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A COMPLEXITY DRAIN ON CELLS IN THE EVOLUTION OF MULTICELLULARITY

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Abstract.—A hypothesis has been advanced recently predicting that, in evolution, as higher-level entities arise from associations of lower-level organisms, and as these entities acquire the ability to feed, reproduce, defend themselves, and so on, the lower-level organisms will tend to lose much of their internal complexity (McShea 2001a). In other words, in hierarchical transitions, there is a drain on numbers of part types at the lower level. One possible rationale is that the transfer of functional demands to the higher level renders many part types at the lower level useless, and thus their loss in evolution is favored by selection for economy. Here, a test is conducted at the cell level, comparing numbers of part types in free-living eukaryotic cells (protists) and the cells of metazoans and land plants. Differences are significant and consistent with the hypothesis, suggesting that tests at other hierarchical levels may be worthwhile.

Key words.—Complexity, evolutionary trends, hierarchy, parts.

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Hierarchical transitions occur when a group of organisms combine to form a higher-level entity (Spencer 1904; Campbell 1958; Simon 1962; Pattee 1970; Eldredge and Salthe 1984; Salthe 1985, 1993; Buss 1987; Bonner 1988; Maynard Smith 1988; Wimsatt 1994; Maynard Smith and Szathmáry 1995; McShea 1996, 2001b; Pettersson 1996; Valentine and May 1996; Michod 1999). In the history of life, salient examples include the origin of the eukaryotic cell from symbiotic associations of prokaryotic cells, of multicellular individuals from clones of free-living eukaryotic cells, and of colonies from associations of multicellular individuals. A hypothesis has been advanced recently that predicts that, as such transitions occur, and as the new higher-level entities acquire the ability to feed, reproduce, defend themselves, and so on, their component organisms will tend to lose many of these functional capabilities and therefore will tend to lose many of their internal parts (McShea 2001a). More precisely, the expectation is that the component organisms will tend to lose part types at the next hierarchical level down (Fig. 1). (Technical understandings of the terms *part* and *level* will be offered shortly; for present purposes, colloquial meanings suffice.)

Concretely, the hypothesis predicts that the cells of multicellular organisms will have fewer part types than free-living eukaryotic cells, that is, nonparasitic protists (hereafter simply protists; sensu Patterson 1999). At a higher level, in colonial marine invertebrates and social insects, the multicellular individuals (e.g., polyps, zooids) that constitute the more individuated colonies—that is, those in which coloniality is more highly developed (Boardman and Cheetham 1973)—should have fewer part types, on average, than those of their less colonial or solitary relatives (Beklemishev 1969). And at a lower level, the former prokaryotes that today function as organelles in eukaryotic cells (e.g., mitochondria, plastids) should have fewer part types than free-living prokaryotes.

Attention to hierarchical level is crucial. A considerable body of evidence and theory has been advanced predicting that part types will be *gained* as functionality emerges, that is as labor is divided and the component organisms become specialized and transformed into parts (e.g., Wilson 1968, 1985; Beklemishev 1969; Schopf 1973; Corning 1983; Bell 1985; Bonner 1988; Harvell 1994; Valentine et al. 1994; Bell and Moores 1997). The hypothesis here targets a lower level, predicting the loss of part types *within* the component organisms.

Some evidence can be found in support of the hypothesis. Mature human hemocytes contain almost no macroscopic structures; nuclei, mitochondria, ribosomes, lysosomes, and Golgi complexes, for example, are all absent (Weiss 1984). In contrast, *Perispira*, a free-living ciliate, contains many such parts, including all of those listed above plus a second type of nucleus (the macronucleus), contractile vacuoles, cilia, and others. These are extreme cases, but even the more typical metazoan and land plant cells seem to be simpler, to contain fewer part types than free-living eukaryotic cells (e.g., Gerhart and Kirschner 1997, p. 242; McShea 2001a). At the colony level, Beklemishev (1969) noted the simplification of the clonal units in marine invertebrate colonies (e.g., zooids in bryozoans) compared with those of related free-living species (also Mackie 1986; Wood et al. 1992). And Oster and Wilson (1978) noted that in highly individuated insect colonies, behavioral repertories of individual insects are simplified.

Thus, the existing evidence is generally supportive of the hypothesis. But it is also highly impressionistic, leaving room for doubt. Here, I offer a test at the cell level, in particular, a comparison of counts of part types in free-living protists with counts in metazoan and land plant cells. (Notice that an empirical treatment of this sort could be described equally well as an attempt to document a pattern, and that the decision to frame it as a test of an hypothesis is somewhat arbitrary.)

The concern here is mainly with empirical issues: developing operational protocols for counting parts in cells, testing for differences in part counts, and exploring possible biases in the data. This paper thus complements an earlier treatment (McShea 2001a), where the focus was on conceptual issues.

Significance

The hypothesis, if true, is relevant to our understanding of one of the most widely recognized trends in the history of life, the apparent increase in organismal complexity (McShea

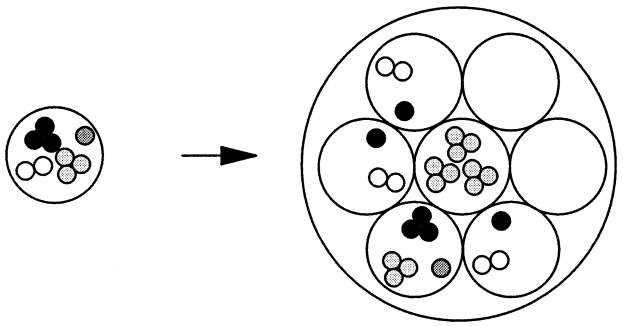

FIG. 1. A schematic representation of the hypothesis (see text). The figure shows the transformation of a free-living cell (medium-sized circle, left) into a higher-level multicellular entity (large circle, right) consisting of seven cells. Here, the free-living cell (left) has four part types (small circles), whereas those within the higher-level entity have (clockwise from leftmost): 2, 2, 0, 0, 2, 3, and the one in the middle has 1, for an average of 1.4.

1991, 1996). It is well established that complexity in the hierarchical sense considered here has increased, in other words, that there has been a trend in the number of levels of nesting of parts within wholes (Spencer 1904; Needham 1943; Stebbins 1969; Buss 1987; Bonner 1988; Maynard Smith 1988; Maynard Smith and Szathmáry 1995; Pettersson 1996; Knoll and Bambach 2000; McShea 2001b). Furthermore, there is some evidence to suggest a trend in a nonhierarchical sense as well, that is, in the number of part types at a given hierarchical level. For example, trends have been documented in number of limb-pair types in aquatic arthropods (Cisne 1974) and in number of cell types in metazoans (Valentine et al. 1994). The hypothesis offered here is significant in that it suggests that this buildup of nonhierarchical complexity in evolution may be partly offset by losses, that is, by a kind of complexity drain. Importantly, the point is not only that complexity decreases occur. Many apparent cases of decrease are well known and routinely cited, especially those thought to have occurred in the evolution of certain parasites (e.g., Gould 1996; although see Brooks and McLennan 1993). Rather, the suggestion is that decreases occur regularly, in conjunction with hierarchical increases, perhaps in a lawlike way.

The larger issue here concerns the structural and organizational consequences of the origin of new hierarchical levels (e.g., Campbell 1958; Simon 1962; Pattee 1970; Salthe 1985, 1993; Bonner 1988; Wimsatt 1994; Anderson and McShea 2001; McShea 2001a). In particular, this study is relevant to the suggestion that all or most origins of new levels—eukaryotic cell from prokaryotic cells, multicellular organisms from free-living cells, colonies from multicellular individuals—entail a common constellation of morphological and physiological correlates (Salthe 1985; Wimsatt 1994; Anderson and McShea 2001). Possible examples of such correlates include increases in size, in connectedness among lower-level organisms, and in partitioning of work into group and team tasks, as well as losses of complexity in the lower-level organisms (for others, see Anderson and McShea 2001).

More generally, this treatment is relevant to the ongoing discussion—dating back at least to Spencer (1900, 1904)—of what have in recent decades been called ''levels of organization'' or ''integrative levels'' (Redfield 1942; Needham 1943; Novikoff 1945; Fiebleman 1955; Polanyi 1968; Guttman 1976; MacMahon et al. 1978; Wimsatt 1994) and also to more contemporary discussions of ''levels of selection'' (Maynard Smith 1988; Sober and Wilson 1994; Brandon 1996, 1999; Gould and Lloyd 1999; Keller 1999; Michod 1999). Importantly, however, the concern is not (at least directly) with the problem of how new higher levels arise and are maintained (Leigh 1991; Maynard Smith and Szathmáry 1995; Michod 1999). Nor is it with the more particular problem of the route by which certain levels arose historically, such as the origin of the eukaryotic cell (e.g., Dyar and Obar 1994; Brown and Doolittle 1997; Roger 1999) or the origin of multicellularity (e.g., Bell 1985; Buss 1987; Bonner 1988, 1998; Gerhart and Kirschner 1997; Kirk 1998).

Terminology

Two clarifications are needed before proceeding. First, a distinction needs to be made between number of part types and absolute number of parts. A specialized muscle cell in a metazoan might have fewer part types than a protist but more total parts, if the muscle cell has, say, an enormous number of mitochondria. The hypothesis predicts a reduction only in number of part types, because it is part types that are expected to be correlated with number of cell functions (see below). However, for economy I will often refer to numbers of parts, omitting the word *types*.

Second, the hypothesis predicts a *tendency* to lose functions and parts. Using this term is designed to cover the possibility that losses are offset by an independent, pervasive evolutionary trend producing an increase in all lineages. No such trend has been demonstrated (McShea 1996), but if it exists, the prediction would be a lesser gain (rather than a loss) in multicellular lineages compared to protist lineages.

CONCEPTUAL ISSUES

Evolutionary Rationales

The hypothesis is supported by a simple evolutionary logic. Consider the cell level. As multicellular entities emerged from associations of free-living cells and as these entities acquired functional capabilities, functional demands on their component cells would have been reduced. Then, the component cells would have been expected to lose parts in the interest of economy, in other words, on account of the reduction in energy and material costs in development and physiology for the higher-level entity. Thus, a mammalian liver cell is expected to have relatively few survival- and reproduction-related demands to meet—because the higher-level entity accomplishes many of these functions for it—and therefore the cell requires few parts. In contrast, a free-living flagellate such as *Euglena* must perform all functions for itself, and so it requires more. More generally, free-living species at any hierarchical level ought to experience a greater number of functional demands than related species that are contained within and participate in larger functional wholes. (This rationale could be understood as a generalization of the standard explanation for the apparent reduction in complexity in parasites.)

A second rationale is that selection on a multicellular entity favors a reduction in the range of behavioral possibilities in its component cells. To play its proper role, a cell must not only behave appropriately, it must be constrained from behaving inappropriately. One way to eliminate inappropriate behaviors is to eliminate the parts that produce them. In cells, behaviors lost would likely include those involved in competition for access to the germ line (Buss 1987), but also any behavior not associated with a cell's specialized function. In effect, the hypothesis can be understood as predicting a decrease in autonomy—that is, in the ability of lower-level organisms to behave independently—as well as in complexity.

Importantly, neither rationale predicts the loss of all cell parts. For metazoan and land plant cells, most specialized functions probably require at least one part and some presumably more. Also, most cells will probably retain a number of housekeeping functions, along with the parts for specialized functions. In any case, the prediction is only that parts will be lost *on average*. Furthermore, the prediction is not that all cells will fit the predicted pattern. Especially in protists, number of functional demands and thus number of cell parts (see below) can be expected to vary with differences in ecology, and as a result, undoubtedly some protists will require very few parts. Again the prediction is only that protists will have more parts on average.

These rationales may be compelling, but reasons can be imagined why they might be wrong or incomplete. For example, it could be that functional demands are actually more numerous at higher levels, so that lower-level organisms actually need more parts than their free-living relatives. Another possibility is that loss of parts is advantageous but often impossible to effect if most parts are highly integrated into development (Saunders and Ho 1976, 1981). Thus, given the theoretical uncertainties, empirical testing is worthwhile.

Hierarchy

The term hierarchy here refers to a structural relationship. Lower-level organisms are physically contained within and partly constitute higher-level entities (Bunge 1959; Simon 1962; Pattee 1970; Salthe 1985, 1993; Wimsatt 1994; McShea 1996, 2001b; Pettersson 1996; Valentine and May 1996). For hierarchy in this sense, genealogy is not directly relevant. A higher-level entity in which the lower-level organisms have different genotypes (e.g., the prokaryotic partners in an early eukaryotic cell) is hierarchical in the same sense as one in which they are clones. Furthermore, the concern here is not (directly) with control hierarchies, such as those that occur in development (Riedl 1978; Wimsatt 1986; Valentine and Erwin 1987; Salthe 1993; Raff 1996; Arthur 1997).

Salthe (1985) argued that three levels are needed ordinarily in a hierarchical analysis: the focal level plus one adjacent level above and one below. The focal level here is occupied by eukaryotic cells, both free-living and not. The lower level is occupied by parts at a level just below the cell. (Parts at still lower levels are called subparts; see below.) And the higher level is occupied by the multicellular entity as a whole. In many multicellular organisms, various levels of nesting intervene (e.g., tissues and organs), but it is the level of the whole organism at which selection presumably acts to produce function, and therefore that is the higher level of interest.

Notice that the hypothesis requires function at two levels, the higher level and the focal level. If the higher-level entity were a mere association of cells with no functional capabilities of its own, then no functional take-over would occur and no loss of parts would be favored at the cell level. And if the focal-level entities, cells, had no preexisting functional capabilities, they would have no functions to lose, and again no loss of parts would be expected. In other words, the assumption is that both levels are present or former levels of selection.

Parts

A part is understood here as a set of elements that are relatively well connected to each other and less well connected to elements outside the set (Campbell 1958; Simon 1962; see discussion and references in McShea and Venit 2001). In other words, parts are entities that are relatively well integrated internally and well isolated externally. Thus, most of the entities we would call objects are likely to be parts (i.e., object parts), including biological objects such as a cell nucleus or a mitochondrion. However, parts may also be spatially distributed. In a cell, a metabolic cycle might be a part (i.e., a physiological part), and in a multicellular organism, a group of nerve and muscle cells that produce a behavior might be a part (i.e., a behavior part), at least while the behavior is being performed.

In this view, there is no logically necessary connection between part types and functions; parts may be functional or not. However, in organisms, a strong empirical correlation between part types and functions is expected and is a necessary assumption here, so that a reduction in number of functional demands on the component organisms will be manifest as a reduction in part types. (For an in-principle argument for the existence of a good correlation, see McShea 2000.) Notice that a good correlation does not require a one-to-one mapping, with each part type performing a single function; indeed, in organisms, the expectation is that multiple functions will often overlap in their use of the same part (Wimsatt 1974).

Two clarifications are in order. First, the level occupied by a part is a function only of topology not of absolute size. Object nested within the outer perimeter of a cell are parts, whereas objects nested within these are subparts (McShea and Venit 2001). Thus, a large object such as a mitochondrion or a plastid is probably a cell part (whereas cristae within mitochondria or thylakoids within plastids are probable subparts). But a smaller object, such as a protein molecule in the cytoplasm, may also be a part if no objects intervene hierarchically between it and the cell as a whole.

Second, the hypothesis predicts only that parts will be lost at the level just below the cell, not necessarily at lower levels. One reason is that the loss of a part type may not entail the loss of a subpart type. For example, microtubules may be subparts of a flagellar apparatus, but they are also present within other cell parts, and therefore may be retained in a cell even if the flagella is lost. More generally, the thinking

is that cell functions will mostly be performed by parts at a level just below the cell, but subparts perform subfunctions; and functions tend to isolate (Salthe 1985) or ''screen off'' (Brandon 1996) subfunctions, meaning that subparts tend to be less visible to selection (McShea and Venit 2001). Thus, in general, selection at the level of the whole may be felt at lower levels, but the effect is expected to attenuate, on average, as the difference in level increases.

A perhaps surprising consequence is that genes may be poor proxies for cell parts. First, in principle, a cell part might be lost with no change (or even with an increase) in number of expressed genes. But more generally, most genes are probably subparts and sufficiently distant hierarchically from the cell level that selection at that level for loss of parts may not affect genes very directly. Notice that for certain evolutionary questions, it may be appropriate to use genes as parts because of their special role in heredity and development, for example, where the issue is the relationship between parts and complexity of development (e.g., Valentine 2000). Here, however, parts are structural (and by assumption functional) entities, not evolutionary or generative ones, and thus the special roles of genes are only indirectly relevant. Of course, the relationship between number of parts and number of genes (generative sense) has been of some interest—for example, in connection with the C-value paradox (Cavalier-Smith 1985)—and could be investigated further using the parts-identification methods developed here.

Parts correspond closely to what Campbell (1958) called entities, what Simon (1962) called subsystems (in his discussion of ''nearly decomposable systems''), and what Hull (1980) called individuals (see also Salthe 1985; Mishler and Brandon 1987; Wilson 1999; Anderson and McShea 2001; McShea 2001b). A part is also a kind of *module*, but not in the sense that has become common in evolutionary-developmental biology in recent years, where module usually refers to a more or less independent unit in the development of an organism (e.g., Wagner and Altenberg 1996). Rather, a part is a module in what might be called the ''operation'' of an organism, for example, in its physiology or behavior, rather than its development. The ''dynamic modules'' of Mittenthal et al. (1992) include both operational and developmental entities.

METHODS

In testing the hypothesis, a major challenge was to devise operational protocols for identifying parts so that the number of parts in a cell could be counted in an objective way (McShea and Venit 2001). The strategy adopted was not to attempt to count all parts, but rather to count a sample of them, ideally in a way that is consistent and unbiased with respect to the hypothesis. To do this, part counts were limited to large object parts, that is, parts that are visible in a cell at a magnification that is low relative to the size of the cell. In effect, large object parts were used as a proxy for all true cell parts, including the smaller, atom- or molecule-sized parts and the spatially distributed parts (i.e., physiological and behavioral parts).

Large Object Parts

The large object parts at the level just below the cell include mitochondria, plastids, Golgi apparatus, nucleus, vacuoles, the one or two types of flagellae, cilia, the plasma membrane, and the various granules, vesicles, and microtubular structures. Thus, cell parts include the objects commonly called organelles but also many other objects.

Four categories of large object parts were distinguished: free-floating, compositional, and shape parts, plus questionable parts, hereafter abbreviated F, C, S, and ? parts (McShea and Venit 2001). F parts are objects within a cell that seem to be unattached, at least at low magnification and in many preparations, such as a mitochondrion or nucleus. In fact, many of these are attached to other objects at the fine-structural level. The nuclear membrane, for example, is actually continuous with the endoplasmic reticulum (Dyer and Obar 1994). However, the assumption is that the integration created by fine or less-visible connections will be weaker, on average, than that created by ultrastructural or more-visible ones. Thus, apparently free-floating objects should be fairly well isolated and are likely to be true parts. Plastids, contractile vacuoles, vesicles, granules, and extrusomes are all considered F parts.

C parts are objects that are contiguous with other objects but differ from them in composition. An example is a desmosome, a cell-cell adhesive junction in metazoans that can be understood as a local modification of the plasma membrane. The membrane region containing a desmosome is contiguous with the rest of the membrane but differs from it in composition by virtue of containing certain transmembrane proteins (Kowalczyk et al. 1999). Due to the contact with another object, the isolation of C parts seems less extreme than for F parts, and thus our confidence that they are true parts should be lower. Examples of C parts include structures enveloping the plasma membrane but compositionally distinct from it, such as cell walls, tests, and sheaths, as well as structures invested in it, such as flagellae, cilia, and cell-cell junctions.

S parts are objects that are contiguous with other objects but differ significantly in shape. A pseudopod of an *Amoeba*, for example, is contiguous with the rest of the plasma membrane but differs in shape from it. The isolation of S parts is considered even less certain than for C parts. Other examples of S parts include microvilli, cytopharynxes (or cytostomes), and other deformations of the plasma membrane.

Present and former levels of selection, including the protistan and multicellular levels, are often occupied relatively discretely and unambiguously (Wimsatt 1974). But parts organization at the level just below the cell is expected to be more complex; to accommodate ambiguities arising, a fourth category was established for questionable parts (? parts). These include objects that do not clearly occupy the proper level (such as an eyespot, which in some taxa may be a subpart of a plastid), or objects that are not clearly distinct types (e.g., in some haptophytes, the second flagellum, which is nearly the same as the first).

For the tests conducted (see below), comparisons were made among taxa using four estimates of numbers of parts: F, F + C, F + C + S, F + C + S + ? (hereafter abbreviated

TABLE 1. "Standard set" of cell part types.

Plasma membrane
Nucleus
Mitochondria, hydrogenosomes, and kinetoplasts
Endoplasmic reticulum (smooth, rough)
Free ribosomes
Golgi apparatus
Various microtubular, microfilamentous, and intermediate-filamentous structures (e.g., ciliary basal bodies, centrioles, microtubule organizing centers, cytoskeleton, flagellar bases)
Various spherical (or subspherical) structures that appear internally undifferentiated at low magnification: e.g., lysosomes, peroxisomes, phagosomes, vesicles, concretions, granules, glycogen bodies, vacuoles (except contractile vacuoles)

F, FC, FCS, and FCS?). The series represents estimates that are increasingly inclusive but that allow increasingly ambiguous parts. In effect, sample size increases, but at the expense of our confidence in the accuracy of the count.

This approach may seem somewhat blinkered in that it ignores much of what is known about function in cell parts, and also quite coarse, in that it overlooks the mechanisms by which parts perform functions, some of which are known in great detail at the molecular level (e.g., Sleigh 1989; Goodsell 1993; Gerhart and Kirschner 1997). However, the narrowness of the approach is appropriate to the hypothesis, which has to do only with *number* of parts, and the coarseness is required by considerations of hierarchical level. The hypothesis refers to the number of parts at a level just below the cell, as discussed, whereas mechanisms tend to reside at lower levels.

Sampling Protocols

Object parts were identified using descriptions and electron micrographs in the cytological literature. Using all available descriptive material for each cell type would radically underestimate part counts for less-well-studied cell types, and therefore to make part counts comparable, only a single journal article (i.e., a single source paper) was used for each cell type. Of course, any single source paper will overlook many parts, especially papers on metazoan and land plant cells in which the focus is typically on cell specializations. For example, a paper on a retinal cell might focus on the light-receiving apparatus and fail to mention the Golgi apparatus, even if present. To correct for this, a standard set of part types was developed (Table 1), and every cell type was assumed to contain a full complement of standard parts, unless its source paper mentioned that a part was absent. Thus, the data here are counts of parts in addition to the standard set. A consequence of using this tactic is that negative part counts are possible in principle and in fact occurred for certain cells, that is, those that contained few specialized parts and were also missing many from the standard set. Notice that the last two entries in the standard set are very broad and unspecific (Table 1), with the result that the actual number of parts the set represents is large and difficult to estimate. However, no estimate is needed to use the set as a common baseline for comparative purposes.

Finally, number of parts often varies over the life of a cell, but here counts are conducted at a single point in time. Thus, a count represents a kind of snapshot of a cell's structure taken at an arbitrary moment in its life cycle. Table 2 gives two sample lists of parts.

This strategy will produce some errors. First, because certain parts are overlooked—that is, physiological parts, behavior parts, and small object parts—some subparts will be misidentified as parts and vice versa. Second, some apparent object parts will not be parts at all, because invisible connections will sometimes cross object boundaries. Finally, many parts occupy a given level only partially, or to some degree (Wimsatt 1974), but here occupation of a given level is discretized. That is, a part is judged to be either present at the proper level or not, thus introducing some error. Importantly, however, none of these limitations is problematic unless it creates a bias, for example, unless it produces systematically higher part counts in protists. At present, I have

TABLE 2. Categorization and counts of part types in a metazoan and a protist.

Rabbit obplacental giant cells (Blackburn et al. 1989)		Pedinella (Dictyochae, Pedinellea) (Swale 1969)	
Part types	Category	Part types	Category
Eosinophilic inclusions (= erythrocytes?)	F?	Yellow-brown chromatophore	F
Desmosomal junctions	C	Contractile vacuole	F
Intracellular microvilli-lined canaliculi	C	Flagellum	C
Elongate cell processes	S	Second flagellum (internal)	C
Nucleus	standard set	Deep indentation at anterior pole	S
Mitochondria	standard set	Shallow indentation at posterior pole	S
Golgi apparatus	standard set	Tentacles	S
Other parts from standard set		Peduncle	S
		Nucleus	standard set
		Mitochondria	standard set
		Food vacuoles	standard set
		Other parts from standard set	
Part counts (in addition to standard set)			
F	0		2
FC	2		4
FCS	3		8
FCS?	4		8

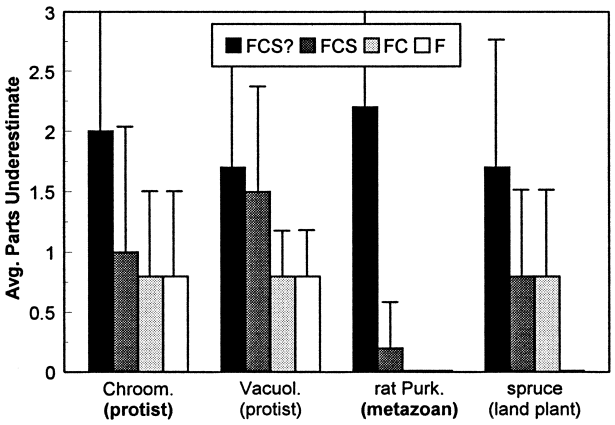

FIG. 2. Literature-bias evaluation. A possible bias arises from the fact that source papers for multicellular organisms tend to focus on cell specializations, whereas papers on protists emphasize systematics. Thus, descriptions of protists might be more complete, biasing counts in favor of the hypothesis. To investigate this, four test cases were selected, four species for which multiple source papers could be found: two protists (*Chroomonas salina*, $n = 6$ source papers; *Vacuolaria virescens*, $n = 6$), a metazoan cell type (*Rattus rattus*, cerebellar purkinji cell, $n = 5$), and a land plant cell type (*Picea abies*, needle mesophyll cell, $n = 6$). For each, number of parts was estimated in two ways. First, counts were conducted using each source paper by itself and then these were averaged to estimate number of parts per source paper. Second, the information in all source papers for a species was combined to generate a kind of best estimate of the actual number of part types in that cell type. The actual minus the average per paper is a measure of the degree to which any single paper will, on average, underestimate the actual. Vertical bars show average underestimates for four part-type estimates (F, FC, FCS, and FCS?; see text); error bars show one standard deviation. The data show that underestimates for the metazoan and land plant cells are not greater, and if anything seem to be mostly less, than for protists.

no reason to think such biases are present, although I will continue to explore the issue. (A possible bias in the source papers is addressed in Fig. 2.)

A Test of the Hypothesis

The test consisted of two comparisons, each conducted in two ways: (1) metazoan cells ($n = 30$) compared first with a diverse paraphyletic group of protists ($n = 26$) and second with the probable sister taxon to the Metazoa among extant species, the choanoflagellates ($n = 5$) (Wainright et al. 1993); and (2) land plant cells ($n = 18$) compared first with the same paraphyletic group of protists, and second with free-living chlorophytes ($n = 10$), a closely related outgroup to the land plants (Friedl 1997). The charophytes are the probable sister taxon among extant species (Bhattacharya and Medlin 1998; Qui and Palmer 1999), but most are not solitary unicells, that is, not truly free-living in the vegetative stage.

The sample of protists was chosen to cover a wide taxonomic range roughly at what has been called the phylum level (sensu Corliss 1994; see also Margulis et al. 1990). This group includes representatives of the eukaryotic crown group (Knoll 1992; Sogin 1997)—the alveolates (e.g., Ciliophora), stramenopiles (e.g., Phaeophyta), and rhodophytes—and some from more basal groups, the Euglenozoa and the diplomonads (Metamonada; see Appendix). Obligate and facultative parasites were excluded, because they participate to some extent in a higher-level entity and thus may be subject to selection for functional loss. Protists that are nontransiently colonial or multicellular (e.g., filamentous) were avoided; those that are also differentiated into multiple cell types in the vegetative stage were excluded. Finally, species whose principal habitat is laboratory cultures, and forms known to be aberrant (e.g., mutants induced in the laboratory) were excluded. Metazoan and land plant cells were also chosen to represent a wide diversity of both higher taxa and cell types, and laboratory and aberrant forms were also ruled out.

For metazoans, most of the variance in number of parts was accounted for by variation among cell types, rather than among taxa (see data and discussion in McShea 2001a). Thus, one can think of the mean part count for metazoans as an estimate for a kind of composite metazoan, for a single, hypothetical, chimeric multicellular organism composed of cell types drawn from various higher taxa within the group. In other words, for purposes of this analysis, the Metazoa can be considered a single datapoint. Presumably the same is true for land plants.

Seemingly, the analysis should nevertheless take into account phylogenetic relationships among the protists (e.g., Sogin et al. 1996a; Pace 1997). However, in this case, it is reasonable to treat them as independent, at least at a high taxonomic level. One reason is that the tree is not yet known with great confidence at a high taxonomic level (e.g., Philippe and Adoutte 1998; Katz 1999; Stiller and Hall 1999; Taylor 1999). Another is that divergences are separated in most cases by enormous stretches of time, allowing many opportunities for gains and losses of parts in most lineages. In other words, phylogenetic constraint on the relevant time scale may be minimal. But more importantly, the character at issue here, number of part types, is not homologous among higher taxa, for the most part. That is, each higher taxon's parts list, over and above the standard set, consists mostly of *unique* part types (with three exceptions, discussed below). Thus, similar part counts among higher taxa are only minimally the result of phylogenetic constraint. (Within higher taxa, the situation is different; see below.)

RESULTS AND DISCUSSION

Results are shown in Table 3; *P*-values are based on bootstrap tests of differences between means (1000 resamplings with replacement) in each of the two groups compared (see Appendix for part counts). With one exception, F parts in choanoflagellates, results were consistent with and supported the hypothesis. Sample sizes were modest, but differences were highly significant and presumably would only become more so with larger samples.

As noted, in protists, parts are not independent within higher taxa. To accommodate this, part counts were averaged within higher taxa and the analysis repeated, yielding similar results (not shown). Also, three parts in particular (plastids, contractile vacuoles, and flagellae) actually are shared among many higher taxa, at least in some cases with some significant degree of homology (e.g., for plastids, see Delwiche 1999). Interestingly, removing these three from the analysis rendered most of the differences in Table 3 insignificant, raising

TABLE 3. Mean part-count comparisons: multicellular versus protists and multicellular versus sister taxa.

	Metazoan cells (n = 30)	versus protists (n = 26)	P	versus choano-flagellates (n = 5)	P
F	0.36	1.69	<.001	0	—
FC	1.50	3.73	<.001	2.80	<.001
FCS	2.39	4.55	<.001	3.98	<.010
FCS?	2.77	5.54	<.001	4.60	<.009
	Land plant cells (n = 18)	versus protists (n = 26)		versus chloro-phytes (n = 10)	
F	0.67	1.69	<.001	1.40	<.013
FC	2.22	3.73	<.002	3.51	<.001
FCS	2.22	4.55	<.001	3.51	<.001
FCS?	2.44	5.54	<.001	3.90	<.001

the possibility that it was mainly the loss of these parts in most metazoan cells (and perhaps the second two in land plant cells) that accounts for the differences between the groups. Consistent with the hypothesis, the interpretation would be that in the evolution of multicellularity, the functions performed by these parts—resource acquisition, osmotic control, and propulsion, respectively—were transferred from the cell level to the level of the whole organism.

Table 3 suggests that part differences between free-living protists and cells in multicellular organisms are quite small. For example, metazoan cells seem to have only about one to three more part types than protists. Of course, this is so only if the standard set is fairly stable (as assumed above), that is, only if it is present as a more or less complete package in most cells (with some obvious exceptions, such as human hemocytes). This might be the case if, for example, the parts of the standard set have mostly housekeeping functions, in which case removing them might be typically difficult or impossible for selection to accomplish.

However, there is a possible alternative assumption, namely that the standard set has undergone the same pattern of reduction as the nonstandard set, or in other words, that the parts counted here are a representative sample of all parts (including the standard set). Under this assumption, the complexity drain could be quite large. Ratios of part counts for metazoans to those for protists range from about 0.2 to 0.5. Concretely, if the standard set contains on the order of hundreds of parts, then absolute parts differences might also number in the hundreds. (Notice that under this assumption, technically, absences from the standard set would not be subtracted from part counts; in fact, however, such absences were rare [see Appendix] and using this approach would not significantly alter the quantitative results reported in Table 3.) A separate study of the standard set would be needed to illuminate this matter further (see below).

Alternative Evolutionary Interpretations

Results are consistent with at least two evolutionary possibilities: (1) an ancestral protist having many parts, with a decrease in cell parts occurring in the multicellular lineages; and (2) an ancestral state with few parts, and an increase in all or most lineages, but a lesser increase in the multicellular lineages. The second possibility is supported somewhat by the low part counts in certain modern amitochondriate protists, on the assumption that they branched basally in the eukaryotic radiation and are relatively underived (e.g., Sogin et al. 1996b; Simpson et al. 1997). Most of these are parasitic, but some are free-living (e.g., the pelobiont *Mastigamoeba schizophrenia*) and are also missing many parts from the standard set (see Simpson et al. 1997). On the other hand, some of the amitochondriates have a moderate number of parts (e.g., the free-living metamonad *Trepomonas agilis* has at least five in addition to the standard set). Also, the euglenozoans, which branched later but still before the crown radiation, have part counts slightly higher than the protist mean. In sum, the primitive eukaryotic state and the evolutionary route by which protists attained more parts remain open questions.

Another explanation of the data, one that is not consistent with the hypothesis, also needs to be considered. Possibly cells in multicellular organisms actually have more parts than protists but the parts are smaller, molecule-sized, and mostly invisible in electron micrographs. The suggestion is that multicellular development and physiology require coordination of cellular activity, most of which is mediated by molecule-sized signals and receptors not required for free-living existence (Kordon 1993; Bonner 2000). However, the argument can be countered. A free-living cell may require little cell-to-cell signaling, but it may still use many molecular signals (or technically, cues) for controlling rate of reproduction, detecting and orienting toward food, avoiding toxic substances and predators, and so on (e.g., Csaba 1996; Rasmussen et al. 1996; Christensen et al. 1998). In other words, it is not obvious that a protist needs fewer molecule-sized parts than, for example, a metazoan cell, keeping in mind that the relevant datum is number of part *per cell*, not number for the whole metazoan. Indeed, total number of signal and cue functions per cell could be about the same: Csaba speculates that in the transition to multicellularity, ''many receptors in one cell, representing all functions, diverged to a few receptors in many cells'' performing different functions (Csaba 1996, p. 22). Still, pending comparative studies of numbers of signal types, this alternative explanation remains a live possibility. (And if true at the cell level, the question arises whether a decrease in the size of parts accompanies the advent of higher levels generally. And, if so, why?)

Alternative Views of the Complexity Drain

If the hypothesis is correct, the hierarchical transition from free-living cell to multicellular entity can be seen in various ways. From the viewpoint of the focal level, the cell, we see a stripping of functional capabilities and a reduction of internal structure, a conversion of independent, multifunctional cells into specialized components. (Of course, the stripping is rarely complete, in that specialized functions and certain housekeeping functions remain.)

Looking down from the vantage point of the multicellular entity, however, we see both gains and losses. Parts are lost within cells, but gained within the multicellular whole. For one thing, the transformation of cells into specialized com-

ponents is a gain. In other words, as cells lose parts internally, they also *become* parts at a higher level, within the multicellular entity. Other changes occurring at the same time are also gains, including the emergence of tissues, organs, and other cell-cell collaborations, which add parts at intermediate levels between cell and multicellular entity (Anderson and McShea 2001). Thus, from this top-down viewpoint, what we see is a shift in parts, a transfer of complexity, from a level below the cell to a higher level, closer to that of the multicellular organism as a whole (McShea and Anderson in press). It is worth noting, however, that the net effect on complexity of this transfer is unknown. That is, we do not know the relative magnitudes of the vectors promoting increases or decreases at the various hierarchical levels.

The multicellular entity's viewpoint raises another possibility, one that might seem to imply that no drain exists. Even if number of part types per cell declines, as the data here show, the total number of cell-part types in a multicellular entity, summed over all cell types, might increase. Concretely, the suggestion is that a mammal as a whole might have more cell-part types than a *Euglena*. This seems plausible, although it has not been demonstrated. In any case, there is no contradiction: The occurrence of losses, a drain, is consistent with a net gain.

Finally, the losses documented here actually suggest a complexity drain in two senses. First, there is the drain on part types at a single level, the level just below the cell, as documented in Table 3. Second, this loss of part types and the accompanying loss of functional capability and autonomy in cells suggests that the emergence of multicellularity may be accompanied by a weakening of the cell level, so to speak, that is, by a reduction in the importance of cells as discrete functional units. Consistent with this view, Beklemishev (1969) described the emergence of coloniality as a weakening of the individuality of the lower-level units (e.g., zooids, castes). Arguably, this weakening of lower levels constitutes a partial loss of hierarchical structure, in effect, a drain on complexity in a hierarchical sense.

The Reality of Parts

In most studies of morphological complexity, the parts compared among taxa are homologous, and thus the biological significance of a count of types is not in doubt (Cisne 1974; McShea 1993, 1996; Valentine et al. 1994). The use I make of the parts notion here—to refer to mainly nonhomologous structures and as a proxy for number of functions—is less common (but see Schopf et al. 1975) and therefore might engender some skepticism. Also, the protocols used to identify them require many assumptions, with the result that parts might seem to be arbitrary or subjective units.

One answer is that the effect of subjectivity can be evaluated. I did this in the present study, asking a student to repeat my counts for a subset of cell types; our counts were well correlated (see data in McShea 2001a). Another answer is that the differences in part counts documented above are highly significant, which strongly suggests that the protocols are not picking out arbitrary units, despite the many assumptions, and that cell parts are biologically significant units (whether or not the hypothesis the counts ostensibly support is correct). In other words, if cell parts were not real units, if they were purely human constructs, then significant differences would not have been found.

Additional Tests

The test here compared only extreme cases. Cells from metazoans and land plants, in which multicellularity is well developed, were compared with free-living cells, in which degree of multicellularity, or degree of individuation at the multicellular level (McShea 2001b), is essentially zero. An extension of this study might investigate whether part counts in cells decline continuously with increase in degree of multicellular individuation. A test case could be the volvocine algae, which show a continuum of individuation from the free-living *Chlamydomonas* to *Volvox*, a multicellular form with thousands of cells and two cell types in the vegetative phase (Kirk 1998). Another useful case might be the cellular slime molds that have both a free-living unicellular phase and a multicellular phase in their life cycles (Bonner 1988).

Also at the cell level, this study raises a number of issues that would be addressable with a larger dataset. First, the assumptions regarding the standard set could be investigated, in particular to discover whether the standard set is especially stable or whether it follows the same pattern of reduction as the parts counted here. Second, other assumptions could be tested; for example, it would be useful to investigate numbers of molecule-sized parts (perhaps using numbers of mRNA transcripts in the cytoplasm as a proxy) to see whether they follow the same pattern as larger parts. Third, larger samples would allow us to investigate possible quantitative relationships. For example, consider cell-part types versus cell types. Changizi (2001) has shown in a number of systems, both natural and artificial, that number of part types at one level (C) and number of part types at the next level (or what he calls ''expressions,'' E) are related by a simple power law with exponent between zero and one ($C \propto E^b$, $0 < b \leq 1$) (for further discussion, see Changizi 2001). For cells, the testable prediction would be that such a relationship holds between total number of cell-part types in a multicellular organism, summed across all cells (C), and number of cell types (E).

Finally, testing the hypothesis at the colony level would be worthwhile. The hypothesis predicts, for example, that ants in the more individuated colonies should be less complex than those in less individuated colonies (Oster and Wilson 1978). Colony size could be used as a proxy for individuation (Anderson and McShea 2001), and ideally part counts for individual ants would include behavior parts as well as object parts. A test is also feasible in bryozoans, where criteria have already been developed for assessing degree of colony individuation (Boardman and Cheetham 1973) and for identifying parts in zooids (McShea and Venit 2001).

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APPENDIX
Taxa and Part-Type Counts

Higher taxon	Species	Cell type	F	C	S	?	FCS?
Metazoans							
Annelida	*Dina lineata*	pear-shaped secretory	0	4	1	0	5
Annelida	*Eisenia foetida*	cocoon-producing	1	0	1	1	3
Annelida	*Glossiphonia complanata*	adipose	1	0	1	0	2
Annelida	*Haementeria ghilianii*	anterior salivary	0	0	2	1	3
Arthropoda	*Drosophila auraria*	early third-instar salivary	0	0	1	0	1
Arthropoda	*Gammarus setosus*	attachment cells of organ of Bellonci	0	5	1	0	6
Arthropoda	*Gaussia princeps*	luminous	0	1	1	1	3
Arthropoda	*Leucania loreyi*	isolated cell of corpora allata	0	0	1	0	1
Arthropoda	*Locusta migratoria*	type B of rectal pad epithelium	0	3	0	1	4
Arthropoda	*Periplaneta americana*	hemocytes	2	0	1	0	3
Chordata	*Acipenser brevirostratus*	basal cells of notochord epithelium	0	1	1	0	2
Chordata	*Dasypus novemcinctus*	acinar cells in mandibular gland	1	0	0	0	1
Chordata	*Eubalaena australis*	lipokeratinocytes	0	2	1	1	4
Chordata	*Gallus gallus*	supporting cells of paratympanic organ	1	1	2	0	4
Chordata	*Gallus gallus*	anterior pituitary	1	0	0	0	1
Chordata	*Homo sapiens*	melanocytes	0	2	1	1	4
Chordata	*Lepidosiren paradoxa*	adenohypophysis agranular	0	4	2	0	6
Chordata	*Mus*	epithelial fundus gland of gastric mucosa	0	1	2	0	3
Chordata	*Oryctolagus cuniculus*	obplacental giant cells	0	2	1	1	4
Chordata	*Sparus aurata*	monocyte-like cytotoxic	0	0	1	0	1
Cnidaria	*Calliactis parasitica*	gastrodermal sensory	0	1	1	1	3
Echinodermata	*Eupentacta quinquesemita*	morula cells	1	0	0	0	1
Mollosca	*Helix pomatia*	cells lining small blood vessels	0	0	1	0	1
Mollosca	*Ilyanassa obsoleta*	oocytes	1	0	0	0	1
Mollosca	*Tridacna crocea*	muscle cells in adductor	0	1	0	0	1
Nemertea	*Riseriellus occultus*	sensory cells	0	3	2	3	8
Platyhelminthes	*Bothrioplana semperi*	sensory receptor	0	3	1	0	4
Porifera	*Oscarella lobularis*	vacuolar cells	1	0	0	0	1
Porifera	*Tedania ignis*	myocytes	1	0	0	0	1
Sipuncula	*Phascolosoma granulatum*	nephridial granular	0	0	1	0	1
		mean	0.37	1.13	0.90	0.37	2.77
		standard deviation	0.56	1.48	0.66	0.67	1.87
Land plants							
Anthocerophyta	*Phaeoceros laevis*	parenchyma cells of tuber	1	1	0	0	2
Anthophyta	*Acacia cornigera*	cortical cells of Beltian body	2	2	0	0	4
Anthophyta	*Cypripedium calceolus*	bulbous terminal trichome	1	1	0	1	3
Anthophyta	*Echinocereus engelmannii*	mature zone shoot apical meristem	1	1	0	0	2
Anthophyta	*Gossypium hirsutum*	parenchymal cells of nectary subgland tissue	1	2	0	0	3
Anthophyta	*Helianthus annuus*	spongy mesohyl cells under palisade	1	2	0	0	3
Anthophyta	*Hoya carnosa*	long cells of root exodermis	0	2	0	0	2
Anthophyta	*Lilium leucanthum*	stigmatoid cells of pistil	1	1	0	1	3
Anthophyta	*Mimosa pudica*	starch sheath cells of primary pulvinus	1	2	0	0	3
Anthophyta	*Thlaspi arvense*	sieve	−6	1	0	0	−5
Anthophyta	*Utricularia monanthos*	terminal cells of external glands	1	2	0	1	4
Bryophyta	*Polytrichum juniperinum*	food conducting cells of sporophyte foot	1	2	0	0	3
Coniferophyta	*Pinus nigra*	mesophyll cells of needle	1	2	0	1	4
Gnetophyta	*Ephedra monosperma*	root apex (columella)	1	2	0	0	3
Gnetophyta	*Gnetum gnemon*	laticifer	2	1	0	0	3
Hepatophyta	*Odontoschisma denudatum*	marginal cells of young leaves	1	1	0	0	2
Lycophyta	*Selaginella kraussiana*	glossopodial cells of ligule	1	1	0	0	2

APPENDIX
Continued.

Higher taxon	Species	Cell type	F	C	S	?	FCS?
Pterophyta	*Osmunda cinnamomea*	promeristem	1	2	0	0	3
		mean	0.67	1.56	0.00	0.22	2.44
		standard deviation	1.71	0.51	0.00	0.43	1.98
Protists							
Charophyta	*Closterium littorale*		2	1	0	1	4
Chlorarachniophyta	*Chlorarachnion reptans*		2	0	1	0	3
Ciliophora	*Perispira ovum*		4	2	2	2	10
Ciliophora	*Prorodon teres*		3	3	2	0	8
Ciliophora	*Stentor polymorphus*		2	3	2	2	9
Cryptomonadida	*Rhodomonas lacustris*		3	3	1	1	8
Dictyochae (Pedinellea)	*Pedinella hexacostata*		2	2	4	0	8
Dinozoa	*Aureodinium pigmentosum*		1	3	0	0	4
Dinozoa	*Woloszynskia micra*		2	4	1	0	7
Euglenozoa	*Khawkinea quartana*		1	4	1	0	6
Euglenozoa	*Paranema trichophorum*		1	3	2	0	6
Glaucophyta	*Glaucocystis nostochinearum*		0	1	0	3	4
Haptophyta	*Chrysochromulina megacylindra*		1	3	0	1	5
Haptophyta	*Diacronema vlkianum*		1	3	1	0	5
Kinetoplastida	*Bodo curvifilus*		1	2	1	1	5
Metamonada	*Trepomonas agilis*		1	2	1	1	5
Phaeophyta (Chrysophyceae)	*Chromulina placentula*		2	2	0	2	6
Phaeophyta (Chrysophyceae)	*Chrysococcus rufescens*		3	3	0	1	7
Phaeophyta (Chrysophyceae)	*Ochromonas tuberculatus*		3	2	0	2	7
Phaeophyta (Eustigmatophyceae)	*Chlorobotrys regularis*		1	0	0	1	2
Phaeophyta (Eustigmatophyceae)	*Pseudocharaciopsis texensis*		2	3	0	0	5
Raphidophyta	*Olisthodiscus luteus*		1	2	0	1	4
Rhizopoda	*Trichosphaerium micrum*		0	1	1	1	3
Rhizopoda (Foraminiferea)	*Globigerinoides ruber*		3	1	0	5	9
Rhodophyta	*Dixoniella grisea*		1	0	0	1	2
Rhodophyta	*Rhodella maculata*		1	0	1	1	3
		mean	1.69	2.04	0.81	1.04	5.58
		standard deviation	1.01	1.22	0.98	1.15	2.23
Choanoflagellates	*Acanthoeca spectabilis*		0	3	0	0	3
	Codosiga botrytis		0	3	2	0	5
	Proterospongia choanojuncta		0	3	2	1	6
	Savillea micropora		0	2	0	1	3
	Stephanoeca diplocostata		0	3	2	1	6
		mean	0.00	2.80	1.20	0.60	4.60
		standard deviation	0.00	0.45	1.10	0.55	1.52
Chlorophytes	*Ankyra* sp.		1	3	0	0	4
	Carteria crucifera		1	2	0	1	4
	Chlamydomonas reinhardi		2	2	0	0	4
	Chlorococcum echinozygotum		2	2	0	1	5
	Chlorogonium elongatum		1	2	0	0	3
	Dunaliella primolecta		1	1	0	2	4
	Nautococcus mammilatus		2	3	0	0	5
	Polytomella agilis		1	2	0	0	3
	Pseudococcomyxa simplex		1	2	0	0	3
	Treubaria setigera		2	2	0	0	4
		mean	1.40	2.10	0.00	0.40	3.90
		standard deviation	0.52	0.57	0.00	0.70	0.74