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# Nascent multicellular life and the emergence of individuality

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The evolution of multicellular organisms from unicellular ancestors involves a shift in the level at which selection operates. It is usual to think about this shift in terms of the emergence of traits that cause heritable differences in reproductive output at the level of nascent collectives. Defining these traits and the causes of their origin lies at the heart of understanding the evolution of multicellular life. In working toward a mechanistic, take-nothing-for-granted account, we begin by recognizing that the standard Lewontin formulation of properties necessary and sufficient for evolution by natural selection does not necessarily encompass Darwinian evolution in primitive collectives where parent-offspring relationships may have been poorly defined. This, we suggest, limits the ability to conceptualize and capture the earliest manifestations of Darwinian properties. By way of solution we propose a relaxed interpretation of Lewontin's conditions and present these in the form of a set of necessary requirements for evolution by natural selection based upon the establishment of genealogical connections between recurrences of collectives. With emphasis on genealogy – as opposed to reproduction – it is possible to conceive selection acting on collectives prior to any manifestation of heritable variance in fitness. Such possibility draws attention to the evolutionary emergence of traits that strengthen causal relationships between recurrences – traits likely to underpin the emergence of forms of multiplication that establish parent-offspring relationships. Application of this framework to collectives of marginal status, particularly those whose recurrence is not defined by genealogy, makes clear that change at the level of collectives need not arise from selection acting at the higher level. We conclude by outlining applicability of our framework to loosely defined collectives of cells, such as those comprising the slugs of social amoeba and microbes that constitute the human microbiome.

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## 1. Introduction

The first multicellular life forms, the cyanobacteria, emerged from free-living photosynthetic cells ~3 billion years ago (Baldauf 2003). Over subsequent eons, transitions from single-celled organisms to multicellular types occurred on multiple

occasions with spectacular examples evident among plants, fungi and metazoans (Bonner 2000; Grosberg and Strathmann 2007).

Each transition began with free-living entities (cells) that by virtue of their capacity for differential reproduction were units of selection (Lewontin 1970). As such, they evolved by Darwinian processes (Godfrey-Smith 2009). During the

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transition to multicellularity, collectives of cells emerged that came to participate in evolutionary processes in their own right (Maynard Smith and Szathmary 1995; Michod 1999; Okasha 2006). This involved a hierarchical shift in the level of selection and with it the emergence of new kinds of biological individuals – individuals whose success very often curtailed the independent evolution of lower level entities (Buss 1987; Godfrey-Smith 2009).

The transition from single cells to multicellularity is but one example of a more general class of phenomena in which autonomously replicating lower level entities are transformed by natural selection to become ‘parts’ of new higher level structures (Bonner 1974; Buss 1987). Together such events have become known as ‘major evolutionary transitions’ (Maynard Smith and Szathmary 1995; Jablonka and Lamb 2006; Okasha 2006). The evolution of chromosomes (from genes) marks one such instance. During this transition, autonomously replicating genes came together to form chromosomes. On completion, genes replicate solely as part of the higher level structure, namely, the chromosome. The evolution of the eukaryotic cell from two once free-living bacterial-like cells exemplifies a further such transition (Maynard Smith and Szathmary 1995).

Our interest is the evolutionary transition from single cells to multicellular life. While there exists a rich theoretical framework (see for example Okasha 2006), this framework says little about the underlying mechanistic and selective causes. What is needed is critical thinking – backed by plausible biological detail – surrounding the earliest events underpinning the evolutionary emergence of Darwinian individuality, that is, the emergence of traits that confer upon nascent multicellular forms the ability to participate in the process of evolution by natural selection (Michod 1999; Griesemer 2000; Godfrey-Smith 2009; Rainey and Kerr 2010; Bouchard and Huneman 2013; Libby and Rainey 2013a; Clarke 2014). From a theoretical perspective the emergence of such characteristics means that collectives manifest some component of heritable variance in fitness (Okasha 2006; Godfrey-Smith 2009; Clarke 2014). From a practical perspective this requires that heritable variance in fitness at the level of collectives be measurable.

Typically, fitness is quantified by reproductive capacity, that is, by the number of viable offspring produced. In paradigmatic biological populations, of sexual or asexual types, parents and offspring are related by causality (Godfrey-Smith 2009): if parents are eliminated, then there are no offspring. In this sense reproduction is the endogenous capacity of parents to leave offspring. Offspring are genetically related to their parents – there exists genetic similarity by descent. We nonetheless recognize that subtle distinctions can be made regarding the role of development and heritability in the context of reproductive capacities (Griesemer 2000; Bourrat 2014a).

Even though the concept of parenthood may be extended to situations in which offspring are the descendants of multiple parents, it lacks intuitive appeal. This is particularly so in instances where successive recurrences of collectives lack any causal connection and arise not through endogenous reproductive capacity of a ‘parent’, but through the action of external factors that, for example, cause particles within a collective to periodically fission and then to randomly (in space and time) fuse with cells from a multiplicity of earlier recurrences, to form a new collective.

Because such fission and fusion events may have been relevant to the earliest types of multicellular life (Bonner 1998; Tarnita *et al.* 2012), we step back from the paradigmatic view of organismal reproduction. Starting with the simplest conceivable collectives of reproducing cells, we consider properties of nascent multicellular organisms from the perspective of the kinds of evolutionary processes in which they may have participated. In minimal form, such collectives are likely to have lacked recognizable organismal characteristics. For example, integration among cells may have been non-existent, the boundaries between generations were likely blurred, heredity minimal, and capacity for collective-level reproduction entirely absent. Given such characteristics, attempts to establish the existence of heritable variance in fitness at the level of collectives is fraught with difficulties, since it requires that there exist parents and offspring that can be identified, measured and counted.

To progress we advocate relaxation of the central ingredients for evolution by natural selection. Rather than variation, differential reproduction and heredity (Lewontin 1970), we suggest a minimal set of necessary conditions that comprise identity, recurrence and genealogy. This minimal recipe, we argue, is applicable to populations of collectives lacking Darwinian status and is thus useful when thinking about the emergence of the kinds of traits and functional innovations necessary for selection to have some chance of working at the level of nascent collectives. Arising from the application of this framework comes the recognition of the importance of traits that strengthen causal relationships between recurrences – traits that ultimately find expression in processes such as reproduction, which are likely to have driven emergence of Darwinian individuality.

## 2. Darwinian individuality

The set of necessary and sufficient conditions for evolution by natural selection requires that entities vary one to another (there is variation), they reproduce (fitness is understood as reproductive output), and offspring show some resemblance to the parent types (there is heritability) (Lewontin 1970; Hull 1980; Godfrey-Smith 2009). Provided that some component of variation affects reproductive success, then a population endowed with these Darwinian properties will evolve

by natural selection: the population is a Darwinian population and its members are Darwinian individuals. The population manifests heritable variance in fitness – a property apparent by virtue of differences in reproductive output.

The notion of Darwinian individuality is central: the evolutionary transition from single cells to multicellularity begins with a population of individual cells replete with Darwinian properties. The transition is complete when the focal population comprises collectives (of cells) that are themselves Darwinian individuals and members of Darwinian populations. In moving from single cells to multicellular types, selection shifts level, from individuals to collectives (Michod 1999; Okasha 2006).

However, selection cannot simply choose to shift. Any shift depends on the emergence of Darwinian properties at the level of nascent collectives. Understanding the evolution of multicellularity therefore requires mechanistic understanding of how Darwinian properties emerge from the most basic of cellular collectives – collectives that on first emergence may have lacked any such properties (Libby and Rainey 2013a). To quote: ‘*what kind of change constitutes a first step in the transition process?*’ (Clarke 2014). Nowak and Ohtsuki (2008) make a similar case for the origin of replication in prebiotic life.

We place particular emphasis on the need for mechanistic thinking. Without such thinking it is possible to fall into the trap, as we and others have emphasized (Griesemer 2000; Rainey 2007; Rainey and Kerr 2010; Libby and Rainey 2013a; Clarke 2014), of invoking Darwinian properties as the cause of their own evolution. Moreover, as experimental incursions into the origins of multicellularity mount (Herron and Michod 2008; Herron *et al.* 2009; Ratcliff *et al.* 2012; Ratcliff *et al.* 2013; Hammerschmidt K, Rose C, Kerr B and Rainey PB, unpublished), attention is increasingly drawn to details, the resolution of which stand to fuel understanding (Rainey and Kerr 2010). Central to this knowledge-gathering process is recognition that Darwinian properties existing at the level of cells cannot be assumed to automatically transition to the level of collectives; rather, Darwinian properties evolve afresh at each transition and, as such, they require evolutionary explanation (Griesemer 2000; Rainey and Kerr 2010).

### 3. Detecting Darwinian properties in nascent collectives

Our focal interest is the nature and properties of the earliest traits (and range of ways) that provide opportunity for selection to transition from cells to collectives. Drawing upon the Lewontin criteria for evolution by natural selection (Lewontin 1970; Godfrey-Smith 2009), and with the aim of identifying first steps on the road to multicellularity, it is necessary to know whether a newly found phenotype does cause progression along this path. This requires an assay by which the potential for evolutionary change at the collective-

level can be judged. The prevailing view is that any such assay should aim to determine whether the trait or behaviour of interest results in heritable variance in fitness – at the level of collectives (for example, Clarke 2014). In principle this is simple and requires nothing more than identifying collectives at one point in time and then counting offspring collectives at some future time point. Assuming that ‘like begets like’, then any difference in reproductive output among collectives constitutes evidence of heritable variance in fitness at the level of collectives.

For paradigm Darwinian populations in which individuals are highly integrated units, where there are clear boundaries and where generations are separated by bottleneck events, demarcation of individuals is straightforward. Observation alone allows identification of parents and offspring: differences in reproductive output among variant types can thus be readily determined. Heritability can be checked via, for example, genetic fingerprinting. While clear-cut for paradigm populations, the earliest phases in the transition to multicellularity may not have involved assemblages conforming to paradigm Darwinian status (Libby and Rainey 2013a).

Imagine groups of cells formed by eddies and currents in a stream. Observation shows a lack of integration among the parts: cells from one group merge with others or are lost. The groups themselves are ephemeral and do not ‘reproduce’ in any recognizable manner. While groups are born and die, groups existing at the present time share little relatedness with groups existing at some earlier time. Determining whether there is any heritable variance in fitness is thus challenging because ‘parents’ and ‘offspring’ are neither easily defined nor counted.

This hypothetical example might seem extreme, but in reality there are numerous extant organisms that are of less than paradigm status. Take, for example, social amoebas, whose life cycles proceed through a multicellular differentiated phase. It is of interest to know the extent to which natural selection acts on slugs of *Dictyostelium discoideum* (as opposed to the cells that comprise the slug stage). The starting position is manageable: slugs can be identified and counted, but slugs are aggregates that contain heterogeneous mixtures of lineages (Kaushik and Nanjundiah 2003; Gilbert *et al.* 2009; Sathe *et al.* 2010). The slug develops into a sessile stalk that becomes capped with spores; spores disperse and eventually germinate into individual cells that exist in isolation for unknown numbers of generations. Depending on environmental cues, cells may aggregate to once again generate genetically heterogeneous slugs. While slugs recur, there is a blurry (at best) parent-offspring relationship. In fact it does not make much sense to talk about slug reproduction given the ambiguity surrounding the nature of the parent-offspring relationship. The answer to whether there exists heritable variance in fitness at the level of slugs seems

uncertain. As such, the question as to whether there exist slug-level adaptations as a consequence of selection operating – even to a marginal extent – at the level of slugs, is difficult to answer.

It is possible though that analysing populations for evidence of heritable variance in fitness is the wrong way to proceed. Perhaps there is a more suitable assay; perhaps, even a more suitable way of posing the question. What if the recipe for evolution by natural selection could be expressed in terms such that heritable variance in fitness was not presented as the defining requirement? Lewontin's formulation, although intended as general and minimalist, is based on observations of extant organisms, most of which are of paradigm Darwinian status. For organisms of non-paradigm status, particularly those where recurrences of a collective phase involves blending of genetically distinct parts, evidence of selection operating at the level of collectives cannot be gained (or refuted) by measuring heritable differences in fitness, because the evolving entities lack the qualities that the definition requires be measured (Bouchard 2008).

One possibility is that collectives that lack heritable variance in fitness simply cannot participate in evolution by natural selection (at the collective-level). This is indeed true when each recurrence of a collective is formed from a random assemblage of cells. Under such circumstances, exemplified by trait group models (Maynard Smith 1964; Wilson 1975), properties of the collectives that are derived from their particle composition are not heritable. But it seems reasonable to envisage situations where recurrences comprise a statistically biased sample of types present in earlier recurrences. Such a bias, repeatedly attained, stands to establish a genealogy, and with it a causal relationship linking occurrences of collectives at different time points (Sober and Wilson 1998). This possibility – and its potential significance – is discussed further below, but at this stage it is necessary to ask whether there are alternative approaches for dealing with the problem we pose, namely, a way of determining whether some newfound trait contributes to the emergence of Darwinian individuality and whether, therefore, a component of selection can be attributed to some evolutionary process taking place at the collective-level.

One approach is to draw upon the Price equation (Price 1970, 1972, 1995): the multilevel formulation allows the partitioning of variance into components attributable to effects at collective- and individual-levels. If the covariance between collective-level fitness and collective-level traits is positive, then a component of fitness is attributed to selection acting at higher levels (Okasha 2006; Clarke 2014).

While the Price equation is a useful means of detecting change, it says nothing about the properties of the entities of interest or the kinds of processes the focal entities might participate in (Simon *et al.* 2012). For instance, the equation

contains no expression for birth or death, at either particle, or collective-levels, and no terms for selection (at either level). Given our advocacy for a mechanistic stance, a statistical approach that pays no concern to properties or process is unlikely to be helpful when it comes to elucidating key causal events in the transition from cells to collectives. Nonetheless, as a way of detecting change due to some higher level process, the Price approach offers possibility. The trouble is, the multi-level Price approach partitions variance in a way that presupposes the existence of reproduction at the collective-level. Fitness may thus be mistakenly attributed to higher level processes when in fact no such higher level processes operate (Okasha 2006; Godfrey-Smith 2009).

Although this shortcoming of the Price approach is recognized (Okasha 2006) – and solutions advocated (see Okasha 2006) – it is helpful to place it in the context of a simple model that represents the most ephemeral of collectives of the kind that could exemplify early stages in the evolution of multicellular life, and whose evolution involves a simple life cycle that parallels that of the social amoeba (Garcia and De Monte 2013). In their model Garcia and De Monte define two types of particle (cell), each of which is endowed with a different propensity to aggregate and form collectives (for example, due to the production of adhesive glues). The more adhesive type is referred to as a 'sticky' type. Sticky particles increase the quality (in terms of coherence) of the collective they belong to, at the cost of an unconditional fitness loss associated with production of the glue. Less sticky particles derive benefit from joining collectives containing sticky types.

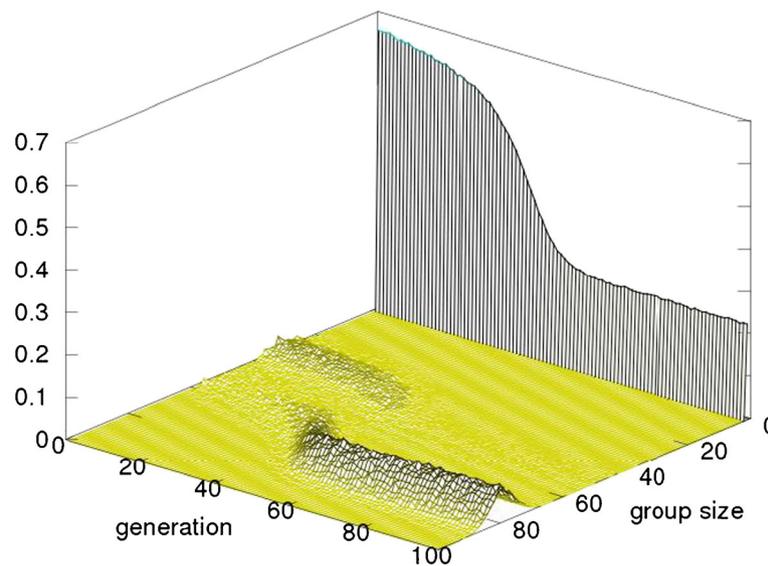
The life cycle involves a period where all particles are randomly distributed, followed by a phase where collectives are formed based on defined rules of aggregation. The model is conceptually similar to standard trait group models (Maynard Smith 1964; Wilson 1975), with the difference being that the 'stickiness trait' influences both the properties of collectives, and the way individual particles are assorted. Once in collectives, particles play a (linear) public-goods game that determines the number of offspring cells (and their aggregation phenotype) based upon the proportion of sticky types within collectives. To complete the cycle, particles within collectives again disperse, so that in the following generation groups are reformed by a random re-sampling from the population of particles. Details of the toy model for aggregation are described elsewhere (Garcia and De Monte 2013).

In the model, random mixing of particles between two successive phases of group formation means that while the collectives may be identified as being of the same kind, the ancestry of particles reveal distributed patterns of descent. If one were to attempt to identify parent-offspring relationships at the collective-level based, for example, on genetic

fingerprinting of particles within groups, then groups derived by random re-sampling of the total (infinite) population would be composed of unrelated particles. Nonetheless, in the model both population composition and group size distribution change in time as a consequence of the coupling of ecological and evolutionary dynamics. An increase in the proportion of sticky particles results in an increase in the average size of collectives. In turn, this further favours sticky types by virtue of the fact that larger groups contain on average a larger proportion of sticky cells. Over evolutionary time, once the fraction of sticky particles exceeds a threshold, group size distribution steadily displaces towards larger group sizes (figure 1). Eventually, a steady-state distribution is attained, where groups of different sizes coexist with a class of lonely individual that do not attach to any group. Different particle types exist both in groups and alone. Such collective-level change, driven by differences in group composition, naturally suggests the operation of selection on some collective-level Darwinian trait, for example, group

cohesiveness. Indeed, particle-level fitness is assessed based on a property of the collective, namely, the fraction of sticky particles within its given group. Looking only at the time-evolution of group size distribution, it would be entirely reasonable to conclude that this was the product of collective-level reproduction, that is, groups of larger size left more group-level offspring relative to groups of smaller size.

Analysis of the evolution of groups of given sizes using the multi-level Price approach also supports the view that change is due to selection operating at the level of collectives. To see this, imagine observing groups following each aggregation phase of the life cycle. If the population of collectives is divided in size classes, it is possible to compute the fitness of classes in terms of variation in the number of groups contained within each class (if groups beget groups of the same size, then such a measure would equate to average reproduction rate). At the same time, each class is characterized by an average fraction of sticky individuals,



**Figure 1.** Dynamics of the distribution of group size frequencies in a population of  $2^{16}$  particles, 15% of which were sticky at the initial time point. At every generation the particles form groups within randomly formed patches of size  $T=100$ : particles are serially given the possibility to stick to a randomly chosen ‘recruiter’ particle and attach to it according to fixed probabilities associated with the nature – sticky or less so – of the particles involved. Particles subsequently reproduce according to the payoff gained by playing a public goods game within each group (with benefit-to-cost ratio of 20). The descendants are eventually pooled together before the aggregation process begins anew in the following generation. Details of the model are provided in Garcia and De Monte (2013). A singleton component of the distribution, corresponding to particles that are not part of any group (along the generation axis, for unitary group size), coexists with a component containing particles that form groups. Groups progressively displace toward higher sizes. If particles were observed exclusively within groups of a given size class, then it would be apparent that in the previous generation such particles may have existed either as members of groups, or as singletons. Were an entire size-class to be eliminated it would not matter: in the next generation the size-class would be re-populated from a mixture of group-forming and singleton-particles, of either type. As a consequence, a parent-offspring relationship between groups cannot be established. A network of descent can be established between particular groups by connecting those sharing particle lineages, but this does not result in a collective-level genealogy. Indeed, groups of one given size will not be preferentially connected to any other size class in the previous generation, since all groups are obtained by random re-sampling of the population of particles: lineages assort randomly.

which can be defined as a ‘collective-level’ trait. The trait value and fitness of such classes co-vary in the population, which reflects the fact that groups of larger size tend to be enriched in sticky particles. This leads to the conclusion that changes in group composition are driven by between-collective selection.

The beauty of mechanism-based studies (including explicit models) is the opportunity provided to gain insight into biological details underpinning transitions. Because properties of particles and the processes governing the life cycle in the Garcia and De Monte (2013) model are explicit, it is possible to look for explanation of collective-level processes. In this example, by construction, while collectives are born and die at every recurrence, there is no parent-offspring relationship at the collective level and heritability at the collective-level is marginal at best. Despite our intuition, and findings arising from a Price analysis, there is no possibility of selection acting at the level of collectives – all changes are a consequence of selection on individual particles and mediated by variation among groups. If the average fitness advantage of sticky types across the entire population were computed, then this would differ from the average fitness of sticky types found solely within groups, an example of what is known as Simpson’s paradox (Haldane 1932; Wright 1945; Wade 1978) (for experimental verification, see Chuang *et al.* 2009). Were groups to beget groups directly, then the evolutionary outcome would be very different. Imagine that collectives generate exact copies of themselves with a rate proportional to the average stickiness of the composing particles. Under this scenario, lonely particles would entirely disappear and only groups containing the largest proportion of sticky particles would survive.

At the heart of disquietude surrounding the appropriate level of selection is the concept of fitness and how it should be measured (Bouchard 2008). Even though fitness is a central concept in evolutionary theory, there is no generally agreed way of quantification, particularly in instances where knowledge of the appropriate spatial and temporal scale necessary to describe a specific scenario is lacking. This is particularly problematic in situations where the distinction between parent and offspring is not clear. Even if exact knowledge of the parent-offspring relationship is substituted by causal relationships revealed by fitness covariance of properties (Godfrey-Smith 2009), there remain difficulties as to the appropriate time scale at which a system should be observed (Bourrat 2014b). In order to progress we suggest focusing attention on properties that are both observable, and measurable, and evident in both organisms with clearly defined parent-offspring relationships, and those of marginal status. This, we suggest, is collective-level genealogy, which sets the basis for evaluating collective-level inheritance.

#### 4. A relaxed formulation of Lewontin’s criteria

In wanting to understand the kind of change and the underlying causes of that change responsible for the earliest manifestations of Darwinian individuality (at the level of collectives), it is necessary to be able to detect such change. It is apparent that the standard Lewontin formulation of properties necessary and sufficient for evolution by natural selection may be impossible to assay in populations of collectives where genealogical connections between recurrences are the result of random mixing among particles. Under such conditions, heritability at the collective-level is minimal. This does not mean that in such population selection necessarily fails to see higher level properties; rather, it raises the possibility that the criteria upon which evidence is sought may be inadequate for the intended purpose. If true, then it is likely that this limits our capacity to conceptualize and capture the earliest manifestations of Darwinian properties.

By way of stimulating thought, we advocate a relaxed formulation of the Lewontin conditions based upon the extent of genealogical connection between recurrences of collectives. With emphasis on genealogy – as opposed to reproduction (where there exists a direct causal relationship between parent and offspring) – it is possible to conceive selection acting on collectives prior to the emergence of distinct collective-level lineages. Accordingly, 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. It is important to note that unlike the Lewontin criteria which are both necessary and sufficient for evolution by natural selection, our formulation establishes a set of conditions that are necessary but not sufficient. This renders our formulation particularly suited to the earliest manifestations of multicellular life.

1. *Identity: a criterion for delimiting collectives.* For a collective to be counted it must be recognizable on the basis of a minimal set of characteristics. The existence of boundaries, or physical proximity, are typical criteria used to identify multicellular aggregates. However, configurations of loosely connected particles, where groups exist as temporary clusters, may also respond to selection by virtue of their collective-level properties (see also the ‘existence’ category of Libby and Rainey 2013a). The identity criterion implies a choice on the spatiotemporal scale appropriate for observing the collective and defining the equivalence class of objects ‘of the same kind’. As such, it incurs in the limitations, and a degree of subjectivity, typical of clustering problems.
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. This makes it possible to compare the number and properties of the collectives at two different time points. Recurrence does not imply the need for collective-level reproduction, but can be a mere consequence of some process re-occurring at the level of interacting particles, even if this is driven by a forcing exogenous to the population. For instance, if particles periodically aggregate and then disperse due to a change in one environmental parameter that drives the population across a phase transition (Newman 2012), they will form collectives time and again without there being any causal dependence on the state of the collectives at a previous time. Such collectives, recognizable by means of the criterion of spatial proximity, will recur, making it possible to compare properties at two different times. In particular, statistics about single-particle properties can be opportunely weighted according to the degree of assortment within groups. It is important to recognize that a recurrence relationship is more general than reproduction and need not imply a genealogical relationship between collectives at two distinct times. The choice of time interval is critical: there may be different ways of quantifying and interpreting collective-level changes, depending on the choice of the observation times. For collectives with an endogenous or exogenous cycle, 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. The case where nothing varies at the collective-level between times  $t$  and  $t'$ , even if conceivable, is unlikely to be interesting from the point of view of the emergence of a higher level of selection. In the general case where the formation of collectives is imperfectly controlled, aleatory effects will naturally result in variability among collectives. This is likely to be especially true in ephemeral groups, where variability is assured given the existence of heritable variation among the constituent cellular particles, and the contingency of aggregation of particles of which collectives are comprised.

3. *Genealogy: the possibility of identifying the precursor(s) of a recurrence, based on the sharing of particle lineages among collectives across successive recurrences.* A causal genealogical relationship can be said to exist whenever there is a nonrandom assortment of particle lineages between recurrences of collectives (there is no requirement for individual particles to have multiplied between recurrences). Such genealogical relationships can arise through numerous simple mechanisms that promote assortment. At the level of one particular group, descendent groups may be represented

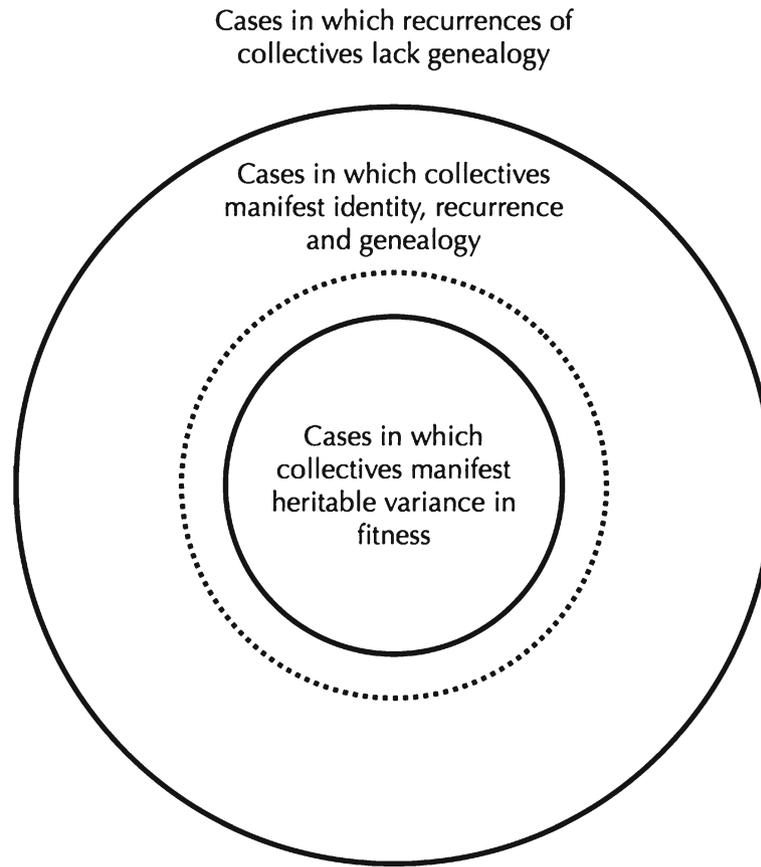
by an edge in a network connecting two collectives that contain particles belonging to the same lineage. Edges can be weighted in order to reflect the number of such shared lineages (for instance, a recurrence may comprise 30% of the cells found in a given recurrence of the previous generation, and by 50% of the cells in another, while the remaining 20% may have originated from particles previously unassociated with any collective). Genealogy is however a property of recurrences, that is of equivalence classes of alike collectives, over which the descendants of individual groups are averaged. The genealogical relationships thus define heritability of collective-level traits.

This formulation of minimal necessary requirements for evolution by natural selection is not intended to compete with, or be counter to, the widely accepted version articulated by Lewontin (1970); rather, it is intended to be fully compatible with this perspective, but more relaxed. Indeed, collectives endowed with the properties of variation, reproduction and heredity, such as paradigmatic metazoan organisms, clearly display identity, recurrence and genealogy. Thus, for paradigmatic metazoans, our genealogically motivated formulation adds nothing new.

One way of conceptualizing the relationship between our relaxed conditions and those of Lewontin is as a Venn diagram in which the Lewontin conditions are nested within those advocated here (figure 2). Collectives that recur, but where there is no genealogical relationship between recurrences (for example, where each recurrence is a random sample of particles from a global pool), fall outside the class of systems encompassed by our criteria. Progression from the outer to the inner circle involves moving from a state where evolution by natural selection may occur given identity, recurrence and genealogy (a set of necessary conditions) to one where evolution by natural selection will occur given heritable variance in fitness (a set of conditions both necessary and sufficient).

It is of interest to consider the possibility that fitness at the higher level might be defined by inclusion of a further (not currently recognized) condition, additional to those proposed here, which is qualitatively similar to higher-level reproduction, but applicable to more general settings. If so, then a sufficient condition to capture fitness heritability would lead the inner and dashed circles to coincide, although such a condition may result in a broadening of scope of the inner circle. If not, then this would suggest that the Lewontin conditions are necessary and sufficient in only those instances where collectives beget collectives and where there is a direct causal relationship between parent and offspring.

The three conditions of identity, recurrence and genealogy have the virtue of being applicable to cases where parent-offspring relationships are poorly defined, such as instances where collectives are formed by aggregation of particles. Moreover, the revised framework can be applied – in a measurable sense – to the most ephemeral and improbable of collectives. Utility lies in the fact that collectives can be



**Figure 2.** Venn diagram showing relationship between the relaxed formulation of conditions necessary for evolution by natural selection advocated here and those of Lewontin, which are both necessary and sufficient. Lying outside the circle are collectives that lack necessary conditions for evolution by natural selection (at the level of collectives), for example, collectives in which the constituent particles bear no genealogical relationship between recurrences. Trait group models with random mixing between recurrences would fall within this category. In the context of the relaxed conditions (outer circle), it is conceivable that inclusion of a further condition, additional to identity, recurrence and genealogy, may allow collective-level fitness to be defined in a qualitatively similar manner to higher-level reproduction. Populations satisfying four such conditions would be contained in the dashed circle. If those conditions were equivalent to Lewontin's ones, but with definition of fitness broader than reproductive success, then this would lead the inner and dashed circles to coincide, but may also result in a broadening of scope of the inner circle.

assayed for the existence of properties necessary for selection to operate among collectives. But perhaps more important is the fact that by emphasizing a genealogical perspective, rather than one that accords sole significance to heritable differences in reproductive output (defined by recognizable parent-offspring relationships), concepts of the earliest collectives expand. No longer is the investigator constrained by the need to think in terms of collectives manifesting evidence of heritable variation in fitness. Indeed, according to a genealogical perspective, any mechanism that promotes genealogical assortment among particles, and where that bias is evident across at least two recurrences of a collective, provides some opportunity for selection to operate at the higher level. Genealogy sets the framework for evaluating heredity at the collective level:

different genealogies support the transmission of collective properties to a different degree.

### 5. Scenarios for Darwinian evolution of nascent collectives

To draw attention to the utility of the formulation of conditions necessary for evolution by natural selection we return to consideration of the simplest possible collectives. A useful starting point is with collectives that satisfy both the existence and recurrence criteria, but lack genealogical connections between recurrences. Such a group might be represented by cells within a stream as discussed earlier and idealized in trait group models (Wilson 1975; Godfrey-

Smith and Kerr 2009; Cramer *et al.* 2012; Garcia and De Monte 2013; Garcia *et al.* 2014). Lack of collective-level genealogy – which can be determined by genetic assay of the particles – means that such groups can evolve by selection acting at the particle level, but possibilities for the evolution of collective-level adaptations is marginal. Although our interest is ultimately in the emergence of collectives with the capacity to evolve by natural selection, we do not treat as insignificant the importance of evolutionary change in groups in the absence of higher level selection. Such change, wrought by external factors, or via evolution of particles within collectives, may have played an important role in the emergence of collectives with Darwinian properties (Lynch 2007; Libby and Rainey 2013a).

Of many potential first steps toward the emergence of collective-level individuality are those that lead to assortment of genotypes and thus the establishment of lineages that persist through multiple recurrences of collectives. Imagine, for instance, the ephemeral aggregates formed in streams, but which when reformed bear some relationship to an earlier recurrence. For instance, it would be possible to modify the model of Garcia and De Monte (2013) so that patches of size  $T$  are formed due to complementation of the group at an earlier occurrence with individuals randomly drawn from singletons that are part of a global population. This choice mimics dispersal of ‘lonely’ particles across patches. When groups are formed again after reshuffling particles within one patch, a fraction of the particles composing groups at two successive times belong to the same lineage: a genealogical relationship thus exists. The genealogy thus established permits the possibility that at least some component of the evolution of collectives is Darwinian in nature.

Analogously, if slugs of *Dictyostelium* occurring at two successive times share a number of particle-level lineages, as a consequence of incomplete mixing of strains, then advantages provided by the slug stage can evolve by natural selection at the collective-level. Numerous routes can be envisaged that bring about assortment of particle-level lineages. Indeed, the existence of genealogical relationships are most likely the rule rather than the exception. Adhesive polymer production is one readily achievable way to ensure that cells of a single genotype have greater probability of remaining together (Spiers *et al.* 2002; Rainey and Rainey 2003; Velicer and Yu 2003). Predation is known to favour group formation (Boraas *et al.* 1998), as is incomplete cell division (Koschwanez *et al.* 2013). The extent to which clumping genotypes are represented in successive recursions might vary considerably. Nonetheless, provided there exists a genealogical connection between aggregates, a component of selection has the opportunity to shift to the level of collectives. Such a shift, although possibly marginal, stands to feed back in a positive manner favouring mutant types that

have enhanced lineage-forming tendency (assuming that glue production and formation of groups delivers some higher level benefit) (Crespi 2004).

Taken to a logical end point, genotypes that comprise recurrences of collectives at one point in time may eventually come to be the sole seed for future recurrences, even to the extent that future recurrences become direct genetic descendants of past recurrences. At such a time it makes sense to refer to future recurrences as offspring, or descendants, of earlier recurrences: heritable variance in fitness may thus eventually manifest at the level of collectives.

## 6. Discussion

There are multiple ways that collectives comprising individual Darwinian entities can evolve. Not all of these ways require, or imply, that collectives need be Darwinian units in their own right. Heritable variation in fitness at the level of the composing entities alone (for example, cells) can lead to changes at the level of collectives – even in the absence of selection working at this level. On the other hand, if collectives are identifiable, recur and there exists a genealogical relationship between recurrences, then collectives stand to be seen, to some extent, by selection. This holds even in those instances where causal parent-offspring relationships at the collective-level are absent.

As soon as a mechanism comes into existence that ensures the recurrence of collectives, it is highly likely that some genealogy is established (only when particles are randomly mixed between two occurrences will genealogy be absent). However, selection will operate at the higher level only if the composing particles provide to the collective a property that affects the rate of reproduction of the particles themselves.

The possibility of identifying a genealogy at the level of collectives depends on knowledge of the individual particles of which collectives are comprised. This is not something that can be deduced by observation and measurement of the properties of collectives alone. Statistical approaches like the Price equation rely, for instance, on successive observations of collectives and not on knowledge of the mechanistic processes underlying their recurrence. They thus treat equally cases where collectives beget collectives, and cases where collectives are re-generated by unrelated particles (where the particles bear no genealogical relationship between recurrences of collectives). The distinction between these scenarios is important and has implications for how collective-level fitness and heredity is defined.

The three factors that we have discussed here (identity, recurrence and genealogy) define a minimal set of conditions that, when manifest at the level of collectives, allows the possibility of Darwinian evolution at the level of collectives. The formulation encompasses both paradigmatic organisms

of asexual and sexual kinds, where the three factors are trivially present, and organisms of more marginal Darwinian status where parent-offspring relations are poorly defined, such as in the cellular slime molds. Moreover, the formulation applies to egalitarian as well as fraternal transitions (Queller 2000): it does not matter if the particles composing the collective are of the same or of different nature, as long as their association is recursive and a genealogy is established.

Collectives formed as ephemeral aggregations of moving particles, although marginal in extant organisms, may have been important in the transition from unicellular to multicellular life. Such intermediate – and indeterminate – life forms, endowed with a degree of heritability much weaker than what is typical among extant organisms, could nonetheless have been raw material upon which selection might have acted (at the collective-level). Progressively more efficient Darwinian machines are likely to have emerged given the potential for positive feedback between natural selection and organismal forms, through innovations such as life cycles, mechanisms for suppressing particle-level conflicts, and perhaps, most of all, development (Buss 1987; Griesemer 2000; Pepper and Herron 2008; Newman 2012). Indeed, it is interesting to consider the possibility that certain organismal configurations might represent stable states that, irrespective of the starting position, will be achieved time and again, given the feedback between form and selection (Crespi 2004). One such stable state would seem to be reproduction via a single-cell stage.

In the context of feedback it seems likely that certain organismal configurations are seen more potently by selection than others. These may simply be a consequence of the nature of the genealogical relationships they underpin. For example, reproduction via single-cell bottlenecks ensures high heritability and minimizes genetics conflicts within collectives (Buss 1987; Wolpert and Szathmari 2002). Nascent organisms that ‘discover’ such modes of reproduction will participate more effectively in evolution by natural selection compared to nascent types that reproduce by fissioning (where heritability is compromised). Similarly, certain early-found innovations may position collectives on particular evolutionary paths, rendering the emergence of higher levels of complexity either more, or less, likely (Libby and Rainey 2013a). For instance, mechanisms generating self-sustained life cycles offer opportunity for temporal compartmentalization and may play a central role in the evolutionary emergence of development. In fact, we would go as far as to suggest that life cycles that proceed via single-cell bottlenecks have been instrumental in driving the evolution of development – development itself driven by the selective advantage of reconstituting the collective phase following transition through a single-cell stage (Griesemer 2000; Libby and Rainey 2013b).

The approach that we have taken here, which is to present a ‘take-nothing-for-granted’ view on the evolutionary transition from single cells to the earliest self-replicating multicellular organisms appears distinctly gradualist. We emphasize, however, that we do not believe that evolution must necessarily go through all the intermediate steps of progressive strengthening of genealogy until paradigmatic modes of reproduction are achieved. Quite the opposite (cf. Clarke 2014). While it is possible to envisage multicellularity emerging in small steps with, for example, additional quanta of heritability being added in successive stages (as outlined above), it is also feasible that certain small steps can radically improve the potency of nascent Darwinian machines. For example, a collective that acquires the capacity to reproduce via a single-cell stage (Rainey and Kerr 2010; Libby and Rainey 2013a) – a possibility demonstrably achievable in a small number of mutational steps (Herron and Michod 2008; Ratcliff *et al.* 2012; Ratcliff *et al.* 2013; Hammerschmidt K, Rose C, Kerr B and Rainey PB, unpublished) – is likely to result in sudden and discontinuous transitions to stronger integration and the emergence of a degree of organismality that allows selection to shift in a significant manner to the higher level. Should collective-level reproduction proceed via a dedicated germ line phase, then the discontinuous gain stands to be even greater (Rainey 2007). In neither of these instances of ‘abrupt’ transition is the capacity for reproduction a derived trait and product of selection acting at the higher level. Neither is it necessary to invoke higher-level selection to explain the evolution of such capacities. Small phenotypic changes at the level of even the earliest groups can have remarkable consequences for selection and its capacity to transition. Indeed, as has been argued previously, life cycles, which can arise in a plausibly small number of steps and from the most basic of ingredients, can (and will) cause selection to move from cell to collective-level in an abrupt manner: the ‘handles’ provided by certain innovations being a ‘gift’ to selection.

Finally, although our aim in suggesting a relaxation of the standard Lewontin conditions has been to facilitate an expanded view of the earliest stages in the evolution of multicellular life, it has more general relevance. Features of early forms of multicellular life in which parent-offspring relationships were poorly defined are found in many extant organisms. The cellular slime molds discussed above are but one example.

Of current relevance is understanding the extent to which the communities of microbes that colonize tissues of multicellular organisms are units of selection. Various microbiome projects (Turnbaugh *et al.* 2007) reveal relatively stable communities of microbes, colonizing, for example, the digestive tract of humans (Turnbaugh *et al.* 2009). To what extent are these communities seen by selection? To

what extent do they manifest adaptations that are a consequence of selection at the level of collectives (Gardner and Grafen 2009; van Baalen 2013)?

A typical stance would be to ask to what extent do these communities manifest heritable variance in fitness. But, as pointed out above, this requires the existence of measurable parent-offspring relationships. While the microbial community of the mother is in part passed to the baby via the birth canal, babies also acquire microbes from a range of other sources. Indeed, acquisition of new members continues throughout life (Ley *et al.* 2008). To some extent the microbial communities of the human gut might be considered units of selection, particularly those that pass through the female line, but how is it possible to know? And, what about the possibility that the unit of selection might encompass households, communities, even nations?

Our relaxation of Lewontin's conditions provides a way forward: the issue is whether there exists a genealogical relationship between recurrences. This can be addressed at different temporal and spatial scales. All that is necessary is a means of sampling and a means of genotyping. Both are possible. Indeed the data likely currently exist.

It is of more than passing interest to know the extent to which selection sees microbial communities and their hosts. For example, if selection does see such collectives, then adaptations at the collective-level are likely to exist (Okasha 2006; Gardner and Grafen 2009; van Baalen 2013). Such collective-level traits may be critical determinants of health and disease (Cho and Blaser 2012). But interest in understanding the extent to which selection sees collectives goes well beyond humans and our gut microbiomes – it sits at the heart of the evolution of biological complexity.

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