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Individuality and the Control of Life Cycles

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Introduction

The units of evolution have themselves evolved. In some cases, one unit of evolution becomes integrated into a larger one and loses much of its autonomy. For instance, single-celled organisms evolved into multicellular organisms, insect colonies evolved out of cooperation among individual insects, and eukaryotes evolved out of a symbiotic relationship that formed after one prokaryote engulfed another (Maynard Smith and Szathmáry 1995). Biologists call these events evolutionary transitions in individuality, and together each transition builds on another to form a compositional hierarchy of the units of evolution (Buss 1987; Maynard Smith and Szathmáry 1995; Calcott and Sterelny 2011). At the most general level, evolutionary transitions matter because the nature of biological individuality is essential to understanding the nature of life and the scope of the biological sciences. The particular challenge posed by the transitions is to explain how new kinds of biological individuality, composed out of modified versions of preexisting individuals, actually evolved and persisted over time.

Any method for approaching this problem faces a number of distinctive challenges, of which I list only three here. One is level neutrality: the method must be able to explain how and why evolutionary transitions happened and persisted at each level of the compositional hierarchy. It must, for instance, work as well for explaining the transition from genes to chromosomes, prokaryotes to eukaryotes, and single-celled to multicellular organisms. Another challenge is that the method must be able to determine whether something is an individual or not.[1] In the context of the evolutionary transitions, this is necessary to evaluating whether a transition has happened and to what degree. A third challenge is to address a range of different explanatory questions. We want to know how and why the transition occurred, along with why

the new kind of individual persisted over time. Moreover, we will also want to explain what happened after the transition in terms of how the transition occurred. For example, some transitions lead to a diversification of subtypes within the new kind of individual, such as after the formation of eukaryotes, while other transitions result in a comparatively static form.

The current, dominant approach to explaining evolutionary transitions, multilevel selection (MLS) theory, answers aspects of these challenges using mathematical models from population genetics. For example, an MLS model can address whether an evolved cooperative trait among single cells in a larger group can become universal and remain stable given its regular loss through mutation as well as competition between cells (Michod 1997). Variations on such a model can also address other problems, such as the benefits and costs of a multicellular individual reproducing using propagules made of one or many cells. Moreover, MLS theory provides a principled way for determining whether individuality exists at some level in terms of whether we can ascribe fitness to groups at that level (Okasha 2006).

However, I will argue that MLS theory does not provide a complete, self-sufficient approach to theorizing about evolutionary transitions. As a formal, mathematical theory about evolution within a population, it presupposes but does not address the material structure of the population that realizes the model. An MLS model might tell us whether a cooperative trait could become fixed in a population, for example, but it won't be able to explain how the cooperation actually works to produce an adaptive effect on the group's fitness. It also won't be able to account for the sources of variety in the possible modes of cooperation available to a population. MLS theory can tell us when fitness has transferred from one level of units to another, but it can give misleading answers unless we have some other, principled guide for picking out units (Clarke 2012; Clarke 2014). Furthermore, it is commonly acknowledged among biologists that actually measuring the fitness of an individual—sometimes even obtaining and interpreting proxy measurements—is difficult in practice and prone to error (Hendry 2005; Orr 2009). Hence even if MLS theory were sufficient in principle, there would still be room for other approaches in practice that avoided the difficulties and limitations of depending on fitness alone.

I will also argue for a positive complement to MLS theory based on the material and causal structures that are responsible for the control of events within an individual's life cycle. I introduce the concept of a demarcator as a material entity or causal process responsible for a biological individual's nature as a complex whole that is composed out of a set of parts.[2] As defined here, a demarcator is a necessary participant in key events of an individual's

life cycle, such as in reproduction, and it also serves as a focal point for the control of the life cycle overall. That is, the causal processes that influence where, when, and how different events in a life cycle occur do so by acting through or on the demarcator. I will show how this perspective allows us to individuate biological entities based on their possession of one or more demarcators and the extent to which these demarcators are focal points for control.

Besides not defining individuality in terms of fitness, one benefit of the demarcator approach is systematizing and explaining the causes of evolutionary variation, including constraints. I will pursue this point in one direction here, focusing on how demarcators provide a novel way of thinking about the control of inheritance. In particular, I show how using demarcators allows us to derive a version of Griesemer and Wimsatt's concepts of material overlap and scaffolding as pathways for heredity (Wimsatt and Griesemer 2007; Griesemer 2000a and b, 2002, 2014). In addition, I show how material overlap and scaffolding can apply to two different cases in the evolution of multicellularity, focusing on how a multicellular group can exert causal control over the functional states of its cellular parts. Although much work remains to be done to establish demarcators as an approach to individuality, in the conclusion I discuss the possibilities for a pluralist stance on biological individuality that is based on commensurate but distinct ways of defining the domain of biology.

The Multilevel Selection Framework

Given that evolutionary transitions pose deep problems for classical evolutionary theory, how can we go about explaining the evolution of the compositional hierarchy of biological individuals? Focusing on the process of a transition itself, Ellen Clarke has recently split this larger issue into three subproblems (Clarke 2014): How does the transition happen? Why does it happen? How is it maintained?[3]

The dominant framework for explaining evolutionary transitions, MLS theory, answers aspects of these subproblems using mathematical models from population genetics. I will not attempt a general review of MLS theory and its place in the larger controversies about group selection, since the general theory and debate have only limited relevance here and would take us too far afield. (For more discussion, see Okasha 2005; Leigh 2010.)

What questions, then, can multilevel selection theory answer about evolutionary transitions? Richard Michod's paradigmatic work on the evolution of multicellularity over the past two decades provides a convenient set of ex-

amples (e.g., Michod 1996, 1997; Michod and Roze 1999; Michod et al. 2003). The most basic question the model can address is the problem of maintaining the higher-level unit: whether evolved cooperation among single cells (the lower-level units) can become fixed in the population given loss of the cooperative trait through mutation and competition between cells (Michod 1997). The multilevel character of the model comes from specifying a life cycle structure, shown in Figure 3.1, which in this case features obligatory multicellular development from a single founder cell and reproduction through the dispersion of gametes. The multicellular individuals produce more gametes when they contain more cooperative cells, but the cooperative cells pay a price of slower reproduction within the group compared to defectors (free-riders or "cheaters" that benefit from the other cells' cooperation but do not contribute themselves). If the defectors come to dominate the group, then the cooperative trait is less frequent in the gametes produced and may be lost in future generations.

Variations on this model can also address other issues relevant to the subproblems identified above, such as the benefits and costs of a distinct germline or unicellular genetic bottleneck (Grosberg and Strathmann 1998, 2007). The issue of the unicellular genetic bottleneck—whether it's easier to maintain cooperation when multicellular organisms reproduce using single-celled zygotes or multicellular propagules—addresses an important dimension of the "How?" problem. The origins of cooperation have received less attention in the literature (Calcott 2007), but MLS modeling can assess how strong a

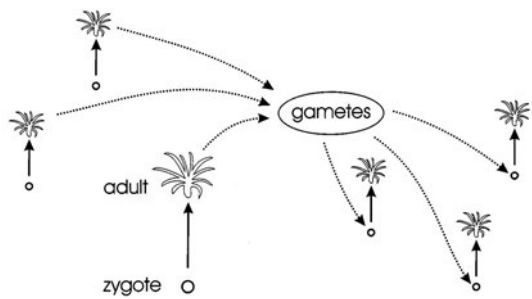

FIGURE 3.1. A multicellular life cycle. Illustration of a life cycle used by Richard Michod to specify a multilevel selection model. The cycle involves an obligatory stage as a zygote, which then develops into a multicellular adult. The adult produces gametes that generate new adults by a process that can vary in the mathematical model (not illustrated here). If reproduction is asexual, for example, these gametes are equivalent to the zygote, while if reproduction is sexual, two gametes would combine to form the zygote. During the life cycle, natural selection can operate at two levels, between single cells during the growth of the adult form and between multicellular adults. Modified from R. E. Michod, "Cooperation and Conflict in the Evolution of Individuality. I. Multilevel Selection of the Organism," *American Naturalist* 149, no. 4 (1997): 609, fig. 1.

source of beneficial cooperation would have to be to drive a transition, for example.

Another, more general function of MLS theory is as a tracking device for measuring the progress of a transition (Griesemer 2007). In other words, it helps us determine what stage a transition is in and how different events advance it as a process. Samir Okasha has shown the theory's utility in this regard using the multilevel Price equation, a formalism widely adopted in quantitative applications of MLS theory (Okasha 2006; also see Clarke 2014): given a choice of the lower- and higher-level individuals, the Price equation allows us to determine at which levels fitness is properly distributed. The idea, then, is that at the start of a transition the higher level is not a unit of selection that carries fitness, while at the end the lower level has lost most or all of its relevance for selection.

Individuation Mechanisms

However, considering Michod's models also illuminates what MLS theory cannot supply for the study of evolutionary transitions: the material structure of any specific case that serves to realize or specify the model. For example, we saw how Michod's model presupposed a particular life cycle structure. Moreover, the models don't tell us anything about the range of possible cooperative traits that might play a role in the transition, or the internal or external conditions under which different mechanisms for generating benefits are available. These are not weaknesses in the modeling work itself, but rather a reminder that MLS theory is not sufficient on its own to explain all of the questions we have about transitions. (For arguments related to the one I make here, see De Monte and Rainey 2014; and Winther 2006, 2009.)

In particular, MLS theory can tell us the degree to which fitness has transferred from one level of units to another, but it doesn't tell us which units to pick out in the first place (Clarke 2012, 2014). "Note that [the Price] equation itself does not tell us anything about how to choose these groups—they must be defined before the multilevel analysis can be applied" (Clarke 2014, 6). In order to interpret the abstract MLS framework and apply it to a concrete case, Clarke suggests that we look for causal mechanisms in the world that give objects the capacity to act as units of selection. Any mechanisms that contribute to an object's capacity in this way serve as "individuating mechanisms" for defining the units in an MLS model.

She goes on to provide criteria for what would qualify as an individuating mechanism based on Richard Lewontin's classic analysis of the units of selection (Lewontin 1970). "Selection can act on a collection to produce

evolution only if its members vary heritably for some trait that affects their fitness," so individuating mechanisms define a collection's capacity for selection by "influencing the amount of genetic variance it contains; influencing the extent to which that genetic variance causes variance in fitness within the collection; [or] influencing the heritability of the genetic variance, or of the fitness effects" (Clarke 2013, 428). A similar set of functional criteria for individuating mechanisms would also apply for non-genetic inheritance processes. Examples of individuating mechanisms include genetic bottlenecks, separation of the germ line from somatic cells, fair meiosis, and the immune system (Clarke 2013).[4]

While the idea of an individuating mechanism is very useful in general, it faces several difficulties. In another paper, I argue that focusing on the capacity for selection as the only type of capacity for individuality is problematic (Sterner 2015). For example, a population of individuals may possess a high capacity for undergoing selection that nonetheless does not translate into adaptation because frequency-dependent effects block the trait from going to fixation.

Another option, which I will explore here, is to focus on the causal structure of the life cycle, including reproduction and development. This approach would draw on methods in molecular cell biology, developmental genetics, and comparative genomics, among others. The approach assumes that the living systems of interest share a general life cycle process whose causal structure can be analyzed without requiring the measurement or estimation of fitness.

Control of Life Cycles

I have argued so far that MLS theory is not sufficient in itself to explain all of the relevant questions scientists have about evolutionary transitions. What alternative to fitness could there be as a foundational concept for a level-neutral approach to defining and studying individuality? We would be looking for one or more functional capacities that can be realized in a variety of ways, such that its multiple realizability serves as its source of generality across levels. Moreover, there needs to be some objective criteria against which we can benchmark the degree of individuality ascribed to an object. The capacity of living things in a population to undergo selection, for example, tracks their importance for explaining and predicting evolutionary change over time.

Ideally, we would also have a precise theory for the degree of individuality that tells us how individuality changes as different mechanisms affecting the relevant functional capacity are gained or lost. Interaction effects between

mechanisms would likely be important, and we should anticipate that nonlinearity will be present as well.

In this section, I will start to develop an alternative theory of individuality using a functional-capacity-and-multiple-realizers schema that does not depend on fitness. The core concept that substitutes for a unit of selection in this manner is what I will call a demarcator, which distinguishes the parts of an individual from non-parts. The main significance of this distinction is that different classes of causal interactions will be possible among parts, among non-parts, and between parts and non-parts. Something will be a biological individual when it possesses at least one demarcator and this demarcator is a focal point for the causal control of key events in its life cycle. A material object or causal process is a focal point for control to the extent that the variation possible within a given life cycle can be explained by the following: a) the focal point's effects on what becomes a part of the object in question; b) its effects on what causal interactions are possible among parts, among non-parts, and between the two; and c) changes in the properties that underlie these two sets of effects that happen during the course of the life cycle. Causal processes will then count as individuating when they contribute to making the demarcator a focal point for control.[5]

My aim here is not to present a self-enclosed, purely theoretical definition of biological individuality. Rather, my goal is to show how the nature of individuality is an empirical problem that goes beyond ascertaining which entities can carry fitness or explaining how cooperation can be adaptive or maintained. I aim to show how one can theorize about biological individuality outside the domain of MLS theory by focusing on certain capacities of biological individuals that MLS theory presupposes but does not address.

In order to take this positive step, we will need to make the key assumption that a definition of biological individuality should allow us to say which things in the world are parts of an individual and which are not. Note that some ability to demarcate parts and wholes is essential to specifying any MLS model. Michod's model above, for example, depends on assigning cell lineages to particular multicellular groups in order to evaluate the effects of within-group conflict. More generally, evolutionary transitions produce new individuals that are composed of parts that were or still are individuals to some degree. Explaining how the higher-level individual evolved must involve some way of specifying the parts of which it is composed. A degree of fuzziness in distinguishing parts from non-parts is allowable so long as some things clearly do count as parts of a given individual and others do not, but I will set this issue aside for now for the sake of simplicity.

Instead of looking at the capacity of a kind of object to undergo selec-

tion over generations, we will examine the causes of variation for key events within the kind's life cycle. Reproduction is a particularly essential event in this regard. (I have something quite minimal in mind for the term reproduction here, so that it includes "making more of" an object by processes like fragmentation, similar to Maynard Smith's notion of multiplication [Griesemer 2000b].) Any new instance of a life cycle begins with a reproduction event that occurs during an already ongoing life cycle process. Generally, the entity we identify with the new life cycle won't yet be capable of reproducing. We can think of this period, between genesis and first reproduction, as the process of development. Griesemer, for example, defines development in terms of the acquisition of the capacity to reproduce (Griesemer 2002). The most familiar way for a life cycle to end is through death, for example by predation, disease, aging, or accident. A life cycle can also end in reproduction, however, if the parent is not preserved as an individual during the event. For example, when a cell divides symmetrically in half, biologists treat both of these cells as daughters of the parent rather than identifying one as the parent and the other as the offspring.

Consider the definition of life cycle the biologist John Tyler Bonner has given for multicellular organisms. Bonner describes four major stages: a single-cell stage, a period of growth and development, a period of maturity, and a period of reproduction (Bonner 1993, 17–18). The last two may coincide, but for many species the period of reproduction ends before the period of maturity, leading to senescence (i.e., aging).[6]

In order to investigate whether an object should count as a biological individual, we need to start by identifying a recurrent pattern of events that plausibly corresponds to a life cycle and then examine whether the pattern can be explained in terms of the effects of one or more demarcators. That is, we start with empirical observations of a repeating sequence of events that are connected together as a causal process. We then hypothesize that the observed phenomenon reflects the life cycle of some biological individual or group of individuals. If this is correct, then we can infer that one or more demarcators must exist for this individual. To get the research process moving, we then have to make a further hypothesis about what these demarcators are as material entities or causal processes.[7] As I'll describe further in a moment, any demarcator must play specific roles in explaining how, when, and where key events in the individual's life cycle occur. Determining whether these conditions hold for our proposed demarcators then becomes the major project for empirical research. As we come to understand what these demarcators do in the life cycle (and whether they are involved at all), we gain theoretical insight into the nature of the biological individual we proposed. If we

can ultimately find no way to demarcate the supposed individual, then we are forced to abandon the original hypothesis and explain the data another way.[8]

What is involved in hypothesizing a demarcator for an individual and how do its properties relate to events in the life cycle? Since reproduction involves the making of more individuals, it must also involve the production of more demarcators, since each individual as a whole will differ in at least some of its parts. Moreover, the generation of new demarcators during reproduction will be essential to this process rather than incidental: whatever we have hypothesized the demarcator to be, it forms the causal basis for any new individual existing as a whole. How the demarcator changes during reproduction should therefore be central to explaining how the reproductive process happens. Plausible examples of demarcators would be the membrane of a cell, the immune system of an animal, successful interbreeding between members of a population, or the covalent bonds that hold together a plasmid as a single molecule.[9] Things that would probably not count as demarcators would be the process of fair meiosis or a unicellular bottleneck during reproduction.[10]

Another role for the demarcator is bringing about the actual specificity of the "contents" of the individual. The parts of the individual must stand in some, possibly complex relationship with the actual, concrete demarcator that marks them off as distinct from other objects in the world. Furthermore, the parts must differ in their capacities for causal interactions compared to things outside the whole. For example, the membrane of a cell is selectively permeable, affecting which molecules can interact inside the cell, and an animal's immune system selectively recognizes tolerable or beneficial entities, while rejecting or attacking potentially harmful ones. Any hypothesis of a demarcator, then, must serve to explain how certain things in the world become parts and how this status affects their causal capacities.

Lastly, there must be criteria for distinguishing between instances of a demarcator. This is a problem distinct from the individuation of biological individuals, since a given demarcator may be necessary but insufficient for characterizing that individuality. Prima facie it is not obvious that there is a general criterion or set of criteria which identify instances of every demarcator type. Plausible examples, though, would be the material continuity of a plasma membrane in a cell, or the contiguity of all covalent bonds in a molecule. Some sort of distinguishing criteria must be included, therefore, in putting forward a demarcator as an hypothesis.

The demarcator thus serves two necessary roles in a life cycle: an individual's demarcator must be able to change and multiply as part of reproduction, and the demarcator is also causally responsible for the specificity of parthood membership. Beyond this, the demarcator may have to undergo changes dur-

ing the development of an individual from birth into reproductive maturity. It may also have to change or transform when the individual transitions to a new ecological niche or when the environment changes, for instance in metamorphosis during the complex life cycle of some insects. Additionally, as I'll discuss in more detail below, the demarcator is a crucial participant in the process of inheritance.

Each of these roles provides a dimension in which to evaluate the quality of the demarcator as an hypothesis. Without a demarcator that serves these two necessary roles, hypothesizing the existence of a biological individual as an explanation for the observed pattern of events must fail (or we have to abandon the demarcator framework). However, there is also another dimension to evaluating the quality of a demarcator: its importance to explaining how, when, and where key events in the life cycle occur. Recall from the earlier discussion that fitness, because it supports the prediction and explanation of demographic changes in a population, affords an objective basis for determining evolutionary individuality. The properties of a demarcator that influence life cycle control play an analogous role here by allowing one to predict and explain particular outcomes for a given life cycle as well as the observed range of variation within a population.

I will formulate this role for demarcators by requiring that they serve as focal points of control for events in the life cycle. By control, I don't necessarily mean to imply a centralized system for manipulating the life cycle.[11] More minimally, I use control to refer to the aggregation of all those causal processes that make a difference to how a life cycle happens over time, considered across the range of relevant conditions. For example, why did an individual reproduce sexually under these circumstances and asexually in another context? Alternatively, why did a free-swimming cell transform into a spore when the puddle it was living in started to evaporate? A material object or causal process is a focal point for control to the extent that the variation possible within the given life cycle can be explained by the following: a) the focal point's effects on what becomes a part of the object in question; b) its effects on what causal interactions are possible among parts, among nonparts, and between the two; and c) changes in the properties of the demarcator responsible for these two sets of effects during the life cycle. Note that the demarcator being a focal point of control does not say anything per se about whether the biological individual is a *locus* of control for its own life cycle (Bechtel and Richardson 1993). That is, the importance of the demarcator for the control of life cycles is neutral with respect to whether the origins of the control are internal or external.

With the basic properties of demarcators in hand, we can turn to ana-

lyze the process of an evolutionary transition in terms of the emergence of a higher-level demarcator that encompasses a set of parts that are or were biological individuals. One starting point for the transition is if preexisting demarcators for the lower-level individuals start to associate in some way, erasing the distinguishing features that established them as separate. For example, a group of cells might associate together by linking their cell membranes or walls through incomplete cell division or the creation of cytoplasmic bridges between membranes. Association between demarcators in this way would likely constrain the independence of events in each individual's life cycle, altering the process of reproduction as a result of the new linkages. At this point, evolution could then proceed to shift the focal point of control to the new, conjoined demarcator from the prior, separate demarcators.

A second pathway could be to evolve an entirely new demarcator without relying on the modification of preexisting ones. A group of cells might evolve a molecular signal they could emit to indicate membership in the group, for instance. This signal could serve as the basis of a new demarcator, one based on behavioral responses to the signal that would complement their cell membranes rather than merge them together.

Analyzing Inheritance in Terms of Demarcators

Although much more needs to be said about demarcators, I will focus for the rest of the paper on showing how they ground a novel perspective on biological heredity. One of the virtues of the demarcator approach is its potential to offer a level-neutral perspective on heredity that breaks free of the dichotomy between development and heredity put forward and entrenched by the Modern Synthesis. Nothing in the demarcator view of individuality presupposes the existence of DNA, genes, or other sorts of replicators as vehicles for heredity (Sterelny, Smith, and Dickison 1996), although it hardly denies their importance. This section and the next will show how we can use demarcators as a foundation for analyzing the nature of heredity and the tradeoffs between different kinds of inheritance processes.

The central insight is understanding the nature of heredity in terms of how one individual can exert causal control over another. Demarcators let us analyze the possible pathways of control into four categories:

1) control is exerted via some material part of one individual becoming part of the other;
2) control is exerted via a material entity that is not a part of either individual;

3) control is exerted via a mixture, where the material entity that starts as a part of the first individual does not become a part of the second;
4) control is exerted via a material entity that is not a part of the first individual but ends up becoming a part of the second.

We can think of heredity in the broadest possible sense as control exerted by one individual on another that causes the recipient to acquire one or more traits similar to the controller. This includes the familiar, vertical sense of heredity between parent and offspring, but also the horizontal transmission of traits between individuals. However, control exerted along the four pathways can also function in the other direction—that is, to produce dissimilarities between individuals. In the following section, for example, we will see how the asymmetric division of a cell into one larger and one smaller cell is essential for generating distinct cell types within a species of multicellular algae. As I will use the term here, then, the control of inheritance tracks both similarity and dissimilarity as outcomes, whereas heredity focuses solely on the extent of similarity between parents and offspring.

As I have defined them, the four pathways for control have a close relationship to the concepts of material overlap and scaffolding used by James Griesemer and William Wimsatt (Griesemer 2000a, 2002, 2014; Wimsatt and Griesemer 2007). Material overlap would correspond to the first category—that is, inheritance through the transfer of parts. Scaffolding would include the remaining three categories. Table 3.1 presents this relationship in a two-by-two matrix.

As an example, consider how one cell might exert control over another. If the controller passes some of its DNA, proteins, cell membrane, or other internal molecules over into the other, this counts as material overlap. Alter-

TABLE 3.1. Classification of mechanisms for the control of inheritance

		Controlled Individual	
		Internal	External
Controlling Individual	Internal	Material Overlap	Scaffolding
	External	Scaffolding	Scaffolding

NOTE. Four pathways along which one individual can exert causal control over the traits of another, based on whether the material entities used to exert this control are parts of either individual. When the material starts as a part of the controlling individual, for example, this counts as material overlap. The terms internal and external here are used simply as shorthand for being or not being a part of the relevant individual.

natively, there are three possible ways for it to use scaffolding. The controller might extrude an extracellular matrix that influences the controlled cell, for example (internal to external control). The controller could digest carbohydrate molecules in the environment that get taken up by the controlled cell (external to internal control). It could also act on an extracellular matrix connecting both cells to deform its shape and change the local pressure or other forces acting on the controlled cell (external to external).

It's worth pointing out, though, that if the controlled entity is actually a physical part of the controller, then the distinctions in Table 3.1 partially collapse. An example would be symbiosis through engulfment, such as mitochondria within eukaryotic cells. In this situation, the inside of the controller overlaps with the outside of the controlled, eliminating one kind of scaffolding. Nonetheless, the overall distinction between scaffolding and material overlap remains viable.

Griesemer and Wimsatt originally introduced material overlap and scaffolding in order to characterize inheritance from a developmental point of view. Griesemer in particular has argued that biological reproduction can be defined as a special kind of multiplication (i.e., making more of) that involves material overlap, which he calls progeneration (Griesemer 2000a). Griesemer suggests that material overlap is critical for biological reproduction because it transfers preexisting organizational structure to the new generation instead of attempting to form the offspring out of unorganized matter. The paradigmatic case would be how a daughter cell inherits one of the original DNA strands from its parent's double helix, along with a newly synthesized copy. (Note, though, that progeneration involves a particular kind of material overlap, in which the part transferred to the offspring produces similarity between parent and offspring because the causal dispositions of the part make the same difference to the offspring's traits as they did for the parent's.) While I don't presuppose his definition of reproduction here, his arguments are crucial to showing why material overlap has distinctive importance as a pathway for inheritance.

Scaffolding then serves as a complementary resource for the development of an individual. "Scaffolding refers to facilitation of a process that would otherwise be more difficult or costly without it, and which tends to be temporary—an element of a maintenance-, growth-, development-, or construction process that fades away, is removed, or becomes 'invisible' even if it remains structurally integral to the product" (Griesemer 2014, 26). Griesemer also writes, "More generally, scaffolds persist on different time scales than what they scaffold. Infrastructure can persist on very long time scales

relative to individuals who use it and thus create correlated environments for organisms of different generations" (Griesemer 2014, 51).

While maintaining the crucial complementary role for scaffolding in relation to material overlap, I depart from Griesemer and Wimsatt's definition by privileging a spatial rather than temporal definition for the concept. This spatial approach also implies a strict dichotomy between material overlap and scaffolding that does not generally hold for Griesemer and Wimsatt's usage. We can see the difference by comparing Griesemer's emphasis on scaffolding as relative to the timescale of an individual and the version I give in Table 3.1, where scaffolding is defined in purely spatial terms. Additionally, Griesemer does not allow certain cases of the transfer of material parts from outside to inside an individual to count as scaffolding: "It would appear that a scaffold per se does not contribute material parts to the developing system, so it cannot count as food" (Griesemer 2014, 30). However, one can recapture some of Griesemer's key types of scaffolding—for example, the notion of infrastructure, by incorporating temporality as a secondary basis for categorization. Hence we could define infrastructure as including material objects that remain external to the controlled entities and that exist on much longer timescales than their lifecycles.

The major utility of material overlap and scaffolding for theorizing about inheritance follows the differing consequences of using one or the other as a pathway for control. As Griesemer has argued, material overlap is generally more reliable as an inheritance mechanism: the offspring acquires the traits of the parent because it has acquired some of the parts of the parent that causally produced those traits in the first place. "Material overlap can increase the robustness and reliability of transmission of capacities, compared to reliance on an unstable and uncertain environment to deliver components in suitable temporal order and spatial configuration, because complex organization can be preserved and propagated in material propagules" (Griesemer 2014, 26). Hence, "if material overlap is an efficient and effective way of propagating and producing developmental order and organization, then it should be favored, entrenched, conserved in evolution or else its absence should require special explanation" (Griesemer 2014, 28).

Indeed, material overlap is not always a possible or advantageous pathway for controlling inheritance. In some cases, for example, the inherited trait may need to vary with environmental circumstances that cannot be prespecified by the genome. The ability of some birds to recognize members of their own species, for instance, is acquired through interaction with other birds during development rather than hardwired into their genetics (Soler and

Soler 1999). Receiving material parts from another cell carries also risks as well as benefits: the sender could transmit its disadvantageous traits as well, or it could use the interaction as an opportunity for predation, parasitism, or competition. Additionally, material overlap requires the evolved capacity to generate and manage the transmission, which may limit the range of control a parent can exert if it requires close physical interaction.

In general, then, the value of scaffolding and material overlap lies in how they illuminate the potential costs and benefits of different combinations of control mechanisms for inheritance. Inheritance through material overlap may be most reliable, but if its ability to vary with environmental circumstances is constrained, then ecological specialization through scaffolding may be the best option. In the next section, I discuss two examples that illustrate how understanding inheritance using demarcators lets us evaluate different pathways toward higher-level individuality in evolutionary transitions. In particular, the demarcator view of individuality gives us a way to theorize about how different ways that lower-level individuals might associate together during a transition generates both affordances and constraints on the possibility for evolution of a new level of individuality.

Material Overlap and Scaffolding in the Inheritance of Cell Differentiation

This section examines how life cycle control interacts with different ways of initiating multicellularity from lineages of bacteria or unicellular eukaryotes. Most work on the benefits and costs of multicellularity has focused on scenarios where multicellularity initiates through the division of a single cell and its descendants remain stuck together through adhesion or incomplete cytokinesis (the failure of cells to separate fully during division).[12] However, multicellularity can also initiate when multiple cells aggregate together, for example, in dictyostelid slime molds (Kessin 2001), myxobacteria (Pathak et al. 2012), and biofilms, which can include multiple species in the same aggregate (Claessen et al. 2014). Obviously, the availability of material overlap cannot be taken for granted when multicellularity starts by aggregation rather than cell division. Does this affect how life cycle control evolves during the transition? For instance, are there ways of policing cheating cells using scaffolding that provide alternatives to strategies based on material overlap, such as a genetic bottleneck? I will look at two cases of mechanisms for the inheritance of cell types, one based on material overlap and the other on scaffolding.

In general, cell differentiation depends on the ability of cells to occupy

discrete overall physiological states in a stable manner. (For a philosophical discussion of cell types, see Slater 2013.) The key difference between cell types is not their DNA but how they use it. Controlling the inheritance of cell differentiation therefore goes beyond the material overlap involved in DNA replication. Although cell differentiation technically refers to the progressive specialization of cells within a multicellular organism, unicellular organisms also transition between discrete physiological states during their lifecycle. For example, many single-celled organisms alternate between states specializing in growth or reproduction, often driven by the presence or absence of nutrients in the environment.

Cell types can also have other crucial features. The type may be irreversible, in the sense that the cell possesses no internal capacity to undifferentiate. One type may also be capable of generating more specialized types. Totipotent cells can generate all other cell types in an organism, while multipotent cells are more limited. Figure 3.2 illustrates some of the various dynamic relationships that are possible between cell types.

MATERIAL OVERLAP: *VOLVOX CARTERI*

The process of cell division can cause material overlap between generations in two ways: first, through directly transmitting DNA and other cellular materials, such as cell membranes and protein complexes; second, by failing to achieve separation, such that partial connections, such as cytoplasmic bridges or conjoined cell walls, ensure ongoing overlap of material components between the cells. Asymmetric cell division—that is, where the two daughter cells receive different inheritances from their parent, appears to be a general mechanism for producing heritable differentiation through direct transmission. It is important for generating a split in germinal and somatic cell

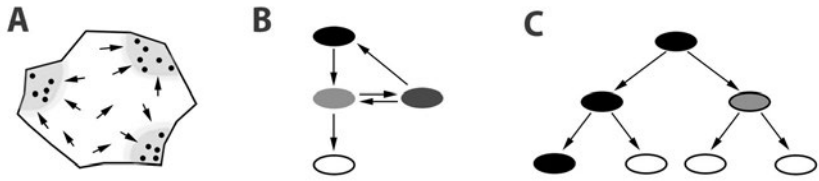

FIGURE 3.2. Networks between modular states. (A) Phenotypic modularity through forcing in the available space of physiological states. States of the cells in a group are represented by dots in the larger physiological space. Arrows could represent underlying dynamic forces in the gene network structure or environmental causes. (B) Four modular differentiated states. Notice that the transition from light gray to white is irreversible, while the other three form a cycle. (C) Cell differentiation during development. Black cells can differentiate into any other state (totipotent), while gray can only differentiate into white.

lineages in some species of the chlorophyte (green algae) Volvocales. However, incomplete cytokinesis is also crucial. In the species *Volvox carteri*, for instance, it plays a central role in shaping the spatial development of the multicellular organism (Kirk 1998).

The chlorophyte Order Volvocales has become a paradigm case study for the evolution of eukaryotic multicellularity (Kirk 1998; Herron and Michod 2008; Michod 2007). The many species within the order are monophyletic and exhibit a range of lifestyles from unicellular to complex multicellular forms. The species *V. carteri* exhibits heritable differentiation into two cell types: flagellated cells incapable of further reproduction that form a spherical boundary around the organism and germ cells that divide within the organism to form small new individuals. (See Figure 3.3.)

V. carteri produces its separation of germ and soma cells through the combined effect of asymmetric cell division and the inhibition of growth. When a germ cell divides, it splits into one large and one small cell. Through a still unknown mechanism, cell size controls the expression of the gene that limits chloroplast activity. As a result, the small cell can divide a few more times but cannot grow and therefore represents a reproductive dead-end. The other

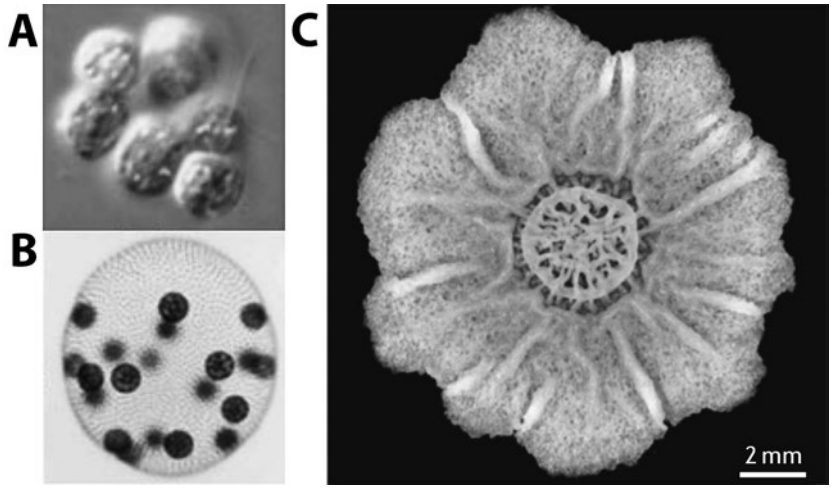

FIGURE 3.3. Cases of multicellularity. Images showing different varieties of multicellularity. (A) A group of cells from *Gonium pectorale*, a colonial species in Volvocales. (B) The mature state of *Volvox carteri*, a relative to *G. pectorale*. Each dot in the sphere is a somatic cell and the dark internal spheres are still-developing, smaller versions of the adult organism. (A) and (B) from Kirk (2005), used by permission of John C. Wiley and Sons. (C) Top-down view of a mature *Bacillus subtilis* biofilm. Reprinted by permission of Macmillan Publishers Ltd.: *Nature Reviews Microbiology*, Hera Vlamakis, Yunrong Chai, Pascale Beauregard, Richard Losick, and Roberto Kolter, "Sticking Together: Building a Biofilm the *Bacillus subtilis* Way," copyright 2013.

cell, however, stays large enough that its chloroplasts remain active, and can therefore continue to divide indefinitely. Heritable control of differentiation is thus produced by material overlap in bulk—that is, by the asymmetric distribution of cell volume—in combination with persistent negative regulatory feedback. Commitment to either trajectory—germ or soma—then invokes further physiological specializations. The somatic cells, for example, remain on the outside of the sphere and are responsible for controlling the locomotion of the group through the joint action of their flagella.

Interestingly, multicellularity in Volvocales appears to have initiated through clonal division and only later evolved incomplete cytokinesis. This suggests that the transition occurred through a sequence of modifications to the cells' demarcators. According to the most recent phylogeny, early colonial forms of multicellularity, lacking differentiation, began through the loss of individual cell walls and the growth of a shared extracellular matrix formed of carbohydrates and proteins (Herron and Michod 2008). At this early point, cell division proceeded to completion. However, one of the next major steps toward complex multicellularity involved the retention of cytoplasmic bridges between cells, effectively linking each cell's demarcator into a larger unit. One function of these linkages was to provide a more determinate geometric structure to the colony that was necessary for *V. carteri* to evolve its distinctive spherical shape. The bridges enable *V. carteri* to undergo inversion during development such that its gonidia (germinal cells) move from the outside to the inside of the sphere. This suggests one way in which the conjoined cell membranes serve as a focal point for control during the life cycle—that is, in order to develop a higher-order demarcator that establishes the gonidia as being "inside" the individual in a novel way.

SCAFFOLDING: *BACILLUS SUBTILIS*

Although material overlap may in general be a more reliable mechanism for controlling the inheritance of differentiation, in some contexts it can be unavailable or ineffective. In this subsection I describe a case of one-way (paracrine) signaling between cells in the bacterium *Bacillus subtilis* that also depends on the production of extracellular matrix to generate heritable differentiation. For a related debate about whether biofilms count as evolutionary individuals, see Ereshefsky and Pedroso (2012) and Clarke (2016).

B. subtilis forms complex multicellular biofilms through a combination of aggregation and clonal reproduction (Aguilar et al. 2007; Vlamakis et al. 2008).[13] Biologists have described a variety of cell types in the biofilms that are also spatially localized. Major types include motile cells, which are usu-

ally found at the bottom of biofilms, sporulating cells at the top, matrix-producing cells in clumps throughout the biofilm, and "miner" cells that secrete chemicals to break down nutrients for use by nearby cells (López and Kolter 2010). Biofilms in general exhibit a temporal program of development from initial formation to dispersal, and *B. subtilis* is a premier model organism in this regard (Monds and O'Toole 2009; Vlamakis et al. 2008). Mobile cells dominate early-stage biofilms in *B. subtilis*, followed by the formation of patches of matrix-producing cells. Matrix cells appear to enter spore formation soonest, although sporulation eventually spreads throughout most of the biofilm. Remaining cells may then disperse and return to a free-living state, while the spores spread from the elevated aerial structures created by the biofilm.

López et al. have found that biofilm development in *B. subtilis* depends on one-way signaling between cells (López et al. 2009; López and Kolter 2010). The sequence of steps goes as follows: cells initiating biofilm development generally produce a signaling molecule, comX. When comX exceeds a certain threshold, some cells begin producing a second signal called surfactin. Surfactin acts along a general pathway to disrupt the permeability of cell membranes in a way that stimulates the cell to differentiate and start producing extracellular matrix. Crucially, López et al. (2009) found that surfactin production and matrix production occurred in different populations of cells. This implied that matrix cells were not responding to comX, and López et al. found that the matrix surrounding these cells was sufficient to block the signal.

As Elizabeth Shank and Roberto Kolter point out in a recent review, "This unidirectional paracrine signaling, where one cell type produces a signal to which another cell type responds, allows the compartmentalization of cellular differentiation and permits cell-type status to be maintained over numerous generations" (Shank and Kolter 2011, 743). Descendent cells near the early matrix producers would inherit the extracellular matrix and its barrier against comX signaling. Hence we would expect that the matrix serves as scaffolding that constrains the range of types available to new cells by preventing their differentiation into surfactin-producers.

This case suggests an interesting alternative to policing mechanisms based on material overlap: when an early signal to initiate multicellularity also imposes a cost on cells that fail to cooperate. Although still speculative, surfactin could be playing both roles for *B. subtilis*. We do know that it causes stress to the membranes of any cells not protected by the extracellular matrix, and also that it activates a general signaling pathway that responds to many different sources, including environmental toxins or secretions from preda-

tors. It is conceivable, then, that *B. subtilis* might have appropriated surfactin from being an externally produced source of membrane stress to serving as an internally produced signal and policer of biofilm development. This dual role would effectively co-opt one of the basic benefits of life in a biofilm, protection from a harsh environment, to also serve as a mechanism for policing cells that don't cooperate in producing this benefit. It shows how an evolutionary transition that starts by aggregation could use scaffolding as an alternative control strategy instead of material overlap based on cell division. Furthermore, it points to how the disruption or breakdown of demarcators may be relevant to evolving a higher-level individuality in addition to their generation or conjunction.

Conclusion

Evolutionary transitions in individuality pose deep problems for biological theory. As a group, the transitions impose a dynamic chronological order to the forms of living things that have existed over time. Attempting to explain how and why the transitions occurred forces us to reckon with the messy and complex emergence of new forms of individuality over time rather than focusing primarily on paradigmatic kinds of individuals, such as sexually reproducing animals, which have already evolved. A successful answer to the problems raised by evolutionary transitions needs to at least address the three issues I raised in the introduction: it must offer a level-neutral account of what individuals are, provide a means for identifying whether some object is an individual, and address a variety of explanatory questions about the transition.

An evolutionary account of biological individuality, based on MLS theory, is arguably the most promising option we currently have for these first two challenges. However, it gains these merits in part by abstracting away from the material and causal properties of the systems under study, and I have argued that this limits its ability to address the full range of explanatory questions we have. This motivates the search for complementary accounts of individuality that are not based on fitness. I have presented one such alternative account here based on the concept of demarcators and argued for its potential—still in need of further development—to be level neutral and support identification of individuals.

Further development of the demarcator account could proceed in several directions. Demonstrating its level neutrality directly would require examining a number of the demarcators I proposed here across the different evolutionary transitions and showing that they have the correct functional

roles and serve as focal points. Another possibility is to apply the concepts of material overlap and scaffolding to analyzing the affordances and constraints for evolution offered by different pathways to evolving higher-level individuality. In the transition to multicellularity, for example, there are a variety of ways for higher-level demarcators to emerge that would also have different tendencies to support the control of inheritance using material overlap or scaffolding—for example, compare aggregation via an extracellular matrix versus adhesion after cell division.

Additionally, the demarcator account, as grounded in the notion of causal control and the specificity of parts versus non-parts, has interesting connections to the topic of biological information. The notion of demarcator is defined here in terms of how much control is exerted via its properties rather than whether control originates among the parts it picks out. More broadly, however, the concept of control I have used here depends on the very sort of causal specificity that is fundamental to biological information (Sterner 2014). When life cycle control depends on environmental cues, inheritance, or communication, we could interpret the material structures carrying out control as information systems. This points to a way in which the comparative study of biological information (Jablonka and Lamb 2005) intersects with the comparative study of evolutionary transitions.

Finally, it may be possible to use a similar theoretical framework as I have here to develop analogous theories of individuality based on metabolism or cooperation. We could set the key functional capacity of a biological individual using an analysis of metabolic autonomy, for example. Mechanisms that contributed to this capacity would qualify as individuating mechanisms, and we would need to describe how the degree of individuality depended on the particular individuating mechanisms present. If this is possible, the domain of biological individuals—that is, the subject matter of biology—could then be defined according to a plurality of perspectives, each focusing on one aspect of the complex phenomena we traditionally group under the heading of "living things." What we commonly think of as competing approaches to individuality—metabolism, fitness, cooperation, life cycles—might then turn out to be epistemically complementary rather than ontologically exclusive.

Acknowledgments

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ume, and also to Matt Herron, Alan Love, and Rick Grosberg for helpful comments and suggestions. Figure 3.1 is an adaptation of Figure 1 (p. 609) in R. E. Michod, "Cooperation and Conflict in the Evolution of Individuality. I. Multilevel Selection of the Organism," *American Naturalist* 149, no. 4 (1997), and is used by permission. This research was supported by NSF postdoctoral grant SES-1153114.

Notes to Chapter Three

1. By "thing" or "object" in this paper I mean simply what would be a plausible candidate for a biological individual.

2. The concepts of "part" and "whole" are complex technical terms in philosophy (Achille 2014), but I will not follow any particular philosophical account here. Instead, we can look to the local theories and practices of biologists to understand how the term should be applied for a particular phenomenon of study (see Winther 2006).

3. For the sake of completeness, I suggest that we also add, "How evolvable is the higher-order unit?" The algae species *Volvox carteri*, for example, evolved multicellularity 50–75 million years ago but has not developed further differentiation among cell types since then (Nedelcu and Michod 2004).

4. Fair meiosis occurs when there is an equal probability for each chromosome to end up in each haploid daughter cell. Fairness matters in situations such as the production of eggs in humans, where only one of the four haploid cells generated by meiosis matures to become a viable gamete.

5. The concept of a mechanism will not do any distinctive work in this paper in contrast to the more general concept of a causal process. Obviously the fact that mechanisms are recurring types of causal processes that reliably produce an effect will make them more interesting and tractable for biologists in general. To my knowledge, nothing in Clarke's account depends on choosing one notion of mechanism or another.

6. The philosopher Robert Wilson has also given a fairly general definition of a life cycle in the context of analyzing what it means to be an organism (Wilson 2005). Using the concept of a "living agent" that comes from a related part of his work, he defines a life cycle as being "comprised of a causal succession of entities, each a living agent, which themselves, together with the processes that mediate their succession, recur across generations" (Wilson 2005, 60).

7. In this paper, I will systematically avoid choosing between a substantive versus processual nature for demarcators. That is, I will allow demarcators to be material objects, enduring over time, and also sequences of events, connected by causal relations. Both ways of framing the underlying ontology behind life cycles have their own heuristic values for actual biological research, e.g., designing experiments or generating explanations.

8. Note that the sequence I describe here starts from a position of relative theoretical ignorance about what the individual is, so an initial empirical description of the target phenomenon is crucial for getting the investigation going.

9. I'm hedging here because being a demarcator is ultimately an empirical matter, as I'll describe in a moment, and I do not intend to stipulate that the concept must include these cases.

10. The demarcator must exist at the time that we wish to determine the parts of the individual. In the case of the unicellular bottleneck, what would matter for demarcation is whatever

generates cohesion among the descendants of the original cell over time, not the mere fact that they are descendants from a single individual. See above for a definition of fair meiosis.

11. I recognize that the word "control" has a number of potentially unfortunate connotations. My original inspiration for focusing on the control of life cycles came from Leigh Van Valen's classic idea that "evolution is the control of development by ecology" (Van Valen 1973). Other relevant sources are John Tyler Bonner's discussion of the control of pattern in development (Bonner 1974), and Bechtel and Richardson's discussion of a locus of control in a functional system (Bechtel and Richardson 1993).

12. Even here, there is a surprising diversity of alternatives to classical binary fission worth recognizing (Angert 2005).

13. In the wild, it's likely that many biofilms are multi-species and include a considerable range of genetic variation within species. Most laboratory cultures, however, focus on single-species biofilms grown from a single strain. That microbial culturing techniques now accommodate biofilm formation reflects a major advance, but the techniques still idealize away a large amount of ecological complexity and population structure present in nature.

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