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Organismal size, metabolism and the evolution of complexity in metazoans

Megan C. McCarthy* and Brian J. Enquist

Department of Ecology and Evolution, University of Arizona, Tucson, AZ 85721, USA

ABSTRACT

Questions: What is the macroevolutionary relationship between body size, number of cell types and metabolism? Furthermore, why does the relationship between body size and the number of cell types hold between major metazoan clades but not within closely related taxa?

Mathematical methods: Expand the allometric relationship between size and metabolism to include (1) the energetic costs of supporting an increased number of cell types and (2) the phylogenetic constraints governing the number of cell types.

Key assumptions: An increase in organismal size selects for additional cell types. This is due to biophysical constraints and transport demands. The increase in cell types allows the organism to perform new functions. The extra cell types also require more intercellular networks. Therefore, the amount of energy required per unit of body mass should increase with the number of cell types. Phylogeny may also constrain the number of cell types within taxa. This constraint will limit the number of cell types to be approximately constant within a bauplan (a unique organismal form comprised of an anatomical and physiological design).

Predictions: Organismal size should be positively correlated to the number of cell types across metazoan taxa. However, this relationship will not hold within clades due to energetic and phylogenetic constraints. The energetic constraint leads to a positive correlation between the number of cell types and metabolic intensity (the mass-specific rate of energy processing standardized to a given body size) across metazoan bauplans. Available data support these predictions. Metabolic intensity is positively related to the number of cell types in metazoan clades.

Keywords: allometry, evolutionary trends, macroevolution, multicellularity, number of cell types, organismal energetics.

INTRODUCTION

Organismal complexity is positively correlated to body size. Both size and complexity have increased throughout the evolutionary history of life (Bonner, 1968, 1988; Valentine *et al.*, 1994; Bell and

* Author to whom all correspondence should be addressed. e-mail: mccarth1@email.arizona.edu

Mooers, 1997; Vermeij, 1999). While these patterns are widely accepted, the mechanisms behind the evolution of organismal complexity are poorly understood (Bonner, 1988; Maynard-Smith and Szathmáry, 1995). For example, why are groups that are orders of magnitude larger than a tree shrew, such as kelp and fungi, considerably less complex? In addition, why do some clades increase in complexity while others are apparently limited?

Part of the problem in understanding the evolution of biological complexity is that there is not a standard definition of complexity. McShea (1996) provides several definitions for biological complexity. These include: the number of different parts within a hierarchy (genes, cells, organs, etc.), the number of interactions between parts in this hierarchy, the number of parts for a particular spatial or temporal scale and the number of interactions between parts in a spatial or temporal scale. A common measure of biological complexity that fits within this definition is the total number of different cell types found within an organism or taxonomic group (Bonner, 1968, 1988; Valentine *et al.*, 1994; Bell and Mooers, 1997; Carroll, 2001). Differences in cell types can be identified by compositional and functional differences. The number of cell types provides a measure of the division of labour without comparing tissue types between diverse organisms (Bonner, 1988).

Some conceptual models have linked the evolution of organismal complexity, measured by the number of cell types, with increases in organismal body size (Bonner, 1968, 1988). Other conceptual models have connected the evolution of metabolic intensity, the mass specific rate of energetic processing for a given body mass, with body size (Vermeij, 1999). However, none of these approaches have considered the mechanistic linkage between the number of cell types, body size and metabolic intensity.

Interestingly, body size, complexity and metabolic intensity have all increased throughout macroevolutionary time (Bonner, 1968, 1988; Valentine *et al.*, 1994; Vermeij, 1999; Carroll, 2001; Witting, 2003). The number of cell types in a clade is positively related to time of origin (Valentine *et al.*, 1994). Metazoan clades that appeared more recently in evolutionary time have about two orders of magnitude more cell types than the earliest clades (see Fig. 1a). Metabolic intensity of metazoan clades is also positively related to time of origin (Lotka, 1956; Zotin and Konoplev, 1984; Vermeij, 1987; Witting, 2003). Clades that appeared later in evolutionary time have about 2.5 orders of magnitude higher field metabolic intensity than the earliest diverged clades (see Fig. 1b). In sum, more recently derived clades have more cell types (Valentine *et al.*, 1994) and increased metabolic intensity than more ancient clades. Together, these macroevolutionary patterns suggest a mechanistic link between metabolic intensity and the number of cell types.

Here we develop a general model that integrates macroevolutionary increases in size, number of cell types and metabolic intensity (Fig. 2, black arrows). We start with the observation that throughout evolutionary time there has been a consistent increase in organismal size (Bonner, 1988, 1998, 2004; Enquist, 2003). We use selection for increased size as the basis for understanding variation in number of cell types. However, we show that selection for size alone does not predict the number of cell types within a given clade. We hypothesize that an increase in the number of cell types is metabolically demanding. A new cell type not only takes on a new function, but also integrates tissue systems and information networks. In support of our hypothesis, we show that the number of cell types is positively correlated to the metabolic intensity of a taxon. This relationship between the number of cell types and metabolic intensity provides a critical mechanistic link that connects macroevolutionary patterns in size, cell type number and metabolic intensity.

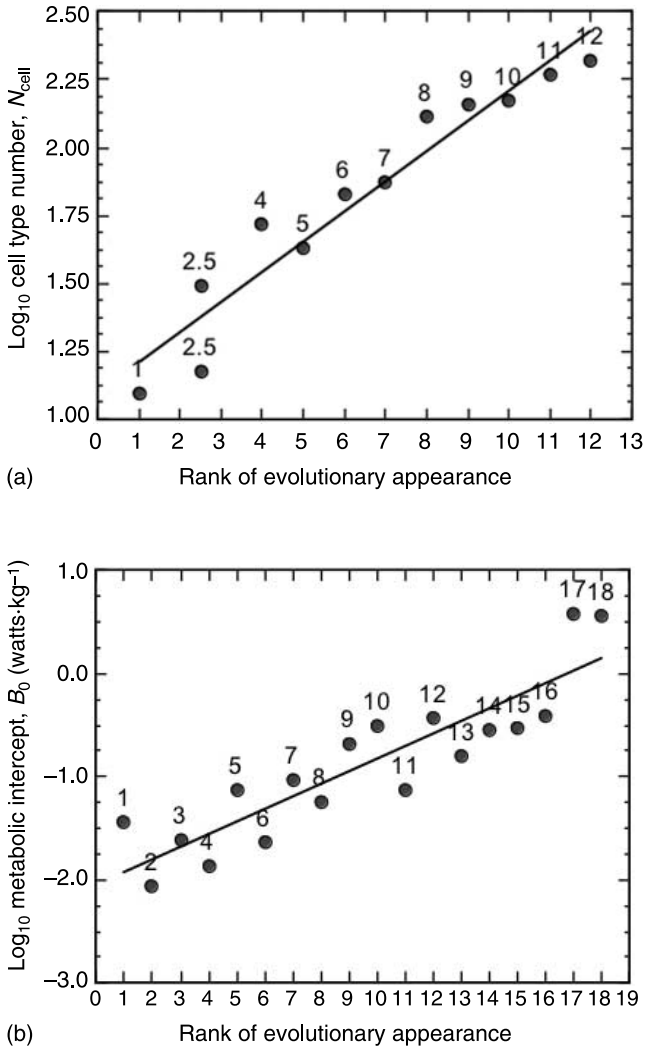

Fig. 1. (a) Relationship between cell type number for metazoan taxa and rank of evolutionary appearance. Small rank represents early evolutionary appearance (data from Valentine *et al.*, 1994). 1 = Porifera, 2.5 = Cnidaria and Haemocoelic Bilaterian, 4 = Arthropoda, 5 = Echinodermata and Annelida, 6 = Agnatha, 7 = Cephalopoda, 8 = Actinopterygii, 9 = Amphibia, 10 = Diapsida, 11 = Aves, 12 = Hominidae. (b) Relationship between metabolic intercept, B_0, for metazoan taxa and rank of evolutionary appearance. Small rank represents early evolutionary appearance (data from Peters, 1983; graph and analysis from B.J. Enquist *et al.*, unpublished). 1 = Unicells, 2 = Protozoa, 3 = Porifera, 4 = Anthozoa, 5 = Scyphozoa, 6 = Nematoda, 7 = Mollusca, 8 = Branchiopoda, 9 = Oligochaeta, 10 = Gymnolaemata, 11 = Malacostraca, 12 = Copepoda, 13 = Arachnida, 14 = Insecta, 15 = Osteichthyes, 16 = Amphibia, 17 = Squamata, 18 = Mammalia.

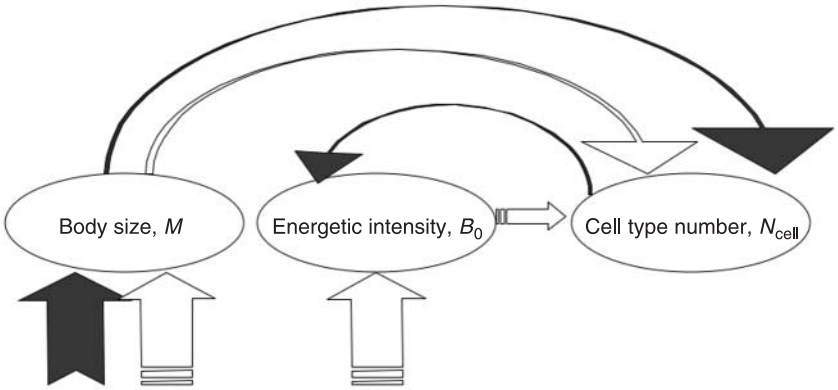

Fig. 2. Flow chart representing the relationship between body size, metabolic intensity, B_0 (the rate of energy processing per unit body size), and cell type number. The large dark, vertical arrow indicates which character selection operates on. The curved black arrows represent how each character requires changes in the other characters. Selection could also operate on metabolic power (white vertical arrows), which would result in a change in both energetic intensity and body size. Selection on metabolic power would then require changes in the number of cell types through both the increase required by additional size (curved white arrow) and the increase required by increased energy processing (white horizontal arrow).

A GENERAL MODEL FOR ASSESSING MACROEVOLUTIONARY PATTERNS IN CELL TYPE NUMBER, ORGANISMAL ENERGETICS AND BODY SIZE

We start by hypothesizing that there has been strong selection for increases in organismal size (Fig. 2, vertical black arrow). Indeed, the maximum size of organisms has increased over evolutionary time (Bonner, 1988). Macroevolutionary increases in body size have been explained by selection (Bonner, 1988, 1993, 1998) and increased variance from a minimum size boundary (McShea, 1994; Gould, 1996). Increased size may be selected for because of increased reproductive output, increased competitive ability, the ability to invade new habitats and exploit previously unavailable resources, predator evasion, and so on (Bonner, 1968, 1988, 1998, 2004; Vermeij, 1987; Kirk, 1998; Kaiser, 2001; Enquist, 2003). This size increase, regardless of the mechanism, requires additional organismal innovations because of biomechanical constraints (to support and move a larger body) and an increased need for long-distance intercellular transport of resources and information (Bonner, 1988, 1998; Kaiser, 2001; Enquist, 2003). We argue selection to overcome these additional constraints requires an increased number of cell types (Fig. 2, large curved black arrow). These cell types are necessary to support and move the larger mass, transport fluids, nutrients and gases to all the cells in the organism, excrete waste material, and relay information from one part of the body to another (Bonner, 1988, 1998; Kaiser, 2001).

We start with the well-known relationship between the basal metabolism of an organism, B (the rate of energy processing per unit time), and its body mass, M. The early work of Rubner (1883) and Kleiber (1932) showed the energy throughput of an organism depends on the size or mass, M, of an organism (see references in Schmidt-Nielsen, 1984). In general, the relationship between B and M is given by the allometric equation

$$B = B_0 M^{3/4} \tag{1}$$

where B is organismal basal metabolism, B_0 is the metabolic intensity that characterizes a taxonomic group (Mammalia, Aves, Insecta, etc., or an individual during ontogeny) and M is body mass. The 3/4 exponent is both empirically and theoretically verified (Kleiber, 1932, 1961; Hemmingson, 1960; Peters, 1983; Schmidt-Nielsen, 1984; West *et al.*, 1997; Savage *et al.*, 2004). Metabolic intensity is the y-intercept of the relationship between whole-body metabolism and size. Organisms with higher values of B_0 have higher rates of energy processing for a given body size. Metabolic intensity is size dependent. When solved for, $B_0 = B/M^{3/4}$, and has units of watts per unit mass$^{-3/4}$ ($\mathrm{W} \cdot M^{-3/4}$). Due to this size dependence, values of B_0 are standardized so they can be compared between clades.

We can link whole-organismal metabolism to the number of cells within the body and the metabolic rate of individual cells using equation (1). Following similar reasoning by West *et al.* (2002), we assume that the organism is composed of N_c cells each having an *in vivo* metabolic rate B_c. We then express whole-organism metabolism as the number of cells multiplied by the average metabolic rate of a cell so that $B = N_c B_c = [(M/M_c)\, B_c]$. Here M_c is the mass of an average cell within a given taxonomic group and $N_c = M/M_c$. From equation (1), we show that the metabolic intensity is influenced by the average mass and metabolic rate of a cell so that

$$B_0 = \frac{\left(\frac{M}{M_c}\right) B_c}{M^{3/4}} \quad \text{or as} \quad B_0 = \left(\frac{B_c}{M_c}\right) M^{1/4} \tag{2}$$

Across most organisms there is little variation in average cell size, M_c, with body size, M (Thompson, 1942). Therefore, equation (2) indicates that for a given body size, M, variation in the empirical value of B_0 between taxa largely reflects changes in rates of cellular metabolism, B_c (Kleiber, 1961; Schmidt-Nielsen, 1984). This is an important point, since below we use measures of B_0 across metazoan taxa to show how variation in the metabolic intensity, B_0, is linked to the number of cell types. Values of B_0 have been shown to vary greatly between metazoan taxonomic groups (Peters, 1983). Values of B_0 are highest for animals such as mammals and birds, and become successively lower for reptiles then amphibians, etc., with unicellular organisms having the lowest value (Hemmingson, 1960). This pattern exists even for temperature-corrected metabolic data (Robinson *et al.*, 1983; Gillooly *et al.*, 2001).

Metabolic cost of supporting an increase in the number of cell types

We assume that the number of cell types within an organism, N_{cell}, is limited by the whole-organismal metabolic rate, B (Fig. 2). In other words, there is a metabolic cost to supporting a given number of cell types, N_{cell}. Each additional cell type allows the organism to perform a new function, or improve the performance of an old function. The net result is that increasing the number of cell types (i.e. having specialized cells) allows the organism to do more work with the same number of cells. Therefore, specialized cells should have higher energy demands. For example, the energetically demanding brain and immune system cells of a human (Buttgereit *et al.*, 2000; Dienel and Hertz, 2003) will do more work than the same number of cells in an earthworm. More cell types will also require an increase in cellular and tissue integration, such as hormones and cell-to-cell signalling. These functions are also energetically intensive (Jequier, 2002; Estienne *et al.*, 2003). Therefore, an increase in the number of cell types, N_{cell}, requires an increase in whole-organism metabolism, B.

The most simplistic model assumes that the number of cell types, N_{cell}, is directly proportional to whole-organismal metabolism, B, such that

$$N_{cell} = c_0 B \quad \text{or} \quad B = N_{cell}/c_0 \tag{3}$$

Equation (3) summarizes the energetic constraints of supporting a greater number of cell types. Here c_0 is the number of cell types that can be supported for a given value of B. The value of c_0 is equal to N_{cell}/B (or N_{cell}/Watts). This is a measure of the efficiency of functions such as tissue integration or cell–cell signalling. If the cost of tissue integration increases with increasing cell types (i.e. the cost per cell is higher for 100 cell types than for 5), the efficiency of tissue integration, c_0, will decrease.

Recall from equation (1) that organismal metabolism is limited by organismal size, such that $B = B_0 M^{3/4}$. Therefore, by substitution from equation (1), the total number of cell types is related to organism size by

$$N_{cell} = c_0(B_0 M^{3/4}) \tag{4}$$

Note that equation (4) states that variation in the number of cell types, N_{cell}, is due to variability in metabolic intensity, B_0, the efficiency of tissue integration, c_0, and body size, M. Because of the assumed energetic cost of maintaining a certain number of cell types, N_{cell}, selection for increases in the number of cell types should lead to a change in at least one of the variables governing whole-organism metabolism (c_0, B_0 and/or M).

However, equation (4) does not incorporate phylogenetic constraints. Several authors have noted that within major metazoan bauplans, the number of cell types is remarkably constant (for a discussion, see Bonner, 1988, 2004). We use the term 'bauplan' to mean a unique organismal form comprised of an integrated and scalable anatomical and physiological design [for a review of the bauplan concept, see Gould (1977)]. We incorporate phylogenetic constraints into our model by limiting the generality of equation (4) within bauplans such that N_{cell} does not scale with body size. For example, the number of cell types in a shrew will be approximately the same as the number of cell types in an elephant (Bonner, 1988). We expect that these phylogenetic constraints on N_{cell} will hold for the major metazoan lineages. Phylogenetic constraints on equation (4) can then be expressed as

$$N_{cell} = c_0 B_0 M^0 \tag{5}$$

where M^0 indicates that within a bauplan the total number of cell types is independent of body size, M [for how temperature and cellular stoichiometry would also modify cellular metabolism expressed in equation (5), see Gillooly *et al.* (2001)]. We now have an expression for how M, B_0 and c_0 influences N_{cell}. In addition, we can combine equation (5) with equations (2) and (3) to show that $B_c = N_{cell}(M_c/c_0)M^{-1/4}$. This equation indicates cellular metabolism, B_c, is not only allometrically related to body mass, M, but also to cell type number, N_{cell}.

It is important to note that the values of c_0 and B_0 may differ between major clades or bauplans. At this stage, reasonable estimates for the efficiency of tissue integration, c_0, are not known. If c_0 is approximately constant across groups (i.e. the cost of tissue integration and cell–cell signalling are similar between mammals and Porifera), then the number of cell types, N_{cell}, will be directly proportional to metabolic intensity, B_0. However, if additional cell types require *increased* intercellular communication and transport, the cost per cell increases. So the efficiency of tissue integration, c_0, will decrease with increases in N_{cell}.

Then the number of cell types, N_{cell}, will still be positively related to metabolic intensity, B_0, but the slope of the relationship (when plotted on log–log axes) will be less than one.

In summary, our model indicates that although selection on body size may require an increase in cell types (Bonner, 1988, 2004), constraints due to the rate of energetic processing and phylogeny will constrain this relationship. The correlation between size and the number of cell types can only occur if accompanied by additional changes in cellular metabolic intensity, B_c, or tissue integration efficiency, c_0.

Specific predictions

Our model makes three specific predictions:

1. Due to phylogenetic constraints, the number of cell types within a bauplan, N_{cell}, will be independent of body mass. However, we expect N_{cell} to vary between bauplans due to differences in tissue integration efficiency, c_0, and the cellular metabolic intensity of cells, B_c (see below).
2. A 'cross-bauplan' correlation between organismal size and the number of cell types may result from differences in the range of body masses between bauplans. Across all metazoans, a positive correlation between body mass, M, and the number of cell types, N_{cell} (such as shown by Bonner, 2004), can occur if two conditions hold. First, there needs to be a directional difference in the body size ranges of metazoans. Specifically, bauplans with a smaller mean body size need to have a lower minimum and maximum body size than the minimum and maximum body size of bauplans with greater mean body size. Second, bauplans with a larger mean body size need to be characterized by a higher metabolic intensity, B_0, and/or value of c_0. If such differences occur, our model would predict a positive relationship between the number of cell types, N_{cell}, and body size, M, across metazoans. However, we do not expect this relationship within bauplans.
3. According to equation (5), a log–log plot across bauplans of the number of cell types, N_{cell}, by metabolic intensity, B_0, will yield a linear relationship with a slope that approximates unity. The slope will be one if the efficiency of tissue integration, c_0, is approximately constant across groups. But, if the efficiency of tissue integration, c_0, decreases (i.e. the cost per cell increases) with more cell types, the slope between the number of cell types, N_{cell}, by metabolic intensity, B_0, will be less than one. An exponent of one indicates that the number of cell types is directly proportional to B_0, which approximates cellular metabolism, B_c. An exponent less than one would indicate that increasing numbers of cell types have additional costs (such as tissue integration).

These predictions were tested by assembling a global database for the average cell type number, $\langle N_{cell} \rangle$, and differences in metabolic intensity, B_0, for a given bauplan, where the bauplans are represented by metazoan taxa. We then compared $\langle N_{cell} \rangle$ per bauplan to values of metabolic intensity, B_0.

METHODS

We calculated the number of cell types for each organism by examining the histology literature (Priest, 1963; Rigby and Tunnell, 1971; Leake, 1975; Robb, 1977; Groman, 1982; Valentine *et al.*, 1994; Bell and

Mooers, 1997). The number of cell types within each tissue type was based on differences in composition and/or function, as described by the authors. Cell types can be identified in histology studies by distinct staining due to compositional differences, or by variation in function. This allowed us to identify the number of cell types in a variety of organisms. Organisms were chosen from many diverse taxa and a wide range of body sizes. The number of cell types was calculated from adult organisms to avoid complications due to differences in ontogeny between organisms. Females were used to calculate the number of cell types unless the organism was hermaphroditic. In this case, only the female sex organs were considered (Bell and Mooers, 1997). For all organisms, the number of cell types was then compared to a body size measure, extracted from the scientific literature. To assess the relationship between B_0 and N_{cell}, we calculated an average number of cell types, $\langle N_{cell} \rangle$, for each taxon represented within our database.

Values for the number of cell types and body size were log transformed and fit with a linear Model II regression (reduced major axis, RMA). This method is recommended when variables are interdependent, functional relationships are being sought and error is expected to be similar between the variables (Sokal and Rohlf, 1995). Each species was classified into a taxon using systematic websites (Tree of Life: http://tolweb.org/tree/phylogeny.html; and Systematics of the Metazoa, UC Berkeley: www.ucmp.berkeley.edu/phyla/metazoasy.html). A phylogenetic tree was used to ensure each data point was independent (i.e. Arthropoda and Insecta could not both be used). Metabolic intensity, B_0, was then compared to the average number of cell types for each taxa (see Table 1 for taxa). The average number of cell types for each taxa was used instead of the y-intercept from a cell type number and body size regression. This is because organisms within the same class (e.g. a mouse and a human) have extremely similar, or even the same, number of cell types. In addition, a large, slightly less complex organism may inflate the cell type number measure if a scaling relationship between cell type number and body size is used. The metabolic y-intercepts, B_0, for the groups were obtained from Peters' (1983) compilation and were normalized to 1 kg. Some taxa that had body size and cell type number values did not have values for B_0 (Annalids, Mesozoa, Hydrozoa, etc.; see Table 1). Therefore, these taxa were not included in our analyses. The number of cell types and values of B_0 were $\log_{10}$ transformed and fit with a Model II regression.

RESULTS

The data support our model's assumption that the number of cell types is phylogenetically constrained within animal bauplans. When the number of cell types is compared to body size within metazoan phyla, the exponent is statistically indistinguishable from zero for Arthropoda, Cnidaria, Mesozoa, Platyhelminthes and Chordata (see Table 2, Fig. 3a). Annelida had an exponent only slightly above zero (95% confidence interval = 0.008, 0.078). These findings mirror those found in other studies showing little or no correlation between the number of cell types and body size within clades (Bonner, 1988, 2004).

In contrast to the relationship between M and N_{cell} within bauplans (see Fig. 3a), there is a significant positive relationship between body size and cell type number for all metazoans. The relationship between M and N_{cell} is described with an exponent of 0.119 ($r^2 = 0.712$, $n = 65$, $P < 0.0001$, 95% confidence interval = 0.103, 0.135; see Fig. 3b). This result supports the notion that complexity, as measured by the number of cell types, increases with an increase in body size (Bonner, 1988; Bell and Mooers, 1997). However, the result is apparently only

Table 1. Taxa used in Figures 3, 4 and 5

Higher taxa	Taxa in Figure 4	Figure 4 label	Species	Figure 3a label
Sedes incertis			*Salinella salve*	
Placozoa			*Trichoplax adhaerens*	
Ctenophora			*Pleurobrachia sp*	
Entoprocta			*Loxosoma sultana*	
Gastrotricha			*Turbanella cornuta*	
Gastrotricha			*Chordodasys antennatus*	
Gnathostomulida			*Rastrognathis macrostoma*	
Gnathostomulida			*Valvognathis pogonostoa*	
Kinorhyncha			*Pycnophyes frequens*	
Acanthocephala			*Pomphorhynchus laevis*	
Rotifera			*Apsilus vorax*	
Rotifera			*Notholca acuminata*	
Mesozoa			*Dicyemmenea lameerei*	1
Mesozoa			*Dicyema typhus*	2
Mesozoa			*Conocyema polymorpha*	3
Mesozoa			*Dicyemmenea abelis*	4
Mesozoa			*Rhopalura granos*	5
Porifera	Porifera	1		
Porifera	Porifera		*Spongilla lacustris*	
Nematoda	Nematoda	2	*Ascaris suum*	
Nematoda	Nematoda		*Rhabditis monhystera*	
Nematoda	Nematoda		*Caenorhabditis elegans*	
Cnidaria			*Hydra attenuata*	6
Cnidaria			*Microhydra rideri*	7
Cnidaria	Scyphozoa	3	*Haliclystus haliclystus*	8
Cnidaria	Anthozoa	4	*Edwardsiella lineata*	9
Cnidaria	Scyphozoa		*Cyanea cyanea*	10
Annelida	Oligochaeta	5	*Aelosoma tenebrarum*	11
Annelida	Oligochaeta		*Nais variabilis*	12
Annelida	Oligochaeta		*Lumbricus terrestris*	13
Annelida			*Nereis virens*	14
Annelida			*Erpodella testacea*	15
Annelida			*Apodotrocha progenerans*	16
Annelida			*Hirudo medicinalis*	17
Annelida			*Diurodrilus westheidi*	18
Annelida			*Dinophilus conklinii*	19
Platyhelminthes			*Dugesia lugubris*	20
Platyhelminthes			*Fasciola hepatica*	21
Platyhelminthes			*Moniezia expansa*	22
Platyhelminthes			*Dugesia mediterranea*	23
Platyhelminthes			*Anaperus sulcatus*	24
Platyhelminthes			*Macrostomum gigas*	25
Platyhelminthes			*Enterostomula graffi*	26
Mollusca	Mollusca	6	*Helix aspersa*	
Mollusca	Mollusca		*Anodonta cygnea*	
Mollusca	Mollusca		*Sepia officinalis*	

Table 1.—*continued*

Higher taxa	Taxa in Figure 4	Figure 4 label	Species	Figure 3a label
Arthropoda	Copepoda	7	*Pseudocharopinus dentatus*	27
Arthropoda	Arachnida	8	*Araneus diadematus*	28
Arthropoda	Insecta	9	*Periplaneta americanna*	29
Arthropoda	Malacostraca	10	*Carcinus maenas*	30
Arthropoda	Malacostraca		*Callinectes sapidus*	31
Echinodermata			*Asterias rubens*	
Echinodermata			*Echinus esculentus*	
Echinodermata			*Holothuria forskali*	
Chordata			*Scyliorhinus canicula*	32
Chordata			*Scaliodon terrae novae*	33
Chordata	Osteichthyes	11	*Morone saxatilis*	34
Chordata	Osteichthyes		*Lebistes reticulatus*	35
Chordata	Amphibia	12	*Bufo regularis*	36
Chordata	Amphibia		*Rana temporaria*	37
Chordata	Reptilia		*Lacerta vivipara*	38
Chordata	Reptilia	13	*Sphenodon punctatus*	39
Chordata	Aves	14	*Gallus domesticus*	40
Chordata	Mammalia	15	*Homo sapiens*	41
Chordata	Mammalia		*Mus musculus*	42

Note: Not all taxa are in all figures. Taxa with a number listed in the column labelled 'Figure 4 label' are in Fig. 4 with the listed number as a label. Species with a number listed in the column labelled 'Figure 3a label' are in Fig. 3a with the listed number as a label. Figure 3b contains all species listed, with and without number labels.

Table 2. Correlation coefficient, sample size, *P*-value and 95% confidence interval for the exponent from regressions of number of cell types by body size for taxa of metazoans used in Fig. 3a

Phyla	r^2	n	*P*-value	95% CI
Arthropoda	0.271	5	0.369	−0.122, 0.270
Cnidaria	0.584	5	0.132	−0.022, 0.146
Platyhelminthes	0.247	7	0.257	−0.076, 0.024
Annelida*	0.538	9	0.024	0.008, 0.078
Chordata	0.006	11	0.820	−0.041, 0.051

* Taxa that are statistically significant at an alpha of 0.05.

observed across bauplans. Together, Figs. 1 and 3 indicate that there is an increase in both B_0 and the maximum and minimum body sizes across bauplans. In fact, empirical data do indicate that there has been an increase in both metabolic intensity, B_0 (Vermeij, 1999), and body size, M (Bonner, 1968), for metazoan taxa.

There is little variation in the number of cell types within closely related groups, which is expected due to phylogenetic constraints (see Fig. 3a). However, each bauplan differs fundamentally in the average number of cell types, $\langle N_{\text{cell}} \rangle$, as outlined by equation (4). For

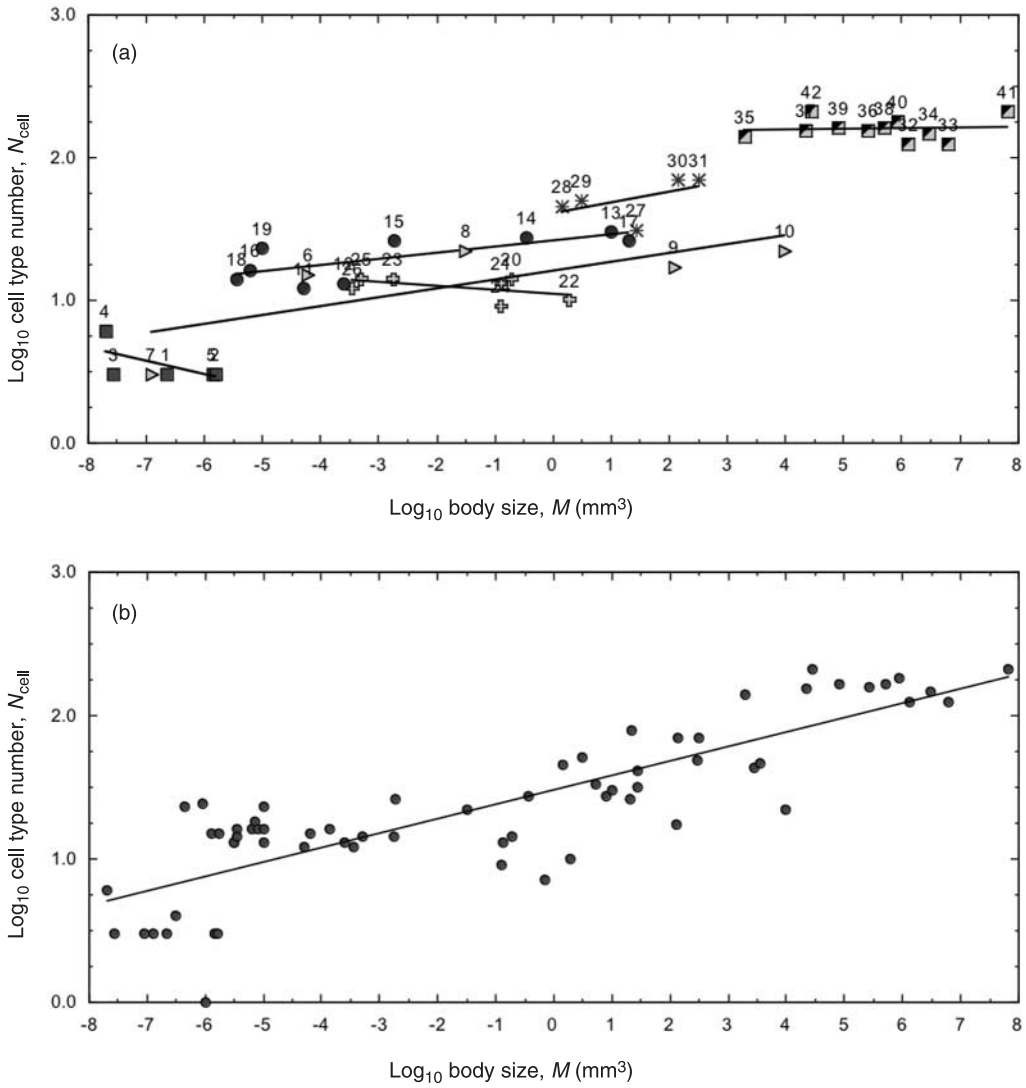

Fig. 3. (a) Number of cell types, N_{cell}, compared with body size, M, for metazoan species (data from histology sources listed in methods). Groups are divided into phyla. Only phyla with cell type number for five or more species are used. Number labels correspond to labels in Table 1. Slopes are not significantly different from zero for Mesozoa, Platyhelminthes, Cnidaria, Arthropoda and Chordata. The slope for Annelida was significant but only slightly above zero. (b) Number of cell types, N_{cell}, compared with body size, M, for all species listed in Table 1 (data from histology sources listed in methods).

a given value of body mass, M, there is approximately two orders of magnitude of variation in the average number of cell types, $\langle N_{cell} \rangle$, between clades. Our model indicates that differences in $\langle N_{cell} \rangle$ should be related to changes in B_0 (which we assume to be governed by the cellular metabolic intensity, B_c) or to variation in c_0 (efficiency of tissue integration). Therefore, a plot of $\langle N_{cell} \rangle$ versus B_0 should yield a linear positive relationship.

As predicted by our model, the average number of cell types, $\langle N_{cell} \rangle$, is significantly correlated with the metabolic intensity of the taxa, B_0 (see Fig. 4). The average number of cell types, $\langle N_{cell} \rangle$, is related to metabolic intercepts according to the equation $\langle N_{cell} \rangle = 2.1 B_0^{0.58}$ ($r^2 = 0.69$, $n = 15$, $P < 0.0001$), with a 95% confidence interval of 0.39 to 0.77 for the exponent. This slope is less than one, suggesting that as the average number of cell types increases, the cost of tissue integration per cell type increases.

DISCUSSION

The use of allometric analyses to make inferences about evolution is not new (see Huxley, 1932 and Gould, 1977 and references therein). There is a rich literature that dates back nearly a century, and recent applications to biomechanics and life history are noteworthy (Charnov, 1993; Niklas, 1994). However, there have been few recent attempts to address links between allometry, natural selection and major trends in evolution (Bonner, 1988). A critical observation is that fitness is ultimately limited by the rate at which organisms acquire and transform energy. This energy is needed to do the work required for survival, growth and reproduction (West *et al.*, 1997). Nevertheless, it is not clear how the energetics of metabolism relates to fitness (Brown *et al.*, 1993). Our central thesis builds upon this general allometric framework.

While the positive relationship between size and complexity has been verified repeatedly (Bonner, 1988, 2004; Bell and Mooers, 1997), the mechanisms behind this relationship are not entirely clear (Bonner, 1988; Maynard-Smith and Szathmáry, 1995). Size is not related to number of cell types within metazoan taxa. This lack of correlation has been observed for several groups, from within canine breeds to within the mammalian clade (Bonner, 1988, 1993). Furthermore, organisms of similar size, such as a shrub and a mammal, have very different numbers of cell types (Bonner, 1988, 2004). Also, changes in size may not be accompanied by changes in cell type number and vice versa (Bonner, 2004). Bonner (2004) discusses these discrepancies and

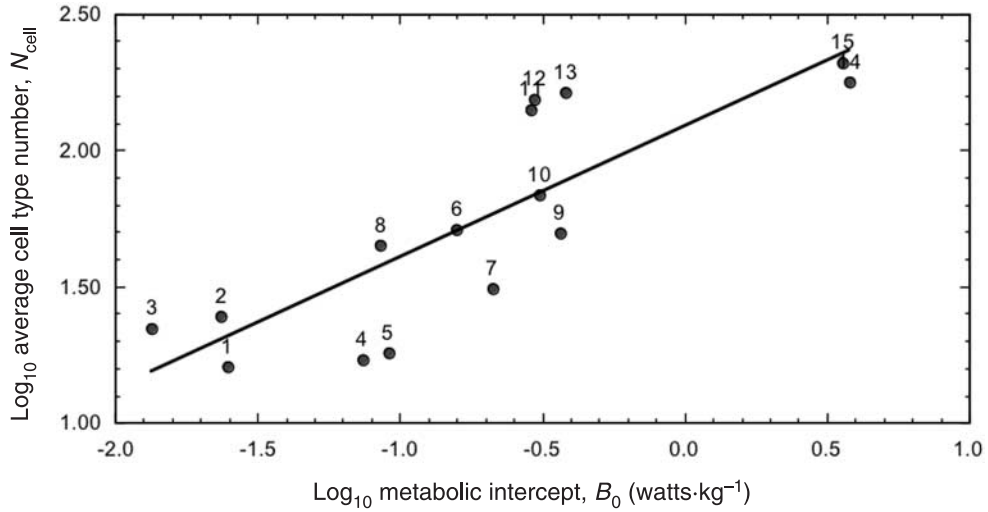

Fig. 4. The average number of cell types, $\langle N_{cell} \rangle$, for each metazoan taxa is compared with the metabolic y-intercept, B_0, for that taxa. The metabolic y-intercept is taken from metabolism and body size regressions (see text). Number labels correspond to labels in Table 1.

suggests that the rule between size and complexity may by influenced by other factors. Our model provides an explanation for the breakdown of the relationship between size and biological complexity. We suggest that the number of cell types is constrained by the rate of energy processing as well as phylogeny.

Phylogeny appears to be an important constraint on cell type number. Within a bauplan, size does not appear to be related to the number of cell types (Fig. 3a). A new mammal is unlikely to evolve with 2 cell types or 2000, because this would require a transformation of the entire bauplan of the organism. This constraint leads to organisms within the same class (e.g. a mouse and a whale) having extremely similar, if not the same, number of cell types. However, organisms in the same phylum (e.g. a sponge and a shrew) have very different numbers of cell types. Phylogeny provides an explanation for the breakdown of the size–complexity relationship within taxa. However, this does not clarify why organisms of similar size can have starkly different numbers of cell types.

The central assertion of our model is that an increase in biological complexity (as measured by the number of cell types) requires an increase in cellular metabolic intensity. Our argument is reminiscent of several authors who have suggested that the greater the gross national product of a country, the greater the number of jobs or degree of specialization due to energy input (Bonner, 1993; Moses and Brown, 2003). For example, Koufopanou and Bell (1993) showed that *Volvox carteri* (a multicellular green algae with reproductive and vegetative cell types) only gains a reproductive advantage over colonies that have one cell type (which switches between reproductive and vegetative functions) in the most resource-rich environments. Furthermore, this division of labour is a disadvantage in low nutrient conditions, suggesting complexity requires additional energy input.

Both the number of cell types (Valentine *et al.*, 1994) *and* metabolic intensity (Vermeij, 1999) have increased over evolutionary time. In fact, the positive correlations between average cell type number and rank of evolutionary appearance, and between metabolic intensity and rank of evolutionary appearance, appear to parallel each other (see Fig. 1). This suggests that the differences in cell type number between groups may be allowed by a directional change in metabolic intensity, B_0. More recently derived bauplans are more specialized and energetically intensive (as reflected by their higher values of metabolic intensity, B_0). It is important to note that different groups of plants do not show variation in B_0 (Peters, 1983; Niklas and Enquist, 2001). Interestingly, plant taxa also show little variation in the number of cell types (Bell and Mooers, 1997). These patterns further support the link between the average cell type number and metabolic intensity suggested by our model.

As predicted, the average number of cell types is positively related to metabolic intensity (which represents cellular metabolic intensity) across metazoan bauplans (Fig. 4). This relationship is described by a power-function with an exponent of 0.58. As previously discussed, each new cell type requires cellular and tissue integration, such as hormones and cell-to-cell signals. These new cell types must interact and communicate with the other cell types. If the number of interactions per cell type does not change with increasing cell types (i.e. the efficiency of tissue integration, c_0, is constant across bauplans), then we would expect a linear relationship between $\langle N_{\text{cell}} \rangle$ and B_0 with an exponent of 1 (prediction #3). However, if the number of interactions increases with each new cell type (i.e. the number of integrations needed for the 100th cell type is much higher than that for the 5th cell type), the efficiency of tissue integration, c_0, decreases. The exponent between $\langle N_{\text{cell}} \rangle$ and B_0 of 0.58 suggests that an increase in tissue integration is necessary to support the extra cell types. This is analogous to how additional links in a food web are

required for an increased number of trophic levels and/or diversity within trophic levels (Martinez, 1992).

In this paper, we hypothesize that the predominant mechanism controlling complexity is selection for increased body size creating a requirement for additional cell types. However, cell type number can only increase if there is a concomitant increase in cellular metabolic intensity. Although we argue this is a predominant mechanism controlling complexity (Fig. 2), we agree that the flow of causation may work in other directions. There has also probably been selection for increased metabolic power, whole-body metabolism of an organism (Vermeij, 1987, 1999; Witting, 2003). An increase in metabolic power would allow organisms to outcompete their neighbours by using the resources more rapidly (Vermeij, 1987, 1999; Brown *et al.*, 1993). Metabolic power, B, can be increased by an increase in size, M, and/or metabolic intensity, B_0. Selection on metabolic power, B, would require an increase in cell type number, N_{cell}, to support the larger body mass, M, as discussed previously (Fig. 2, white curved arrow). In addition, selection for increased metabolic intensity, B_0 (which is a component of metabolic power), may require the addition of specialized cells to maximally supply resources (Fig. 2, white horizontal arrow). Due to our hypothesized link between body size, the number of cell types and metabolic intensity, any selection for increased metabolic power would also require an increase in the number of cell types. There are likely several routes for an increase in the number of cell types. The connections presented in our model indicate that an increase in size, metabolic intensity and complexity can result from selection on any one of these traits.

Cell type number, metabolic intensity and body size have all increased by jumps throughout evolutionary history. There are many reasons as to why these traits are closely related to each other (Bonner, 1968, 1988; Vermeij, 1987, 1999; Valentine *et al.*, 1994; Carroll, 2001). This paper hypothesizes the key connections that govern the relationships between these prominent macroevolutionary patterns. Several additional hypotheses have attempted to explain the evolution of complexity. These include: larger organisms supporting a higher division of labour (Bell and Mooers, 1997), random drift from a minimum size boundary leading to increased variance (Gould, 1996; McShea, 1994; Valentine *et al.*, 1994), and increased modularity (Carroll, 2001).

We agree that larger organisms may require more cell types to maintain fitness (Bonner, 2004). However, we argue that the metabolic intensity of cells limits the number of cell types. Additional cell types increase the energetic demand because they often perform new tasks. Also, new cell types require further tissue integration, communication and transport.

Our model suggests that complexity will increase only in environments that can supply the required energy. The metabolic rate of metazoan cells appears to play a major role – perhaps a central role – in evolutionary changes in size and complexity (see also Pfeiffer and Bonhoeffer, 2003). Limits caused by metabolism, in addition to size and phylogeny, may explain why certain groups have become complex. Additionally, our model provides a basis for understanding the mechanistic linkages between organismal size, the number of different cell types and metabolic intensity. Because of the interrelatedness of these three traits, increases in size are not only limited by the number of cell types but also metabolic intensity. Therefore, size, complexity and metabolic intensity may increase due to just selection for increased size. This interconnectedness elucidates the macroevolutionary trends in size, complexity and metabolic intensity, which have paralleled each other throughout the history of metazoans.

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