote reproduction (72), improve dispersal (20, 21), support reliable development (73), or because it is simply associated with one of the ancestral life stages (e.g., syngamy) (74); this would lead to strict genetic homogeneity as an inevitable side-effect, even when it is not strictly required to prevent within-group conflict (75).

If trade-offs between group properties are deleterious to the life cycle, such as the one between group formation and propagule production, selection could favor mutations that overcome these trade-offs. This was demonstrated experimentally in *Pseudomonas aeruginosa* (21) by exposing it to a life-cycle regime in which cells had to alternate between two life stages: one in which group formation (i.e., adhesive cells) was favored and one in which propagule production (i.e., nonadhesive cells) was favored. Under this selection regime, cells evolved a surprising molecular trick to overcome the trade-off between group formation and propagule production. They increased mutation rates that—via frameshift mutations in a specific genomic region—facilitated the alternation between adhesive and nonadhesive phenotypic states. Consequently, groups always produced nonadhesive propagules, while a fraction of the propagules always reverted to group formation. Another solution to overcome this group formation–propagule production trade-off is regulation, as is the case in many strains of *V. cholerae*. These strains regulate matrix production based on nutrient availability (76): cells stimulate matrix production and stick together in good conditions, but inhibit matrix production and secrete enzymes that digest the remaining matrix to allow dispersal when conditions deteriorate.

The properties of the first groups are not only expected to be interdependent, but also to vary considerably across generations (4, 63, 77). In the relative absence of developmental control, groups are likely to be sensitive to small environmental perturbations. For example, a small change in the shear stress could affect the group size, group shape, and rate of propagule production. An important selective target might therefore be the reproducibility of group properties (4): selection in favor of developmental mechanisms that improve the reproducibility of beneficial group properties across generations (77). Selection for reproducibility is, in effect, selection for developmental control, since reproducible properties can evolve only to the extent that group formation is under the control of a heritable developmental program (78), whether it be encoded by a single or multiple genomes. Importantly, our bottom-up approach emphasizes that reproducibility of group properties can evolve after the origin of group formation, which only requires the act of group formation, and not the group properties, to be reproducible across generations (see discussion of Q1). Beneficial properties that might first be triggered by specific ecological conditions (i.e., facultatively expressed), can—via the evolution of new developmental mechanisms—become part of the developmental program, and therefore be expressed under a much wider range of conditions (i.e., genetic assimilation) (79, 80). For example, selection might favor groups that produce stress-resistant propagules. Initially, these might only be produced under starvation, which triggers sporulation as part of the ancestral developmental program.

However, additional mutations might allow for quorum-sensing signaling (81), which could facilitate sporulation to also be triggered by high cell densities (i.e., bigger groups), even in the relative absence of starvation signals [in some colony-forming bacteria sporulation indeed depends on quorum-sensing signals (for example, see refs. 82 and 83)]. In the end, any developmental mechanism that facilitates the robust expression of a beneficial group property, over a large range of ecological conditions, is a mechanism that improves reproducibility via a form of developmental canalization.

Developmental mechanisms that promote reproducibility can also evolve in the presence of genetic diversity. For example, in the case of symbiosis, a group property (e.g., cross-feeding) might rely on the presence of two symbiotic partners, but might be difficult to reproduce if these partners dissociate after group formation and cannot re-establish a new group. Developmental mechanisms that prevent genotypes from dissociating (e.g., mechanisms that promote vertical transmission of the symbiotic partners) or promote their reestablishing a new group (e.g., partner-choice mechanisms) could improve the reproducibility of group properties (37). There might also be selection against the association of some genotypes. For example, if cooperation gives rise to a group property, noncooperative genotypes could reduce the fidelity with which this property is propagated across generations (e.g., noncooperating cells could undermine the development of the group property by exploiting cooperating cells). In that case, selection might favor developmental mechanisms that prevent noncooperative cells from joining the group [e.g., assortment mechanisms, such as kin discrimination and bottlenecks (84)]. The extent to which within-group conflict leads to reproducibility issues depends on the grouping mechanism and the ecological context in which groups are formed (28, 50). For example, groups formed by ST are less prone to internal conflict than those formed by CT (49), because in ST conflicts can only arise through mutations. Importantly, even if noncooperative cells might occasionally join a CT group, strong spatial assortment, if present in the environment, could still prevent those cells from parasitizing other groups, and therefore from reducing the reproducibility of group properties in the population.

Since within-group conflict is just one of many factors that could reduce the reproducibility of group properties, a lack of conflict does not guarantee accurate reproducibility of group properties. Conversely, the presence of within-group conflict does not automatically reduce reproducibility either, since there might be mechanisms that suppress within-group selection; for example, it could be physically impossible for the noncooperative cells to spread within the group, as is the case for cancerous tissues in plants (85). Thus, mechanisms that prevent within-group conflict (i.e., assortment mechanisms) and inhibit within-group selection [i.e., individuating mechanisms (see *SI Appendix, Text S2*) (86)] are merely a subset of the many developmental mechanisms that could influence the reproducibility of group properties.

(Q5 and Q6) New Organizing Principles and Organizing Complexity. New organizing principles are those that underlie the organization of a group but that were not present in the ancestor. In the previous section we already alluded to some of these principles (e.g., quorum-sensing signaling). There are many organizing principles, which act at different spatial scales, ranging from the organization of single cells to that of organs. Some of these organizing principles are shared across a wide-range of multicellular organisms: for example, cell differentiation, cell-to-cell communication, pattern formation, lateral inhibition, induction, determination, regional differentiation, differential adhesion, segmentation, germ–soma differentiation, boundary formation, and tissue formation (10, 27, 64, 87). However, not all of these organizing principles are unique to multicellular groups: for example, in some cases, the solitary ancestor might already express cell differentiation or communication. Only when organizing principles evolved after the origin of the first groups do we consider them to be new organizing principles of the group.

We have relatively little understanding of the origin of most organizing principles (e.g., germ–soma differentiation, tissue formation, pattern formation). However, there is accumulating evidence for the important role of the ancestor in the evolution of new organizing principles (12). For example, the aquatic and colonial green alga *Volvox carteri* exhibits germ–soma differentiation, with biflagellated somatic cells at the periphery of the spherical colony and dividing germ cells in the interior (Fig. 2) (34). Differentiation of somatic cells is regulated by RegA, a protein that suppresses photosynthesis and thereby prevents division (88). Interestingly, phylogenetic studies revealed that a close homolog of RegA is involved in photoacclimation, a plastic response that can be triggered

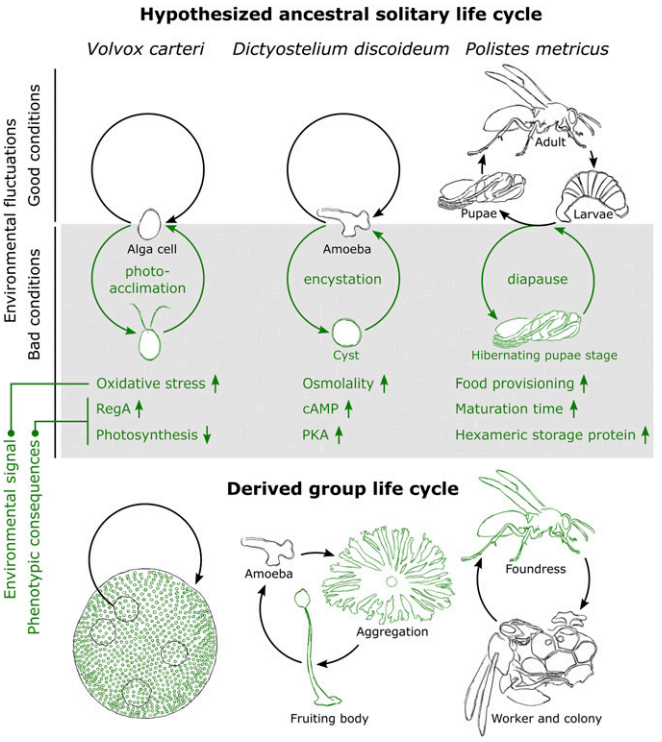

Fig. 2. Relationship between life stages in hypothesized life cycles of solitary ancestors and group formation in derived group life cycles. (*Upper*) Simplified depiction of hypothesized ancestral solitary life cycles of *V. carteri* (33, 88, 89), *D. discoideum* (90), and *Polistes metricus* (103–105). Life cycles here consist of a life stage expressed under good conditions (black) and a life stage expressed under adverse conditions (green). For the latter life stage, we show an environmental signal that might trigger it and some phenotypic consequences. For *P. metricus*, high food provisioning at the end of the breeding season is hypothesized to be a cue for the upcoming winter season. (*Lower*) Simplified depiction of group life cycles of: *V. carteri*, corresponding to fifth life cycle in Fig. 1 (ST group and nondividing unicellular life stage; zygote, not shown); *D. discoideum*, corresponding to first life cycle in Fig. 1 (CT group and dividing unicellular life stage); and *P. metricus*, corresponding to seventh life cycle in *SI Appendix*, Fig. S3 (ST group and nonreproducing solitary life stage). Developmental program underlying life stages in solitary ancestor is co-opted for group formation (shown in green): differentiation of somatic cells (*V. carteri*), fruiting body formation (*D. discoideum*), and appearance of foundress phenotype (*P. metricus*).

by light deprivation (39, 89). In the unicellular ancestor, photoacclimation was likely required for cells to adjust to the diurnal light cycle: inhibiting photosynthesis during light limitation prevents oxidative stress. Thus, the regulatory protein involved in a switch between life stages in the solitary ancestor was co-opted for germ–soma differentiation in its multicellular descendant (Fig. 2). An even more striking case of co-option is found in the phagocytic and soil-dwelling amoeba *Dictyostelium discoideum* (90). This social amoeba is exposed to feast–famine cycles resulting from fluctuating resource levels in the soil. Upon starvation, cells aggregate into fruiting bodies that mediate spore dispersal. Cell aggregation, fruiting body formation, and sporulation depend on cAMP, which exerts its effect by activating cAMP receptors and the cAMP-dependent protein kinase (PKA) (54, 90). Interestingly, in species of solitary amoebae, encystation—which can be triggered by osmotic stress (e.g., due to soil dehydration)—also relies on cAMP-mediated activation of PKA (91). The disruption of cAMP receptors in the social amoeba *Polysphondylium pallidum*—a relative of *D. discoideum*—results in malformed fruiting bodies that are filled with cysts instead of spores (92). *P. pallidum* normally only forms cysts in the unicellular life stage (by comparison, *D. discoideum* never forms cysts). Supported by phylogenetic studies, these results indicate that the developmental program underlying fruiting body formation is derived from the encystation program (Fig. 2). In fact, one could argue that cysts in solitary amoebae and fruiting bodies in *D. discoideum* are distant homologies, in much the same way as fins and arms are homologies (93): they are different functional realizations of a (partly) conserved developmental program.

This mounting evidence for the importance of the ancestral developmental program to the emergence of new organizing principles in its multicellular descendants (see also refs. 12, 36, 94, and 95) also emphasizes the need for caution when referring to HET in multicellularity as transitions in complexity. Many solitary organisms have intricate regulatory pathways—such as the encystation program in solitary amoebae—that could potentially support multicellular organization. In fact, multiple phylogenetic studies have shown that the regulatory complexity of solitary organisms, when focusing on specific regulatory pathways, can be comparable to that of their multicellular relatives. For example, the choanoflagellate *Monosiga brevicollis*, the closest unicellular relative of the metazoans, has a repertoire of phosphotyrosine signaling comparable to that of metazoans (96–98). This is particularly striking since phosphotyrosine signaling—involved in cell differentiation, adhesion, and the control of cell proliferation in metazoans (99)—was long considered to be unique to metazoan development. Along similar lines, Clarke et al. (100) showed that the solitary amoeba, *Acanthamoeba castellanii*, displays a rich repertoire of sensory receptors, transcription factors, and phosphotyrosine signaling, comparable to that of *D. discoideum*. The regulatory complexity in these solitary organisms likely reflects the complex ecology to which they are exposed—cells have to find food, avoid predation, and withstand many environmental changes (16, 19)—and therefore reveals that the life cycle of the solitary organisms can in many ways be more complex than that of their multicellular relatives. Hence, the full complexity of an organism cannot be adequately captured by measuring group properties alone (e.g., group size, number of differentiated cell types); one must also account for the properties of its life cycle (12, 101).

Even though we focus in our bottom-up approach largely on questions underlying the very origin of HET (Q1–Q4), we believe that this approach nevertheless can provide a valuable starting point toward understanding the kinds of organizational complexity that can emerge subsequently, which constitutes an important research challenge (Q5 and Q6). We are surrounded by an incredible diversity of multicellular organization, from filamentous algae to metazoan development, but it remains unclear what determines the organizational outcomes of these HET. Even though we have some intuitive understanding (e.g., filamentous organisms might be unlikely to evolve 3D structures), there are no theoretical or empirical studies yet that systematically approach this question. This is problematic, because intuition often fails. A salient example is the assumption that organizing principles arise in a certain intuitive order, from less to more complex, which has been disproven by phylogenetic studies in both volvocine green algae (102) and social amoebae (17). Traditional classifications based on phenotypic complexity do not match phylogenetic history; species that are phenotypically alike (i.e., similar complexity) are often far apart on the phylogenetic tree, while species that are phenotypically different are often closely related. Just as counterintuitively, many species with a relatively simple organization (e.g., small group sizes, few cell types, simple morphology) are derived from ones with more complex organization [e.g., the *Acytosteliums*, social amoebae that lack stalk cells, are derived from an ancestor with stalk cells (17)]. These phylogenetic studies further reveal that many organizing principles are invented multiple times (e.g., germ-soma differentiation) (102), which suggests that the developmental program underlying group formation strongly potentiates the evolution of some organizing principles more than others. A systematic, bottom-up approach to the study of HET could reveal what is possible, not only what seems intuitively probable. And by understanding how the earliest organizing principles came about, we could identify questions that help us understand the evolution of more advanced ones.

Other HET. Although here we focused on the transition to multicellularity, the above questions can also be applied to other HET, both fraternal and egalitarian. Each HET has its own peculiarities that need to be accounted for. For example, in the case of animal sociality, a group cannot be defined in the same way as for multicellularity (*SI Appendix, Text S4* and Fig. S3). However, despite these differences, the six questions we outline here help to identify commonalities and parallels among the various HET. For example, as for multicellularity, there is strong evidence that the ancestral life cycle plays an important role in the emergence of animal groups. This is exemplified in *Polistes* wasps, for which the bivoltine life cycle of the solitary ancestor was hypothesized to constitute a stepping stone to eusociality and caste differentiation (103–105). Wasps with a bivoltine life cycle have two reproductive broods a year (Fig. 2): the first brood occurs at the start of the breeding season and undergoes normal development; the second brood occurs in the summer and intercedes development by a diapause stage to survive winter. The phenotypic differences between the spring and summer brood result from a developmental switch, in which larvae can follow one of two possible developmental trajectories depending on the cues they experience (i.e., food provisioning). Substantiated by empirical evidence (105), the diapause ground-plan hypothesis (103, 104) states that this developmental switch is co-opted for caste differentiation in *Polistes*, in the same way that photoacclimation in the green algae and encystation in the amoebae were co-opted in the transition to multicellularity (89, 90) (Fig. 2). Recent work has further suggested that the bivoltine life cycle might also facilitate the transition to eusociality by allowing for the joint evolution of sex ratios and helping (106). Taken together, these studies highlight the paramount importance of the ancestral life cycle in the HET to animal sociality and reinforce the similarity across HET.

Conclusion

In this report, we proposed an integrative, bottom-up approach to study the dynamics underlying HET in biological organization. Starting from the solitary ancestor and its life cycle, we discussed how the first life cycles with a group life stage could originate (Q1); what properties characterize the first groups (Q2); how selection could act on those properties (Q3) and subsequently alter the organization of the groups (Q4); and, finally, how new organizing principles could evolve (Q5) and influence future organizational complexity (Q6). We argue that only by starting with the solitary ancestor and its life cycle, and studying these six questions, can we derive an understanding of the causal factors underlying HET. Then, by comparing different instantiations of the same transition (e.g., the multiple origins and transitions to multicellularity), we can determine whether the same causal factors underlie different transitions and which causal factors explain the different organizational outcomes of those transitions.

Acknowledgments

We thank John T. Bonner, whose pioneering work on multicellularity has been an inspiration to us and who provided invaluable feedback on this manuscript; Eörs Szathmáry and two anonymous reviewers for insightful comments and criticism; and Rob Pringle and the C.E.T. laboratory for discussion. J.v.G. received support from The Netherlands Organization for Scientific Research Rubicon Grant 2015-2. C.E.T. received support from the Alfred P. Sloan Foundation.

1 Bonner JT (1974) *On Development: The Biology of Form* (Harvard Univ Press, Cambridge, MA).
2 Buss LW (1987) *The Evolution of Individuality* (Princeton Univ Press, Princeton, NJ).
3 Maynard Smith J, Szathmáry E (1995) *The Major Transitions in Evolution* (Freeman, Oxford).
4 Szathmáry E (2015) Toward major evolutionary transitions theory 2.0. *Proc Natl Acad Sci USA* 112:10104–10111.
5 Michod RE (2007) Evolution of individuality during the transition from unicellular to multicellular life. *Proc Natl Acad Sci USA* 104:8613–8618.
6 Griesemer J (2000) The units of evolutionary transition. *Selection* 1:67–80.
7 Clarke E (2014) Origins of evolutionary transitions. *J Biosci* 39:303–317.
8 De Monte S, Rainey PB (2014) Nascent multicellular life and the emergence of individuality. *J Biosci* 39:237–248.
9 Fontana W, Buss LW (1994) The arrival of the fittest: Toward a theory of biological organization. *Bull Math Biol* 56:1–64.
10 Gerhart J, Kirschner M (1997) *Cells, Embryos, and Evolution: Toward a Cellular and Developmental Understanding of Phenotypic Variation and Evolutionary Adaptability* (Blackwell, Oxford).
11 Szathmáry E (2001) The origin of the human language faculty: The language amoeba hypothesis. *New Essays on the Origin of Language*, eds Trabant J, Ward S (Mouton de Gruyter, Berlin), pp 55–81.
12 Sebé-Pedrós A, Degnan BM, Ruiz-Trillo I (2017) The origin of Metazoa: A unicellular perspective. *Nat Rev Genet* 18:498–512.
13 Jacob F (1982) *The Possible and the Actual* (Washington Univ Press, Seattle, WA).
14 Hogeweg P (2000) Shapes in the shadow: Evolutionary dynamics of morphogenesis. *Artif Life* 6:85–101.
15 King N, Carroll SB (2001) A receptor tyrosine kinase from choanoflagellates: Molecular insights into early animal evolution. *Proc Natl Acad Sci USA* 98:15032–15037.
16 King N (2004) The unicellular ancestry of animal development. *Dev Cell* 7:313–325.
17 Schaap P, et al. (2006) Molecular phylogeny and evolution of morphology in the social amoebas. *Science* 314:661–663.
18 Rokas A (2008) The molecular origins of multicellular transitions. *Curr Opin Genet Dev* 18:472–478.
19 Wolpert L (1994) The evolutionary origin of development: Cycles, patterning, privilege and continuity. *Dev* (Suppl):79–84.
20 Ratcliff WC, et al. (2013) Experimental evolution of an alternating uni- and multicellular life cycle in *Chlamydomonas reinhardtii*. *Nat Commun* 4:2742.
21 Hammerschmidt K, Rose CJ, Kerr B, Rainey PB (2014) Life cycles, fitness decoupling and the evolution of multicellularity. *Nature* 515:75–79.
22 Nadell CD, Drescher K, Foster KR (2016) Spatial structure, cooperation and competition in biofilms. *Nat Rev Microbiol* 14:589–600.
23 Wilson DS (1975) A theory of group selection. *Proc Natl Acad Sci USA* 72:143–146.
24 Michod RE (1997) Cooperation and conflict in the evolution of individuality. I. Multilevel selection of the organism. *Am Nat* 149:607–645.
25 Furusawa C, Kaneko K (1998) Emergence of multicellular organisms with dynamic differentiation and spatial pattern. *Artif Life* 4:79–93.
26 Queller DC (2000) Relatedness and the fraternal major transitions. *Philos Trans R Soc Lond B Biol Sci* 355:1647–1655.
27 Forgacs G, Newman SA (2005) *Biological Physics of the Developing Embryo* (Cambridge Univ Press, Cambridge, UK).
28 Nowak MA (2006) Five rules for the evolution of cooperation. *Science* 314:1560–1563.
29 Fletcher JA, Doebeli M (2009) A simple and general explanation for the evolution of altruism. *Proc Biol Sci* 276:13–19.
30 Bonner JT (1965) *Size and Cycle: An Essay on the Structure of Biology* (Princeton Univ Press, Princeton, NJ).
31 Koschwanez JH, Foster KR, Murray AW (2013) Improved use of a public good selects for the evolution of undifferentiated multicellularity. *eLife* 2:e00367.
32 Alegado RA, et al. (2012) A bacterial sulfonolipid triggers multicellular development in the closest living relatives of animals. *eLife* 1:e00013.
33 Kirk DL (1999) Evolution of multicellularity in the volvocine algae. *Curr Opin Plant Biol* 2:496–501.
34 Kirk DL (2005) A twelve-step program for evolving multicellularity and a division of labor. *BioEssays* 27:299–310.
35 Sathe S, Durand PM (2016) Cellular aggregation in *Chlamydomonas* (Chlorophyceae) is chimaeric and depends on traits like cell size and motility. *Eur J Phycol* 51:129–138.
36 Sebé-Pedrós A, et al. (2013) Regulated aggregative multicellularity in a close unicellular relative of metazoa. *eLife* 2:e01287.
37 Estrela S, Kerr B, Morris JJ (2016) Transitions in individuality through symbiosis. *Curr Opin Microbiol* 31:191–198.
38 Libby E, B Rainey P (2013) A conceptual framework for the evolutionary origins of multicellularity. *Phys Biol* 10:035001.
39 Herron MD, Nedelcu AM (2015) Volvocine algae: From simple to complex multicellularity. *Evolutionary Transitions to Multicellular Life*, eds Ruiz-Trillo I, Nedelcu AM (Springer, Amsterdam), pp 129–152.
40 West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Syst* 20:249–278.
41 Khersonsky O, Tawfik DS (2010) Enzyme promiscuity: A mechanistic and evolutionary perspective. *Annu Rev Biochem* 79:471–505.
42 Sakaguchi M, Murakami H, Suzaki T (2001) Involvement of a 40-kDa glycoprotein in food recognition, prey capture, and induction of phagocytosis in the protozoon *Actinophrys sol*. *Protist* 152:33–41.
43 West SA, Fisher RM, Gardner A, Kiers ET (2015) Major evolutionary transitions in individuality. *Proc Natl Acad Sci USA* 112:10112–10119.
44 Kuranda MJ, Robbins PW (1991) Chitinase is required for cell separation during growth of *Saccharomyces cerevisiae*. *J Biol Chem* 266:19758–19767.
45 Chai Y, Norman T, Kolter R, Losick R (2010) An epigenetic switch governing daughter cell separation in *Bacillus subtilis*. *Genes Dev* 24:754–765.
46 Herron MD, Rashidi A, Shelton DE, Driscoll WW (2013) Cellular differentiation and individuality in the 'minor' multicellular taxa. *Biol Rev Camb Philos Soc* 88:844–861.
47 Tarnita CE, Washburne A, Martinez-Garcia R, Sgro AE, Levin SA (2015) Fitness tradeoffs between spores and nonaggregating cells can explain the coexistence of diverse genotypes in cellular slime molds. *Proc Natl Acad Sci USA* 112:2776–2781.
48 Bonner JT (1998) The origins of multicellularity. *Integr Biol* 1:27–36.
49 Grosberg RK, Strathmann RR (2007) The evolution of multicellularity: A minor major transition? *Annu Rev Ecol Evol Syst* 38:621–654.

50 Tarnita CE, Taubes CH, Nowak MA (2013) Evolutionary construction by staying together and coming together. *J Theor Biol* 320:10–22.
51 Angert ER (2005) Alternatives to binary fission in bacteria. *Nat Rev Microbiol* 3:214–224.
52 Flärdh K, Buttner MJ (2009) *Streptomyces* morphogenetics: Dissecting differentiation in a filamentous bacterium. *Nat Rev Microbiol* 7:36–49.
53 Berk V, et al. (2012) Molecular architecture and assembly principles of *Vibrio cholerae* biofilms. *Science* 337:236–239.
54 Loomis WF (2014) Cell signaling during development of *Dictyostelium*. *Dev Biol* 391:1–16.
55 Hölscher T, et al. (2015) Motility, chemotaxis and aerotaxis contribute to competitiveness during bacterial pellicle biofilm development. *J Mol Biol* 427:3695–3708.
56 Duran-Nebreda S, Solé R (2015) Emergence of multicellularity in a model of cell growth, death and aggregation under size-dependent selection. *J R Soc Interface* 12:20140982.
57 Pande S, et al. (2015) Metabolic cross-feeding via intercellular nanotubes among bacteria. *Nat Commun* 6:6238.
58 Momeni B, Brileya KA, Fields MW, Shou W (2013) Strong inter-population cooperation leads to partner intermixing in microbial communities. *eLife* 2:e00230.
59 Stewart PS, Franklin MJ (2008) Physiological heterogeneity in biofilms. *Nat Rev Microbiol* 6:199–210.
60 Schlichting CD (2003) Origins of differentiation via phenotypic plasticity. *Evol Dev* 5:98–105.
61 Mikhailov KV, et al. (2009) The origin of Metazoa: A transition from temporal to spatial cell differentiation. *BioEssays* 31:758–768.
62 Gierer A, Meinhardt H (1972) A theory of biological pattern formation. *Kybernetik* 12:30–39.
63 Newman SA, Forgacs G, Muller GB (2006) Before programs: The physical origination of multicellular forms. *Int J Dev Biol* 50:289–299.
64 Newman SA, Bhat R (2009) Dynamical patterning modules: A "pattern language" for development and evolution of multicellular form. *Int J Dev Biol* 53:693–705.
65 Alpkvist E, Klapper I (2007) Description of mechanical response including detachment using a novel particle model of biofilm/flow interaction. *Water Sci Technol* 55:265–273.
66 Rainey PB, Kerr B (2010) Cheats as first propagules: A new hypothesis for the evolution of individuality during the transition from single cells to multicellularity. *BioEssays* 32:872–880.
67 van Gestel J, Nowak MA (2016) Phenotypic heterogeneity and the evolution of bacterial life cycles. *PLoS Comput Biol* 12:e1004764.
68 Nadell CD, Bassler BL (2011) A fitness trade-off between local competition and dispersal in *Vibrio cholerae* biofilms. *Proc Natl Acad Sci USA* 108:14181–14185.
69 Garcia T, De Monte S (2013) Group formation and the evolution of sociality. *Evolution* 67:131–141.
70 Ghosh P, Mondal J, Ben-Jacob E, Levine H (2015) Mechanically-driven phase separation in a growing bacterial colony. *Proc Natl Acad Sci USA* 112:E2166–E2173.
71 Serra DO, Richter AM, Hengge R (2013) Cellulose as an architectural element in spatially structured *Escherichia coli* biofilms. *J Bacteriol* 195:5540–5554.
72 Pichugin Y, Pena J, Rainey P, Traulsen A (2017) Fragmentation modes and the evolution of life cycles. *bioRxiv*, https://doi.org/10.1101/120097.
73 Wolpert L, Szathmáry E (2002) Multicellularity: Evolution and the egg. *Nature* 420:745.
74 Grosberg RK, Strathmann RR (1998) One cell, two cell, red cell, blue cell: The persistence of a unicellular stage in multicellular life histories. *Trends Ecol Evol* 13:112–116.
75 Akçay E, Van Cleve J (2012) Behavioral responses in structured populations pave the way to group optimality. *Am Nat* 179:257–269.
76 Yan J, Nadell CD, Bassler BL (2017) Environmental fluctuation governs selection for plasticity in biofilm production. *ISME J* 11:1569–1577.
77 Nanjundiah V (2016) Cellular slime mold development as a paradigm for the transition from unicellular to multicellular life. *Multicellularity: Origins and Evolution*, eds Niklas KJ, Newman SA (MIT Press, Cambridge, MA), pp 105–130.
78 Griesemer J (2000) Development, culture, and the units of inheritance. *Philos Sci* 67:S348–S368.
79 Waddington CH (1952) Selection of the genetic basis for an acquired character. *Nature* 169:278.
80 Pigliucci M, Murren CJ, Schlichting CD (2006) Phenotypic plasticity and evolution by genetic assimilation. *J Exp Biol* 209:2362–2367.
81 Miller MB, Bassler BL (2001) Quorum sensing in bacteria. *Annu Rev Microbiol* 55:165–199.
82 Lazazzera BA (2000) Quorum sensing and starvation: Signals for entry into stationary phase. *Curr Opin Microbiol* 3:177–182.
83 van Gestel J, Nowak MA, Tarnita CE (2012) The evolution of cell-to-cell communication in a sporulating bacterium. *PLoS Comput Biol* 8:e1002818.
84 Powers ST, Penn AS, Watson RA (2011) The concurrent evolution of cooperation and the population structures that support it. *Evolution* 65:1527–1543.
85 Sussex IM (1973) Do concepts of animal development apply to plant systems. *Brookhaven Symp Biol* 25:145–151.
86 Clarke E (2013) The multiple realizability of biological individuals. *J Philos* 110:413–435.
87 Niklas KJ, Newman SA (2013) The origins of multicellular organisms. *Evol Dev* 15:41–52.
88 Kirk MM, et al. (1999) *regA*, a *Volvox* gene that plays a central role in germ-soma differentiation, encodes a novel regulatory protein. *Development* 126:639–647.
89 Nedelcu AM, Michod RE (2006) The evolutionary origin of an altruistic gene. *Mol Biol Evol* 23:1460–1464.
90 Schaap P (2011) Evolutionary crossroads in developmental biology: *Dictyostelium discoideum*. *Development* 138:387–396.
91 Ritchie AV, van Es S, Fouquet C, Schaap P (2008) From drought sensing to developmental control: Evolution of cyclic AMP signaling in social amoebas. *Mol Biol Evol* 25:2109–2118.
92 Kawabe Y, et al. (2009) Activated cAMP receptors switch encystation into sporulation. *Proc Natl Acad Sci USA* 106:7089–7094.
93 Shubin N, Tabin C, Carroll S (1997) Fossils, genes and the evolution of animal limbs. *Nature* 388:639–648.
94 Lee JH, Lin H, Joo S, Goodenough U (2008) Early sexual origins of homeoprotein heterodimerization and evolution of the plant KNOX/BELL family. *Cell* 133:829–840.
95 Hanschen ER, et al. (2016) The *Gonium pectorale* genome demonstrates co-option of cell cycle regulation during the evolution of multicellularity. *Nat Commun* 7:11370.
96 King N, et al. (2008) The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature* 451:783–788.
97 Manning G, Young SL, Miller WT, Zhai Y (2008) The protist, *Monosiga brevicollis*, has a tyrosine kinase signaling network more elaborate and diverse than found in any known metazoan. *Proc Natl Acad Sci USA* 105:9674–9679.
98 Pincus D, Letunic I, Bork P, Lim WA (2008) Evolution of the phospho-tyrosine signaling machinery in premetazoan lineages. *Proc Natl Acad Sci USA* 105:9680–9684.
99 Hunter T (2009) Tyrosine phosphorylation: Thirty years and counting. *Curr Opin Cell Biol* 21:140–146.
100 Clarke M, et al. (2013) Genome of *Acanthamoeba castellanii* highlights extensive lateral gene transfer and early evolution of tyrosine kinase signaling. *Genome Biol* 14:R11.
101 Bell G, Koufopanou V (1991) The architecture of the life cycle in small organisms. *Philos Trans R Soc Lond B Biol Sci* 332:81–89.
102 Herron MD, Michod RE (2008) Evolution of complexity in the volvocine algae: Transitions in individuality through Darwin's eye. *Evolution* 62:436–451.
103 Hunt JH (2012) A conceptual model for the origin of worker behaviour and adaptation of eusociality. *J Evol Biol* 25:1–19.
104 Hunt JH, Amdam GV (2005) Bivoltinism as an antecedent to eusociality in the paper wasp genus *Polistes*. *Science* 308:264–267.
105 Hunt JH, et al. (2007) A diapause pathway underlies the gyne phenotype in *Polistes* wasps, revealing an evolutionary route to caste-containing insect societies. *Proc Natl Acad Sci USA* 104:14020–14025.
106 Quiñones AE, Pen I (2017) A unified model of Hymenopteran preadaptations that trigger the evolutionary transition to eusociality. *Nat Commun* 8:15920.
107 Claessen D, Rozen DE, Kuipers OP, Søgaard-Andersen L, van Wezel GP (2014) Bacterial solutions to multicellularity: A tale of biofilms, filaments and fruiting bodies. *Nat Rev Microbiol* 12:115–124.
108 van Gestel J, Vlamakis H, Kolter R (2015) From cell differentiation to cell collectives: *Bacillus subtilis* uses division of labor to migrate. *PLoS Biol* 13:e1002141.
109 Litman GW, Dishaw LJ (2013) Histocompatibility: Clarifying fusion confusion. *Curr Biol* 23:R934–R935.
110 Adler CE, Seidel CW, McKinney SA, Alvarado AS (2014) Selective amputation of the pharynx identifies a FoxA-dependent regeneration program in planaria. *eLife* 3:e02238.

Supplementary information

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

Jordi van Gestel & Corina E. Tarnita

Content **Page**

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.

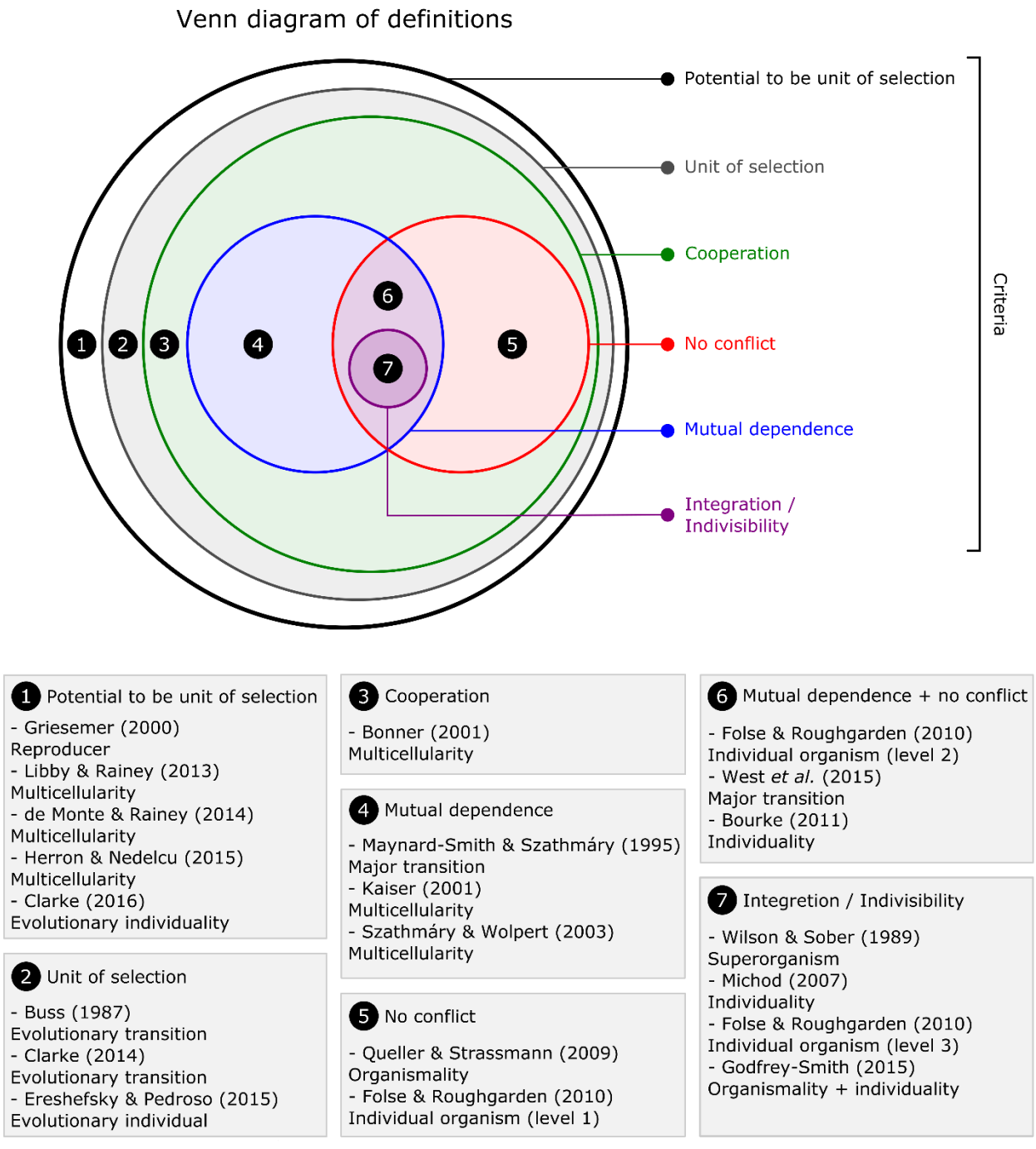

Figure S1. Criteria and definitions for the unit of biological organization. Venn diagram that categorizes definitions for the unit of biological organization based on six criteria: potential to be unit of selection (black); unit of selection (grey); cooperation (green); no conflict (red); mutual dependence (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

Reference	Griesemer J (2000) The units of evolutionary transition. *Selection* 1(1-3): 67-80.
Term	**Reproducer**
Definition	"Reproducers are entities that multiply by material overlap of propagules conferring the capacity to develop." (see also Text S1)

Reference	Libby E, Rainey PB (2013) A conceptual framework for the evolutionary origins of multicellularity. *Physical biology* 10(3): 035001.
Term	**Multicellularity**
Definition	"(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."

Reference	de Monte S, Rainey PB (2014) Nascent multicellular life and the emergence of individuality. *Journal of Biosciences* 39(2): 237-248.
Term	**Multicellularity**
Definition	"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'>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."

Reference	Herron MD, Nedelcu AM (2015) Volvocine algae: from simple to complex Multicellularity. *Evolutionary Transitions to Multicellular Life*, eds Ruiz-Trillo I, Nedelcu AM (Springer), pp. 129-153.
Term	**Multicellularity**
Definition	"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"

Reference	Clarke E (2016) A levels-of-selection approach to evolutionary individuality. *Biology & Philosophy* 31(6): 893-911.
Term	**Evolutionary individuality**
Definition	"A collection of living parts which has some capacity for responding to selection at the between-collection level, because of the action of individuating mechanisms"

2. Unit of selection

Reference	Buss LW (1987) *Evolution of individuality* (Princeton University Press)
Term	**Evolutionary transition**
Definition	"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

	by the external environment alone, following the transition it became selected by traits expressed by the higher unit."

Reference	Clarke E (2014) Origins of evolutionary transitions. *Journal of Biosciences* 39(2): 303-317.
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	Ereshefsky M, Pedroso M (2015). Rethinking evolutionary individuality. *Proceedings of the National Academy of Sciences* 112(33): 10126-10132.
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	Bonner JT (2001) *First Signals: The Evolution of Multicellular Development* (Princeton University Press)
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	Maynard Smith J, Szathmáry E (1995) *The Major Transitions in Evolution* (Oxford University Press).
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	Kaiser D (2001) Building a multicellular organism. *Annual Review of Genetics* 35(1): 103-123.
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	Szathmáry E, Wolpert L (2003) The transition from single cells to multicellularity. *Genetic and Cultural Evolution of Cooperation*, eds. Hammerstein P (MIT Press), pp. 271-290.
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

Reference	Queller DC, Strassmann JE (2009) Beyond society: the evolution of organismality. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364(1533): 3143-3155.
Term	**Organismality**
Definition	"The organism is simply a unit with high cooperation and very low conflict among its parts"

Reference	Folse HJ, Roughgarden J (2010) What is an individual organism? A multilevel selection perspective. *The Quarterly Review of Biology* 85(4): 447-472.
Term	**Individuality** (This definitions falls in multiple categories simultaneously)
Definition	"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."

6. Mutual dependence + no conflict

Reference	West SA, Fisher RM, Gardner A, Kiers ET (2015) Major evolutionary transitions in individuality. *Proceedings of the National Academy of Sciences* 112(33): 10112-10119.
Term	**Major transition**
Definition	"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."

Reference	Bourke (2011) *Principles of Social Evolution* (Oxford University Press)
Term	**Individuality**
Definition	"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"

7. Integration / Indivisibility

Reference	Wilson DS, Sober E (1989) Reviving the superorganism. *Journal of Theoretical Biology* 136(3): 337-356.
Term	**Superorganism**
Definition	"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."

Reference	Michod RE (2007) Evolution of individuality during the transition from unicellular to multicellular life. *Proceedings of the National Academy of Sciences* 104(1): 8613-8618.
Term	**Evolutionary individuals**
Definition	"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."

Reference	Godfrey-Smith (2015) Individuality and Life Cycles. *Individuals Across the Sciences*, eds. Pradeu T, Guay A (Oxford University Press), pp. 85-102.
Term	**Organismality + individuality** (combination of both organismality and individuality)
Definition	"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."

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, "progeneration 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 underpinnings[1], 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[2]: "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[3]. 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

---

preceding reproduction as a distinct phase; rather, developmental processes are embedded in reproduction and reproductive processes are embedded in development. They are entwined aspects of life made coherent by their intertwining." [p. 805].

[2] 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)).

[3] "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?[4] Only traits that reoccur sufficiently often with respect to an organism´s life cycle have the potential to evolve by natural selection[5] (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)).

[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.

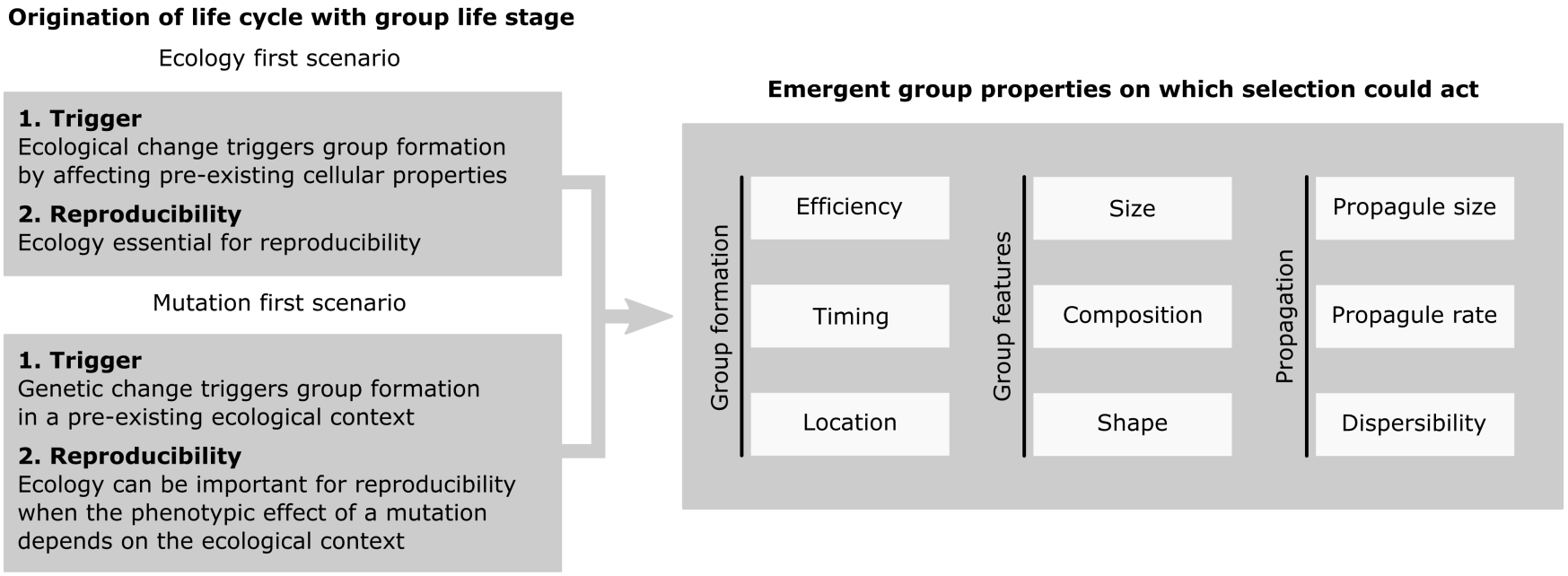

Figure S2. The origination of life cycles with a group life phase and emergent group properties that could be selected for. Left: two alternative scenarios that lead to the origination of the first group life cycles: the ecology first scenario and the mutation first scenario. Right: possible properties 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.

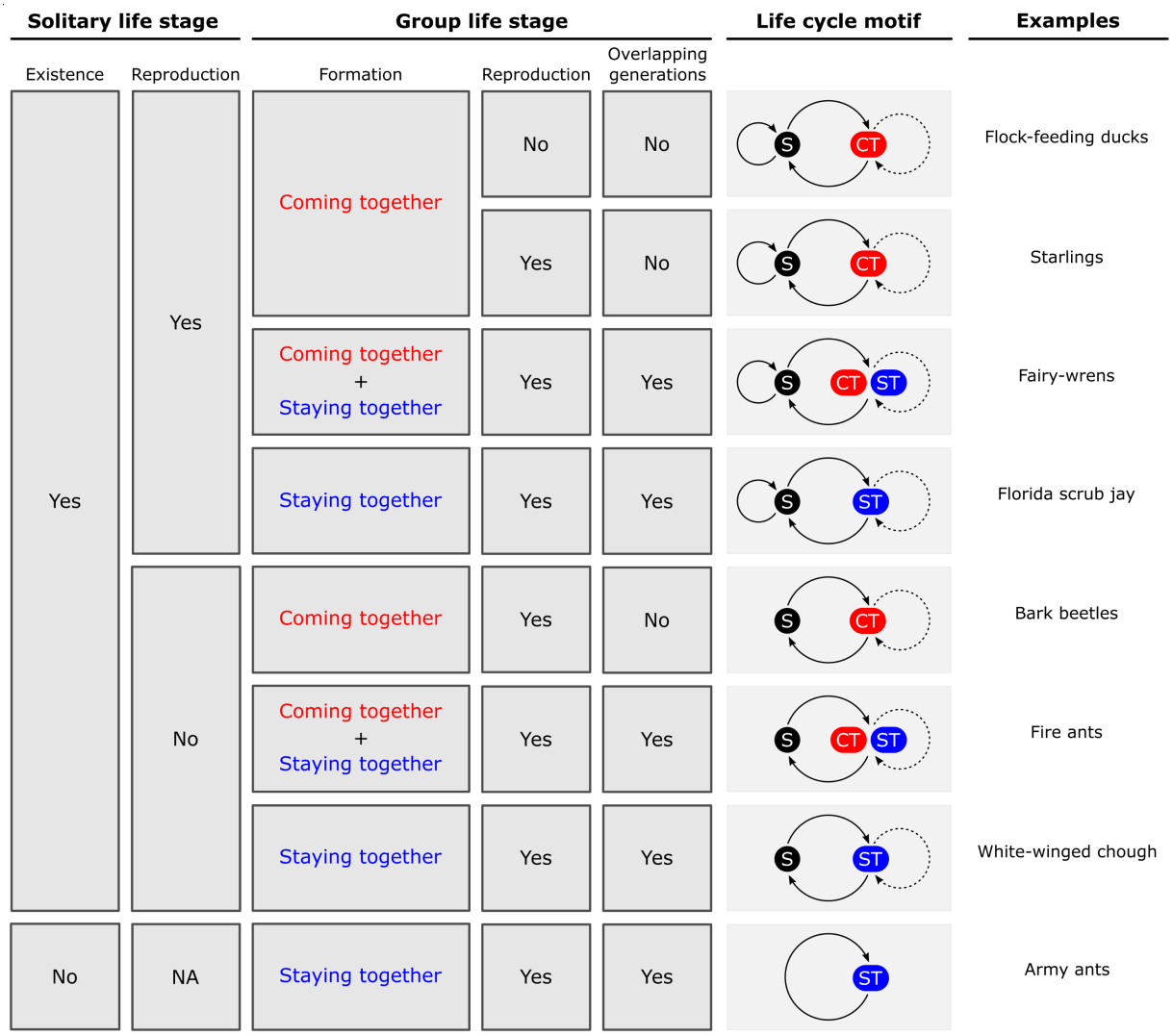

Figure S3. Potential animal life cycles that could emerge upon the origin of the first animal groups (figure shows *possible* life cycles, irrespective of their likelihood of emerging). The life cycle motifs give a simple and schematic representation of the transitions that could occur within the first 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

1. Santelices B (1999) How many kinds of individual are there? *Trends Ecol Evol* 14(4):152–155.
2. Pepper JW, Herron MD (2008) Does biology need an organism concept? *Biol Rev* 83(4):621–627.
3. Maynard Smith J, Brookfield JFY (1983) Models of evolution. *Proc R Soc Lond B Biol Sci* 219(1216):315–325.
4. Maynard Smith J (1987) How to model evolution. *The Latest on the Best: Essays on Evolution and Optimality*, ed Dupre J (MIT Press, Cambridge, MA), pp 119–131.
5. Szathmáry E, Maynard Smith J (1993) The origin of genetic systems. *Abstr Bot* 17:197–206.
6. Griesemer J (2000) Development, culture, and the units of inheritance. *Philos Sci* 67(3):S348–S368.
7. Griesemer J (2000) The units of evolutionary transition. *Selection* 1:67–80.
8. Lewontin RC (1970) The units of selection. *Annu Rev Ecol Syst* 1(1):1–18.
9. Monte SD, Rainey PB (2014) Nascent multicellular life and the emergence of individuality. *J Biosci* 39(2):237–248.
10. Bonner JT (2001) *First Signals: The Evolution of Multicellular Development* (Princeton Univ Press, Princeton).
11. Buss LW (1999) Slime molds, ascidians, and the utility of evolutionary theory. *Proc Natl Acad Sci USA* 96(16):8801–8803.
12. Michod RE (2000) *Darwinian Dynamics: Evolutionary Transitions in Fitness and Individuality* (Princeton Univ Press, Princeton).
13. Queller DC, Strassmann JE (2009) Beyond society: The evolution of organismality. *Philos Trans R Soc Lond B Biol Sci* 364(1533):3143–3155.
14. Maynard Smith J, Szathmáry E (1995) *The Major Transitions in Evolution* (Freeman, New York).
15. Costa JT, Fitzgerald TD (1996) Developments in social terminology: semantic battles in a conceptual war. *Trends Ecol Evol* 11(7):285–289.
16. Schaap P, et al. (2006) Molecular phylogeny and evolution of morphology in the social amoebas. *Science* 314(5799):661–663.
17. Herron MD, Michod RE (2008) Evolution of complexity in the volvocine algae: Transitions in individuality through Darwin's eye. *Evolution* 62(2):436–451.
18. Grau-Bové X, et al. (2017) Dynamics of genomic innovation in the unicellular ancestry of animals. *eLife* 6:e26036.
19. Sebé-Pedrós A, Degnan BM, Ruiz-Trillo I (2017) The origin of Metazoa: a unicellular perspective. *Nat Rev Genet* 18(8):498–512.
20. King N, Carroll SB (2001) A receptor tyrosine kinase from choanoflagellates: Molecular insights into early animal evolution. *Proc Natl Acad Sci USA* 98(26):15032–15037.
21. King N (2004) The unicellular ancestry of animal development. *Dev Cell* 7(3):313–325.
22. King N, et al. (2008) The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature* 451(7180):783–788.
23. Rokas A (2008) The molecular origins of multicellular transitions. *Curr Opin Genet Dev* 18(6):472–478.
24. Sebé-Pedrós A, et al. (2013) Regulated aggregative multicellularity in a close unicellular relative of metazoa. *eLife* 2:e01287.
25. Goldman BS, et al. (2006) Evolution of sensory complexity recorded in a myxobacterial genome. *Proc Natl Acad Sci USA* 103(41):15200–15205.
26. Sebé-Pedrós A, et al. (2016) The dynamic regulatory genome of *Capsaspora* and the origin of animal multicellularity. *Cell* 165(5):1224–1237.
27. Ellers J, Kiers ET, Currie CR, McDonald BR, Visser B (2012) Ecological interactions drive evolutionary loss of traits. *Ecol Lett* 15(10):1071–1082.

28. Moreau CS, Bell CD, Vila R, Archibald SB, Pierce NE (2006) Phylogeny of the ants: Diversification in the age of angiosperms. *Science* 312(5770):101–104.
29. Sperling EA, et al. (2013) Oxygen, ecology, and the Cambrian radiation of animals. *Proc Natl Acad Sci USA* 110(33):13446–13451.
30. Sathe S, Durand PM (2016) Cellular aggregation in *Chlamydomonas* (Chlorophyceae) is chimaeric and depends on traits like cell size and motility. *Eur J Phycol* 51(2):129–138.
31. Ratcliff WC, Denison RF, Borrello M, Travisano M (2012) Experimental evolution of multicellularity. *Proc Natl Acad Sci USA* 109(5):1595–1600.
32. Koschwanez JH, Foster KR, Murray AW (2013) Improved use of a public good selects for the evolution of undifferentiated multicellularity. *eLife* 2:e00367.
33. Hammerschmidt K, Rose CJ, Kerr B, Rainey PB (2014) Life cycles, fitness decoupling and the evolution of multicellularity. *Nature* 515(7525):75–79.
34. Bonner JT (1998) The origins of multicellularity. *Integr Biol* 1(1):27–36.
35. Camazine S, et al. (2003) *Self-organization in Biological Systems* (Princeton Univ Press, Princeton).
36. Forgacs G, Newman SA (2005) *Biological Physics of the Developing Embryo* (Cambridge Univ Press, Cambridge, UK).
37. Flores E, Herrero A (2010) Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nat Rev Microbiol* 8(1):39–50.
38. van Gestel J, Vlamakis H, Kolter R (2015) From cell differentiation to cell collectives: *Bacillus subtilis* uses division of labor to migrate. *PLoS Biol* 13(4):e1002141.
39. Fletcher JA, Doebeli M (2009) A simple and general explanation for the evolution of altruism. *Proc R Soc B Biol Sci* 276(1654):13–19.
40. Nowak MA (2006) Five rules for the evolution of cooperation. *Science* 314(5805):1560–1563.
41. Bourke AFG (2011) *Principles of Social Evolution* (Oxford Univ Press, Oxford).
42. Wilson DS (1975) A theory of group selection. *Proc Natl Acad Sci USA* 72(1):143–146.
43. Damuth J, Heisler IL (1988) Alternative formulations of multilevel selection. *Biol Philos* 3(4):407–430.
44. Okasha S (2006) *Evolution and the Levels of Selection* (Clarendon, Oxford).
45. Clarke E (2013) The multiple realizability of biological individuals. *J Philos* 110(8):413–435.
46. Clarke E (2016) A levels-of-selection approach to evolutionary individuality. *Biol Philos* 31(6):893–911.
47. Gardner A, Grafen A (2009) Capturing the superorganism: a formal theory of group adaptation. *J Evol Biol* 22(4):659–671.
48. Akçay E, Van Cleve J (2012) Behavioral responses in structured populations pave the way to group optimality. *Am Nat* 179(2):257–269.
49. Graner F, Glazier JA (1992) Simulation of biological cell sorting using a two-dimensional extended Potts model. *Phys Rev Lett* 69(13):2013–2016.
50. Hogeweg P (2000) Evolving mechanisms of morphogenesis: On the interplay between differential adhesion and cell differentiation. *J Theor Biol* 203(4):317–333.
51. Gierer A, Meinhardt H (1972) A theory of biological pattern formation. *Kybernetik* 12(1):30–39.
52. Furusawa C, Kaneko K (1998) Emergence of multicellular organisms with dynamic differentiation and spatial pattern. *Artif Life* 4(1):79–93.
53. Queller DC (1997) Cooperators since life began. *Q Rev Biol* 72(2):184–188.
54. Grosberg RK, Strathmann RR (2007) The evolution of multicellularity: A minor major transition? *Annu Rev Ecol Evol Syst* 38(1):621–654.
55. Tarnita CE, Taubes CH, Nowak MA (2013) Evolutionary construction by staying together and coming together. *J Theor Biol* 320:10–22.

56. Szathmáry E (2015) Toward major evolutionary transitions theory 2.0. *Proc Natl Acad Sci USA* 112(33):10104–10111.
57. Bonner JT (1965) *Size and Cycle: An Essay on the Structure of Biology* (Princeton Univ Press, Princeton).
58. Watson JD, Crick FH (1953) Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. *Nature* 171(4356):737–738.
59. Watson JD, Crick FH (1953) Genetical implications of the structure of deoxyribonucleic acid. *Nature* 171(4361):964–967.
60. Gottesman S, Beckwith JR (1969) Directed transposition of the arabinose operon: a technique for the isolation of specialized transducing bacteriophages for any *Escherichia coli* gene. *J Mol Biol* 44(1):117–127.
61. Min Jou W, Haegeman G, Ysebaert M, Fiers W (1972) Nucleotide sequence of the gene coding for the bacteriophage MS2 coat protein. *Nature* 237(5350):82–88.
62. Oyama S (1985) *The Ontogeny of Information: Developmental Systems and Evolution* (Cambridge Univ Press, Cambridge, UK).
63. Griffiths PE, Gray RD (1994) Developmental systems and evolutionary explanation. *J Philos* 91(6):277–304.
64. Oyama S, Griffiths PE, Gray RD (2003) *Cycles of Contingency: Developmental Systems and Evolution* (MIT Press, Cambridge, MA).
65. Bonner JT (1974) *On Development: The Biology of Form* (Harvard Univ Press, Cambridge, MA).
66. Griesemer J (2016) Reproduction in complex life cycles: Toward a developmental reaction norms perspective. *Philos Sci* 83(5):803–815.
67. Dawkins R (1976) *The Selfish Gene* (Oxford Univ Press, Oxford).
68. Szathmáry E, Maynard Smith J (1997) From replicators to reproducers: The first major transitions leading to life. *J Theor Biol* 187(4):555–571.
69. Clarke E (2014) Origins of evolutionary transitions. *J Biosci* 39(2):303–317.
70. Herron MD, Rashidi A, Shelton DE, Driscoll WW (2013) Cellular differentiation and individuality in the 'minor' multicellular taxa. *Biol Rev* 88(4):844–861.
71. Godfrey-Smith P (2016) Complex life cycles and the evolutionary process. *Philos Sci* 83(5):816–827.
72. Schneider JM, Bilde T (2008) Benefits of cooperation with genetic kin in a subsocial spider. *Proc Natl Acad Sci USA* 105(31):10843–10846.
73. West SA, Fisher RM, Gardner A, Kiers ET (2015) Major evolutionary transitions in individuality. *Proc Natl Acad Sci USA* 112(33):10112–10119.
74. Gadagkar R, Bonner JT (1994) Social insects and social amoebae. *J Biosci* 19(2):219–245.
75. Riehl C (2013) Evolutionary routes to non-kin cooperative breeding in birds. *Proc R Soc Lond B Biol Sci* 280(1772):20132245.
76. Nowak MA, Tarnita CE, Wilson EO (2010) The evolution of eusociality. *Nature* 466(7310):1057–1062.
77. Wang J, et al. (2013) A Y-like social chromosome causes alternative colony organization in fire ants. *Nature* 493(7434):664–668.
78. Trible W, et al. (2017) *orco* mutagenesis causes loss of antennal lobe glomeruli and impaired social behavior in ants. *bioRxiv*:112532.
79. Kocher SD, et al. (2014) Transitions in social complexity along elevational gradients reveal a combined impact of season length and development time on social evolution. *Proc R Soc Lond B Biol Sci* 281(1787):20140627.
80. Schwarz MP, Richards MH, Danforth BN (2007) Changing paradigms in insect social evolution: insights from halictine and allodapine bees. *Annu Rev Entomol* 52:127–150.

81. Avilés L, Fletcher JA, Cutter AD (2004) The kin composition of social groups: trading group size for degree of altruism. *Am Nat* 164(2):132–144.
82. West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Syst* 20:249–278.
83. West-Eberhard MJ (2003) *Developmental Plasticity and Evolution* (Oxford Univ. Press, Oxford).
84. Hunt JH (2012) A conceptual model for the origin of worker behaviour and adaptation of eusociality. *J Evol Biol* 25(1):1–19.
85. Quiñones AE, Pen I (2017) A unified model of Hymenopteran preadaptations that trigger the evolutionary transition to eusociality. *Nat Commun* 8:15920.
86. Schlichting CD (2003) Origins of differentiation via phenotypic plasticity. *Evol Dev* 5(1):98–105.
87. Newman SA, Bhat R (2009) Dynamical patterning modules: a 'pattern language' for development and evolution of multicellular form. *Int J Dev Biol* 53(5-6):693–705.
88. Schaap P (2011) Evolutionary crossroads in developmental biology: *Dictyostelium discoideum*. *Development* 138(3):387–396.
89. Kawabe Y, et al. (2015) The evolution of developmental signalling in Dictyostelia from an amoebozoan stress response. *Evolutionary Transitions to Multicellular Life*, eds Ruiz-Trillo I, Nedelcu AM (Springer, Netherlands), pp 451–467.
90. Nanjundiah V (2016) Cellular slime mold development as a paradigm for the transition from unicellular to multicellular life. *Multicellularity: Origins and Evolution*, eds Niklas KJ, Newman SA (MIT Press, Cambridge, MA), pp 105–130.
91. Wittenberger JF (1981) *Animal Social Behavior* (Duxbury Press, Boston).
92. Raffa KF, Berryman AA (1987) Interacting selective pressures in conifer-bark beetle systems: A basis for reciprocal adaptations? *Am Nat* 129(2):234–262.
93. Ehrlich P, Dobkin DS, Wheye D (1988) *Birder's Handbook* (Simon and Schuster, New York).
94. Bernasconi G, Strassmann JE (1999) Cooperation among unrelated individuals: The ant foundress case. *Trends Ecol Evol* 14(12):477–482.
95. Kronauer DJC, Johnson RA, Boomsma JJ (2007) The evolution of multiple mating in army ants. *Evolution* 61(2):413–422.
96. Cornwallis CK, West SA, Davis KE, Griffin AS (2010) Promiscuity and the evolutionary transition to complex societies. *Nature* 466(7309):969–972.