The Evolution of Multicellularity: A Minor Major Transition?

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Abstract

Benefits of increased size and functional specialization of cells have repeatedly promoted the evolution of multicellular organisms from unicellular ancestors. Many requirements for multicellular organization (cell adhesion, cell-cell communication and coordination, programmed cell death) likely evolved in ancestral unicellular organisms. However, the evolution of multicellular organisms from unicellular ancestors may be opposed by genetic conflicts that arise when mutant cell lineages promote their own increase at the expense of the integrity of the multicellular organism. Numerous defenses limit such genetic conflicts, perhaps the most important being development from a unicell, which minimizes conflicts from selection among cell lineages, and redistributes genetic variation arising within multicellular individuals between individuals. With a unicellular bottleneck, defecting cell lineages rarely succeed beyond the life span of the multicellular individual. When multicellularity arises through aggregation of scattered cells or when multicellular organisms fuse to form genetic chimeras, there are more opportunities for propagation of defector cell lineages. Intraorganismal competition may partly explain why multicellular organisms that develop by aggregation generally exhibit less differentiation than organisms that develop clonally.

INTRODUCTION

Beneath the outward harmony of living organisms lies an often contentious history of transitions to ever more inclusive, hierarchically nested levels of biological organization (Bonner 1988, Buss 1987, Carroll 2001, Leigh 1977, Maynard Smith 1988, Maynard Smith & Szathmáry 1995, McShea 2001, Michod 1999). Although views differ on what defines a major evolutionary transition, almost everyone agrees that the following transitions qualify as major (Buss 1987; Maynard Smith & Szathmáry 1995; Queller 1997, 2000): (*a*) the compartmentalization of replicating molecules, yielding the first cells; (*b*) the coalescence of replicating molecules to form chromosomes; (*c*) the use of DNA and proteins as the fundamental elements of the genetic code and replication; (*d*) the consolidation of symbiotic cells to generate the first eukaryotic cells containing chloroplasts and mitochondria; (*e*) sexual reproduction involving the production (by meiosis) and fusion of haploid gametes; (*f*) the evolution of multicellular organisms from unicellular ancestors; and (*g*) the establishment of social groups composed of discrete multicellular individuals.

In every major transition, selection favoring increased levels of biological complexity is opposed by genetic conflicts acting within and across levels of biological organization. As new levels of organization of replicators emerge from previously independently replicating units, how do the fitness interests of lower and higher levels of biological replication become aligned? In other words, what keeps selection acting on the ancestral level of biological organization (within-group selection) from disrupting the integration of the derived level (Maynard Smith & Szathmáry 1995; also see Buss 1987; Frank 2003, Michod 1999, 2003)? In particular, how are defectors or cheaters that selfishly improve their own fitness kept at bay so that the transition becomes established (Buss 1987, Frank 1998, 2003; Griesemer 2000, Leigh 1977, 1991; Maynard Smith 1988; Maynard Smith & Szathmáry 1995; Michod & Nedelcu 2003, Queller 1997, 2000)?

Here, we focus on the evolutionary transition from unicellular to multicellular organization. The first evidence of this transition comes from fossils of prokaryotic filamentous and mat-forming Cyanobacteria-like organisms, dating back 3 to 3.5 billion years (Knoll 2003, Schopf 1993), with signs of cell differentiation more than 2 billion years ago (Tomitani et al. 2006). Multicellular eukaryotes may have existed 1 billion years ago (Knoll et al. 2006), but a major burst of metazoan diversification occurred about 600–700 Mya, at a time of dramatic increases in atmospheric and oceanic oxygen (Carroll 2001, King 2004, Knoll 2003, Maynard Smith & Szathmáry 1995, Pfeiffer et al. 2001).

History has often repeated itself: Multicellular organisms independently originated at least 25 times from unicellular ancestors (**Figure 1**) (Bonner 1998, 2000; Buss 1987; Carroll 2001; Cavalier-Smith 1991; Kaiser 2001; Maynard Smith & Szathmáry 1995; Medina et al. 2003). Multicellularity appears to have originated once for the Metazoa (King 2004), but multiple times (with secondary losses) in plants, fungi, and the Eubacteria (Bonner 2000, Kaiser 2001, Kirk 1998, Medina et al. 2003, Shapiro 1998). Indeed, multicellular organisms continue to evolve from unicellular ancestors (Boraas et al. 1998), and sometimes continue to revert to a

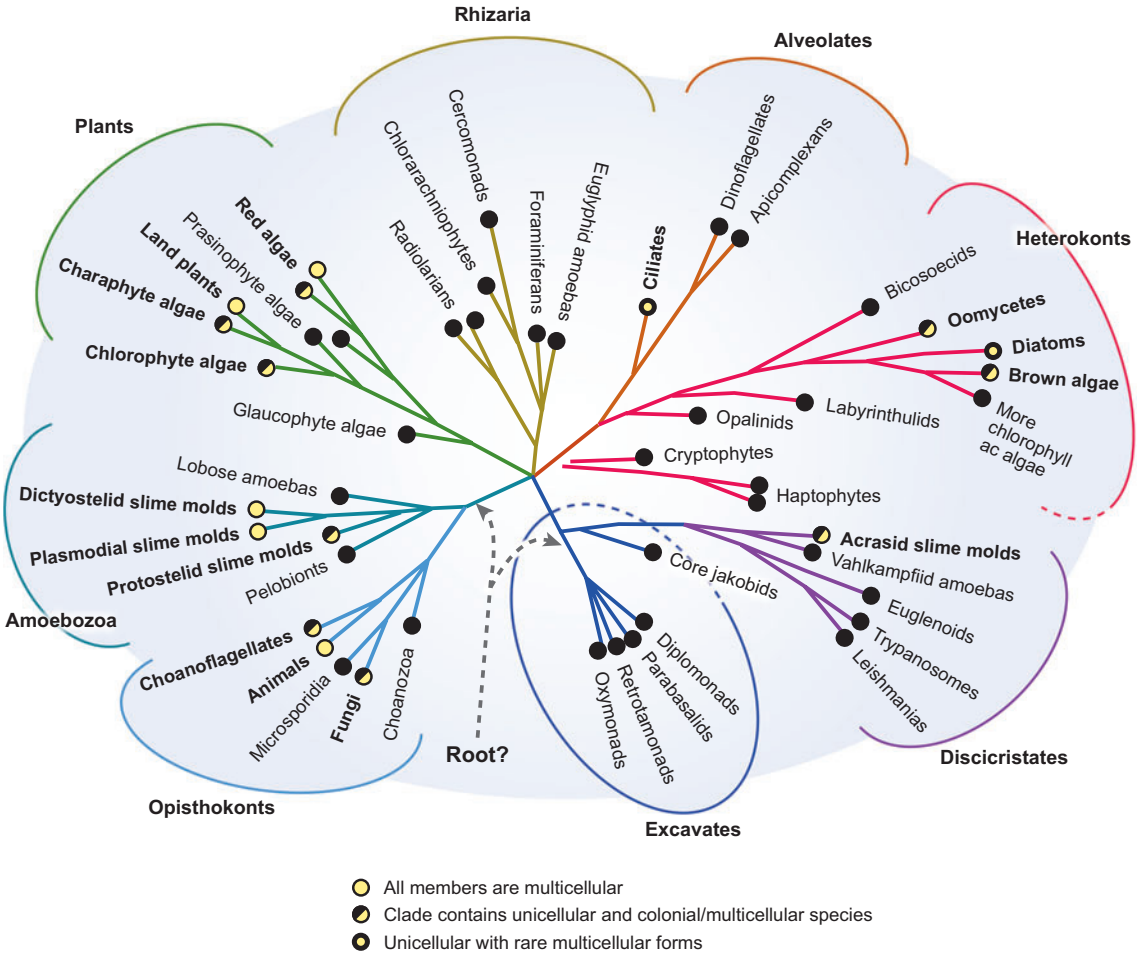

Figure 1

The phylogenetic distribution of multicellularity among eukaryotes. Multicellularity also arose multiply in prokaryotes. Taxa in boldface include at least some multicellular representatives. (After King 2004, from Baldauf 2003). Figure adapted with permission from Baldauf 2003.

unicellular state [e.g., bacteria (Velicer et al. 1998), mammals (Murgia et al. 2006, Strathmann 1991)].

At least in developmental terms, the transition from uni- to multicellular organization may be easy. In some bacteria (Branda et al. 2001), algae (Lürling & Van Donk 2000), and in numerous myxobacteria, myxomycetes, and cellular slime molds (Bonner 1998, Kaiser 2001), the transition to multicellular organization is an inducible response to environmental stimuli. Many of the developmental requirements for multicellular organization, including cell adhesion, cell-cell communication and coordination, and programmed cell death (PCD) likely existed in ancestral

unicellular organisms (reviewed in Bonner 1974, 2000; Kaiser 2001; Keller & Surette 2006; King 2004; Lachmann et al. 2003; Miller & Bassler 2001; Shapiro 1998). Moreover, epigenetic modification of patterns of gene expression, a hallmark of cellular differentiation in multicellular organisms, also characterizes many unicellular organisms (e.g., Ausmees & Jacobs-Wagner 2003).

In contrast to rare (or even singular) evolutionary transitions, the developmental and evolutionary lability of the transition to multicellularity permits analysis of both the selective forces favoring the evolution of a major transition and the adaptive mechanisms that control defectors and stabilize the transition. We review (*a*) the sources and nature of genetic conflicts of interest that could challenge a shift in the unit of selection from the cell to the multicellular organism; (*b*) the circumstances under which each source of conflict would have the greatest impact on the transition; and (*c*) the mechanisms that control defecting cells and align the fitness interests of the cells that cooperate to form multicellular organisms. We argue that the most common ways that multicellular organisms develop and propagate align the fitness interests of the replicators that constitute multicellular organisms. Thus, the transition to multicellularity is relatively easy—a minor major transition. Nevertheless, some modes of formation and propagation of multicellular organisms increase the scope for conflicts among replicators and the evolutionary consequences of deleterious mutations.

Finally, we turn to the question posed by Szathmáry & Wolpert (2003, p. 301):

> Is then the evolutionary transition to multicellularity a difficult one or not? The blunt answer is: not at all, since multicellularity has arisen more than twenty times in evolution . . . . However, there are only three lineages that produced complex organisms: plants animals, and fungi. Three hits in 3.5 billion years are not that many.

Three hits is almost certainly an underestimate, because plants include independently derived, complex multicellular organisms, like red and brown algae (Niklas 2000). Nevertheless, although the transition to simple multicellularity may be relatively easy, there appear to be less well-understood obstacles to the evolution of multicellular complexity.

DEVELOPMENT OF MULTICELLULAR ORGANISMS

If there is genetic variation among the cells that constitute a multicellular organism, then selection within its life span can favor the increase of cells of one genotype at the expense of others (Buss 1983a,b, 1987; Grosberg 1988; Hughes 1989; Klekowski 1988; Michod 1997, 1999, 2003; Orive 2001; Otto & Hastings 1998; Otto & Orive 1995). Some modes of development and propagation of multicellular organisms limit the scope for selection to occur within and across generations, whereas others may promote it (**Figure 2**) (Bell & Koufopanou 1991; Crespi 2001; Grosberg & Strathmann 1998; Hamilton 1964b, 1987a,b; Keller & Surette 2006; Kondrashov 1994; Michod 2003; Michod & Roze 1999; Otto & Orive 1995; Queller 2000; Roze & Michod 2001; Seger 1988).

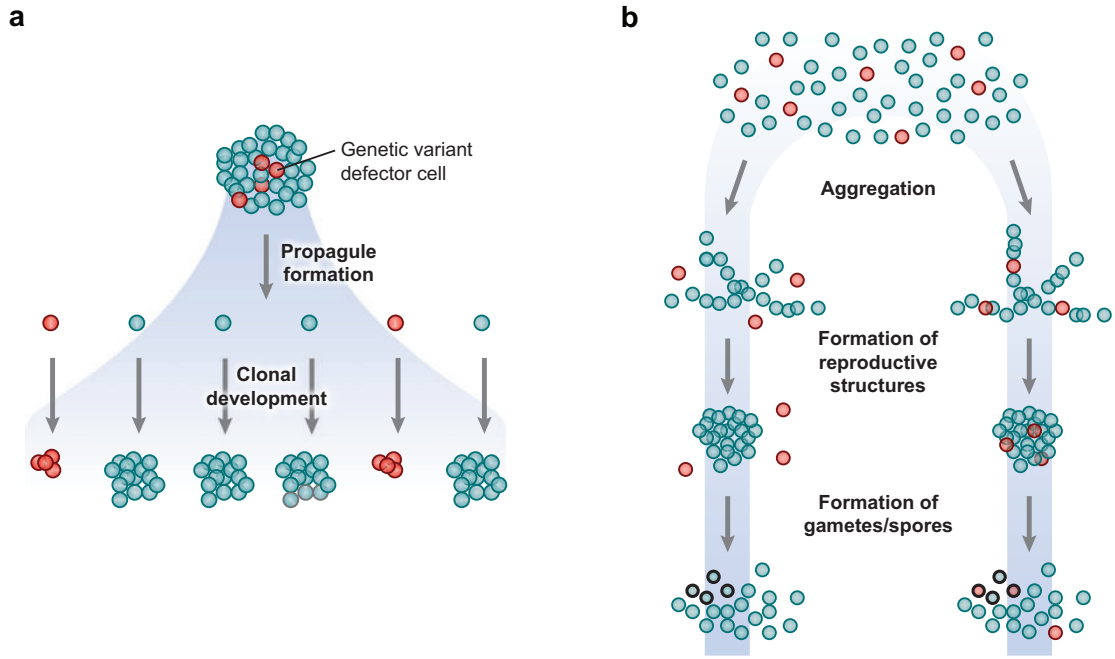

Figure 2

Generalized life cycles of multicellular organisms and their genetic consequences. (*a*) Clonal development from unicellular spore or zygote. Genetic variant defectors (red cells) that arise from somatic mutation within an organism are redistributed among organisms each generation. (*b*) Aggregative development. Defector cells are denoted in red. In the left pathway, a recognition system excludes cheaters joining an aggregation; in the right pathway, cheaters gain access to the spore population. Cells that become spores have black outlines. (Adapted from Keller & Surette 2006.)

Unicellar/Clonal Development

Clonal development from a unicellular spore or zygote (unitary development, sensu Queller 2000) characterizes virtually all multicellular aquatic organisms, as well as the majority of terrestrial forms (**Figure 2*a***) (Bonner 1998, 2000). Multicellularity arises when cells stay connected to (or even encased within) one another following division.

Under strictly clonal development, genetic variation among cell lineages within an organism is low and can arise only through somatic mutation, intergenotypic fusion, or pathogen infection (Dawkins 1976, Grosberg & Strathmann 1998, Hamilton 1964b, Maynard Smith 1988, Seger 1988). Consequently, epigenetic changes in patterns of gene expression (including methylation and chromatin diminution) or post-transcriptional modification, rather than genetic variation among cells within the organism, are the main sources of specialization of individual cells or groups of cells (King 2004). Furthermore, when clonal development regularly features passage through a unicellular stage, any genetic variation that has arisen within the

parent becomes redistributed among offspring in the next generation. This regular redistribution of genetic variation from the within- to the among-organism level will further reduce opportunities for within-organism selection. It should also deter defector mutant lineages that enhance their own propagation at the expense of the multicellular organism because advantages based on defection will be curtailed when defectors constitute whole organisms in the next generation (**Figure 2*a***).

Vegetative, Multicellular Development

Some organisms that develop clonally also produce multicellular vegetative propagules, with important consequences for the accumulation and distribution of genetic variation within and among individuals (Kondrashov 1994, Michod & Roze 1999, Roze & Michod 2001). (To our knowledge, no multicellular organisms that develop by aggregation also produce multicellular propagules.) The recency with which vegetative propagules passed through a unicellular developmental stage is a fundamentally important determinant of the distribution of within- and among-organism genetic variation (Grosberg & Strathmann 1998, Kondrashov 1994, Roze & Michod 2001). Kondrashov (1994) modeled the effects of propagule size, relatedness of cells, and spatial patterns of cell division on mutation load in vegetative propagules at the equilibrium between mutation and selection against deleterious mutants. These analyses, like those of Roze & Michod (2001), show that at one extreme, if all cells in a vegetative propagule are recently descended from a single ancestral cell, then within-organism genetic variation (in terms of mutation load) does not appreciably increase with the number of cells that constitute the propagule. At the other extreme, if distantly related cells initiate a propagule, mutation load and within-organism genetic variation can dramatically increase. Additionally, if selection acts on the cell lineages that potentially contribute to a multicellular propagule before the propagule is formed, mutation load should decline (Avise 1993, Crow 1988, Otto & Orive 1995).

In many clades of multicellular organisms, including land plants, most groups of algae, Eubacteria, and Archaebacteria, the cells have rigid walls (Niklas 2004). Consequently, mutant cell lineages cannot migrate from where they originate (Buss 1983b, Pineda-Krch & Lehtilä 2004, Sussex 1973), and opportunities for cheaters to invade propagules are reduced. Vegetative propagation can genetically approximate development from a unicell, without reduction to a single-cell propagule, as in a unicellular meristem (Kondrashov 1994). Similarly, in mitotically generated chains of immobile cells, as in diatoms and Cyanobacteria, cells of vegetative fragments will often share a recent common ancestor. Animal cells are far more mobile; consequently, variants can more readily spread through the individual (Buss 1983a,b). If mobile cells join a stolon, bud, fragment, gemmule, or other multicellular propagule, then mutations that have accumulated over repeated mitotic cycles throughout the organism could be included in the propagule. Analogous processes can occur among syncytial nuclei encased within the rigid walls of fungal hyphae; however, a variety of mechanisms, including clamp connections in Basidiomycota, may limit the spread of mutant nuclei (Buss 1987). Thus, control of mutational load and defector cell lineages in animals

may require more frequent unicellular bottlenecks than in most multicellular bacteria and plants.

Aggregative Development

Aggregative development of multicellularity occurs in a few groups of terrestrial or semiterrestrial microorganisms (Bonner 2000), including myxobacteria (Dworkin 1972, Shimkets 1990), some ciliates (e.g., Blanton & Olive 1983), myxomycetes (Olive 1975), and cellular slime molds (**Figure 2*b***). In the aggregative mode, cells live independently for most of their life cycles, but episodically associate to become multicellular (reviewed in Bonner 1998, 2000; Crespi 2001; Keller & Surette 2006).

In contrast to the clonal mode of development, genetic variation in aggregating multicellular forms can arise through the association of genetically distinct lineages (**Figure 2*b***). Depending upon the mating system and population viscosity, these lineages may or may not be closely related. Thus, the primary source of genetic heterogeneity within aggregating forms arises from the association of different genotypes, rather than mutation.

Aggregating life cycles greatly increase the opportunity for cheating cell lineages to exploit cooperating lineages, and to spread and persist in populations (Armstrong 1984; Buss 1982; Crespi 2001; De Angelo et al. 1990; Gadagkar & Bonner 1994; Hamilton 1964b, 1987a,b; Keller & Surette 2006, Matsuda & Harada 1990; Maynard Smith & Szathmáry 1995; Michod & Roze 1999). Furthermore, the aggregative mode will result in more within-organism genetic variance than in organisms that develop in the clonal mode, increasing the scope for within-organism selection relative to among-organism selection (Michod 2003). This may be the reason why differentiation in most obligately aggregating multicellular organisms is limited to the production of multicellular fruiting bodies or foraging slug-like structures (Bonner 2000), the main exceptions being organisms such as colonial marine animals and fungi that facultatively (and perhaps accidentally) fuse following extensive multicellular development (Grosberg 1988). It may also explain why multicellular vegetative propagules are rarely, if ever, formed by aggregation.

ADVANTAGES OF MULTICELLULARITY

The frequent origination and spread of multicellularity suggests that selection favoring the transition is pervasive and that the genetic and developmental obstacles to this transition are relatively easy to overcome. Multiple processes could favor the evolution and persistence of multicellularity and differentiation.

Size-Related Advantages

Morphological, paleontological, and experimental evidence all suggest that the advantages of increased size initially favored, and can still promote, the origination and persistence of multicellularity (Bonner 1965, 1998, 2000, 2004; King 2004). The first multicellular organisms were likely filaments or clusters of undifferentiated

cells (Knoll 2003), and the initial size-related benefits of forming such structures were probably ecological (Bonner 2000). The most likely selective agents were phagotrophic organisms that consumed unicellular prey (Stanley 1973). Trade-offs between susceptibility and other aspects of performance (say, buoyancy, physiological exchange, or growth rate) limit size increase for unicellular prey. An apparently easy response was to increase size by increasing the number of cells by cloning and adhesion. Once multicellular predators and suspension feeders evolved, the size race was on between consumers and their victims (Bell 1985).

Experiments using both unicellular and multicellular predators have led either to the facultative induction of multicellularity or to the evolution of multicellular descendants from unicellular ancestors. For example, as the freshwater green alga *Scenedesmus acutus* divides, it retains its daughter cells within its cell walls; these daughter cells can remain attached and form colonies, or they can separate and live as unicells (Van den Hoek et al. 1995). *S. acutus* is normally colonial in field populations, but unicellular in lab cultures (Lürling & Van Donk 2000). Exposure of unicellular *S. acutus* cultures to water from cultures of *Daphnia*, a cladoceran predator on *Scenedesmus*, significantly increased colony formation over controls. The colonial morphs grew and photosynthesized at the same rate as unicells, but sank more rapidly. In a similar experiment, cultures of the unicellular alga *Chlorella vulgaris* repeatedly evolved to form stable, self-replicating colonies within 100 generations in the presence of a phagotrophic predatory flagellate, with the colonies nearly invulnerable to predation (Boraas et al. 1998). The colonial state heritably persisted after removal of the predator.

Even in the absence of phagotrophs (the first fossils of which date back to 750 Mya; see Porter et al. 2003), increased size may have been favored to (*a*) give a competitive advantage in benthic forms, perhaps via overgrowth (Buss 1990); (*b*) provide storage reserves when nutrients are limiting (Koufopanou & Bell 1993, Szathmáry & Wolpert 2003); (*c*) expand feeding opportunities [e.g., group-feeding myxomycetes (Dworkin 1972) and myxobacteria (Shimkets 1990) that produce extracellular digestive enzymes]; (*d*) generate an internal environment protected by an external layer of cells (Gerhart & Kirschner 1997); (*e*) allow novel metabolic opportunities (e.g., Pfeiffer & Bonhoeffer 2003); or (*f*) enhance motility for dispersal or foraging (Foster et al. 2002).

Functional Specialization and Division of Labor

Unicells are capable of differentiation, producing diverse phenotypes in response to environmental cues. However, they can divide labor only in time. Multicellular organisms can simultaneously partition complementary tasks among different cells. By providing a larger pool of laborers, selection for increased size of multicellular organisms can supply both the opportunity for increasing division of labor and the incentive for increasing specialization through economies of scale (e.g., Bell 1985, Bonner 2004, Queller 2000). Moreover, when development occurs clonally, the benefits of division of labor are shared primarily among close relatives, fueling the evolution of division of labor (Hamilton 1964b, 1987b).

The proteobacterium *Caulobacter crescentus* exhibits one of the most basic forms of division of labor: a flagellated swarmer cell attaches to a substrate, loses its flagellum, and becomes a functional stalk, then repeatedly divides to produce more swarmers that detach (England & Gober 2001, Martin & Brun 2000). To the extent that *Caulobacter* life cycle approximates the earliest phases of multicellular differentiation, it implies that constraints at the cellular level promoted the initial phases of division of labor in multicellular organisms. Here we examine two proposed cell-level constraints: metabolic incompatibilities and trade-offs between motility and cell division.

Metabolic cooperation. Some key metabolic processes cannot concurrently take place within a cell. For example, photosynthesis interferes with nitrogen fixation because nitrogenase does not effectively catalyze fixation in the presence of oxygen (reviewed in Kaiser 2001). Unicellular Cyanobacteria must either forgo nitrogen fixation, or fix nitrogen at night and photosynthesize by day. However, the nitrogen-fixing heterocysts of some filamentous multicellular Cyanobacteria differentiate in response to a shortage of fixed nitrogen. Heterocysts have less permeable cell walls than photosynthetic cells and so can effectively remain anaerobic and fix N_2, even when adjacent to photosynthesizing cells in a filament. Heterocyst-like structures appeared in the fossil record perhaps 2 billion years ago (Giovannoni et al. 1988). This antiquity, along with the regular spacing of heterocysts along filaments, suggests that genetic machinery for cell-cell interactions and developmental coordination—requirements for differentiation and division of labor—existed in some of the very first multicellular prokaryotes (reviewed in Kaiser 2001, Wolk 2000, Zhang et al. 2006).

Motility-mitosis trade-offs. Comparative and experimental studies of the evolution of germ and soma in volvocacean green algae indicate advantages from division of labor, with the benefits increasing with size (Bell 1985, Bell & Koufopanou 1991, Koufopanou & Bell 1993, reviewed in Kirk 2003, Michod 2003). The clade encompasses unicellular forms (e.g., *Chlamydomonas*), as well as small (<64 cells), undifferentiated colonies (e.g., *Gonium*, *Eudorina*), and larger (up to 50,000 cells) colonies (e.g., *Volvox*) with cellular differentiation. The larger spherical colonial forms consist of two types of cells: (*a*) internal, unflagellated germ cells that can divide and give rise to new colonies, and (*b*) flagellated, external somatic cells that keep the colony suspended (Kirk 1998) and promote nutrient exchange (Solari et al. 2006). The somatic cells appear to be terminally differentiated and have a limited capacity to divide once they form. This pattern of germ-soma differentiation evolved multiple times (and likely was lost several times, too) in the Volvocales (Kirk 1997, 1998, 1999; Coleman 1999). A small number of genetic changes appears to control this transition (Kirk 1997, 2003).

The loss of mitotic activity in the somatic cells of large volvocaceans is associated with a peculiar trade-off between cell division and locomotion (Johnson & Porter 1968, Koufopanou 1994). Some green algae lack rigid cell walls and can divide and swim at the same time (Kirk 1997, 1998), but in volvocaceans, the flagella are fixed to the cell wall so that their basal bodies cannot move to act as centrioles during

mitosis while still attached to the flagella. The constraint is not absolute: Flagella can continue beating without their basal bodies for up to five cycles of cell division. Beyond that, mitosis or locomotion must stop. However, by differentiating a nonmotile, internal set of cells (the germ line), a colony can continue swimming while its germ cells divide to form new colonies. Additionally, this arrangement of germ and soma improves the efficiency of nutrient uptake through a source-sink effect (Bell 1985, Koufopanou & Bell 1993, Solari et al. 2006). The beating of the external flagella also enhances nutrient flux to the colony and waste removal from the colony (Solari et al. 2006).

A similar trade-off between motility and cell division appears in metazoans, although with a different structural cause. Metazoan cells, like their protozoan ancestors, have a single microtubule organizing center that serves either in the flagellar basal body or in the mitotic spindle. Consequently, the cells cannot divide while they have a functional flagellum or cilium (Buss 1987, King 2004, Margulis 1981). Buss (1987) argued that this limitation had profoundly affected the evolution of metazoan development and differentiation:

> The blastula can hardly continue to develop while moving, for its surface is covered with ciliated cells, each of which is unable to divide. In this respect the ciliated embryo has inherited a severe liability from its protist ancestors. . . . A mechanism must exist by which the embryo may simultaneously continue to move, yet escape its protistan past. How then can an embryo, covered with cells incapable of dividing, continue to develop while moving?
>
> Buss 1987, pp. 42–44

According to Buss (1987), this ancestral metazoan constraint favored differentiation of a population of flagellated somatic cells (altruistically sacrificing their own reproduction) to propel a group of internal, unflagellated, proliferating cells. In turn, these internal cells were the forerunners of the germ line (Buss 1983a, 1987; King 2004).

Many groups of metazoans, notably taxa that vegetatively propagate, do not sequester a germ line (Blackstone & Jasker 2003; Buss 1983a,b, 1987), but still produce motile embryos and larvae. Do developing embryos or larvae pay a price in terms of motility because of this constraint? Perhaps, but the price should be low because flagellated or ciliated cells need not divide synchronously or shed their cilia or flagella for a long period. For example, the cells of sea urchin blastulae lose cilia briefly ($\leq$30 min) and asynchronously (Masuda & Sato 1984); the blastulae do not become bald and they continuously swim while developing. Thus, the cell-level trade-off embodied in the flagellation constraint does not necessarily force the evolution of internal, mitotically active cells (germ) and external, ciliated or flagellated cells (soma).

GENETIC CONFLICTS IN THE TRANSITION

The advantages of multicellularity depend upon cooperation among cells, but cooperation invites cheating. The most widely cited threats to intercellular cooperation

during the transition to multicellularity arise from conflicts between genetically distinct cell lineages, some of which may be devoted to the cooperative production of an efficient multicellular organism and others of which are committed to selfish proliferation at the expense of the integrity and performance of the organism (reviewed in Buss 1987, Michod 2003). Mutation, aggregation or fusion with other genotypes, and infection by intracellular symbionts or pathogens are the key sources of defector cell lineages in multicellular organisms.

Kin selection theory predicts that cooperation among cell lineages will be favored when $rb > c$, where r is the genetic relatedness of recipient to actor, b is the fitness benefit to the recipient, and c the cost to the actor (Hamilton 1964a,b). Recent descent of all cells by clonal development from an ancestral unicell provides the greatest assurance of high relatedness ($r \approx 1$). Selection can then favor genes that result in some cells of the organism ceasing division (or dying) to enhance survival and reproduction of the remaining cells that constitute the organism. In Hamilton's (1964b p. 25) words, "... our theory predicts for clones a complete absence of any form of competition which is not to the overall advantage and also the highest degree of mutual altruism. This is borne out well enough by the behavior of clones which make up the bodies of multicellular organisms." However, in multicellular groups that develop by aggregation, close kinship of cells is far from assured.

Genetic conflicts that arose during lower-level transitions remain unsettled in the transitions to multicellularity (Burt & Trivers 2006, Dawkins 1976, Maynard Smith & Szathmáry 1995). Some of these conflicts involve fungible (or interchangeable) units; others occur between nonfungible units (Lachmann et al. 2003, Queller 2000). Fungible units share the same gene pool and directly compete for transmission, and kinship is a key factor influencing cooperation. Organelles within cells, cells within organisms, and conspecific multicellular individuals in societies all represent fungible units. Nonfungible units do not share the same gene pool and compete indirectly; kinship is not a factor in the evolution of cooperation between nonfungible units. Examples of potential conflicts between nonfungible units in the transition to multicellularity include interactions between genes within chromosomes, and—for eukaryotes—interactions between nuclei and organelles. In addition, many multicellular organisms depend upon higher-level, nonfungible cooperative interactions with different species.

Conflicts between Fungible Units

Cell-lineage interactions. When costs of cooperation reduce the growth rate of a cell lineage, mutant noncooperative cells could gain a competitive advantage over cooperating cells, at least within the organism's life span (Michod 1997, Michod & Roze 1999, Orive 2001, Otto & Orive 1995). If these cells gain access to reproductive tissues, then their influence could extend across generations. Buss (1982, 1983a,b, 1987) proposed that cell lineages that evolve behaviors giving them a disproportionately large share of a host's reproductive output represent a major long-term threat to the integrity of multicellular individuals. Germ-line parasites gain their advantage by directing differentiation toward reproductive cells, using the host for somatic

support. Somatic cell parasites multiply more rapidly than their host's cells, biasing access to germ lines by a numerical advantage. In the long run, a transmission advantage for either form of defector requires access to the germ line. Thus, the defectors must invade organisms that do not sequester a germ line (and which have mobile cells), strike before a host sequesters a germ line (Buss 1982, Michod 1999), or arise in a germ line or meristem.

Defectors in clonal developers. Genetic variants, presumably owing to in situ mutation, arise with detectable frequency in many multicellular organisms with clonal development (reviewed in Pineda-Krch & Lehtilä 2004). Cancers, primarily in vertebrates, constitute the clearest evidence for defecting cell lineages in clonal developers, but very few are transmitted across generations and manage to re-infect other hosts or succeed on their own. Consequently, cancers rarely have any evolutionary potential beyond the life span of the individual they threaten (Frank 2003, Nunney 1999). The exceptions are interesting and may be more numerous than currently thought. Canine transmissible venereal tumor is the best documented example. Strong genetic evidence indicates that the infectious agent is itself a tumor cell that originated between 200 and 2500 years ago from a wolf or East Asian dog breed (Murgia et al. 2006). The tumor cells are apparently transmitted venereally, as well as by allogrooming and bites (Das & Das 2000). Although the genetic data are less comprehensive, devil facial tumor disease of Tasmanian devils has a similar natural history, and the "infective agent is a rogue cell line that initially evolved in a tumor of unknown origin" (Pearse & Swift 2006). Transmission of sarcoma cells among hamsters by mosquitoes may also occur (Banfield et al. 1965). HeLa cells, originally isolated from a human cervical carcinoma, now thrive by providing social benefits to those who maintain them as unicells in tissue culture (Strathmann 1991).

The general lack of transgenerational success partly reflects the challenges for a defector gaining access to a host's germ line (Buss 1983a, 1987). For example, defector mutants in organisms with rigid cell walls, unless they appear in meristematic tissue, will only succeed in damaging their host (Klekowski 1988). The same is true of defectors in metazoans that sequester their germ line: Mutants that appear in somatic cells have little hope for a future of their own (Buss 1983a,b, 1987, 1990).

Just as importantly, any obligate defector (such as a cancer) that rapidly divides and fails to cooperate with the other cells of the organism is unlikely to perform well on its own. Yet, a unicellular bottleneck would force it to do just that (**Figure 2*a***) (Bell 1989, Grosberg & Strathmann 1998, Raff 1988, Van Valen 1988, Wolpert 1990). A multicellular organism composed entirely of defector cells would, at best, have no advantage relative to cooperating genotypes in the next generation and would more likely be at a functional disadvantage (Strathmann 1991). For example, in the social myxobacterium *Myxococcus xanthus*, most defector strains can persist only in the company of normal strains; in isolation, defectors produce abnormal multicellular structures, and perish (Fiegna & Velicer 2003). The same situation should apply to isolated defector mutants in the cellular slime mold *Dictyostelium* (Buss 1982), unless they facultatively produce normal stalks when they develop by themselves (Strassmann et al. 2000).

The more differentiated and integrated a multicellular organism is, the greater the potential that a defector will compromise organismal function (Roberts 2005) and—in the case of clonal development from a unicell—its own future, were it to produce spores or zygotes. As multicellular organisms become more differentiated, a defector cell lineage is more likely to be a defective cell lineage once it leaves the company of its host organism. Facultative defection represents one escape from this quandary, with defectors cooperating with cells carrying the same mutation (Queller 2000). Mutations for such facultative defection seem less probable than a purely defector mutant; moreover, if there is a cost associated with being a facultative defector, then this strategy would lose its advantage once it had produced an organism composed entirely of cells with that mutation.

Defectors in aggregating developers. Multicellular organisms that develop by aggregation offer more opportunities for cheaters to prosper, especially when kinship among the genotypes that co-aggregate is low (**Figure 2*b***). It should therefore not be surprising that the best evidence for successful, persistent defecting cell lineages comes from organisms that become multicellular by aggregation.

Several recent reviews have examined evidence for cheating in cellular slime molds, myxobacteria, and pseudomonad Eubacteria (Ackerman & Chao 2004, Crespi 2001, Keller & Surette 2006, Pál & Papp 2000, Strassmann & Queller 2004, Travisano & Velicer 2004, Velicer 2003). Cellular slime molds aggregate when starved, forming a multicellular slug (the pseudoplasmodium) that eventually differentiates into a fruiting body, or sorocarp. About 20% of the sorocarp consists of sterile stalk cells that distally support a mass of spores (the sorus). These spores germinate to form the next generation of free-living amoebae.

Filosa (1962) first clearly documented mutants in natural populations of *Dictyostelium mucoroides* that could act as defectors. In cultures initiated from individual spores isolated from a single sorus, he regularly found a large range of phenotypes in the next generation of sorocarps. At one extreme were cultures with normal stalks and sori; at the other were cultures that lacked stalks and produced only sori. Stalkless mutants would presumably perform poorly in nature because of the stalk's presumed role in dispersal (Bonner 1967, 1982; Huss 1989). However, when aggregating with stalk-producing forms, these stalkless mutants, in principle, could persist.

Buss (1982) isolated a naturally occurring stalkless mutant that could co-aggregate with normal stalked forms. By experimentally varying the ratio of stalkless to stalked spores that initiated a culture, Buss showed that stalkless forms, as expected, consistently produced a greater-than-expected fraction of the spores from fruiting bodies. These mutants, some of which result from a simple deficiency in an F-box protein, appear to be guided into prespore developmental pathways and may also direct normal cells to become part of the sterile stalk (Ennis et al. 2000). All else being equal, the success of such obligately stalkless mutants should exhibit negative frequency dependence in natural populations because, as the frequency of stalkless mutants increased (given their advantage in producing spores), they would increasingly co-aggregate with other stalkless mutants and be ineffective dispersers (Armstrong 1984, Buss 1982, Gadagkar & Bonner 1994).

Recent studies of *Dictyostelium discoideum* have added novel insights to these findings. Strassmann et al. (2000) used microsatellite markers to confirm that clones isolated from field populations combined to form chimeric sorocarps. They identified a high frequency of cheaters from natural isolates, some of which facultatively adjusted their allocation to stalk and spore, depending upon whether they grew in isolation or as a chimera.

In many myxobacteria, there are two social phases of the life cycle in which cheating can occur (Dworkin 1996). During the group-foraging phase, cells secrete an array of chemicals that coordinate movement and digest prey (reviewed in Dworkin 1996, Shimkets 1990). Social parasites could respond to these chemicals, and consume partially digested food, without paying the price to make these substances. In addition, swarming requires motility and coordination, behaviors mediated by extracellular pili and fibrils (Shi & Zusman 1993) that are costly to produce and can potentially be parasitized (Velicer & Yu 2003).

In the second social phase, starved myxobacterial cells aggregate to form fruiting bodies that, like those of many cellular slime molds, consist of supportive somatic cells, destined to die, and cells that will become spores. Nonspore cells lyse, potentially feeding cells that will become spores. The studies of *M. xanthus* by Velicer and colleagues have identified mutants that in mixed-cell cultures obtain a disproportionately high representation of spores, or failed to make spores but outgrew spore-making competitors (e.g., Fiegna & Velicer 2003, Velicer et al. 1998, 2000). Some of these sporeless mutants retained the capacity to make spores, but only when mixed with spore formers (Velicer et al. 2000). These sporeless mutants exploited their spore-making hosts, and some eventually lost the ability to make spores themselves, going extinct (Fiegna & Velicer 2003, Velicer et al. 2000). Remarkably, at least one of these sporeless mutants eventually recovered the ability to make spores, and the recovery required a single point mutation at the locus encoding an acetyltransferase (Fiegna et al. 2006).

Finally, in selection experiments performed on the eubacterial plant pathogen *Pseudomonas fluorescens*, wild-type unicells, when grown in unshaken medium, eventually die from oxygen starvation, but novel cells often evolve that colonize the surface of the culture medium and form a multicellular biofilm that can both respire and access nutrients (Rainey & Rainey 2003). The production of the extracellular adhesive polymer that supports the film is costly to individual cells. Defectors do not produce the matrix of the biofilm and at high frequencies can sink the entire structure.

Selection on the evolution of cooperation in organisms that develop by aggregation depends upon whether the cells that form multicellular structures are clone mates or close kin (Crespi 2001). As Ackerman & Chao (2004) note, the production of adhesives is the foundation of multicellularity in *Myxococcus* and *Pseudomonas*. These environmental adhesives could also increase population viscosity, uniting clone mates or kin as cells divide before differentiating into a fruiting body or biofilm, and providing an incentive for cooperation (Rainey & Rainey 2003, Velicer et al. 2000). Nevertheless, several laboratory studies on the genetic composition of fruiting bodies in *D. mucoroides* and *D. discoideum* suggest that multiple genotypes can co-aggregate and

that defectors sometimes join these aggregations (Buss 1982, Filosa 1962, Strassmann et al. 2000).

The opportunities for co-aggregation of multiple clones of microorganisms in the rhizosphere may be substantial. Buss (1982) found at least two strains of *D. mucoroides* in closely spaced soil samples. Francis & Eisenberg (1993) identified multiple clones of *D. discoideum* in spatially restricted samples. A microsatellite-based study by Fortunato et al. (2003) revealed multiple genotypes of *D. discoideum* within samples separated by as little as 6 mm. (Nevertheless, average r among isolates from a single soil sample was approximately 0.5, partly owing to multiple isolates being from the same clone but also because $r \approx 0.15$ for different clones in a sample.) Vos & Velicer (2006) isolated 78 clones of *M. xanthus* containing 21 unique genotypes in a 225-cm^2 soil sample, suggesting that members of different clones co-occur on spatial scales that would allow them to join the same aggregation. Whether different clones naturally co-aggregate and cooperate in feeding swarms or to form fruiting bodies is not yet settled. *M. xanthus* clones isolated from remote locations are antagonistic, with reduced spore production in forced mixtures (Fiegna & Velicer 2005). It is not yet known whether naturally co-occurring clones behave comparably poorly (Vos & Velicer 2006).

In sum, a great deal remains to be discovered about spatial scales of interaction in natural habitats and the importance of kinship in the evolution of social behavior in aggregating microorganisms (reviewed in Crespi 2001). *D. discoideum* apparently do not distinguish close from distant kin when they aggregate (Strassmann et al. 2000). However, some clones of *D. mucoroides* do not mix (e.g., Buss 1982), and Mehdiabadi et al. (2006) recently showed a strong effect of kinship on the formation and composition of sorocarps in *Dictyostelium purpureum*. Kaushik et al. (2006) reported substantial levels of interclonal incompatibility in *Dictyostelium giganteum*. Even with low levels of relatedness, if the fitness of defector mutants on their own is sufficiently low, kin selection can maintain altruistic behavior (Hudson et al. 2002). Thus, in some species, kinship alone may control conflict in multicellular structures produced by aggregation, whereas in other species, alternative defenses, including greenbeard recognition (Queller et al. 2003) and policing (Frank 1995, 2003; Sachs et al. 2004), may also be important. Moreover, despite the costs from defectors, there may be synergistic benefits to co-aggregation and cooperation that outweigh the costs (Foster et al. 2002), especially when there are diminishing returns to escalating investment in either competition or cooperation (Foster 2004).

Defectors in fusion chimeras. Many sponges, as well as colonial cnidarians, bryozoans, and ascidians, occasionally fuse with genetically distinct conspecifics, forming genetically chimeric individuals (reviewed in Buss 1987, 1990; Grosberg 1988; Hughes 1989, 2002). Intergenotypic fusion also occurs among the hyphae of some fungi (Wu & Glass 2001), the sporelings of red and green algae (Gonzáles & Correa 1996, Santelices 2004), and the roots of some plants (reviewed in Pineda-Krch & Lehtilä 2004). As with aggregative development, intergenotypic fusion opens the door to horizontal transfer of defectors, especially when cells are mobile. Moreover, because virtually all metazoans capable of intergenotypic fusion do not sequester

a germ line (Blackstone & Jasker 2003; Buss 1982, 1983a,b, 1987; Nieuwkoop & Sutasurya 1981), and because their cells are mobile, they are especially vulnerable to defectors that invade germ lines and therefore can be vertically transmitted across generations.

Several studies of invertebrate chimeras provide circumstantial evidence for the existence of defector genotypes in natural populations (Pancer et al. 1995, Sabbadin & Zaniolo 1979; reviewed in Buss 1987, 1990; Grosberg 1988). The clearest demonstration of intraspecific cheating in fusion chimeras comes from the colonial ascidian *Botryllus schlosseri*, where some genotypes are predictably overrepresented in the gametic output of chimeric colonies (e.g., Stoner & Weissman 1996, Stoner et al. 1999). Although chimeric organisms may have some synergistic performance advantages over single genotypes that offset the individual cost of being parasitized (Buss 1982, 1990; Foster 2004; Rinkevich & Weissman 1992), they may also often pay substantial functional costs (e.g., Barki et al. 2002, Frank et al. 1997, Maldonado 1998, Rinkevich & Weissman 1992, Santelices et al. 1999). Nevertheless, with some notable exceptions (e.g., Bishop & Sommerfeldt 1999), almost all metazoans that fuse with conspecifics (reviewed in Grosberg 1988), including *Botryllus* (Oka 1970, Scofield et al. 1982), possess highly polymorphic, genetically based self/nonself recognition systems that limit fusion to clone mates and close kin (reviewed in Grosberg 1988, Grosberg et al. 1996). Consequently, the fitness costs of such parasitism should be correspondingly reduced.

Conflicts between Nonfungible Units

Multicellular organisms include diverse intracellular and extracellular symbionts that are nonfungible evolutionary units. As Hamilton (1987b) suggested for insect societies composed of close kin, development from unicells could be costly in that multicellular organisms composed of a clone of cells could be more vulnerable to pathogens. Multicellular organisms have, however, evolved a variety of cellular and humoral defenses against horizontally transmitted pathogens. In addition, host organisms also select among mutualistic partners: Squids choose specific light-emitting *Vibrio* spp. (Fidopiastis et al. 1998), anthozoan cnidarians accept or reject specific algal strains as photosynthetic partners (Baker 2003, Muller-Parker & Secord 2005), and leguminous plants punish or exclude less-productive nitrogen-fixing rhizobia (Denison 2000). Finally, a unicellular bottleneck aids control of uncooperative extracellular symbionts because unicellular offspring cannot harbor large extracellular parasites (Grosberg & Strathmann 1998).

Eukaryotic cells contain vertically transmitted intracellular symbionts, notably mitochondria and chloroplasts, but often also less helpful ones. Selection among replicators within cells can differ from selection between cells, generating conflicts that can increase the fitness of symbionts at the expense of host function (Burt & Trivers 2006). In addition, asexually multiplying endosymbionts are subject to Muller's ratchet, accumulating deleterious mutations. This misalignment of selection on host cells and endosymbionts originated in unicellular organisms and continues, incompletely resolved, in multicellular organisms. Moreover, for a multicellular organism, genetic

differences among endosymbionts could be the basis for genetic differences among its cells, leading to competition among its cell lineages.

Uniparental inheritance of mitochondria (or other internal symbionts) partially limits opportunities for conflicts within the cells of a multicellular organism (Birky 1995). There also appears to be a within-cell germ-line bottleneck that further restricts the number of transmitted endosymbionts, limiting the scope for diversification within host cells and thus maintaining performance at the cellular and multicellular levels (Bergstrom & Pritchard 1998, Krakauer & Mira 1999). Although the within-cell bottleneck is not as complete as that for nuclei, it can serve to redistribute variation within a cell to variation between cells, and thus between multicellular offspring (Bergstrom & Pritchard 1998, Sekiguchi et al. 2003).

DEFENSES AGAINST DEFECTORS

Michod (2003) distinguished between two kinds of defense against defectors. The first limits the prospects for transmission or survival of defectors from generation to generation, restricting the opportunity for defectors to spread through a population. The second controls within-organism variation and threats that arise within the life cycle of a multicellular organism. There is a sharp divide between those who argue that the primary conflict mediator in the evolution of multicellularity was and remains a unicellular bottleneck (e.g., Dawkins 1982; Keller & Surette 2006; Maynard Smith 1988; Maynard Smith & Szathmáry 1995; Queller 1997, 2000; Seger 1988; Van Valen 1988; reviewed in Grosberg & Strathmann 1998), and those who either omit the bottleneck entirely or who acknowledge its significance, albeit as a secondary one. For example, Buss (1987) emphasized the roles of maternal control, germ-line sequestration, and self/nonself recognition systems as conflict mediators during transitions to multicellularity, but did not explicitly consider the impacts of a unicellular bottleneck, a point noted in several reviews of his landmark book (e.g., Bell 1989, Seger 1988, Van Valen 1988). These contrasting views persist. Michod & Nedelcu (2003, p. 66) state, "In the case of multicellular groups, conflict mediation may involve the spread of conflict modifiers producing self-policing, maternal control of cell fate, decreased propagule size, determinate growth of the organism, apoptotic responses, or germ line sequestration . . . " In contrast, Queller (2000, p. 1648) stated, "Relatedness has such a simple role in the transition to multicellularity, that for a long time it received little attention. When the multicellular form develops from a single cell undergoing mitotic divisions . . . it is a clone of genetically identical cells . . . "

Unicellular Bottlenecks/Propagule Size

The life cycles of the vast majority of multicellular organisms regularly include a unicellular stage. There may be some ecological advantages to being a unicell, especially with respect to dispersal, and most of the proposed advantages of sexual reproduction require a unicellular stage; however, the developmental and ecological risks must be considerable. Indeed, many organisms expend substantial resources to protect their offspring while they are small (reviewed in Grosberg & Strathmann 1998,

Strathmann 1998). Protection, in turn, often intimately brings together cells of parents and their offspring, as well as those of siblings, heightening the scope and intensity of parent-offspring and sibling conflict (Clutton-Brock 1991, Haig 1993, Grosberg & Strathmann 1998, Parker et al. 2002).

The effects of a unicellular bottleneck on genetic conflicts could offset these risks in several ways. First, a unicellular bottleneck ensures that each generation begins with a group of cells that shares all of their genes by descent (Dawkins 1976, 1982; Grosberg & Strathmann 1998; Hamilton 1964b; Maynard Smith 1988; Seger 1988). When Buss (1987, p. 53) asked, "Why, then, should any cell in a dividing embryo become ciliated, or otherwise differentiated, in a fashion which limits its own capacity for increase?" he answered, "It should not" (Buss 1987, p. 53). However, with clonal development, $r = 1$ in Hamilton's inequality ($rb > c$), and it seems a cell should become differentiated if $b > c$.

Second, in each generation, the variation among replicators that arises within the parental life cycle is partitioned among offspring, rather than continuing within offspring. Some offspring are more fit and some less, rather than all being compromised (Crow 1988; Dawkins 1982; Griesemer et al. 2005; Grosberg & Strathmann 1998; Keller & Surette 2006; Kondrashov 1994; Maynard Smith 1988; Michod & Roze 2001; Queller 1997, 2000). This process not only reduces mutational load, it also limits the prospects for successful survival and transmission of defector cell lineages that fail to produce a multicellular organism that is as fit as organisms produced by cooperator genotypes (Lachmann et al. 2003). Finally, passage through a unicellular bottleneck, in addition to uniparental inheritance of organelles, reduces (but does not eliminate) the potential for conflict within and among host cells carrying genetically heterogeneous populations of organelles (Bergstrom & Pritchard 1998).

Whether a unicellular bottleneck is sufficient to protect multicellular organisms against defector cell lineages depends upon the frequency and types of variation that arise among cell lineages. In particular, if the frequency of mutations that (*a*) increase their own replication rate, (*b*) damage their multicellular host, and (*c*) can be transmitted across generations is high enough, then other mechanisms of control—including germ-line sequestration and policing—may be important (Roze & Michod 2001). However, there is little evidence that defector cell lineages arise by somatic mutation at a frequency that renders a unicellular bottleneck ineffective (Queller 2000). Indeed, Michod (2003, pp. 298–99) concluded, "So long as mutations are selfish, smaller propagule size may be selected, including single cell reproduction."

There are several cases in which multicellular organisms appear to lack a unicellular bottleneck for multiple generations, or in which single cells contain multiple nuclei. In either situation, genetic uniformity of the cell lineages that constitute a clonally developing multicellular organism could be cumulatively eroded.

1. Attine ants farm a variety of basidiomycete fungi as asexual, multicellular hyphae in their nests (reviewed in Mueller 2002, Mueller et al. 2005). Ant foundresses transport their fungal symbionts to new nests as a multicellular bolus held in an infrabuccal pouch (Chapela et al. 1994, Mueller 2002). Although the fungal symbionts of attines appear to be able to form normal basidiocarps and

produce spores, they may rarely, if ever, actually develop from a unicellular spore (Mueller 2002). However, phylogenetic and population genetic data suggest that the fungal symbiont population could be regularly feralized, passing through a standard bottleneck by germinating through spores and then being redomesticated by ant hosts (Mueller 2002).

2. Lichens, some of which produce multicellular propagules that contain both fungal and algal cells (Kondrashov 1994), may include other examples of long-term vegetative propagation without a bottleneck and may also be stabilized by partner fidelity feedback. However, many fungi in the lichen symbiosis periodically reacquire new algal partners (Honegger 1993, Yahr et al. 2004).
3. Laboratory studies of some members of the delta subgroup of magnetotactic proteobacteria suggest that adult colonies produce multicellular daughter colonies, without any intervening unicellular stage (Keim et al. 2004). Nevertheless, the fact that these normally anaerobic organisms were aerobically cultured cautions against accepting the absence of a bottleneck without further study.
4. Arbuscular mycorrhizal fungi are symbionts of terrestrial plants, facilitating the uptake of nutrients for their hosts and receiving carbon in return (Smith & Read 1997). They are thought to be exclusively asexual and to date from the Ordovician. Their unicellular spores contain hundreds of nuclei, which, in the absence of a uninucleate bottleneck, could lead to the accumulation of genetic differences within and among the cells of the developing fungus. However, recent genetic analyses indicate that all nuclei within a spore are genetically uniform (Pawlowska & Taylor 2004), although the mechanism that produces such homokaryosis is not yet understood.

We know of no other documented instances where multicellular or multinucleate vegetative propagation continues for more than a few generations without a unicellular stage.

Self/Nonself Recognition and Policing

Virtually all multicellular organisms—including most metazoans, plants, fungi, many algae, and bacteria—use genetic, environmental, or physiological cues to distinguish conspecific self from nonself cells or tissues (reviewed in Buss 1983a,b, 1987; Grosberg 1988; Mydlarz et al. 2006). By directly recognizing defector cells or the carriers of defector cells through their phenotypes and genotypes (greenbeard recognition) or by kinship, multicellular organisms can limit the opportunity for invasion by a selfish genotype.

An individual can most efficiently protect itself by detecting and excluding defectors before they can gain the benefits of cooperative multicellularity. The allorecognition systems of many clonal marine invertebrates represent this form of defense, but do not recognize defectors per se, instead limiting cooperative interactions to close relatives with whom they are likely to share genes throughout their genome. The sequencing and annotation of the *D. discoideum* genome has revealed several additional ways that recognition and policing may control defectors in aggregating

multicellular organisms. First, in studies of *dimA*, a gene that controls reception of a molecule (DIF-1) necessary for differentiation into sterile prestalk cells, knockout mutants produce no *dimA* product and cells differentiate only into prespore cells. Such mutants should be very effective cheaters in chimeras. However, aggregations of wild-type cells exclude *dimA* mutants from spores (Foster et al. 2004). This pleiotropic effect of *dimA* means that although mutants should have an advantage in chimeras, they rarely get to exercise it. Second, cells with knockouts of the *csA* gene (which encodes an adhesion protein, gp80) join aggregates with wild-type cells on agar media (Queller et al. 2003), but their weaker intercellular binding properties leave them lagging behind normal amoebae and congregating toward the posterior end of pseudoplasmodia. Cells in this location are very likely to become spores, and so *csA*-deficient mutants should be successful defectors. However, on natural substrates like soil, csA^- cells have such weak intercellular binding strength that they are generally not entrained into developing pseudoplasmodia or sorocarps. The *csA* locus may qualify as a greenbeard gene (sensu Dawkins 1982) and represents a way that csA^+ (wild-type) amoebae can exclude potential defectors from joining sorocarps. Similar greenbeard effects may also influence interactions between fire ants (Keller & Ross 1998, Krieger & Ross 2002) and bacteria (Haig 1997).

Alternatively, with policing, a recognition system can detect cheaters after they appear within an organism, priming an effector mechanism that sequesters, kills, punishes, or encourages the suicide or PCD of the defectors (Frank 1998, 2003; Michod 2003; Sachs et al. 2004). The vertebrate immune system epitomizes this form of policing, although social hymenoptera exhibit similar behaviors (reviewed in Frank 2003, Sachs et al. 2004).

Programmed Cell Death and Apoptosis

PCD encompasses several mechanisms of cellular suicide in both prokaryotes and eukaryotes (Koonin & Aravind 2002). The occurrence of caspase orthologs and other proteins related to PCD in diverse unicellular organisms (Bidle & Falkowski 2004, Gordeeva et al. 2004) implies that some of the functions of PCD in multicellular organisms existed in unicellular ancestors. Apoptosis is a specialized form of PCD, where a cell commits suicide in response to external or internal signals and dies in a way that avoids release of materials damaging to other cells (reviewed in Lodish et al. 2000). In eukaryotes, mitochondria play an important role in apoptosis, suggesting that apoptosis originated with the manipulation of host cells by ancestral protomitochondria. These protomitochondria may have released caspases or cytochrome *c*, perhaps either to maintain their symbiosis by killing host cells upon disturbance (Burt & Trivers 2006, Kobayashi 1998) or to trigger genetic recombination of the host cell in response to stress (Blackstone & Green 1999).

Michod (1999) argued that cell death "should only increase within-organism conflict" because it "increases the number of cell divisions necessary for an organism to reach a given adult size," which consequently increases "the opportunity for within organism change and variation." However, PCD plays critical roles in normal development and the repair or removal of damaged or infected cells or tissues (Meier et al.

2000). Indeed, cancers often result from mutations that disrupt or prevent apoptosis (Kaufmann & Gores 2000). In destroying mutant defector or defective cells that arise during development, apoptosis is a form of policing that represents an extreme form of altruism—an unselfish suicidal role that is expected among clone mates (Hamilton 1964a,b). Apoptosis and other forms of PCD do not necessarily involve conflict; rather, PCD illustrates the ease of policing in a clone of cells.

Maternal Control in Early Development

Buss (1987) proposed that selection to limit conflicts among cell lineages favored the evolution of maternal control of early embryonic development of metazoans. It is true that in the earliest stages of embryogenesis of many metazoans, the maternal genome is often well represented as messenger RNA and maternal gene products dominate developmental processes. However, the cells of an embryo are recent descendents from the zygote, with the least potential for mutation to have influenced genetic divergence among cells. Thus, conflicts among cell lineages should be negligible at early stages, and selection for control of conflicts should be minimal (Queller 2000).

There are alternative hypotheses. Multicellular organisms, including metazoans, differ greatly in the extent to which the maternal genome controls events in early development. The importance of maternal transcripts appears to vary with development time and degree of parental care or protection in metazoans. Reliance on maternal mRNA rather than embryonic transcription may therefore reflect selection for speedy development (Strathmann et al. 2002).

Germ-Line Sequestration

Buss (1983a,b, 1987) also argued that sequestration of a germ line is a defense against the origination and proliferation of defector cell lineages and their transmission across generations. Once a cell, or group of cells, is set aside as the progenitor(s) of all reproductive cells, mutations that arise in the much larger group of nonreproductive (somatic) cell lineages are excluded from being incorporated into gametes and transmitted to the next generation. Germ-line sequestration then becomes one of the key breakthroughs allowing the evolution of differentiated, integrated multicellular organisms. Michod (2003) has modeled the influence of the timing of sequestration, the number of cells sequestered, and the number of mitotic cycles on the distribution of mutational load and fitness variance at the level of the cell lineage and multicellular organism. In these models, the main cost of sequestering a germ line is that sequestered cells and their descendants can no longer provide somatic support. The earlier a germ line is sequestered, and the fewer progenitor cells that initiate it, the less likely it is that defector mutants can arise and come to dominate the gamete pool before the germ line is sequestered. Germ lines are typically set aside as groups of cells, often after multiple cycles of cell division. However, at the extreme, when a single cell initiates the germ line, only mutations arising in that cell and its descendants can appear in gametes. Also, germ line cells typically go through fewer division cycles than most somatic cells, the difference being magnified as organisms grow

somatically (Michod & Roze 2001). Thus, even if all cells in an organism have the same per-division mutation rate to defectors, gamete cells, during their ontogeny, are less likely to be hit by a defector mutant than the remaining somatic cells. Moreover, germ lines may experience lower mutation rates than somatic cell lineages (Drake et al. 1998, Maynard Smith & Szathmáry 1995), perhaps because they have lower metabolic rates than most somatic cells (Michod 2003).

The assumptions in these models raise a fundamental question: How often do defector mutants arise during the ontogeny of a multicellular organism? Such mutants would need to have a remarkable combination of features if they were to survive beyond the generation in which they arose. They could not so thoroughly damage the soma of the organism in which they arose that the host no longer functioned well enough to reproduce competitively. The mutation would have to occur in a cell or cell lineage that produced gametes. In organisms that develop clonally from a unicell, defectors would inevitably have to produce a competitive soma on their own. Such mutants are conceivable, but seem most likely in organisms with minimal differentiation and integration and that develop by aggregation rather than clonally.

Although most models for the evolution of germ-line sequestration (e.g., Michod 1996, 1997; Michod & Roze 1999) consider only mutants that are obligate defectors (unconditional sensu Queller 2000), facultative defectors could arise that produce a normal soma when developing as a clone but defect when they arise in a population of cooperating cells (Hudson et al. 2002, Strassmann et al. 2000). In this case, if there were no costs to being a facultative defector, then the mutant should spread to fixation in a population (Matsuda & Harada 1990). If there were a cost, then a substantial increase in the frequency of the mutant should require a low cost or a high mutation rate. Thus, defector mutants that both significantly disrupt the integrity of clonally developing multicellular organisms and that can also spread through subsequent generations should be rare, reaching an equilibrium determined by the rate at which they arise by mutation, the benefits they gain in a chimeric state, and the costs of developing in isolation (Queller 2000).

All told, germ-line sequestration can reduce the threat of defectors in multicellular organisms being incorporated into gametes and vertically transmitted. Although there are no decisive studies, organisms that sequester germ lines might produce gametes with lower deleterious mutational loads than organisms in lineages that do not sequester their germ lines. Nevertheless, many lineages of multicellular organisms, even those with motile cells, do not sequester germ lines but still manage to produce a diversity of organisms with highly differentiated body plans (Blackstone & Jasker 2003, Extavour & Jakam 2003). Moreover, many of the multicellular organisms that do not sequester a germ line early in ontogeny have scattered pluripotent cells (e.g., plant meristems, stem cells in cnidarians, circulating cells in colonial ascidians), some of which may later become epigenetically modified and develop into germ cells. Thus, germ-line sequestration is not an essential defense for the evolution of multicellular differentiation, even in lineages with motile cells.

The question of what selective factors promote the evolution of a sequestered germ line remains unresolved and may have little to do with the control of defectors. The germ-soma distinction may variously be a response to constraints on motility as

aquatic multicellular organisms increased in size (Solari et al. 2006), a means of avoiding the challenges of dedifferentiating highly modified cells into totipotent gametes by setting aside undifferentiated cells (Jablonka & Lamb 1995), or simply employing as the germ line those cells left after other cell lineages have begun to differentiate. As Queller (2000, p. 1653) suggested, "the germ line might have originated as a consequence of other cell lineages altruistically removing themselves from the reproductive line, to perform some somatic benefit to the organism." From this perspective, populations of germ and stem cells might be kept small, not so much because of the risk of a mutation occurring, but because minimizing the number of germ cells and stem cells limits the number of cells that are in a less differentiated and less useful state.

EVOLUTION OF COMPLEXITY IN MULTICELLULAR ORGANISMS

To conclude, we return to the question of what limits the complexity, specifically the number of different kinds of cells, of multicellular organisms once the transition has occurred. We ignore the arrangements of cells, although much of the diversity of multicellular organisms lies in how cells are arranged. We also ignore the complexity of individual cells, although cells of multicellular organisms appear to have fewer kinds of visible cell parts than unicellular organisms (McShea 2002).

One limit on the number of kinds of cells is suggested by lower levels of cellular differentiation in organisms developing by aggregation than in many comparably sized clonal developers (Wolpert & Szathmáry 2002). Conflict control was the fundamental role of a unicellular bottleneck; cooperation results whether cells differentiate or not. Nonetheless, the bottleneck subsequently facilitated the evolution of extensive differentiation and integration, perhaps because the scope for coordinated development among cells is greater when cooperation is assured by genetic identity among cells. (Differentiation of cells is often influenced by symbionts, but the differentiating cells within each partner species are usually clone mates.)

What are the limits on number of cell types in organisms with clonal development from a unicell? Carroll (2001) noted that because "very few regulatory proteins can orchestrate markedly different cell physiologies, it is curious that more multicellular forms have not evolved." The answer may simply be a lack of selective pressure or trade-offs. For example, the vertebrate immune system generates different cells in indefinite variety because variety is favored by selection. We expect that functional requirements limit the number of cell types in organisms that develop from a single cell. Evolving a change in a regulatory pathway that produces additional variation among cells should require few mutational steps, and few mutational steps could then remove the regulatory change. Once the major functional specializations are satisfied, there is a presumably diminishing return in added capabilities from each additional cell type.

This argument implicitly concerns the role of small variants (within cell types) in the generation of cellular diversity, but trends for more distinctively different cell types support this line of reasoning. For example, the number of cell types increases with body length (Bonner 1965) and with log cell number (estimated from body volume), although in the latter case the slope is only 0.056 (Bell & Mooers 1997).

Bell & Mooers (1997) explain this pattern by the small effect of a single cell on the performance of a large organism, a tissue of many similar cells being the smallest effective unit for a task.

Different kinds of organisms appear to require different numbers of cell types. For a given size, number of cell types is greater for animals than for green plants and greater for green plants than for phaeophytes (Bell & Mooers 1997). The difference in number of cell types between animals and plants suggests a relation between complexity (in terms of variety of cells) and motility of either the constituent cells or the whole organism. This difference is also consistent with the apparent ease of evolving multicellularity for phototrophs versus the difficulties for phagotrophs (Cavalier-Smith 1991). Nevertheless, the barriers to the evolution of multicellular phagotrophy are not obvious. For example, several groups of multicellular phagotrophs (sponges, placozoans, and vorticellid colonies) lack a multicellular mouth, suggesting that the need for a mouth was not a serious obstacle. Perhaps importantly, at least for metazoans, both the number of cell types (Valentine et al. 1994) and body lengths (Bonner 1965) appear to have increased from approximately 600 Mya to the present.

Taken together, the available data suggest that just as the evolution of multicellularity itself is a minor major transition, the same may be true for complexity of multicellular organisms, at least in terms of cellular diversity (also see Vermeij 2006). Although there may be genetic, developmental, and phylogenetic constraints on the evolution of cellular diversity, the evidence so far suggests that cellular diversity can evolve easily when functionally called for by selection.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

Ackerman M, Chao L. 2004. Evolution of cooperation: two for one? *Curr. Biol.* 14:R73–74

Armstrong DP. 1984. Why don't cellular slime molds cheat? *J. Theor. Biol.* 109:271–83

Ausmees N, Jacobs-Wagner C. 2003. Spatial and temporal control of differentiation and cell cycle progression in *Caulobacter crescentus*. *Annu. Rev. Microbiol.* 57:225–47

Avise J. 1993. The evolutionary biology of aging, sexual reproduction, and DNA repair. *Evolution* 47:1293–301

Baker AC. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annu. Rev. Ecol. Evol. Syst.* 34:661–89

Baldauf SL. 2003. The deep roots of eukaryotes. *Science* 300:1703–1706.

Banfield WG, Woke PA, MacKay CM, Cooper HL. 1965. Mosquito transmission of a reticulum cell sarcoma of hamsters. *Science* 148:1239–40

Barki Y, Gateño D, Graur D, Rinkevich B. 2002. Soft-coral natural chimerism: A window in ontogeny allows the creation of entities comprised of incongruous parts. *Mar. Ecol. Prog. Ser.* 231:91–99

Bell G. 1985. The origin and early evolution of germ cells as illustrated in the Volvocales. In *The Origin and Evolution of Sex*, ed. H Halvorson, A Monroy, pp. 221–56. New York: Liss

Bell G. 1989. Darwin and biology. *J. Heredity* 80:417–21

Bell G, Koufopanou V. 1991. The architecture of the life cycle in small organisms. *Philos. Trans. R. Soc. London Ser. B* 332:81–89

Bell G, Mooers AO. 1997. Size and complexity among multicellular organisms. *Biol. J. Linn. Soc. London* 60:345–63

Bergstrom CT, Pritchard J. 1998. Germline bottlenecks and the evolutionary maintenance of mitochondrial genomes. *Genetics* 149:2135–46

Bidle KD, Falkowski P. 2004. Cell death in planktonic, photosynthetic microorganisms. *Nat. Rev. Microbiol.* 2:643–55

Birky CW. 1995. Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proc. Natl. Acad. Sci. USA* 92:11331–38

Bishop JDD, Sommerfeldt AD. 1999. Not like *Botryllus*: indiscriminate postmetamorphic fusion in a compound ascidian. *Proc. R. Soc. London Ser. B* 266:241–48

Blackstone NW, Green DR. 1999. The evolution of a mechanism of cell suicide. *BioEssays* 21:84–88

Blackstone NW, Jasker BD. 2003. Phylogenetic considerations of clonality, coloniality, and mode of germline development in animals. *J. Exp. Zool. B* 297:35–47

Blanton RS, Olive LS. 1983. Ultrastructure of aerial stalk formation by the ciliated protozoan *Sorogena stoianovitchae*. *Protoplasma* 116:125–35

Bonner JT. 1965. *Size and Cycle*. Princeton, NJ: Princeton Univ. Press. 219 pp.

Bonner JT. 1967. *The Cellular Slime Molds*. Princeton, NJ: Princeton Univ. Press. 205 pp. 2nd ed.

Bonner JT. 1974. *On Development: The Biology of Form*. Cambridge, MA: Harvard Univ. Press. 282 pp.

Bonner JT. 1982. Evolutionary strategies and developmental constraints in the cellular slime molds. *Am. Nat.* 119:530–52

Bonner JT. 1988. *The Evolution of Complexity by Means of Natural Selection*. Princeton, NJ: Princeton Univ. Press. 272 pp.

Bonner JT. 1998. The origins of multicellularity. *Integr. Biol.* 1:28–36

Bonner JT. 2000. *First Signals: The Evolution of Multicellular Development*. Princeton, NJ: Princeton Univ. Press. 146 pp.

Bonner JT. 2004. Perspective: the size-complexity rule. *Evolution* 58:1883–90

Boraas ME, Seale DB, Boxhorn JE. 1998. Phagotrophy by a flagellate selects for colonial prey: a possible origin of multicellularity. *Evol. Ecol.* 12:153–64

Branda SS, González-Pastor JE, Ben-Yehuda S, Losick R, Kolter R. 2001. Fruiting body formation by *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* 98:11621–26

Burt A, Trivers R. 2006. *Genes in Conflict: The Biology of Selfish Genetic Elements*. Cambridge, MA: Harvard Univ. Press. 602 pp.

Buss LW. 1982. Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. Natl. Acad. Sci. USA* 79:5337–41

Buss LW. 1983a. Evolution, development, and the units of selection. *Proc. Natl. Acad. Sci. USA* 80:1387–91

Buss LW. 1983b. Somatic variation and evolution. *Paleobiology* 9:12–16

Buss LW. 1987. *The Evolution of Individuality*. Princeton, NJ: Princeton Univ. Press. 201 pp.

Buss LW. 1990. Competition within and between encrusting colonial marine invertebrates. *Trends Ecol. Evol.* 5:352–56

Carroll SB. 2001. Chance and necessity: the evolution of morphological complexity and diversity. *Nature* 409:1102–9

Cavalier-Smith T. 1991. Cell diversification in heterotrophic flagellates. In *The Biology of Free-living Heterotrophic Flagellates*, ed. DJ Patterson, J Larsen, pp. 113–31. Oxford, UK: Clarendon

Chapela IH, Rehner SA, Schultz TR, Mueller UG. 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* 266:1691–94

Clutton-Brock TH. 1991. *The Evolution of Parental Care*. Princeton, NJ: Princeton Univ. Press. 352 pp.

Coleman AW. 1999. Phylogenetic analysis of "Volvocaceae" for comparative genetic studies. *Proc. Natl. Acad. Sci. USA* 96:13892–97

Crespi BJ. 2001. The evolution of social behavior in microorganism. *Trends Ecol. Evol.* 16:178–83

Crow JF. 1988. The importance of recombination. In *The Evolution of Sex: An Examination of Current Ideas*, ed. RE Michod, BR Levin, pp. 56–73. Sunderland, MA: Sinauer

Das U, Das AK. 2000. Review of canine transmissible venereal sarcoma. *Vet. Res. Commun.* 24:545–56

Dawkins R. 1976. *The Selfish Gene*. Oxford, UK: Oxford Univ. Press. 224 pp.

Dawkins R. 1982. *The Extended Phenotype*. San Francisco: Freeman. 307 pp.

DeAngelo MJ, Kish VM, Kolmes SA. 1990. Altruism, selfishness, and heterocytosis in slime molds. *Ethol. Ecol. Evol.* 2:439–43

Denison RF. 2000. Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *Am. Nat.* 156:567–76

Drake JW, Charlesworth B, Charlesworth D, Crow JF. 1998. Rates of spontaneous mutation. *Genetics* 148:1667–86

Dworkin M. 1972. The myxobacteria: new directions in studies of prokaryotic development. *Crit. Rev. Microbiol.* 2:435–52

Dworkin M. 1996. Recent advances in the social and developmental biology of the myxobacteria. *Microbiol. Rev.* 60:70–102

England JC, Gober JW. 2001. Cell cycle control of morphogenesis in *Caulobacter*. *Curr. Opin. Microbiol.* 4:674–80

Ennis HL, Dao DN, Pukatzki SU, Kessin RH. 2000. *Dictyostelium* amoebae lacking an F-box protein form spores rather than stalk in chermas with wild type. *Proc. Natl. Acad. Sci. USA* 97:3292–97

Extavour CG, Akam M. 2003. Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. *Development* 130:5869–84

Fidopiastis PM, von Boletzky S, Ruby EG. 1998. A new niche for *Vibrio logei*, the predominant light organ symbiont of squids in the genus *Sepiola*. *J. Bacteriol.* 180:59–64

Fiegna F, Velicer GJ. 2003. Competitive fates of bacterial social parasites: persistence and self-induced extinction of *Myxococcus xanthus* cheaters. *Proc. R. Soc. London Ser. B* 266:493–98

Fiegna F, Velicer GJ. 2005. Exploitative and hierarchical antagonism in a cooperative bacterium. *PLoS Biol.* 3:1980–87

Fiegna F, Yu Y-TN, Kadam SV, Velicer GJ. 2006. Evolution of an obligate social cheater to a superior cooperator. *Nature* 441:310–14

Filosa MF. 1962. Heterocytosis in cellular slime molds. *Am. Nat.* 96:79–91

Fortunato A, Strassmann JE, Santorelli L, Queller DC. 2003. Co-occurrence in nature of different clones of the social amoeba, *Dictyostelium discoideum*. *Mol. Ecol.* 12:1031–38

Foster KR. 2004. Diminishing returns in social evolution: the not so tragic commons. *J. Evol. Biol.* 17:1058–72

Foster KR, Fortunato A, Strassmann JE, Queller DC. 2002. The costs and benefits of being a chimera. *Proc. R. Soc. London Ser. B* 269:2357–62

Foster KR, Shaulsky G, Strassmann JE, Queller DC, Thompson CRL. 2004. Pleiotropy as a mechanism to stabilise cooperation. *Nature* 431:693–96

Francis D, Eisenberg R. 1993. Genetic structure of a natural population of *Dictyostelium discoideum*, a cellular slime mold. *Mol. Ecol.* 2:385–91

Frank SA. 1995. Mutual policing and repression of competition in the evolution of cooperative groups. *Nature* 377:520–22

Frank SA. 1998. *Foundations of Social Evolution*. Princeton, NJ: Princeton Univ. Press. 268 pp.

Frank SA. 2003. Repression of competition and the evolution of cooperation. *Evolution* 57:693–705

Frank U, Reun U, Loya Y, Rinkevich B. 1997. Alloimmune maturation in the coral *Stylophora pistillata* is achieved through three distinctive stages, 4 months post-metamoprhosis. *Proc. R. Soc. London Ser. B* 264:99–104

Gadagkar R, Bonner JT. 1994. Social insects and social amoebae. *J. Biosci.* 19:219–45

Gerhart J, Kirschner M. 1997. *Cells, Embryos, and Evolution: Toward a Cellular and Developmental Understanding of Phenotypic Variation and Evolutionary Adaptability*. Oxford, UK: Blackwell Sci. 642 pp.

Giovannoni SJ, Turner S, Olsen GJ, Barns S, Lane DJ, Pace NR. 1988. Evolutionary relationships among Cyanobacteria and green chloroplast. *J. Bacteriol.* 170:3584–92

González P, Correa JA. 1996. Fusion and histocompatibility in Rhodophyta. *Hydrobiologia* 326/327:387–92

Gordeeva AV, Labas YA, Zvyagilskaya RA. 2004. Apoptosis in unicellular organisms: mechanisms and evolution. *Biochemistry* 69:1055–66

Griesemer J. 2000. The units of evolutionary transition. *Selection* 1:67–80

Griesemer J, Haber MH, Yamashita G, Gannett L. 2005. Critical notice: cycles of contingency—developmental systems and evolution. *Biol. Philos.* 20:517–44

Grosberg RK. 1988. The evolution of allorecognition specificity in clonal invertebrates. *Q. Rev. Biol.* 63:377–412

Grosberg RK, Levitan DR, Cameron BB. 1996. The evolutionary genetics of allorecognition in the colonial hydrozoan *Hydractinia symbiolongicarpus*. *Evolution* 50:2221–40

Grosberg RK, Strathmann RR. 1998. One cell, two cell, red cell, blue cell: the persistence of a unicellular stage in multicellular life histories. *Trends Ecol. Evol.* 13:112–16

Haig D. 1993. Genetic conflicts in human pregnancy. *Q. Rev. Biol.* 68:495–532

Haig D. 1997. The social gene. In *Behavioural Ecology: An Evolutionary Approach*, ed. JR Krebs, NB Davies, pp. 284–304. Oxford, UK: Blackwell Sci. 4th ed.

Hamilton WD. 1964a. The genetical evolution of social behaviour. Part I. *J. Theor. Biol.* 7:1–16

Hamilton WD. 1964b. The genetical evolution of social behaviour. Part II. *J. Theor. Biol.* 7:17–52

Hamilton WD. 1987a. Discriminating nepotism: expectable, common, overlooked. In *Kin Recognition in Animals*, ed. DJC Fletcher, CD Michener, pp. 417–37. New York: Wiley

Hamilton WD. 1987b. Kinship, recognition, disease, and intelligence: constraints of social evolution. In *Animal Societies: Theories and Facts*, ed. J Itô, JL Brown, J Kikkawa, pp. 81–102. Tokyo: Jpn. Sci. Soc.

Hammerstein P, ed. 2003. *Genetic and Cultural Evolution of Cooperation.* Cambridge, MA: MIT Press

Honegger R. 1993. Tansley review No. 60. Developmental biology of lichens. *New Phytol.* 125:695–77

Hudson RE, Aukema JE, Rispe C, Roze D. 2002. Altruism, cheating, and anticheater adaptations in cellular slime molds. *Am. Nat.* 160:31–43

Hughes RN. 1989. *A Functional Biology of Clonal Animals*. New York: Chapman & Hall. 331 pp.

Hughes RN. 2002. Genetic mosaics and chimeras. In *Reproductive Biology of Invertebrates*, Vol. XI. *Progress in Asexual Reproduction*, ed. RN Hughes, pp. 159–73. New York: Wiley

Huss MJ. 1989. Dispersal of cellular slime molds by two soil invertebrates. *Mycologia* 81:677–82

Jablonka E, Lamb MJ. 1995. *Epigenetic Inheritance and Evolution*. Oxford, UK: Oxford Univ. Press

Johnson UG, Porter KR. 1968. Fine structure of cell division in *Chlamydomonas reinhardi*. *J. Cell Biol.* 38:403–25

Kaiser D. 2001. Building a multicellular organism. *Annu. Rev. Genet.* 35:103–23
Kaufmann SH, Gores GJ. 2000. Apoptosis in cancer: cause and cure. *BioEssays* 22:1007–17
Kaushik S, Katoch B, Nanjundiah V. 2006. Social behaviour in genetically heterogeneous groups of *Dictyostelium giganteum*. *Behav. Ecol. Sociobiol.* 59:521–30
Keim CN, Martins JL, Abreu F, Rosado AS, de Barros HL, et al. 2004. Multicellular life cycle of magnetotactic prokaryotes. *FEMS Microbiol. Lett.* 240:203–8
Keller L, Ross KG. 1998. Selfish genes: a green beard in the red fire ant. *Nature* 394:573–75
Keller L, Surette MG. 2006. Communication in bacteria: an ecological and evolutionary perspective. *Nat. Rev. Microbiol.* 4:249–58
King N. 2004. The unicellular ancestry of animal development. *Dev. Cell* 7:313–25
Kirk D. 1997. The genetic program for germ-soma differentiation in *Volvox*. *Annu. Rev. Genet.* 31:359–80
Kirk D. 1998. *Volvox*. Cambridge, UK: Cambridge Univ. Press. 381 pp.
Kirk D. 1999. Evolution of multicellularity in the volvocine algae. *Curr. Opin. Plant Biol.* 2:296–501
Kirk D. 2003. Seeking the ultimate and proximate causes of *Volvox* multicellularity and cellular differentiation. *Integr. Comp. Biol.* 43:247–53
Klekowski EJ Jr. 1988. *Mutation, Developmental Selection, and Plant Evolution*. New York: Columbia Univ. Press. 373 pp.
Knoll AH. 2003. *Life on a Young Planet*. Princeton, NJ: Princeton Univ. Press. 277 pp.
Knoll AH, Javaux EJ, Hewitt D, Cohen P. 2006. Eukaryotic organisms in Proterozoic oceans. *Philos. Trans. R. Soc. B* 361:1023–38
Kobayashi I. 1998. Selfishness and death: raison d'être of restriction, recombination and mitochondria. *Trends Genet.* 14:368–74
Kondrashov AS. 1994. Mutation load under vegetative reproduction and cytoplasmic inheritance. *Genetics* 137:311–18
Koonin EV, Aravind L. 2002. Origin and evolution of eukaryotic apoptosis. *Cell Death Differ.* 9:394–404
Koufopanou V. 1994. The evolution of soma in the Volvocales. *Am. Nat.* 143:907–31
Koufopanou V, Bell G. 1993. Soma and germ: an experimental approach using *Volvox*. *Proc. R. Soc. London Ser. B* 254:107–13
Krakauer DC, Mira A. 1999. Mitochondria and germ-cell death. *Nature* 400:125–26
Krieger MJB, Ross KG. 2002. Identification of a major gene regulating complex social behavior. *Science* 295:328–32
Lachmann M, Blackstone NW, Haig D, Kowald A, Michod RE, et al. 2003. Group report: cooperation and conflict in the evolution of genomes, cells, and multicellular organisms. See Hammerstein 2003, pp. 327–56
Leigh EG. 1977. How does selection reconcile individual advantage with the good of the group? *Proc. Natl. Acad. Sci. USA* 10:4542–46
Leigh EG. 1991. Genes, bees, and ecosystems: the evolution of a common interest among individuals. *Trends Ecol. Evol.* 6:257–62
Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. 2000. *Molecular Cell Biology*. New York: Freeman. 4th ed.

Lürling M, Van Donk E. 2000. Grazer-induced colony formation in *Scenedesmus*: Are there costs to being colonial? *Oikos* 88:111–18

Maldonado M. 1998. Do chimeric sponges have improved chances of survival? *Mar. Ecol. Prog. Ser.* 164:301–6

Margulis L. 1981. *Symbiosis in Cell Evolution*. San Francisco: Freeman. 419 pp.

Martin ME, Brun YV. 2000. Coordinating development with the cell cycle in *Caulobacter*. *Curr. Opin. Microbiol.* 2:496–501

Masuda M, Sato H. 1984. Reversible resorption of cilia and the centriole cycle in dividing cells of sea urchin blastulae. *Zool. Sci.* 1:445–62

Matsuda H, Harada Y. 1990. Evolutionarily stable stalk to spore ratio in cellular slime molds and the law of equalization of net incomes. *J. Theor. Biol.* 147:329–44

Maynard Smith J. 1988. Evolutionary progress and levels of selection. In *Evolutionary Progress*, ed. MH Nitecki, pp. 219–30. Chicago: Univ. Chicago Press

Maynard Smith J, Szathmáry E. 1995. *The Major Transitions in Evolution*. Oxford, UK: Freeman. 346 pp.

McShea DW. 2001. The minor transitions in evolution and the question of a directional bias. *J. Evol. Biol.* 14:502–18

McShea DW. 2002. A complexity drain on cells in the evolution of multicellularity. *Evolution* 56:441–52

Medina M, Collins A, Taylor J, Valentine JW, Lipps J, et al. 2003. Phylogeny of Opisthokonta and the evolution of multicellularity and complexity in Fungi and Metazoa. *Int. J. Astrobiol.* 2:203–11

Mehdiabadi NJ, Jack CN, Farnham TT, Platt TG, Kalla SE, et al. 2006. Kin preference in a social microbe. *Nature* 442:881–82

Meier P, Finch A, Evan G. 2000. Apoptossis in development. *Nature* 407:796–801

Michod RE. 1996. Evolution of the individual. *Am. Nat.* 150:S5–21

Michod RE. 1997. Cooperation and conflict in the evolution of multicellularity. I. Multilevel selection of the organism. *Am. Nat.* 149:607–45

Michod RE. 1999. *Darwinian Dynamics. Evolutionary Transitions in Fitness and Individuality.* Princeton, NJ: Princeton Univ. Press. 262 pp.

Michod RE. 2003. Cooperation and conflict during the origin of multicellularity. See Hammerstein 2003, pp. 291–307

Michod RE, Nedelcu AM. 2003. On the reorganization of fitness during evolutionary transition in individuality. *Integr. Comp. Biol.* 43:64–73

Michod RE, Roze D. 1999. Cooperation and conflict in the evolution of individuality. III. Transitions in the unit of fitness. In *Mathematical and Computational Biology: Computational Morphogenesis, Hierarchical Complexity, and Digital Evolution*. ed. CL Nehaniv, pp. 47–92. Providence, RI: Am. Math. Soc.

Michod RE, Roze D. 2001. Cooperation and conflict in the evolution of multicellularity. *Heredity* 86:1–7

Miller MB, Bassler BL. 2001. Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55:165–99

Mueller UG. 2002. Ant vs fungus vs mutualism: ant-cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. *Am. Nat.* 160:S67–98

Mueller UG, Gerardo NM, Aanen DK, Six DL, Schultz TR. 2005. The evolution of agriculture in insects. *Annu. Rev. Ecol. Evol. Syst.* 36:563–95

Muller-Parker G, Secord D. 2005. Symbiont distribution along a light gradient within an intertidal cave. *Limnol. Oceanogr.* 50:272–78

Murgia C, Pritchard JK, Kim SY, Fassati A, Weiss RA. 2006. Clonal origin and evolution of a transmissible cancer. *Cell* 126:477–87

Mydlarz LD, Jones LE, Harvell CD. 2006. Innate immunity, environmental drivers, and disease ecology of marine and freshwater invertebrates. *Annu. Rev. Ecol. Evol. Syst.* 37:251–88

Nieuwkoop PD, Sutasurya LA. 1981. *Primordial Germ Cells in the Invertebrates: From Epigenesis to Preformation*. Cambridge, UK: Cambridge Univ. Press. 258 pp.

Niklas KJ. 2000. The evolution of plant body plans. *Ann. Bot.* 85:411–38

Niklas KJ. 2004. The cell walls that bind the tree of life. *BioScience* 54:831–41

Nunney L. 1999. Lineage selection and the evolution of multistage carcinogenesis. *Proc. R. Sci. London Ser. B* 266:493–98

Oka H. 1970. Colony specificity in compound ascidians. In *Profiles of Japanese Science and Scientists*, ed. M Yukawa, pp. 195–206. Tokyo: Kodansha

Olive LS. 1975. *The Mycetozoans*. New York: Academic. 293 pp.

Orive ME. 2001. Somatic mutations in organisms with complex life histories. *Theor. Popul. Biol.* 59:235–49

Otto SP, Hastings IM. 1998. Mutation and selection within the individual. *Genetica* 102/103:507–24

Otto SP, Orive ME. 1995. Evolutionary consequences of mutation and selection within an individual. *Genetics* 141:1173–87

Pál C, Papp B. 2000. Selfish cells threaten multicellular life. *Trends Ecol. Evol.* 15:351–52

Pancer Z, Gershon H, Rinkevich B. 1995. Coexistence and possible parasitism of somatic and germ-cell lines in chimeras of the colonial urochordate *Botryllus schlosseri*. *Biol. Bull.* 189:106–12

Parker GA, Royle NJ, Hartley IR. 2002. Intrafamilial conflict and parental investment: a synthesis. *Philos. Trans. R. Soc. London Ser. B* 357:295–307

Pawlowska TE, Taylor JW. 2004. Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature* 427:733–37

Pearse A-M, Swift K. 2006. Transmission of devil facial-tumour disease. *Nature* 439:549

Pfeiffer T, Bonhoeffer S. 2003. An evolutionary scenario for the transition to undifferentiated multicellularity. *Proc. Natl. Acad. Sci. USA* 100:1095–98

Pfeiffer T, Schuster S, Bonhoeffer S. 2001. Cooperation and competition in the evolution of ATP-producing pathways. *Science* 292:504–7

Pineda-Krch M, Lehtilä K. 2004. Costs and benefits of genetic heterogeneity within organisms. *J. Evol. Biol.* 17:1167–77

Porter SM, Meisterfield R, Knoll AH. 2003. Vase-shaped microfossils from the Neoproterozoic Chuar Group, Grand Canyon: a classification guided by modern testate amoebae. *J. Paleontol.* 77:409–29

Queller DC. 1997. Cooperators since life began. A review of *The Major Transitions in Evolution*. *Q. Rev. Biol.* 72:184–88

Queller DC. 2000. Relatedness and the fraternal major transitions. *Philos. Trans. R. Soc. London Ser. B* 355:1647–55

Queller DC, Ponte E, Bozzaro S, Strassmann JE. 2003. Single gene greenbeard effects in the social amoeba *Dictyostelium discoideum*. *Science* 299:105–6

Raff RA. 1988. The selfish cell lineage. *Cell* 54:445–46

Rainey PB, Rainey K. 2003. Evolution of cooperation and conflict in experimental bacterial populations. *Nature* 425:72–74

Rinkevich B, Weissman IL. 1992. Chimeras vs genetically homogeneous individuals: potential fitness costs and benefits. *Oikos* 63:119–24

Roberts G. 2005. Cooperation through interdependence. *Anim. Behav.* 70:901–8

Roze D, Michod RE. 2001. Mutation, multilevel selection, and the evolution of propagule size during the origin of multicellularity. *Am. Nat.* 6:638–54

Sabbadin A, Zaniolo G. 1979. Sexual differentiation and germ cell transfer in the colonial ascidian *Botryllus schlosseri*. *J. Exp. Zool.* 207:289–304

Sachs J, Mueller IG, Wilcox TP, Bull JJ. 2004. The evolution of cooperation. *Q. Rev. Biol.* 79:135–60

Santelices B. 2004. Mosaicism and chimerism as components of intraorganismal genetic heterogeneity. *J. Evol. Biol.* 17:1187–88

Santelices B, Correa JA, Aedo D, Flores V, Hormazábal M, Sanchez P. 1999. Convergent biological processes in coalescing Rhodophtya. *J. Phycol.* 35:1127–49

Schopf JW. 1993. Microfossils of the Archean apex chert: new evidence of the antiquity of life. *Science* 60:640–46

Scofield VL, Schlumpberger JM, West LA, Weissman IL. 1982. Protochordate allorecognition is controlled by a MHC-like gene system. *Nature* 295:499–502

Seger J. 1988. Review of *The Evolution of Individuality* (LW Buss). *Q. Rev. Biol.* 63:336–37

Sekiguchi K, Kasai K, Levin BC. 2003. Inter- and intragenerational transmission of a human mitochondrial DNA heteroplasmy among 13 maternally-related individuals and differences between and within tissues in two family members. *Mitochondrion* 2:401–14

Shapiro JA. 1998. Thinking about bacterial populations as multicellular organisms. *Annu. Rev. Microbiol.* 52:81–104

Shi W, Zusman DR. 1993. The two motility systems of *Myxococcus xanthus* show different selective advantages on various surfaces. *Proc. Natl. Acad. Sci. USA* 90:3378–82

Shimkets LJ. 1990. Social and developmental biology of the myxobacteria. *Microbiol. Rev.* 54:473–501

Smith SE, Read DJ. 1997. *Mycorrhizal Symbiosis*. San Diego: Academic

Solari CA, Ganguly S, Kessler JO, Michod RE, Goldstein RE. 2006. Multicellularity and the functional interdependence of motility and molecular transport. *Proc. Natl. Acad. Sci. USA* 103:1353–58

Stanley SM. 1973. An ecological theory for the sudden origin of multicellular life in the late Precambrian. *Proc. Natl. Acad. Sci. USA* 70:1486–89

Stoner DS, Rinkevich B, Weissman IL. 1999. Heritable germ and somatic cell lineage competitions in chimeric colonial protochordates. *Proc. Natl. Acad. Sci. USA* 96:9148–53

Stoner DS, Weissman IL. 1996. Somatic and germ cell parasitism in a colonial ascidian: a possible role for a polymorphic allorecognition system. *Proc. Natl. Acad. Sci. USA* 93:15,254–59

Strassmann JE, Queller DC. 2004. Sociobiology goes micro. *ASM News* 70:526–32

Strassmann JE, Zhu Y, Queller DC. 2000. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* 408:965–67

Strathmann RR. 1991. From metazoan to protist via competition among cell lineages. *Evol. Theory* 10:67–70

Strathmann RR. 1998. Peculiar constraints on life histories imposed by protective or nutritive devices for embryos. *Am. Zool.* 35:426–33

Strathmann RR, Staver JM, Hoffman JR. 2002. Risk and the evolution of cell cycle durations of embryos. *Evolution* 56:708–20

Sussex IM. 1973. Do concepts of animal development apply to plant systems. *Brookhaven Symp. Biol.* 25:145–51

Szathmáry E, Wolpert L. 2003. The transition from single cells to multicellularity. See Hammerstein 2003, pp. 285–304

Tomitani A, Knoll AH, Cavanaugh CM, Ohno T. 2006. The evolutionary diversification of the Cyanobacteria: molecular-phylogenetic and paleontological perspectives. *Proc. Natl. Acad. Sci. USA* 103:5442–47

Travisano M, Velicer GJ. 2004. Strategies of microbial cheater control. *Trends Microbiol.* 12:72–78

Valentine JW, Collins AG, Meyer CP. 1994. Morphological complexity increase in metazoans. *Paleobiology* 20:131–42

Van den Hoek E, Mann DG, Jahns HM. 1995. *Algae: An Introduction to Phycology*. Cambridge, UK: Cambridge Univ. Press. 623 pp.

Van Valen LM. 1988. Is somatic selection an evolutionary force? *Evol. Theory* 8:163–67

Velicer GJ. 2003. Social strife in the microbial world. *Trends Microbiol.* 11:330–37

Velicer GJ, Kroos L, Lenski RE. 1998. Loss of social behaviors by *Myxococcus xanthus* during evolution in an unstructured habitat. *Proc. Natl. Acad. Sci. USA* 95:12376–80

Velicer GJ, Kroos L, Lenski RE. 2000. Developmental cheating in the social bacterium *Myxococcus xanthus*. *Nature* 404:598–601

Velicer GJ, Yu Y-TN. 2003. Evolution of novel cooperative swarming in the bacterium *Myxococcus xanthus*. *Nature* 425:75–78

Vermeij GJ. 2006. Historical contingency and the purported uniqueness of evolutionary innovations. *Proc. Natl. Acad. Sci. USA* 103:1804–9

Vos M, Velicer GJ. 2006. Genetic population structure of the soil bacterium *Myxococcus xanthus* at the centimeter scale. *Appl. Environ. Microbiol.* 72:3615–25

Wolk CP. 2000. Heterocyst formation in *Anabaena*. In *Prokaryotic Development*, ed. YV Brun, LJ Shimkets, pp. 83–104. Washington, DC: ASM Press

Wolpert L. 1990. The evolution of development. *Biol. J. Linn. Soc. London* 39:109–24

Wolpert L, Szathmáry E. 2002. Evolution and the egg. *Nature* 420:745

Wu J, Glass NL. 2001. Identification of specificity determinants and generation of alleles with novel specificity in the *het-c* heterokaryon incompatibility locus of *Neurospora crassa*. *Mol. Cell. Biol.* 21:1045–57

Yahr R, Vilgalys R, Depriest PT. 2004. Strong fungal specificity and selectivity for algal symbionts in Florida scrub *Cladonia* lichens. *Mol. Ecol.* 13:3367–78

Zhang C-C, Laurent S, Sakr S, Peng L, Bédu S. 2006. Heterocyst differentiation and pattern formation in Cyanobacteria: a chorus of signals. *Mol. Microbiol.* 59:367–75