

OPERATIONALIZING MAJOR EVOLUTIONARY TRANSITIONS

Carmel, Yohay¹ and Shavit, Ayelet²

¹Faculty of Civil and Environmental Engineering, Technion – Israel Institute of Technology, Haifa, 32000, Israel. E-mail: yohay@technion.ac.il . Phone: +972-502515487.

²Department of Environmental Sciences, Tel Hai College, 12208, Israel.

Corresponding author: Yohay Carmel

Abstract

Major Evolutionary Transitions in Individuality (hereafter, METIs), such as the transition to multicellularity and the transition to eusocial communities, have been at the center of evolutionary research, but not much has been done to operationalize this concept. Here, we devise a set of complexity indicators intended specifically for assessing progression along METIs. We propose four indicators that together capture the nature of the process: system size, indivisibility, reproductive specialization, and nonreproductive specialization. We then conduct a first quantitative comparison across taxa and transition types. Our analysis revealed that indivisibility has a crucial role in the process; it seems irreversible and may mark the exact point where a group of individuals becomes a new individual at a higher hierarchical level. Finding that disparate groups demonstrated a similar pattern of progression along METI allowed us to devise a general predictive model for the progression of major evolutionary transitions in individuality.

Keywords: major evolutionary transitions; multicellularity; eusociality;

Introduction

The emergence of novel levels of individuality is a recurrent theme in the history of life. Biological units that previously existed as independent individuals are incorporated within a higher level of organization, which becomes a higher-level entity, i.e., a new individual [1–8]. For example, multicellular organisms are comprised of cells whose ancestors were individual unicellular organisms [3,9,10]. Other Major Evolutionary Transitions in Individuality (hereafter, METIs) include the formation of proto-cells, and the formation of eukaryotic cells [6], among others. The most recent METI has been the transition of individual organisms into a eusocial colony, as illustrated by ants and bees (and a handful of other eusocial species). The whole colony is arguably a single individual, and the ants (including the queen) can be viewed as the mobile equivalents of cells in an organism [11,12]. Two crucial features justify the labeling of these insect colonies as a kind of individual. First, a single ant could not survive on its own, not even the queen. Only the colony as a whole is capable of survival and reproduction. Second, the vast majority of the colony members do not reproduce.

Another group, large yet much less known, exemplifies several lines of transition from individual multicellular organisms into fully integrated colonies; this group consists of a wide range of taxa from various phyla of marine invertebrates (e.g., corals, hydrozoa, bryozoa, and tunicates [13]. The diversity of internal structure and function within this group attracted zoologists to distinguish stages in the transition 'from aggregates to integrates' (sensu [14] within these phyla; alternative 'colony individuality' scales were proposed [1,14,15]. The view of the integrated colony as a higher-level individual [1,13–17], as well as the parallels between marine invertebrate colonies and eusocial insects [17–19] were noted long ago. A common feature of METIs is that, as the division of labor within the newly established higher-level organism develops, its lower level units become more specialized, and lose much of the capabilities and behavioral repertory of their free-living precursors, as shown for ant castes [20], for zooids in marine invertebrate colonies [21], and for metazoan cells [22].

The notion of evolution as a process of change in the level of individuality was expressed by Dendy as early as 1924: 'Evolution consists to a very large extent, if not mainly, in the progressive merging of individualities of a lower order in others of a higher order' ([2], quoted in [13]). The criteria for being considered an evolutionary individual (Appendix 1) and the processes leading to evolutionary transitions in individuality are topics of sustained discussion in evolutionary theory. How these transitions came to be is a matter of much engagement and research. The mechanisms that drive METIs and, in particular the relative roles of kin selection vs. group selection during a transition to a new level of individuality, are still intensely discussed, and this topic is briefly reviewed in Appendix 2. A separate discussion concerns the proposition that these transitions resulted from passive- rather than active evolution (meaning, without the involvement of forces such as selection [23–25]). Our goal here is not to investigate mechanisms, but rather to provide a systematic and robust method to evaluate METIs, and compare between them. We wish to characterize the fuzzy concept of METI using a set of measurable criteria, or, in other words – to operationalize METIs.

Operationalization of METIs may serve at least two goals: (1) it is a prerequisite for achieving a quantitative understanding of METIs and the factors affecting them, and (2) it may enable concrete comparisons between different METIs. Using a set of measurable parameters will make it possible to identify and evaluate biological entities in terms of their degree of change along the transitional stages

between one level of individuality and another. **Hence, the goals of this study were: (1) to propose an operationalization scheme for METIs based on a concise and robust set of complexity indices; (2) to apply this operationalization scheme to three groups of extant organisms at various stages along a transition, and use the results of this exercise to compare patterns of change during METIs between these groups and between two METI types; and, (3) to propose a general schematic framework for METIs based on the results.**

Operationalization schemes for transitions in individuality

Some comparative studies of METIs classify transition arbitrarily into two or three stages [9,26–28]. Few attempts were made to study METIs using a predefined set of quantitative parameters (=to operationalize METIs) [5,7,29]. McShea [7,12,30] proposed to characterize METIs using vertical complexity (sensu Sterelny [31]), recording the number of nested hierarchical levels present in an organism, and 'horizontal complexity' that determines the degree of individuation for the top hierarchical level. For horizontal complexity, McShea [7,32] proposed three parameters (connectedness, differentiation, and existence of intermediate parts), of which we adopt connectedness and differentiation. Queller and Strassman [33] proposed a plane that represents the level of organismality, whose axes are the level of cooperation and the (inverse of) level of conflict. Recently, Hanschen et al [29] proposed an operationalization scheme that characterized individuality based on eight parameters, targeting specifically the transition to multicellularity, and applied to the volvocine group as a case study. We adopt here two of their parameters, namely reproductive division of labor, and indivisibility. Two other criteria, genetic uniqueness and genetic homogeneity, do not to change during transition [29]. Two additional criteria, physiological unity and integration, and spatiotemporal boundaries, fit the transition to multicellularity, but application to insect social colonies is difficult since lower-level units are motile. Finally, two additional criteria, group-level adaptations and multi-level selection, are valuable as indicators of METIs in general; yet we did not include them in our scheme since they cannot be estimated directly but rather inferred from other, directly observable phenomena. Thus, our scheme for operationalizing METIs combines elements from the schemes of McShea [7] and of Hanschen et al [29].

Many scholars discuss the relations between evolution and complexity, particularly in the context of METIs, for example [3,5,6,25,30,34]. Apparently, **METIs are characterized by increasing complexity** along most of their various stages [3,6,34]. We therefore organize our scheme for operationalizing METIs around the concept of complexity, based on the assumption of an increase in complexity during a

METI. We hypothesize that an operationalization scheme based on a concise set of robust parameters may yield meaningful insights on processes that are common across different evolutionary lines and even across different METI types. The parameters selected for our scheme had to satisfy three criteria: (a) be general and applicable to any transition, (b) could be robustly estimated for various taxa, and (c) cover a unique type of complexity, unaccounted for by other parameters.

Methods

Combining elements from the schemes of McShea [7], Herrera-Paz [35], and Hanschen et al [29], we propose that four parameters can serve as a general operationalizable set for measuring complexity in biological systems: (a) the number of levels in a system [7,35], (b) the number of units in a level [30,36], (c) the number of different types of units, or the variation between units [35,37], and (d) the connectivity between units [35].

Each of these parameters represents an independent concept [30]. Taken together, they capture, at least partially, the elusive and multi-facet notion of complexity in biological systems. However, adjustments are required when adapting this set to any specific circumstances. In the case of major transitions, the following adaptations are needed: The first above-mentioned parameter, i.e., the number of levels in the system, is not relevant to our purposes, since this study concerns only two hierarchical levels that mark the beginning and the end of the transition. Hence, in our scheme, the first parameter is the *number of lower-level units* (indicative of the size of the system). Examples of this parameter are the number of cells in an organism, or the number of organisms in a eusocial colony. The next of the above-mentioned parameters, connectivity between components, is difficult to quantify directly; following [29], we propose *indivisibility*, which is an aspect of connectivity, as a corresponding complexity measure in the proposed operationalization scheme. Finally, the variation between units in a system was evaluated in our scheme using two separate parameters: reproductive specialization [29] (e.g., the distinction between somatic cells and gametes, or between workers and queen), and nonreproductive specialization [7] (e.g., the distinction between various tissues in an organism). We separated these two types of specialization because they can appear at very different stages during a transition and thus could be indicative of different degrees of complexity along the METI continuum. Nonreproductive specialization can be further quantified as a continuous variable, using the number of unit types as an indicator (e.g., the number of cell types). This measure of the variation between units has been commonly used to quantify complexity in living systems [25,36,38], in spite of conceptual- and technical issues, that were only partially solved [39]. While useful in documenting the increased

complexity during advanced stages of individuation, it is less informative in the context of the critical stages of a METI, when the new hierarchical level is being established. We thus do not include the number of unit-types in our scheme.

In the present study, the size of the system was considered a continuous variable. For the three other parameters - indivisibility, reproductive specialization, and nonreproductive specialization - estimating a specific continuous value across taxonomic groups and transition types is highly uncertain, given the present state of our knowledge. Thus, these parameters were recorded as present or absent.

- (a) **System size** (the size of the individual): The number of units within a given system, for example, the number of cells in an organism or the number of individual organisms in a eusocial insect colony.
- (b) **Indivisibility** is the incapacity of some of the system's components (cells, individuals, subgroups) to survive and complete their life cycle separately, independent of the more complex (higher-level) entities (e.g., the organism when separated from the colony). Ideally, this measure could be a continuous variable between zero and unity. However indivisibility is a binary variable here: very low probability (or frequency) of independent survival of lower-level entities is interpreted as complete indivisibility; otherwise complete divisibility (zero indivisibility) is inferred.
- (c) **Reproductive specialization** means that only certain units of the system (cells in an organism / individuals in a colony) specialize in reproduction. Here, reproductive specialization is marked as present when some units are capable of reproduction, while other units are entirely and irreversibly incapable of reproduction.
- (d) **Nonreproductive specialization** is the variability among units that is unrelated to reproduction. In an organism, it is the degree to which different cells specialize in different nonreproductive tasks. This feature is considered present if there are at least two distinct somatic cell types. In eusocial organisms it is considered present if there are at least two types of nonreproductive morphs.

Application to two types of METIs

We applied this operationalization scheme to characterize the location of specific taxa along a METI. We looked for taxa that could be grouped together coherently, in order to characterize intermediate stages along a major transition. Extant organisms representing intermediate stages of a transition pertain to only two types of METI: the transition to multicellularity, and the transition to eusociality. Both transitions are fraternal (sensu Queller [40]); the new higher-level individual is composed of similar and related units. The term 'intermediate' implies that organisms may have gone through part of the transition without completing it; we do not imply that these intermediates are precursors of other

organisms along the transition. A taxon was added to Table 1 if it satisfied two conditions: (1) it differs in at least one feature from all other records in its group already existing in the table. The rationale for this condition is to avoid inflating the table with records that are identical for all columns except the name of the species. (2) Enough data exists to reliably classify that taxon in all criteria.

The volvocines form “a league of their own,” with several species at various stages of “embryonic” multicellularity [41,42]. Thus, the volvocines were treated here as one general group. All other multicellular organisms constituted a second “general group.” From this second group, we selected four specific case studies (taxa), all of which have extant organisms that correspond to the entire range of the transition to multicellularity: (a) **Slime molds** live most of their life cycle as unitary cells, but they may aggregate and form multicellular reproductive structures [43–46]. (b) **Trichoplax** is a basal group of multicellular animals that lack organs and internal structure [47–49]. Recent studies provided conflicting evidence regarding the origins of Trichoplax, being either distant to all other animals [47], or closer than sponges to other animals [49]. (c) **Sponges** are often considered the most primitive multicellular organisms. (d) **Mammals**, which (in contrast to the other three taxa) developed relatively recently. [In spite of 1.4 billion years of divergence, all the organisms included in this general group have a single common ancestor, a eukaryotic cell, and a common structure, multicellularity \(in at least part of their life-cycle\). It is thus reasonable to include disparate organisms in the same group.](#)

Plenty of formations that correspond to intermediate stages of the transition to eusociality currently exist. We selected seven different taxa as case studies for the transition to eusociality Table 1 follows notions and specific examples from Table 1 in Bourke's 'Principles of social evolution' [26]. The large group of colonial marine invertebrates was omitted since an entire article would be required to seriously cover its great taxonomical, structural, and functional diversity; thus, their inclusion would render this paper a zoological survey rather than a conceptual proposition.

Results and Discussion

Some general features in the progression of METIs emerged from our analysis. System size increases across many orders of magnitude. The profound relations between system size and complexity was discussed before, concerning the inherent jump in organism size following a major transition [50] and concerning the increasing complexity within the top hierarchical level with increasing size [36,51–53]. There appears to be a strong relationship between system size and each of the other complexity parameters. For volvocine algae, for example, there are strong positive relations between the number of

cells and soma/germ ratio [54]. These relations between colony size and reproductive specialization were found to be related to the capacity for motility of the colony [55]. In our study, the relations between size and other indicators of complexity are demonstrated by the pattern of +/- signs that emerges in Table 1. Without exception, once a specific parameter (indivisibility, reproductive specialization, or nonreproductive specialization) appears in an organism, it also appears in all larger organisms within the same general group (Table 1). If the relationship between system size and each of the other parameters was weak or nonexistent, then the vertical sequence of +/- signs in each column in Table 1 would be random; as this sequence is perfectly ordered, it appears that the size of a biological system is an important predictor of the location of that system along a METI. Previous claims that the size of a colony is a major driver of the transition to multicellularity [4,36] and to eusociality [56], were made from a theoretical perspective; here we found a surprising and general empirical pattern that seems to confirm their hypothesis. Although size does not relate linearly to time, it is plausible that smaller systems appear earlier than larger systems, and thus it may be assumed that the order of appearance of systems is correlated with system size.

Table 1. Using complexity indicators to identify the location of biological entities along the METIs of multicellularity and eusociality.

Description of table columns appears at the beginning of the methods section.

General group	Case studies	System size (size of the individual)	Indivisibility	Reproductive specialization	Nonreproductive specialization
Volvocine Algae	Tetrabaena ^a	4	-	-	-
	Gonium sp. ^b	4-32	-	-	-
	Pandorina	8-16	+	-	-
	Eudorina	32-64	+	-	-
	Pleodorina ^b	128	+	+	-
	Volvox ^c	10 ⁴	+	+	-
Other Organisms	Slime molds	10 ⁶ ^d	- ^e	- ^f	-
	Trichoplax	10 ⁷ ^g	+ ^h	+ ⁱ	+ ^j
	sponges	10 ¹⁰ -10 ¹² ^k	+ ^l	+ ^m	+ ^m
	mammals	10 ¹⁰ -10 ¹⁵ ⁿ	+	+	+

^a Information on size, indivisibility, and specialization of Tetrabaena was derived from [99].

^b Information on Gonium and Pleodorina was derived from [100].

^c Size: up to 3000 for Volvox carteri [41] and up to 50,000 for Volvox sp [42]. Information on indivisibility and specialization of Volvox was derived from the same references.

^d The number of cells in fruiting bodies of slime molds is between 10,000 and 2,000,000 [43].

^e Some slime mold cells that do not aggregate, still survive and may recover when conditions improve [44], implying that aggregation is not mandatory for survival, and thus no indivisibility (but see 18).

^f During the aggregation phase, some of the cells form the stalk, while others become spores [43]. Yet, each cell has the potential to become either stalk or spore [46].

^g Average cell size ~ 10 micron, body size ~ 2X3X0.015 mm [101], yielding ~ 10⁸ cells.

^h Even a few cells can regenerate into a full organism [101].

ⁱ There is some evidence of bisexual reproduction [48,101].

^j Six different types of somatic cells were found in Trichoplax [102].

^k Sponges are a diverse group with highly variable sizes and shapes, and a large range of sizes is required to describe this group (10¹⁰ – 10¹³ cells per individual; Gitai Yahel and Sally Leys, personal communication).

^l Sponges have a complex body plan; a single somatic cell cannot regenerate to reconstruct a whole sponge; a piece of sponge containing all tissue types may regenerate in some species [103].

^m Sponges have separate germ- and somatic cell lines, and six distinct somatic tissues [104].

ⁿ This estimate is based on the number of cells in a human body, ~3.72*10¹³ [105], considering that mammal sizes vary over eight orders of magnitude, and cell sizes vary over one order of magnitude.

Table 1. continued

General group	Case studies	System size (size of the colony)	Indivisibility ^o	Reproductive specialization ^p	Nonreproductive Specialization ^q
Eusocial colonies ^r	Allodapine bees ^s	10 ¹	-	-	-
	Halictine bees ^s	10 ²	-	-	-
	Naked mole rats ^t	10 ²	-	-	-
	Social spiders ^u	10 ² -- 10 ³	-	-	-
	Bumble bees ^v	10 ²	+	+/-	-
	Vespinae wasps	10 ³	+	+	-
	Honey bees	10 ⁴ -10 ⁵	+	+	-
	Termites ^w	10 ⁶	+	+	+
	Ants	10 ⁶ -10 ^{9x}	+	+	+

^o In eusocial colonies, indivisibility is inferred when queen-replacement is not possible.

^p In eusocial colonies, reproductive specialization is inferred from the degree of queen-worker caste dimorphism and presence of sterile workers [26].

^q In eusocial colonies, nonreproductive specialization is inferred from worker polymorphism.

^r Data on eusocial colonies are based on Table 1 in [56], except where stated otherwise.

^s In Allodapine- and Halictine bees, an incomplete reproductive specialization exists, where workers may produce eggs in some circumstances [56,106].

^t In naked mole rats, reproduction is typically monopolized by a single queen in colonies of 100-300 individuals [57,58]. A queen can be replaced by any of the workers [59]. In spite of some task specialization, task switching is common [107], and in spite of size heterogeneity, there are no castes [108,109].

^u There are no known species of spiders with indivisibility or specialization [Lubin chapter in Shavit's book].

^v The queen Bumble bee is the sole producer of gynes, while queen and workers produce males [110]; reproductive specialization is therefore partial.

^w Data on termites taken from [111–113].

^x Ant megacolony may contain up to 10⁹ ants [114].

With the increase in system size, the three other complexity indicators appear gradually, in a similar (but not identical) order in the three groups inspected here (Table 1): In all three groups, indivisibility and reproductive specialization appear rather early (in systems of 10^2 units). In volvocine algae, indivisibility appears before reproductive specialization, while in the two other groups they appear together. In contrast, nonreproductive specialization appears much later (in systems $> 10^6$ units). This pattern is repeated in the two types of METI, suggesting that even this preliminary operationalization yields insights into the general causal *processes* that produced major evolutionary transitions. Moreover, this repeated empirical pattern allows us to propose a general theoretical scheme for the progression of METIs, consisting of four successive stages; any organism can then be identified as corresponding to one of these stages, using simple and easy-to-apply criteria (see below).

Indivisibility, the incapacity of single units to regain independence, is found even in an organism as simple as the 16-cell algae *Gonium*. It is found in all larger algae, and in all extant multicellular organisms. Indivisibility also appears in all insect colonies larger than 10^2 . Michod [9] claims that "Reproductive specialization is a major factor in the conversion of cell groups into true multicellular individuals. Once cells specialize in fitness components, they cannot survive and reproduce on their own; the group becomes indivisible, and hence, an individual". We fully agree with Michod, that indivisibility marks the stage in which a group of lower-level units becomes a higher-level entity ('individual'). . Once indivisibility is achieved, heritability and variation are automatically transferred to the higher level; selection at the lower level is meaningful only in the context of the newly established individual; the fate of this individual becomes the only determinant of the survival and fitness of each lower-level unit. Thus, indivisibility plays an important causal role in driving a METI from a collection of individuals into a new collective level of individuality. Indivisibility was used to define a transition before [6], but the view of indivisibility as a driver of the transition has been seldom mentioned in evolutionary transition models. A second proposition in the above quote is that reproductive specialization translates inevitably to indivisibility, since specializing cells cannot survive on their own. We agree that reproductive specialization must result in indivisibility, except in cases where indivisibility precedes reproductive specialization, as in the case of *Gonium* (Table 1) and possibly in the case of human societies (Carmel, forthcoming). In such cases, indivisibility In eusocial colonies, incomplete indivisibility (a worker may become a queen in rare occasions) and incomplete reproductive specialization are present in mole rat colonies [57–59], suggesting that a possible positive feedback loop between these two elements may have driven transitions forward to the irreversible point of complete indivisibility.

Therefore, we suggest that indivisibility is better understood as a causal facilitator of reproductive specialization rather than its byproduct.

The early appearance of indivisibility, as observed here, suggests that the issue of cheating does not necessarily play a crucial role in METIs. During a transition, if enough units act as free-riders it may dissolve the emerging cooperative system, yet cheating becomes more risky and less likely if the cheater cannot survive alone. Thus, our findings support Calcott's claim [60], that too much of the science of social evolution concerns the problem of cheating. Once indivisibility is attained, the fitness of cheating individuals essentially corresponds to the fitness of the new, higher-level individual, and the advantage gained through cheating behavior is either neutralized or becomes a disadvantage. There are other crucial issues that need to be resolved in order for the prospective entity to survive, chiefly the synchrony of replication [3,6][61,62]. Once indivisibility is established, a massive selection pressure develops towards solving these conflicts. The fact that most cancer types develop at old age could be attributed to selection pressure towards replicative synchrony.

METI progression scheme as a predictive model

Across three general groups and two METI types, both indivisibility and reproductive specialization were present in all systems $> 10^2$ (with the exception of slime molds, that are not strictly multicellular organisms), while nonreproductive specialization was found only in systems $\geq 10^6$. These findings allow us to outline a general scheme for progression of METIs, dividing the process into four distinct stages (Table 2). Indivisibility and reproductive specialization appear before nonreproductive specialization. Clearly, nonreproductive specialization could not appear prior to reproductive specialization since it would affect cell growth and division rate, translating immediately to reproductive specialization. Thus, organisms possessing the trait of nonreproductive specialization should also possess indivisibility and reproductive specialization. At least in the case of volvocine algae, indivisibility precedes reproductive specialization. It turns out that the size of the organism/colony can reliably indicate its METI stage in terms of the presence / absence of indivisibility and specialization (Table 2). This operationalized model can be applied to additional case studies (such as corals and bryozoans), for validation and comparison purposes.

Conclusion

The concept of “major evolutionary transitions” has been at the center of evolutionary research for the past three decades, yet not much has been done to practically operationalize this concept. This study

attempts to fill this lacuna. The parameters selected to operationalize METIs revealed a sequential chain of development, consistent across three large groups and two METI types. Indivisibility appeared in all cases either before- or together with reproductive specialization, indicating its crucial role in the process; it marks the turning point where a group of individuals becomes a new individual of a higher hierarchical level; moreover, by tying together the fitness of all inseparable lower-level units, and assigning it to the fitness of the newly emerged higher-level individual, indivisibility dictates conflict resolution, and may be a major driving force through the transition. This paper shows that operationalizing METIs makes it possible to compare different systems within the same transition type, as well as different transition types, yielding new and important insights. Finally, such operationalization allows us also to evaluate additional questions in a new way, such as the specific case of humanity in the context of METIs (Carmel, forthcoming).

Table 2. A general four-stage model for the progression of METIs.

Stage	definition	Size	Multicellular organisms		Eusocial insects		Proposed terminology
			description	Examples	description	Examples	
0	No indivisibility no specialization	10 ¹ -10 ²	Any cell may detach from the colony and revert back to unicellular life	Tetra-baena	Any individual in the colony may become a queen	Allodapine / Halictine bees	Aggregation
1	Indivisibility, no specialization	10 ¹ -10 ²	All cells can reproduce but cannot detach from the organism	Gonium	Not applicable	None	Colony
2	Indivisibility + reproductive specialization	10 ² -10 ⁶	Germ/soma separation Cells cannot detach from the organism	Pleodorina, Volvox	Queen-worker separation; neither phenotype can detach from the colony	Bumblebees, Vespinae, Wasps, Honey bees	Proto-organisms, individual
3	Indivisibility + reproductive and nonreproductive specializations	≥10 ⁶	Cells are completely dependent on- and controlled by the organism	All multicellular organisms	Same as above + differentiation into two or more nonreproductive phenotypes	Termites, Ants	Organism, individual

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References

1. Perrier E. 1880 New views of animal transformations. *Pop. Sci. Mon.* **40**, 625–640.
2. Dendy A. 1924 *Outlines of Evolutionary Biology*. New York: D. Appleton and Company.
3. Buss LW. 1987 *Evolution of individuality*. Princeton University Press, Princeton NJ.
4. Jablonka E. 1994 Inheritance systems and the evolution of new levels of individuality. *J. Theor. Biol.* **170**, 301–309.
5. Pettersson M. 1996 *Complexity and Evolution*. Cambridge, UK: Cambridge University Press.
6. Maynard Smith J, Szathmari E. 1995 *The major transitions in evolution*. Oxford, UK: Oxford University Press.
7. McShea DW. 2001 The hierarchical structure of organisms: a scale and documentation of a trend in the maximum. *Paleobiology* **27**, 405–423.
8. Calcott B, Sterelny K. 2011 *The major transitions in evolution revisited*. MIT Press.
9. Michod RE. 2007 Evolution of individuality during the transition from unicellular to multicellular life. *Proc. Natl. Acad. Sci.* **104**, 8613–8618. (doi:10.1073/pnas.0701489104)
10. Libby E, Rainey P. 2013 A conceptual framework for the evolutionary origins of multicellularity. *Phys. Biol.* **10**, 035001. (doi:10.1088/1478-3975/10/3/035001)
11. Hölldobler B, Wilson EO. 1990 *The ants*. Harvard University Press.
12. McShea DW, Changizi MA. 2003 Three puzzles in hierarchical evolution. *Integr. Comp. Biol.* **43**, 74–81.
13. Boardman RS, Cheetham AH, Oliver Jr WA, Coates AG, Bayer FM. 1973 Introducing coloniality. In *Animal colonies: development and function through time* (eds R Boardman, S Cheetham, WJ Oliver), pp. v–ix. Stroudsburg, Pa.: Dowden, Hutchinson and Ross, Inc.
14. Thomson JA, Geddes P. 1931 *Life: Outlines of General Biology*. London and New York: Harper & Brothers.
15. Beklemishev VN. 1970 *Principles of comparative anatomy of invertebrates*. Edinburgh.
16. Mackie GO. 1963 Siphonophores, bud colonies, and superorganisms. In *The Lower Metazoa* (ed E Dougherty), pp. 329–337. Berkeley, California: University of California Press.
17. Rosen BR. 1979 Modules, members and communes: a postscript introduction to social organisms.

- In *Biology and systematics of colonial organisms*. (eds G Larwood, B Rosen), pp. xiii–xxxv. London: Academic Press.
18. Wilson EO. 1953 The origin and evolution of polymorphism in ants. *Q. Rev. Biol.* **28**, 136–156.
 19. Wilson EO. 1975 *Sociobiology*. Cambridge, Massachusetts: MIT Press.
 20. Oster GF, Wilson EO. 1978 *Caste and ecology in the social insects*. Princeton, New Jersey: Princeton University Press.
 21. McSHEA DW. 2001 Parts and integration: consequences of hierarchy. In *Evolutionary patterns: growth, form, and tempo in the fossil record* (eds JBC Jackson, S Lidgard, FK McKinney), pp. 27–60. University of Chicago Press.
 22. McShea DW. 2002 A complexity drain on cells in the evolution of multicellularity. *Evolution (N. Y.)*. **56**, 441–452.
 23. McShea DW. 1994 Mechanisms of large-scale evolutionary trends. *Evolution (N. Y.)*. **48**, 1747–1763.
 24. Gould SJ. 1996 *Full House: The Spread of Excellence from Plato to Darwin*. London: Jonathan Cape.
 25. McShea DW. 2016 Three trends in the history of life: an evolutionary syndrome. *Evol. Biol.* **43**, 531–542.
 26. Bourke AFG. 2011 *Principles of Social Evolution*. Oxford, UK: Oxford University Press.
 27. Clarke E. 2014 Origins of evolutionary transitions. *J. Biosci.* **39**, 303–317. (doi:10.1007/s12038-013-9375-y)
 28. West SA, Fisher RM, Gardner A, Kiers ET. 2015 Major evolutionary transitions in individuality. *Proc. Natl. Acad. Sci.* **112**, 10112 LP – 10119.
 29. Hanschen ER, Davison DR, Grochau-Wright ZI, Michod RE. 2017 Evolution of individuality: a case study in the volvocine green algae. *Philos. Theory, Pract. Biol.* **9**.
 30. McShea DW. 1996 Perspective metazoan complexity and evolution: is there a trend? *Evolution (N. Y.)*. **50**, 477–492.
 31. Sterelny K. 1999 Bacteria at the High Table. *Biol. Philos.* **14**, 459–470.
 32. Anderson C, McShea DW. 2001 Intermediate-level parts in insect societies: adaptive structures that ants build away from the nest. *Insectes Soc.* **48**, 291–301.
 33. Queller DC, Strassmann JE. 2009 Beyond society: the evolution of organismality. *Philos. Trans. R. Soc. B Biol. Sci.* **364**, 3143–3155.
 34. Brooks DR, Wiley EO. 1988 *Evolution as entropy*. University of Chicago Press Chicago.
 35. Herrera-Paz EF. 2014 *Evolution to complexity. From unanimated matter to the superorganism*. CreateSpace Independent Publishing Platform.
 36. Bonner JT. 1988 *The evolution of complexity by means of natural selection*. Princeton University Press.

37. McShea DW, Brandon RN. 2010 *Biology's first law: the tendency for diversity and complexity to increase in evolutionary systems*. University of Chicago Press.
38. Valentine JW, Collins AG, Meyer CP. 1994 Morphological complexity increase in metazoans. *Paleobiology* **20**, 131–142.
39. McShea DW, Venit EP. 2001 WHAT IS A PART? In *The character concept in evolutionary biology* (ed GP Wagner), p. 259. San Diego, California: Academic press.
40. Queller DC. 2000 Relatedness and the fraternal major transitions. *Philos. Trans. R. Soc. London B Biol. Sci.* **355**, 1647–1655.
41. Kirk DL. 2005 A twelve-step program for evolving multicellularity and a division of labor. *BioEssays* **27**, 299–310. (doi:10.1002/bies.20197)
42. Herron MD, Hackett JD, Aylward FO, Michod RE. 2009 Triassic origin and early radiation of multicellular volvocine algae. *Proc. Natl. Acad. Sci.* **106**, 3254–8. (doi:10.1073/pnas.0811205106)
43. Bonner JT. 2009 *The social amoebae: the biology of cellular slime molds*. Princeton University Press.
44. Rainey PB. 2015 Precarious development: the uncertain social life of cellular slime molds. *Proc. Natl. Acad. Sci.* **112**, 2639–40. (doi:10.1073/pnas.1500708112)
45. Kuzdzal-Fick JJ, Foster KR, Queller DC, Strassmann JE. 2007 Exploiting new terrain: an advantage to sociality in the slime mold *Dictyostelium discoideum*. *Behav. Ecol.* **18**, 433–437.
46. Strassmann JE, Queller DC. 2011 Evolution of cooperation and control of cheating in a social microbe. *Proc. Natl. Acad. Sci.* **108**, 10855 LP – 10862. (doi:10.1073/pnas.1102451108)
47. Dellaporta SL, Xu A, Sagasser S, Jakob W, Moreno MA, Buss LW, Schierwater B. 2006 Mitochondrial genome of *Trichoplax adhaerens* supports Placozoa as the basal lower metazoan phylum. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 8751–8756. (doi:10.1073/pnas.0602076103)
48. Pearse VB, Voigt O. 2007 Field biology of placozoans (*Trichoplax*): distribution, diversity, biotic interactions. *Integr. Comp. Biol.* **47**, 677–692.
49. Srivastava M *et al.* 2008 The *Trichoplax* genome and the nature of placozoans. *Nature* **454**, 955.
50. Heim NA *et al.* 2017 Hierarchical complexity and the size limits of life. *Proc. R. Soc. B Biol. Sci.* **284**, 20171039.
51. Bell G, Mooers AO. 1997 Size and complexity among multicellular organisms. *Biol. J. Linn. Soc.* **60**, 345–363. (doi:10.1111/j.1095-8312.1997.tb01500.x)
52. Anderson C, McShea DW. 2001 Individual versus social complexity, with particular reference to ant colonies. *Biol. Rev.* **76**, 211–237.
53. Bonner JT. 2004 Perspective: the size-complexity rule. *Evolution (N. Y.)* **58**, 1883–1890.
54. Koufopanou V. 1994 The evolution of soma in the Volvocales. *Am. Nat.* **143**, 907–931.
55. Solari CA, Kessler JO, Michod RE. 2006 A hydrodynamics approach to the evolution of multicellularity: flagellar motility and germ-soma differentiation in volvoclean green algae. *Am. Nat.* **167**, 537–554.

56. Bourke AFG. 1999 Colony size, social complexity and reproductive conflict in social insects. *J. Evol. Biol.* **12**, 245–257.
57. Jarvis JU. 1981 Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. *Science (80-.)*. **212**, 571–573.
58. Faulkes CG, Bennett NC. 2013 Plasticity and constraints on social evolution in African mole-rats: ultimate and proximate factors. *Philos. Trans. R. Soc. London B Biol. Sci.* **368**, 20120347.
59. Clarke FM, Faulkes CG. 1997 Dominance and queen succession in captive colonies of the eusocial naked mole-rat, *Heterocephalus glaber*. *Proc. R. Soc. London. Ser. B Biol. Sci.* **264**, 993–1000.
60. Calcott B. 2011 Alternative patterns of explanation for major transitions. In *The major transitions in evolution revisited* (eds B Calcott, K Sternly), pp. 35–51. Cambridge, MA: MIT Press.
61. Maliet O, Shelton DE, Michod RE. 2015 A model for the origin of group reproduction during the evolutionary transition to multicellularity. *Biol. Lett.* **11**, 20150157. (doi:10.1098/rsbl.2015.0157)
62. Michod RE. 2011 Evolutionary transitions in individuality: multicellularity and sex. In *The major transitions in evolution revisited* (eds B Calcott, K Sterelny), pp. 169–197. Cambridge, Massachusetts: MIT Press.
63. Nussbaum MC. 1985 *Aristotle's De Motu Animalium: Text with translation, commentary, and interpretive essays*. Princeton, New Jersey: Princeton University Press.
64. Nachtomy O, Shavit A, Smith J. 2002 Leibnizian organisms, nested individuals, and units of selection. *Theory Biosci.* **121**, 205–230.
65. Wilson J. 1999 *Biological individuality: the identity and persistence of living entities*. Cambridge, UK: Cambridge University Press.
66. Clarke E. 2013 The multiple realizability of biological individuals. *J. Philos.* **110**, 413–435.
67. Pradeu T. 2016 Organisms or biological individuals? Combining physiological and evolutionary individuality. *Biol. Philos.* **31**, 797–817. (doi:10.1007/s10539-016-9551-1)
68. Strassmann JE, Queller DC. 2010 The social organism: congresses, parties, and committees. *Evolution (N. Y.)*. **64**, 605–616. (doi:10.1111/j.1558-5646.2009.00929.x)
69. Lewontin RC. 1970 The units of selection. *Annu. Rev. Ecol. Syst.* **1**, 1–18.
70. Hull DL. 1980 Individuality and selection. *Annu. Rev. Ecol. Syst.* **11**, 311–332.
71. Griesemer J. 2014 Reproduction and scaffolded developmental processes: an integrated evolutionary perspective. In *Towards a theory of development* (eds A Minelli, T Pradeu), pp. 183–202. Oxford University Press Oxford.
72. Dawkins R. 1976 *The selfish gene*. Oxford, UK: Oxford University Press.
73. Okasha S. 2006 *Evolution and the levels of selection*. Oxford University Press.
74. Oyama S. 2000 *The ontogeny of information: Developmental systems and evolution*. Durham, NC: Duke university press.
75. Jablonka E, Lamb MJ. 1999 *Epigenetic inheritance and evolution: the Lamarckian dimension*.

- Oxford University Press on Demand.
76. Odling-Smee FJ, Laland KN, Feldman MW. 2003 *Niche construction: the neglected process in evolution*. Princeton university press.
 77. Gilbert SF, Rosenberg E, Zilber-Rosenberg I. 2018 The holobiont with its hologenome is a level of selection in evolution. In *Landscapes of Collectivity in the Life Sciences* (eds S Gisis, E Lamm, A Shavit), p. 305. Cambridge, Mass.: MIT Press.
 78. Pradeu T. 2016 The many faces of biological individuality. *Biol. Philos.* **31**, 761–773. (doi:10.1007/s10539-016-9553-z)
 79. Hull DL. 1992 Individual. *Keywords Evol. Biol.* , 180–187.
 80. Godfrey-Smith P. 2009 *Darwinian Populations and Natural Selection*. Oxford University Press.
 81. Maynard Smith J. 1988 Evolutionary progress and the levels of selection. In *Evolutionary progress* (ed MH Nitecky), pp. 219–230. University of Chicago Press.
 82. Clarke E. 2014 Origins of evolutionary transitions. *J. Biosci.* **39**, 303–317.
 83. Shelton DE, Michod RE. 2014 Group selection and group adaptation during a major evolutionary transition: insights from the evolution of multicellularity in the volvocine algae. *Biol. Theory* **9**, 452–469. (doi:10.1007/s13752-014-0159-x)
 84. Gavrillets S. 2010 Rapid transition towards the division of labor via Evolution of developmental plasticity. *PLOS Comput. Biol.* **6**, e1000805.
 85. Simon B, Fletcher JA, Doebeli M. 2013 Towards a general theory of group selection. *Evolution (N. Y.)*. **67**, 1561–1572.
 86. Hamilton WD. 1964 The genetical evolution of social behaviour I, II. *J. Theor. Biol.* **7**, 1–52. (doi:10.1016/0022-5193(64)90039-6)
 87. Birch J. 2017 *The philosophy of social evolution*. Oxford, UK: Oxford University Press.
 88. Strassmann JE, Queller DC. 2007 Insect societies as divided organisms: the complexities of purpose and cross-purpose. *Proc. Natl. Acad. Sci.* **104**, 8619–8626.
 89. Michod RE. 2000 *Darwinian dynamics : evolutionary transitions in fitness and individuality*. Princeton University Press.
 90. Queller DC, Strassmann JE. 1998 Kin selection and social insects. *Bioscience* **48**, 165–175.
 91. Wilson EO, Hölldobler B. 2005 Eusociality: origin and consequences. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 13367–71. (doi:10.1073/pnas.0505858102)
 92. Wade MJ. 1978 A critical review of the models of group selection. *Q. Rev. Biol.* **53**, 101–114.
 93. Wilson EO, Hölldobler B. 2005 Eusociality: origin and consequences. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 13367–71. (doi:10.1073/pnas.0505858102)
 94. Hughes WOH, Oldroyd BP, Beekman M, Ratnieks FLW. 2008 Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science (80-.)*. **320**, 1213–1216.
 95. Queller DC. 1992 Quantitative genetics, inclusive fitness, and group selection. *Am. Nat.* **139**, 540–

- 558.
96. 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. (doi:10.1038/ncomms11370)
 97. Avital E, Jablonka E. 2000 *Animal traditions: Behavioural inheritance in evolution*. Cambridge University Press.
 98. Jablonka E, Lamb MJ. 2006 The evolution of information in the major transitions. *J. Theor. Biol.* **239**, 236–246. (doi:10.1016/J.JTBI.2005.08.038)
 99. Arakaki Y, Kawai-Toyooka H, Hamamura Y, Higashiyama T, Noga A, Hirono M, Olson BJSC, Nozaki H. 2013 The simplest integrated multicellular organism unveiled. *PLoS One* **8**, e81641. (doi:10.1371/journal.pone.0081641)
 100. Pennak RW. 1978 *Fresh-water invertebrates of the United States*. New York: John Wiley.
 101. Schierwater B, Eitel M, Osigus H-J, von der Chevallerie K, Bergmann T, Hadrys H, Cramm M, Heck L, DeSalle R. 2010 Trichoplax and Placozoa: one of the crucial keys to understanding metazoan evolution. In *Key transitions in animal evolution* (eds R Desalle, B Schierwater), p. 326. Boca Raton, Florida: CRC Press.
 102. Smith CL, Varoqueaux F, Kittelmann M, Azzam RN, Cooper B, Winters CA, Eitel M, Fasshauer D, Reese TS. 2014 Novel cell types, neurosecretory cells, and body plan of the early-diverging metazoan *Trichoplax adhaerens*. *Curr. Biol.* **24**, 1565–1572. (doi:https://doi.org/10.1016/j.cub.2014.05.046)
 103. Eerkes-Medrano D, Feehan CJ, Leys SP. 2015 Sponge cell aggregation: checkpoints in development indicate a high level of organismal complexity. *Invertebr. Biol.* **134**, 1–18. (doi:10.1111/ivb.12072)
 104. Bergquist PR. 1978 *Sponges*. University of California Press.
 105. Bianconi E *et al.* 2013 An estimation of the number of cells in the human body. *Ann. Hum. Biol.* **40**, 463–471. (doi:10.3109/03014460.2013.807878)
 106. Schwarz MP, Richards MH, Danforth BN. 2006 Changing paradigms in insect social evolution: insights from Halictine and Allodapine bees. *Annu. Rev. Entomol.* **52**, 127–150. (doi:10.1146/annurev.ento.51.110104.150950)
 107. Mooney SJ, Filice DCS, Douglas NR, Holmes MM. 2015 Task specialization and task switching in eusocial mammals. *Anim. Behav.* **109**, 227–233. (doi:10.1016/j.anbehav.2015.08.019)
 108. Thorley J, Mendonça R, Vullioud P, Torrents-Ticó M, Zöttl M, Gaynor D, Clutton-Brock T. 2018 No task specialization among helpers in Damaraland mole-rats. *Anim. Behav.* **143**, 9–24. (doi:10.1016/j.anbehav.2018.07.004)
 109. Van Daele PAAG, Desmet N, Adriaens D. 2018 Social organization and biting performance in *Fukomys* mole-rats (Bathyergidae, Rodentia). *bioRxiv*, 325720.
 110. Amsalem E, Grozinger CM, Padilla M, Hefetz A. 2015 The Physiological and Genomic Bases of Bumble Bee Social Behaviour. *Adv. In Insect Phys.* **48**, 37–93. (doi:10.1016/bs.aiip.2015.01.001)

111. Thorne BL. 1997 Evolution of eusociality in termites. *Annu. Rev. Ecol. Syst.* **28**, 27–54. (doi:10.1146/annurev.ecolsys.28.1.27)
112. Engel MS, Grimaldi DA, Krishna K. 2009 Termites (Isoptera): their phylogeny, classification, and rise to ecological dominance. *Am. Museum Novit.* **3650**, 1–27. (doi:10.1206/651.1)
113. Nalepa CA. 2015 Origin of termite eusociality: trophallaxis integrates the social, nutritional, and microbial environments. *Ecol. Entomol.* **40**, 323–335. (doi:10.1111/een.12197)
114. Giraud T, Pedersen JS, Keller L. 2002 Evolution of supercolonies: the Argentine ants of southern Europe. *Proc. Natl. Acad. Sci.* **99**, 6075–6079.

Appendix 1. Defining individuality

The concept of biological individuality and the physical organization and dynamics it denotes has been debated among scientists and philosophers for centuries, from Aristotle's "Parts of Animals" [63] to Leibniz's "monad" (explained in [64], to the "Biological Individuality" [65]. Some aspects of these aforementioned debates found their way to discussions of METIs [27,66], such as the major division between functional and evolutionary definitions of an "individual" [67]. Functional definitions focus on physiological aspects of the individual as a distinct, well-delineated and cohesive unit. Functional individuality focuses on the question of unity: how do distinct components constitute a cohesive unified whole, functioning collectively as a single and regulated unit that persists through time [67,68]. Individuality is often defined in an evolutionary context [16,69,70]. In this context, an individual is a unit characterized by its capability to reproduce [71], and by its degree of heritability and fitness [69], with a possible extension to multi-level selection perspective [Folse and Roughgarden 2010]. Some evolutionary views of individuals focus only on one of the above-mentioned aspects, e.g., individuals as replicators [72], or individuals as interactors [66,73]. A special class of views concerns individuals as interactive parts of a collective system – including a developmental system [74,75], an ecological niche [76], and a holobiont [77]. Queller and Strassman (2009) define an organism as a unit with high cooperation and very low conflict among its parts [x]. Other approaches to individuality also exist [78][Santelices 1999]. For the purpose of the current discussion, **an individual is defined here broadly as a unique entity that is capable of autonomous survival and reproduction**. This definition is meant to serve as a convenient and tentative proposition; it combines functional- and evolutionary elements. It follows from this definition that individuality may appear simultaneously in more than one hierarchical level (such as in the organism and its cells, [69,78,79]. Also, it should be noted that individuality is a quantitative trait [80] rather than a binary (present or absent) trait. We realize that this definition is too narrow to capture all aspects of individuality, and too vague to be useful in precise mathematical models or philosophical definitions, but we believe it is a useful definition in the context of METIs. A comprehensive understanding and integration of the various facets of individuality is yet to be developed [78].

Appendix 2: Social evolution and METIs

How these transitions came to be is a matter of much engagement and debate. A central question concerns the problem of individuality in the sense of multicellular organisms: Organisms live to reproduce, and giving up prospects of reproduction contradicts the organism's most basic interests [81]. In Maynard Smith's words, *'How did natural selection bring about the transitions from one stage to another, since at each transition, selection for 'selfishness' between entities would tend to counteract the change... how is it that selection at the lower level does not disrupt integration at the higher level?'* [81]. In view of this difficulty, several explanations were proposed, based on conceptual descriptions [9,10,26,73,82], on empirical observations and experiments, mainly on volvocine algae [41,83], and on mechanistic mathematical models [84,85]. The relative weight of kin selection vs. group selection is still hotly debated.

The inclusive fitness theory [86] along with its many adjustments and applications for the praxis of scientific modeling is currently one of the most widely accepted explanations of social behavior in general [87,88], including the change in individuality that occurs through major evolutionary transitions [9,26,89]. An ongoing debate is the role of genetic relatedness between colony members in the transition process; more specifically, the question raised is whether genetic relatedness causes [26,90] or is a consequence of [91] METIs to eusociality. Some researchers suggest that in the initial phases of eusociality in insects group selection was crucial [92,93], and others maintain that kin selection played an essential role in the formation of insect societies [26,94]. Yet, most researchers agree that the mathematical models of kin and group selection are mutually translatable [95], while differences concern the spatial and genetic setting within and between organisms [87,92]. It is difficult to obtain evidence of processes that started 10^7 years ago and continued for 10^6 years or more (but see [96] for an indication of strong group selection in the early stages of the transition to multicellularity). In general, it seems that the transition to a stable social group requires that the individuals that form it must inherit the same behavioral information; this information need not be transmitted through DNA; it can also be transmitted through social learning [97,98].