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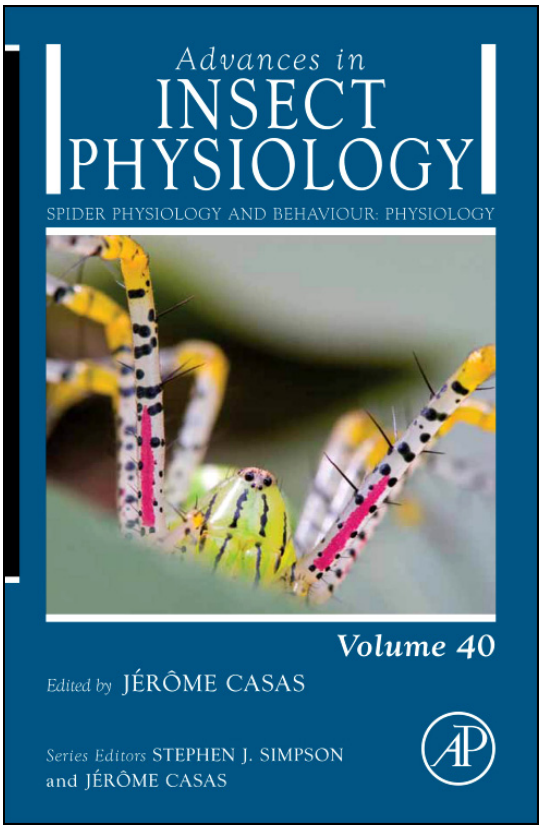

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Grade Changes in Brain–Body Allometry: Morphological and Behavioural Correlates of Brain Size in Miniature Spiders, Insects and Other Invertebrates

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1 Problems of absolute and relative brain size in small animals 156
2 Aims of this review 162
3 Generality of the miniaturization problem 162
4 Possible solutions to miniaturization problems 164
  4.1 The ''size limitation'' option 164
  4.2 The ''over-sized brain'' option 165
  4.3 The ''economy of design'' option 165
5 Predictions derived from possible solutions to the miniaturization problem 167
  5.1 Size limitation option 169
  5.2 Over-sized brain option 169
  5.3 Economy of design option 169
6 Data testing the predictions 169
  6.1 Morphology 170
  6.2 Behaviour 174
7 Discussion 187
  7.1 Morphology 187
  7.2 Behaviour 190
  7.3 The mystery of ''grade changes'' in brain allometry 192
  7.4 General importance and consequences of brain scaling 195
  7.5 Limitations of current data and questions for the future 199
  7.6 Conclusions 203
  Acknowledgements 204
  References 205

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Abstract

We use a recent wave of data to confirm that Haller's rule of brain–body allometry, for smaller species to have relatively larger brains, holds for invertebrates as well as vertebrates. But different invertebrate taxa fall on several different allometric lines (grades). In the smallest animals in several grades, the brain occupies a large fraction (up to approximately 16%) of the total body mass. The brain and the structures enclosing it show morphological alterations suggesting a lack of housing capacity in the head for the brain (e.g. the brain extends into other parts of the body such as the legs or thorax), and other structures normally enclosed in the same area are displaced. Miniature animals may thus sacrifice some morphological aspects of body design to accommodate their disproportionately large CNS. The smallest animals of one such group, orb web spiders, do not show signs of behavioural limitation in web construction compared with larger relatives. We speculate that, because of selection resulting from the high metabolic costs of nervous tissue, grade changes may involve substantial modifications of how brains function, and help explain differences between neuron-profligate vertebrates and invertebrates having far fewer neurons (as few as approximately 200–500 neurons in two groups). Scaling problems associated with small size are of general importance, because many moderate-sized animals have very small free-living immature stages.

> ''. . . one experiences an extraordinary surprise [on comparing the brains of a bee and a dragonfly with those of a fish or an amphibian]. The excellence of the psychic machine does not increase with zoological hierarchy; instead one realizes that in fish and amphibians the nervous centers have undergone an unexpected simplification. Of course their grey matter has increased considerably in mass; but when the structure of their brains is compared with that of bees or dragonflies, they are excessively plain, coarse and rudimentary. It is as if one were to pretend to hold as equals the merits of a rough grandfather clock with the quality of a fine pocket watch, a marvel of fineness, delicacy and precision. As always, in building her marvelous works, nature distinguishes herself much more in her tiny creations than in the large.''
>
> Cajal and Sanchez (1915); transl.

1 Problems of absolute and relative brain size in small animals

Very small animals confront special problems in nervous system design. These problems arise for several reasons: the lower limits of the size at which axons can reliably transmit potentials (Faisal *et al.*, 2005); the relatively large volumes of the nucleus compared with the cytoplasm in very small nerve cells may place lower limits on neuron size (e.g. Grebennikov, 2008; Roth *et al.*, 1994); the general scaling trend known as ''Haller's Rule'' (Rensch, 1948) for smaller animals to have relatively larger brains (e.g. Beutel and Haas, 1998; Beutel *et al.*, 2005;

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Bonner, 2006; Polilov, 2008; Seid *et al.*, 2011; Striedter, 2005; Wehner *et al.*, 2007); the possibly severe metabolic costs of building, maintaining and operating relatively large amounts of nervous tissue and neural networks (e.g. Cherniak, 1995; Hasenstaub *et al.*, 2010; Niven and Laughlin, 2008); and the lower size and complexity of dendrites in smaller brains (Wittenberg and Wang, 2007). Haller's Rule is well established for vertebrates (Striedter, 2005), and we show here that it also applies to invertebrates, but its causal basis is still debated (e.g. Gonzalez-Voyer *et al.*, 2009). The metabolic costs of maintaining nerve tissue are especially high, so an automatic consequence of Haller's Rule is that smaller animals pay disproportionately large metabolic costs. Furthermore, the density of metabolic activity in smaller brains is likely to be greater if they have comparable information-processing capabilities, and smaller neurons transmit information more slowly (Niven, 2010; Niven *et al.*, 2007). Within a given taxonomic group, species with smaller brains tend to have smaller numbers of smaller neurons (Niven, 2010).

Size matters, because to a first approximation, an animal's behavioural abilities should correlate positively with the numbers of neurons and their connectivities (see below). The special problems in nervous system design faced by small animals are of general significance, because species with miniature or small-bodied adults are common (e.g. Gaston *et al.*, 2001; Hanken and Wake, 1993), and because many metazoans with large-bodied adults have free-living immatures that are extremely small which have many of the same behavioural needs as adults.

When brain allometries are compared among different taxonomic groups, the allometric line of one group is often displaced upward or downward with respect to the lines of other groups in brain weight versus body weight plots (Fig. 1A) or to the left or right in % brain versus body weight plots (Fig. 1B). These differences are called ''grade changes'' (Striedter, 2005). For instance, the lines describing the percentage of the body dedicated to the brain versus body weight in amphibians is substantially to the left of that for mammals (Martin, 1981; Striedter, 2005; Fig. 1B).

Here, we discuss the biological significance of these design problems. We survey various arthropods and other invertebrate taxa to empirically demonstrate the constraints that follow from these design problems, and how different taxa may have circumvented them in different ways. Do derived miniature forms conserve comparable behavioural abilities by reducing the size of neurons in order to maintain similar numbers of neurons and their connections? Have they evolved new designs to do more processing with fewer neurons, and so conserve behavioural abilities? Or do smaller animals suffer impaired behavioural performance?

In the size range of insects and spiders, there are reasons to expect that gram for gram the brains of very small species may be functionally inferior. The size of the neuron cell body appears to reach a minimum (about 2 μm in diameter) near the lower end of the range of insect body sizes (body length about 0.3 mm—Grebennikov, 2008), and then does not decrease further in smaller individuals; so very small species probably have reduced numbers of neurons (Beutel *et al.*, 2005, and references therein, Quesada *et al.*, in review), as also

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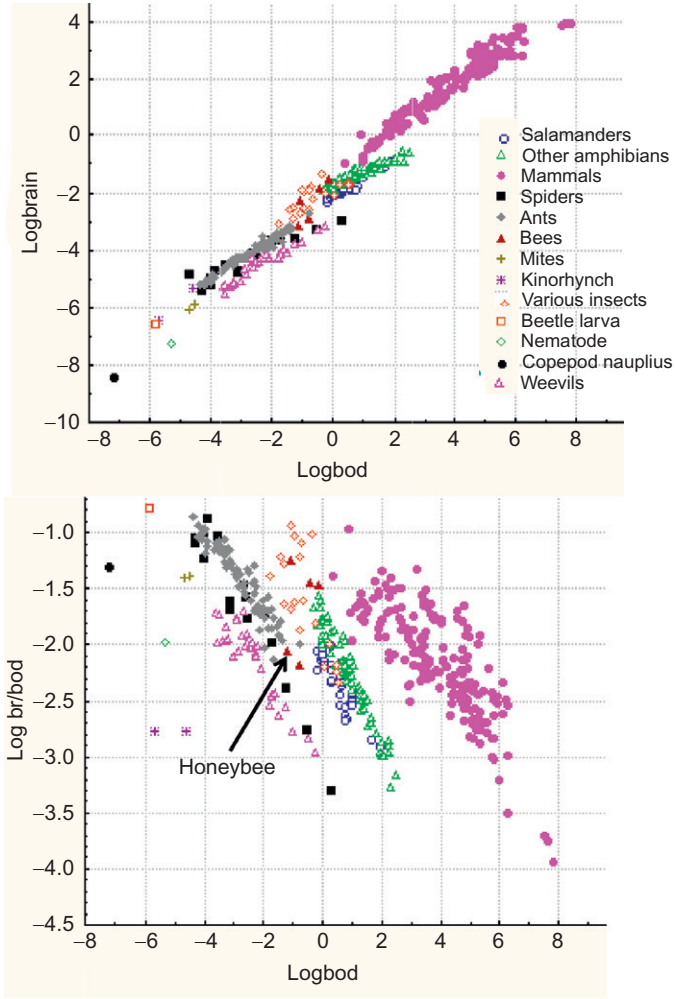

FIG. 1 Brain weight and body weight in vertebrates and assorted invertebrates are given in log–log plots of brain weight versus body weight (A) and of the fraction of the body weight devoted to the brain versus body weight (B) (which permits clearer contrasts between different groups). Sources of data are the following. Vertebrate data were read from graphs in Striedter (2005). Bee weights are from Mares *et al.* (2005), ant weights are from Wehner *et al.* (2007) and Seid *et al.* (2011); weevil weights are from M. Seid, H. Stockwell and W. Wcislo (in preparation). Spider and mite weights were measured directly and from reconstructions of brains from serial sections (Quesada *et al.*, in review, G. Vargas, unpublished). A drawing of a ''typical'' kinorhynch body and brain (Barnes *et al.*, 1993) was deconstructed into simple geometric forms and volumes were estimated based on the cross-sectional body outline in Fig. 296 of Higgins (1983). Two points are given, reflecting uncertainty regarding body weight. Estimated nervous system volume may be underestimated more seriously in this species due to its having a relatively large nerve cord (Bullock and Horridge, 1965). Similar deconstructions and analyses utilized a

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occurs in miniature salamanders (Roth *et al*., 1990). Cells of this size, such as the Kenyon cells of insects, are mostly composed of nucleus, surrounded by only a thin layer of cytoplasm (the nucleus of a neuron of a ptiliid beetle occupies up to 80–90% of the cell—Grebennikov, 2008). Thus a lower limit on cell size may be imposed by nucleus size (Bonner, 2006; Fankhauser *et al*., 1955), and nucleus size in turn correlates with genome size (Gregory, 2002; Hanken and Wake, 1993; Roth *et al*., 1988). In addition, there is a lower limit of approximately 0.1 μm on the diameter of functional unmyelinated axons, because stochastic opening of sodium channels in axon membranes can generate action potentials and thus increase noise in very small axons (at least if ion channels are the same in smaller neurons; Faisal *et al*., 2005). The internal substructures of the brains of very small insects and spiders may also be simpler or fewer in number, though data are scarce (Babu, 1975; Beutel *et al*., 2005). In absolute terms, smaller insects and spiders tend to have fewer sensory receptors, including chemosensory and tactile setae as well as ommatidia in their compound eyes (Chapman, 1982; Foelix, 1996; Jander and Jander, 2002; Kelber *et al*., 2006; Mares *et al*., 2005; Rutowski, 2000; Spaethe and Chittka, 2003; Wcislo, 1995), and their smaller numbers of motor neurons presumably correlate with reduced precision of motor control.

As a consequence of Haller's Rule, miniature animals confront a dilemma. They might maintain information-processing capabilities and behaviours that are comparable to those of larger animals, but either pay the disproportionately high energetic costs (Niven *et al*., 2007), or evolve more efficient nervous systems that require less tissue to perform comparable tasks (e.g. Wehner, 1987). Alternatively, they could adopt life styles that require reduced behavioural capabilities, which would enable reduced investments in nervous tissue. We describe these alternatives in more detail below (see Section 4).

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drawing of the anterior end of the nematode *Caenorhabditis elegans* and a photograph of its entire body (by White *et al*., 1975), and a SEM photo of the entire body plus a cross-section of the body of the first nauplius larva of the copepod *Dactylopusia* sp. (Lacalli, 2009, Figs. 1A and 1D) (this volume may be an underestimate, as the bulbous outline of the nauplius was not obvious in the SEM photo—T. Lacalli, personal communication). The brain volume of this nauplius was calculated by reconstructing sections of the neuropil examined under the TEM (Lacalli, 2009), and an estimate that the cell bodies occupy a volume a bit larger than that of the neuropil (T. Lacalli, personal communication). The body volume of the beetle larva *Mikado* sp. was estimated by deconstruction from Fig. 5 of Polivov and Beutel (2009), assuming a cylindrical cross-section. Their graph (Fig. 20) indicates that the brain occupied about 16.5% of the body volume, so this value was used to estimate the brain volume. All calculations assumed that one cc of brain tissue weighs one gram. All points, other than the copepod nauplius, the beetle larva, and four spiders refer to adults (thus for most groups, additional values for smaller body sizes are lacking). The ''brains'' of spiders and mites include tissue that in a vertebrate or an insect would be included in the spinal cord or ventral nerve cord, and are thus somewhat higher than they should be to make proper comparisons (for instance, the allometric line for spiders in (b) should probably be below that for ants).

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We focus on the relative mass and volumes of the nervous systems rather than the numbers of neurons they contain, because overall size is more directly related to the metabolic costs of a nervous system, thus simplifying deductions with respect to natural selection. Mass is of course only a proxy for energetic cost, because energy consumption is also determined by other factors such as levels of neuron activity and relative amounts of membrane that must be maintained polarized. Pragmatically, data on relative weights are much more common than data on relative numbers of neurons, and data on neuron number frequently lack clarity regarding the methods used to avoid possible biases in stereology (Howard and Reed, 1998). Thus, we analyse alternatives in terms of natural selection to minimize costs, an approach that has provided important insights at the ''macro'' level of morphology (i.e. sizes of different portions of the brain); the organization of behaviour (Clark, 1998); and the ''micro'' level (i.e. distributions of different types of ion gates in neural membranes; see Hasenstaub *et al*., 2010).

We will assume that to a first approximation that brain size is related to behavioural abilities. There is controversy, however, regarding the relations between brain weight or volume and behavioural capabilities (e.g. Shettleworth, 2010). In a particularly clear statement of the position that the correlation is weak or non-existent, Miklos (1998) surveyed the wide range of numbers of neurons in a variety of organisms (from 302 neurons in a nematode, to 300,000 in a salamander 850,000 in a honey bee, 40 million in the lab mouse, 520 million in an octopus, 85,000 million in a human, and 200,000 million in a whale or an elephant). He concluded that the common belief that ''bigger is better'' (having more neurons enables more complex behaviour) is not supported by facts: ''. . .no simple relationships exist between gene and neuron number and apparent morphological and behavioral complexity, and the numbers themselves cannot be used in a predictive sense to come to terms with the behavioral complexities of organisms in different evolutionary lineages (p. 203).'' We believe that this conclusion is probably overly pessimistic. In the first place, a lack of a correlation would imply that some animals have excess neural capacity relative to their behavioural needs, and others have insufficient capacity. Such a pattern is unlikely, because the increased cost of maintaining excessive neural capacity is expected to be severely penalized by natural selection (Niven and Laughlin, 2008). In addition, if neural deficiencies lead to behavioural deficiencies, those individuals with insufficient capacity also will be removed by natural selection. Brain-to-body ratios are subject to selection, and can potentially change quickly, as evidenced for example by the fact that the relative brain volumes of many domesticated animal species are relatively smaller than those of their wild-type ancestors (Bernays and Wcislo, 1994; Campi and Krubitzer, 2010), and brains of laboratory-reared guppies are smaller than those of wild guppies (Burns *et al*., 2009).

Miklos' argument also fails to take into account much data showing that ''more IS better,'' at least with respect to gathering sensory input and information processing (Bonner, 2006; Chittka and Niven, 2009). Absolute size and numbers

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of sensory receptors, rather than relative size and numbers, set the information-processing capabilities of the nervous system and its efficiency (Herculano-Houzel, 2010). For instance, increased resolution, as well as more subtle or detailed processing of visual information, is clearly related to the numbers of sensory cells in the eye, and the amount of nervous tissue in the portions of the brain that process stimuli from the eye (e.g. Kiltie, 2000; Land and Nilsson, 2002). Compared with diurnal bees, nocturnal bees have relatively larger ommatidia and photoreceptors, which increase the numbers of photons captured to sustain vision at extremely low light levels (Warrant, 2008; Warrant *et al*., 2004; Wcislo and Tierney, 2009). Similarly, the motor abilities of a structure correlate with the numbers of associated nerves and muscles (Chittka and Niven, 2009). For instance, both the sensory and motor areas of the brain that are devoted to the front paws are greatly enlarged in the dexterous raccoon compared with those in related mammals (Striedter, 2005); the added mobility of the human thumb is associated with extra muscles and presumably extra nerves innervating them, as compared with the thumb of a chimpanzee (Coyne, 2009).

Failures to find correlations between brain and behavioural measurements have probably stemmed from overly broad classifications of behaviour, and overly inclusive measurements of nervous tissue. For instance, the lack of correlation between the total number of neurons in the brains of salamanders and the acrobatic abilities of those living in terrestrial versus arboreal habitats (Miklos, 1998) may have resulted from a lack of focus on the particular portions of the CNS that are involved in this particular behaviour. When questions regarding correlations between behaviour and relative amount of brain tissue are posed in sufficiently specific terms, correlations are often clear (though it is important to remember that correlations do not demonstrate causality in shaping brain evolution—Healy and Rowe, 2007). In pea aphids (*Acyrthosiphon pisum)*, for example, there are no overall qualitative differences in brain structure between winged and apterous female morphs, but the winged parthenogenetic females (which have ocelli) have larger central bodies than the apterous females (which lack ocelli; Kollmann *et al*., 2011); the central body is a brain region thought to be important for visual spatial orientation (Homberg, 2008). Other examples include positive correlations between hovering flight in hummingbirds and the relative size of the pretectal nucleus lentiformis mesencephali (Iwaniuk and Wylie, 2007), between the relative size of a visual processing area (superior colliculus) and the degree of dependence on sight in diurnal, nocturnal, and burrowing rodents (Campi and Krubitzer, 2010), and between and the relative size of the hippocampus and the relative importance of spatial memory in the ecology of many (Sherry *et al*., 1993) but not all (Brodin, 2005) bird species. In a survey of 18 marsupial species, Iwaniuk *et al*. (2000) showed that ability to execute ''finely coordinated forelimb movements'' was not correlated with overall brain size, but rather with the sizes of particular brain regions. In addition, the different expected correlations between volume and behavioural capabilities are likely to be only approximate for other reasons: these include

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different proportions of glia in different species (they are apparently less abundant in some groups such as nematodes—Bullock and Horridge, 1965), and the need in larger species for longer dendrites and longer and larger diameter axons (to maintain speed of reaction), resulting in a smaller fraction of the brain volume being devoted to information analysis.

Some researchers have proposed that brain size does not correlate with behavioural abilities, but evolves simply through correlated responses to changes in body size (Lande, 1979; Riska and Atchley, 1985). Theoretically we find this argument untenable because it neglects the costly expense of maintaining excess nervous tissue (Hasenstaub *et al*., 2010; Niven and Laughlin, 2008). And empirically, there are many examples of mosaic evolution of different regions of the brain (Chittka and Niven, 2009). As just mentioned, the claim that there is no evolutionary relationship between brain size and behavioural abilities is probably the result of lumping all CNS tissue into a single category ''brain size,'' and of using such vaguely specified traits as ''behaviour'' or ''intelligence.''

One consequence is that the basic tenet of Jerison's (1983) ''principle of proper mass''—that the mass of nervous tissue involved in control of a particular function is appropriately sized to the amount of information processing needed for that function—is likely to be at least generally correct (see also Niven, 2010). The fact that brains are highly integrated, so that a given behaviour may involve activity in many different portions, does not detract from this argument, but only makes it more difficult to focus on all of the appropriate portions of the brain. Evolutionary patterns of mosaic brain evolution, which show that different brain regions are differentially enlarged (or reduced) in different lineages (Streidter, 2005), are consistent with the basic assumption that the most useful comparisons for understanding specific behaviours will involve particular brain regions rather than whole brain measurements.

2 Aims of this review

We survey recent evidence from very small insects and spiders regarding their behavioural abilities, and the relative sizes of their brains. We put these morphological data into as large a context as possible, and thus compare insects and spiders with much larger vertebrates, and also with much smaller invertebrates such as kinorhynchs, nematodes and larval crustaceans.

3 Generality of the miniaturization problem

Small-bodied animals are ubiquitous, and in any given habitat, the adults of most animal species are relatively small (e.g. Gaston *et al*., 2001). In addition, juveniles universally have smaller sizes. Excluding developmental biologists, most scientists working on arthropods concentrate their attention on adults,

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because they are easier to observe, handle, identify, etc. This bias leads one to forget that most arthropods must function independently just after emerging from the egg, when they are much smaller than an adult. For instance, the adult female of the giant spider *Nephila clavipes* weighs on the order of 2,000 mg, but the nymphs that leave the egg sac and build independent orbs to capture their prey weigh only approximately 0.7 mg (Quesada *et al*., submitted). For animals in general, biologists tend to think about size-related problems in terms of adult size. Indeed, when discussing ''limits to smallness'' in the context of body size evolution, Calder (1984, p. 351) wondered whether we are ''missing the truth by limiting our concern to *adult* physiology and ecology?'' (emphasis added).

Of course there are exceptional species with extensive maternal investment prior to hatching, including tsetse flies, scorpions, cockroaches that nurture their offspring internally (Roth and Willis, 1958), and some others, such as trap-door spiders or solitary bees, that lay very large eggs (Coyle, 1971; Iwata and Sakagami, 1966; Main, 1976). In addition, groups such as many holometabolous insects may have benefited from reducing the behavioural challenges faced by their larvae, since eggs are often deposited in or on a food source, which would shield larvae from possible problems in finding food and defending against predators and parasites. Here the major behavioural problems may involve only relatively simple tasks such as masticating and ingesting food. Even in such cases, larvae may nevertheless actively search their environment, as seen in the *Drosophila* rover morphs (e.g. Osborne *et al*., 2001). Nothing is known about relative brain size comparing sitter and rover morphs (M. Sokolowski, personal communication). The fact that the environment is less demanding for larvae of holometabolous insects is reflected by the relative numbers of sensory receptors in adults and larvae; after correcting for body size, adult holometabolous insects have far more sensory receptors than do their immature forms; in contrast, numbers are roughly comparable for hemimetabolous species (Bernays and Wcislo, 1994; Chapman, 1982). But even the larvae of tiny ptiliid beetles, which emerge from eggs laid in or on their food have large brains relative to their body size (Polilov and Beutel, 2010), as do many tiny hymenopteran egg parasitoids (Grebennikov, 2008). The important point is that the problems of miniaturization are clearly not limited to species with tiny adults, but are widespread in the animal kingdom.

Hanken and Wake (1993) argued that the historical process of miniaturization involves a phylogenetic hypothesis, and defined miniaturization as the evolution of extremely small body size relative to other species in the same lineage (they referred only to adults). They recognized that ''extreme'' is a relative term, and that there ''. . . is no strict criterion for distinguishing miniaturization from . . . less severe instances of size reduction'' (*op cit*. p. 502); thus ''miniaturization'' occurs at a different absolute body size for beetles than it does for salamanders, for example. Using this definition (which we also use), they showed that miniaturization of adults has evolved independently in numerous living and extinct invertebrates and vertebrates, and in the latter it seems

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especially common in frogs and fishes. They noted that ''...it is difficult to propose any major metazoan taxa that doesn't offer an example'' (*op cit*. p. 503). Within arthropods, miniaturization of adults has also evolved independently numerous times (e.g. Beutel *et al*., 2005; Grebennikov, 2008; Polilov, 2005; Polilov and Beutel, 2010), but it is presently not possible to estimate the actual number because of the lack of lower-level phylogenies in many taxa. Among stingless bees (Meliponini), for example, miniaturization of adults has evolved independently in 11 different genera (Michener, 2001). The importance of phylogenetic information for identifying the directionality of body size change is illustrated by the case of giant orb-weaving spiders *Nephila*, with huge females and small males; phylogenetic studies demonstrate that *Nephila* evolved gigantism in females, rather than dwarfism (miniaturization) in males (Coddington *et al*., 1997; Hormiga *et al*., 2000).

The physiological problems associated with small size that were discussed above must be confronted by small animals in general, whether or not their small size is derived. Nevertheless, the most interesting small animals for understanding brain miniaturization are those that arise phylogenetically or ontogenetically from larger animals, and thus have a suite of potential behavioural capacities which they must have either conserve or reduce. In this context, species with relatively small ontogenetic changes in body design and life style (e.g. hemimetabolous insects, most arachnids) are of special interest. In a group without complete metamorphosis, any evolutionary reduction in adult body size is preceded ontogenetically by immature forms that already function at smaller body sizes. Thus the earliest immature stages, soon after they emerge from the egg and begin to fend for themselves, must often constitute the ''leading edge'' of evolutionary transitions toward smaller body size.

4 Possible solutions to miniaturization problems

We see three extreme options that animals could use to solve the possible brain scaling and behavioural problems that are associated with very small size. These extremes are not mutually exclusive, and different types of adjustments to small size could occur even in a single animal. We first outline these options, and then review the available behavioural and morphological data to evaluate the extent to which different options are exploited by different taxa.

4.1 THE ''SIZE LIMITATION'' OPTION

One solution to miniaturization problems would be to evolve life styles that are less demanding with respect to behavioural capabilities. This in principle should reduce metabolic costs by reducing the need for expensive brain tissue. Selection should favour reduction in a behavioural capability when the costs associated with this capability outweigh the benefits it confers. The size-limitation hypothesis

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predicts that very small individuals may be more limited behaviourally, because the greater costs of a CNS that confers equal behavioural capabilities have made it advantageous for these animals to settle for reduced behavioural abilities.

For instance, miniaturization in salamanders has evolved independently 10 times (Hanken and Wake, 1993). In these small salamanders, which depend heavily on vision to capture their prey, the relative sizes of different portions of the brain are altered, and the major visual and motor centres increase in relative size (Roth *et al*., 1990). Miniaturized salamanders thus appear to have partially redesigned their brains, reducing the relative sizes of some portions. This implies that some as yet unidentified behavioural functions (sensory, analytical, motor?) may have been lost in miniature salamanders, or that they have other compensatory neural mechanisms in these portions of their brains.

4.2 THE ''OVER-SIZED BRAIN'' OPTION

The selective advantage of particular behavioural capabilities may be great enough to compensate for the relatively high cost of the nervous tissue needed to produce these abilities. Thus smaller animals may evolutionarily opt to maintain these abilities, despite their costs. As a result, the animal will have a relatively large brain.

This hypothesis is at least superficially in accord with the empirically well-established allometric trends that smaller animals generally have relatively larger brains (see Section 1). There is, however, no general consensus regarding the cause(s) of Haller's Rule. Proposed explanations include the scaling relationship between body surface area and volume (Jerison, 1973), and metabolic constraints limiting brain size (Martin, 1981). In *Cataglyphis* ants, colony size correlates positively with larger relative brain size (Wehner *et al*., 2007), but the generality of this finding is uncertain. There are reasons to expect that the more behaviourally specialized workers in larger social insect colonies will generally have relatively smaller brains when compared with solitary individuals or with less specialized workers in smaller societies (see Gronenberg and Riveros, 2009; Wcislo and Riveros, in preparation).

4.3 THE ''ECONOMY OF DESIGN'' OPTION

Typically there is massive redundancy in the inputs that animals gather from their environment, and a large discrepancy between the enormous capacities of sensory systems that gather these inputs and the much more limited capacity of the CNS to process them and generate and retain biologically meaningful information (Dusenbery, 1992; Mercer, 1999). This discrepancy can lead to opportunities for removal of redundant information from receptors before it is sent to central processors (Niven, 2010), and for economizing on information processing (Bernays and Wcislo, 1994) by making adjustments that allow smaller numbers of neurons or lower connectivities to produce at least approximately comparable

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behavioural abilities. Adjustments to improve economy could include a wide variety of possible mechanisms. The list we give below is only preliminary; some adjustments are well documented while others are highly speculative.

The behaviour of some insects (e.g. ants), for instance, is guided by neural mechanisms that are less flexible than those of many vertebrates but that are more efficient in terms of the numbers of neurons involved (the ''matched filters'' of Wehner, 1987; Collett and Collett, 2002). In insects and spiders, much of the processing of inputs occurs at relatively peripheral neural levels, including both the sensory and the motor processes (Barth, 2002; Chapman, 1982), which offers further opportunities to economize on brain tissue (economies of this sort also occur in vertebrates—e.g. the mechanoreceptors in the skin). One such mechanism is illustrated by the sense organs of some spiders, which are built of materials whose properties cause them to respond best to relevant stimuli in contexts in which it is biologically advantageous for the spider to respond (Franzl and Barth, 2009). Some of the fundamental spatial aspects of a navigational problem can be incorporated into the spatial design of a sensory receptor (Wehner, 1987), such as the alignment of rhodopsin in microvillar membranes of photoreceptors to detect polarized light (Homberg *et al*., 2011). Although this may limit the versatility with which input can be handled, it decreases the total input that needs to be centrally processed to generate information. These ''tuned'' sense organs are constructed so as to screen inputs that would otherwise have to be processed by the CNS (Dusenbery, 1992; Franzl and Barth, 2009).

Another possibility to economize would be to make increased use of graded depolarizations rather than action potentials for transmitting messages, as seems to be especially common in insects (Chittka and Niven, 2009) and nematodes (Bullock and Horridge, 1965). Energetic savings would accrue because analogue signals transmit information more efficiently than digital signals; action potentials require larger ion fluxes (e.g. Clark and Häusser, 2006; Niven *et al*., 2008; Sarpeshkar, 1998). Another type of efficiency to achieve a behavioural goal involves ordering behavioural sub-routines so that difficult tasks are divided into series of simpler ones. For instance, if a task such as grasping an object is always preceded by preliminary sensory or motor behaviour such as turning to face the object, grasping is much less demanding with respect to sensory and motor control (Clark, 1998). This organization can dramatically increase efficiency by reducing the sensory and motor abilities needed (Clark, 1998; Miller *et al*., 1960). Miniaturized animals can also realize savings from utilizing the advantageous properties of ''small world neural networks'' (Watts and Strogatz, 1998), but it is not known if such networks are deployed more extensively in miniature animals relative to large-bodied ones.

At a more microscopic level, savings could come from a variety of adaptations: using muscle plates that extend from muscles toward neurons that allow a single synaptic process to stimulate multiple muscles (Fig. 2; Altun and Hall, 2008; Bullock and Horridge, 1965); using the same neurons for multiple

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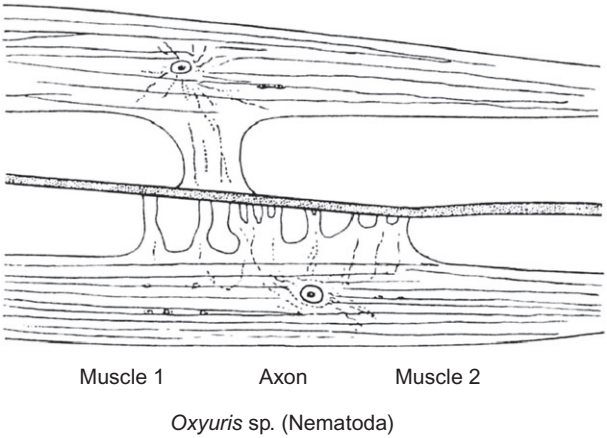

FIG. 2 In nematodes, it is common that a given neuron innervates several muscles, and, as seen in this figure, that muscles send projections to neurons rather than vice versa (Bullock and Horridge, 1965). This could be an efficiency of design that minimizes the numbers and sizes of motor neurons in these tiny animals (after Martini, 1916, from Bullock and Horridge, 1965).

functions; using neuromodulation of networks to produce different outputs from the same neural circuitry (Anderson, 2010; Chittka and Niven, 2009; Katz, 1999); reducing the relative numbers of inter-neurons as opposed to sensory and motor neurons, as is typical in nematodes (Bullock and Horridge, 1965); positioning of neurons and their connections to ''save wire'' and minimize the total length of axons and dendrites (Cherniak, 1995; Cherniak *et al.*, 2004), which is said to be near the theoretical minimum in the nematode, *Caenorhabditis elegans* (Niven, 2010; Fig. 3); or by indirectly controlling cilia through muscles (Keshmirian and Nogrady, 1987). The general importance of minimizing energetic costs is illustrated by the fact that neurons with multiple functions, and neurons that employ analogue signals for transmission over short distances, occur in insects (Bullock and Horridge, 1965) as well as in such ''neuron-profligate'' animals as primates (Niven, 2010; Van Essen and Gallant, 1994). In fact, multiple functionality is probably the ancestral state for neurons (Bullock and Horridge, 1965).

5 Predictions derived from possible solutions to the miniaturization problem

The three options just outlined are expected to be associated with different combinations of behaviour and morphology. These are predictions of possible extreme versions of the options, and actual animals are likely to have mixes of these predicted traits.

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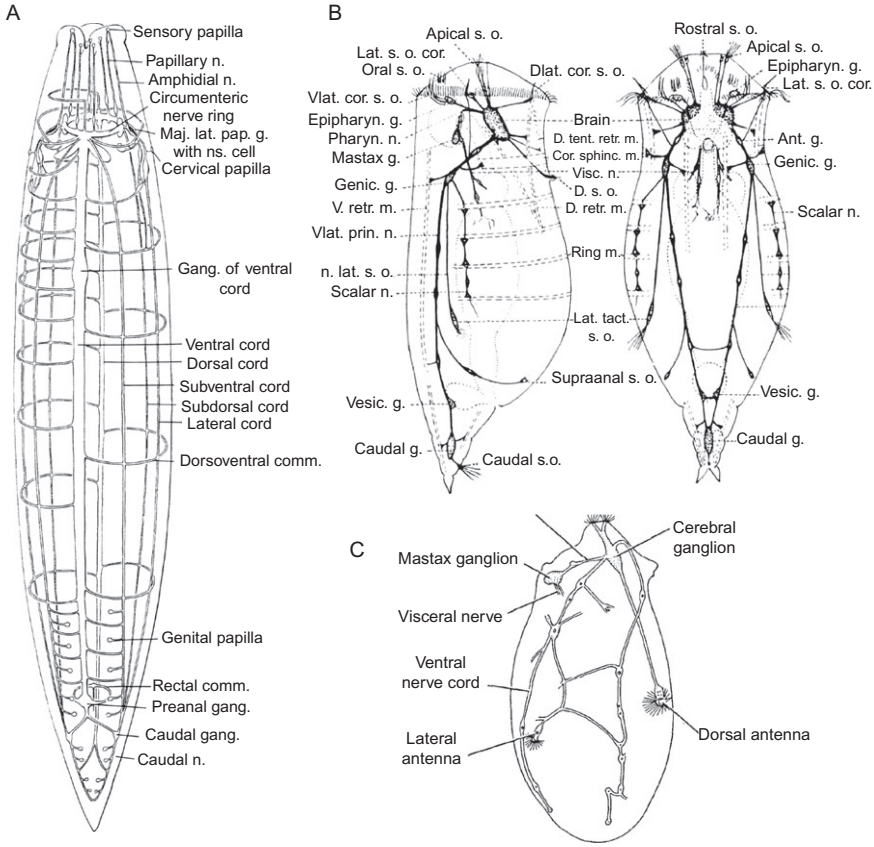

FIG. 3 A possible dimension for efficiency of design is the gross anatomy of nervous systems, illustrated here for two groups of small invertebrates, nematodes (A) and rotifers (B, C). (A) Shows the highly ordered arrangement of the relatively condensed nervous system of the nematode *Ascaris*. In *A. lumbricoides* 63.8% of the animal's total of 254 neurons are in the CNS, and 85.8% of these are connected directly to either muscles or sense organs (Bullock and Horridge, 1965). In another nematode with a somewhat similar design, *C. elegans*, the distribution of neurons brings the length of the connecting processes of neurons close to a theoretical minimum. B and C show the less centralized but nevertheless highly organized nervous systems of two rotifers, a Monogonontan (B) and *Asplanchia* sp. (C). In *A. priodonta*, only about 43% of the approximately 520 neurons are in the brain (Bullock and Horridge, 1965). Can selection for reduced metabolic costs explain these differences in general nervous system designs? (A and B from Bullock and Horridge, 1965; C from Brusca and Brusca, 1990).

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5.1 SIZE LIMITATION OPTION

The most directly testable prediction relates to behaviour: smaller organisms should have more limited or impaired behavioural capacities. Morphologically, the hypothesis predicts that smaller animals will not have ''special'' (derived) morphological compensations in the sizes and shapes of their brains or of the parts of their bodies that house them.

5.2 OVER-SIZED BRAIN OPTION

The behavioural prediction of this hypothesis is that smaller animals will not have impaired behavioural capacities. On the morphological side, smaller animals should have relatively large brains. These could, at least in some cases, either extend into additional areas of the body, or be housed in areas that are expanded or deformed to increase the volume available for the brain.

5.3 ECONOMY OF DESIGN OPTION

The behavioural prediction is that smaller animals will not have reduced behavioural capacities. The morphological prediction is that smaller animals will not have ''special'' morphological compensations that can be seen at the level of overall CNS volumes or the structures housing them. The compensations will occur instead at levels finer than overall brain size.

These options are not mutually exclusive. The basic questions are not theoretical, but rather empirical: Do very small animals tend to sacrifice their behaviour capacities due to allometric constraints on the relative sizes of their nervous systems? Do they make especially substantial sacrifices to devote unusual amounts of energy and material to build and maintain disproportionately large nervous systems? Or have they evolved special designs that allow them to reduce the amount of neural tissue needed to maintain particular behavioural capabilities? Testing these predictions against data will not determine which option is ''correct'' in a hypothetico-deductive sense, but will instead point to which solutions have been adopted by which lineages of animals that have faced the problems of miniaturization.

6 Data testing the predictions

Evolutionary adjustments in behaviour and morphology to miniature size have evolved independently in different lineages of insects and spiders, so we discuss data from the two groups separately.

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6.1 MORPHOLOGY

6.1.1 *Insects*

Recent data on insects and spiders (Beutel *et al*., 2005; Douglass *et al*., in preparation; Grebennikov, 2008; L. Jiménez, unpublished; Polilov, 2005; Polivov and Beutel, 2009; Quesada *et al*., submitted; Seid *et al*., 2011; Wehner *et al*., 2007) indicate that Haller's Rule also holds for arthropods, though they are typically much smaller in size than vertebrates (Fig. 1A). Thus, the brains of smaller insect species are smaller in absolute terms, but are proportionally larger than those of larger insects (Fig. 1B). In addition to being proportionally large, the brains of smaller insects may have smaller neurons that are more densely packed (Beutel *et al*., 2005; Wehner *et al*., 2007). The tiny first instar larva of the strepsipteran *Mengenilla chobauti* (body about 0.2 mm long), has a brain (supra- and sub-esophageal ganglia) that, relative to its body volume, is ~250 times larger than that of a large water beetle (Beutel *et al*., 2005). The brain of the first instar larva of a tiny beetle, *Mikado* sp. (body mass, approximately 0.0016 mg—see caption of Fig. 1), constitutes about 16% of its body mass (Polivov and Beutel, 2009), while that of a tiny ant, *Brachymyrmex* sp. (body mass, ~0.04 mg), constitutes approximately 15% of its biomass (Seid *et al*., 2011); for comparison, the brain of a large beetle constitutes only about 0.02% of its body mass, and a human brain constitutes only about 2% (Beutel *et al*., 2005).

One set of dramatic morphological features of very small insects that fit predictions of the over-sized brain option concern the morphological distortions that are apparently associated with fitting their brains into limited space in their head capsules. For instance, in both tiny first instar strepipteran larvae and tiny ptiliid beetle larvae, the brain extends into the prothorax (Fig. 4). In the strepsipteran, *Mengenilla* sp. ''[t]he whole central nervous system is extremely compact and completely shifted to the posterior thoracic region and abdomen (Beutel *et al*., 2005, p. 308).'' The brains of these tiny animals are so disproportionally large that they no longer fit where they are normally housed (the head capsule), and extend into other nearby portions of the body.

6.1.2 *Spiders*

In *Argiope aurantia* the relative brain size of tiny spiderlings is more than 10 times that of adults (compared with cephalothorax volume), and the proportion of a spiderling's brain mass dedicated to cortex versus neuropil was double that for the adult (Babu, 1975) [Here, we use ''brain'' to refer to the combination of supra- and sub-esophageal ganglia in spiders (see Table 3); Babu uses ''brain'' to refer only to the supra-esophageal ganglion]. Further data on relative brain size for 10 species in four additional orbicularian families, which included spiders substantially smaller (< 0.005 mg body mass) than those studied by Babu (1975), showed a pattern of brain–body scaling consistent with Haller's

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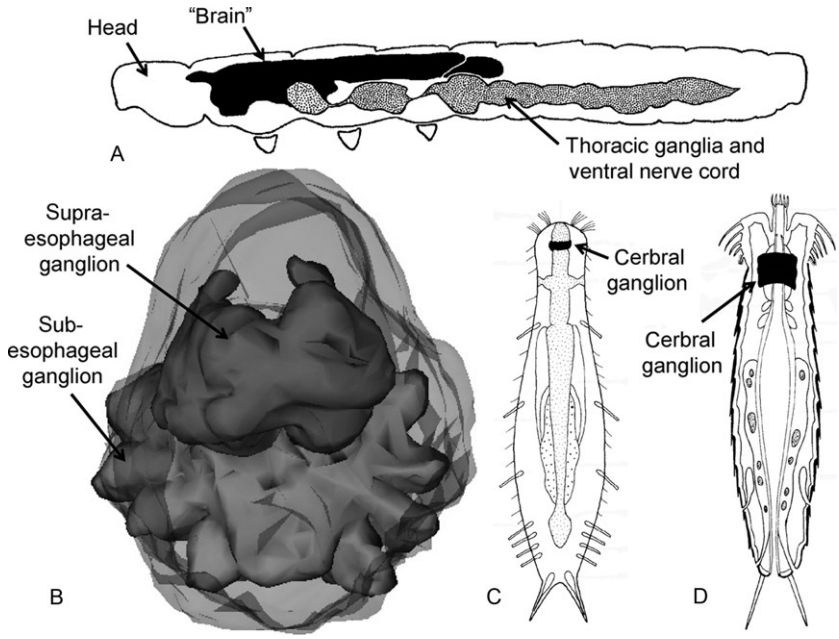

FIG. 4 Morphological correlates of grade changes. The larval beetle (A) and the spider nymph (B) are among the smallest representatives of their respective groups (they are 0.5 and 0.6 mm long respectively, and weigh about 0.002 and < 0.005 mg respectively). They both show signs of running out of room in which to house their relatively gigantic nervous systems. The beetle's brain is larger than its entire head, and has moved into its thorax and abdomen; in the spider, approximately 78% of the cephalothorax is occupied by the brain, which extends into the coxae of the legs and also bulges ventrally (not visible). In contrast, kinorhynchs (C) and gastrotrichs (D) are similar or even smaller in size (respectively they are about 0.3 and 0.2 mm long, and weigh approximately 0.002 mg), but their brains are relatively modest in size. (A from Polivov and Beutel, 2009; B from data in Quesada *et al*., in review; C and D after Barnes *et al*., 1993).

Rule (Fig. 1; Quesada *et al*., in review). As with insects, smaller spiders also have smaller diameter neuron cell bodies (*op. cit*.). There was no obvious difference between the scaling of brain size of three kleptoparasitic species in this group and that of seven orb-weaving species.

The brains of very small spiders showed morphological distortions analogous to those in tiny insects. In this case, the brain extended into the coxae (Fig. 5), a design never seen in the larger spiders of this same study (Quesada *et al*., in review) or in other species (Babu and Barth, 1984; Comstock, 1967; Hill, 1975; Kaestner, 1968). In addition, the relatively large brains of tiny spiders were associated with an apparent deformation of the sternum to accommodate the brain. The sternum of a first instar nymph of *Leucauge mariana* has a conspicuous ventral bulge, in contrast to the nearly flat sternum of the mature female

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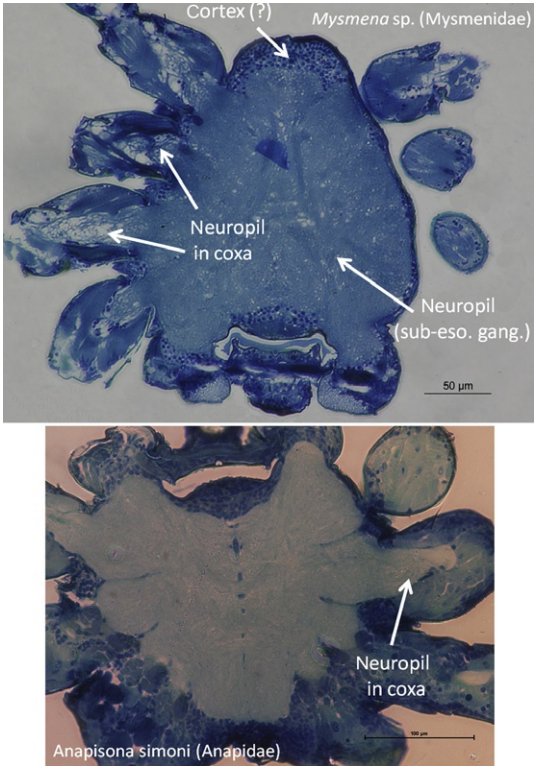

FIG. 5 Sections of tiny spiders showing extensions of the brain neuropil into their coxae.

(Fig. 6; Quesada *et al*., in review). In serial sections, nearly the only tissue that occupies this bulge was the brain (the nerve cell bodies of the sub-esophageal ganglion). Tiny adult spiders in a variety of families, and tiny nymphs of species with medium and large-sized adults, also have ventrally bulging sterna (R. Madrigal, in preparation). Internal deformation is suggested in the early instar nymphs of the salticid *Phidippus clarus*; the digestive cecae that are abundant in the cephalothorax of the adult are nearly completely missing from the cephalothorax of a second instar nymph, which is instead largely occupied by the brain (Hill, 1975).

6.1.3 *Other invertebrates*

The huge relative sizes of the brains of tiny insects and spiders, and the deformed shapes of their brains and the body structures that house them (Fig. 4A and B), lead to speculation that relative brain size may impose lower limits on the body sizes of these groups (Beutel *et al*., 2005; Grebennikov,

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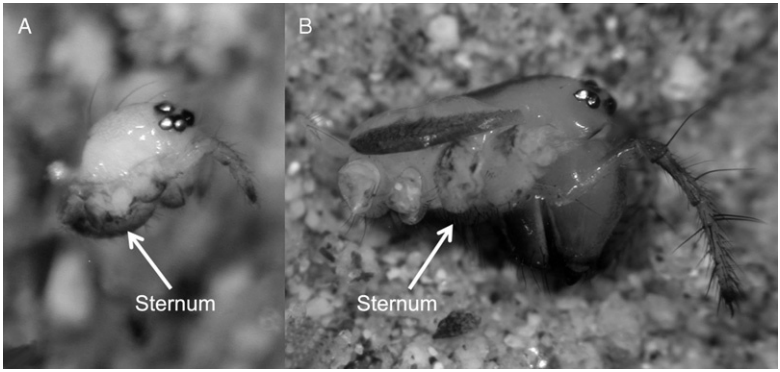

FIG. 6 Lateral views of an early instar nymph (left) and an adult female (right) of *Leucauge mariana* (legs removed), showing the distinctly greater ventral bulge of the sternum of the nymph.

2008). Extrapolating from arthropod brain allometries (Fig. 1), an animal that is an order of magnitude smaller than minute arthropods (e.g. on the order of 0.001 mg in weight) would have a brain that is about 25% of its body weight, a proportion which is seemingly prohibitive (Fig. 1B). Nevertheless, there are many animals whose adults are substantially smaller than the smallest insects and spiders, including tardigrades, gastrotrichs, kinorhynchs, rotifers, nematodes and some crustaceans (''small invertebrates'' in what follows), and the immature stages of these groups and others are smaller still.

How do the relative brain sizes of small invertebrates compare with those of insects and spiders? Measurements of the weights of their brains and bodies that would allow direct comparisons with data from other groups are generally not available. We have instead used published drawings of the nervous systems of a few small invertebrates with geometrically regular forms to obtain preliminary answers to this question. The answer we have found, that the brains of at least some are remarkably *un*exceptional in relative size and design (Fig. 4C and D), may eventually help illuminate the significance of the data from other taxa.

As a first approximation, we used classic two-dimensional drawings illustrating dissections, and deconstructed the bodies and brains of a few morphologically simple animals into multiple geometrically simple objects whose volumes could be easily calculated. For example, many kinorhynchs are more or less rectangular in dorsal view (with the introvert withdrawn; Fig. 4D); the body is ''... flattened ventrally, arched dorsally,'' and ''... triangular or nearly so in cross-section'' (Higgins, 1983; it forms an isoceles triangle with corner angles of 47° in *Kinorhynchus stenopygus* in Fig. 296 of Higgins, 1983). The area enclosed in a tracing of this triangular outline from the figure was 42% that of a circle whose diameter was equal to the length of the base of the triangle (i.e. the width of the animal in dorsal view). The brain has the form of a thickened sheet

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that surrounds the muscular pharynx, which is also triangular in cross-section (Barnes *et al*., 1993). Similar volume estimates were made by deconstructing a nematode, a mite, and the first nauplius larva of a small copepod (see legend of Fig. 1).

Relative brain sizes in the tiny invertebrates are substantially smaller than would be predicted by simple extensions of the allometric lines of insects and spiders (Fig. 1). In terms of overall morphology, their nervous systems occupy only moderate fractions of their body volumes (Fig. 1B), and they show no morphological signs of being physically constrained by the volumes of the areas of their bodies where they are housed (Fig. 4). These species are typical rather than exceptional among very small invertebrates in not showing signs of disproportionately large brains, as judged by published drawings of gastrotrichs, tartigrades, ostracods, loriciferans, copepods and nematodes (Fig. 4; Barnes *et al*., 1993; Brown, 1950; Brusca and Brusca, 1990; Kristensen and Higgins, 1984; Stachowitsch, 1992).

It is not clear whether the displacement of allometric lines to the left (Fig. 1B) in these groups is associated with behavioural inferiority and conforms to the size limitation option, or whether their CNSs possess economies of design that permit greater behavioural capability for a brain of a given size. Given the huge numbers of neurons thought to occur in even the tiny insects (e.g. Beutel *et al*., 2005), it is striking that some of these other, smaller invertebrates function with very small numbers of neurons—302 in the hermaphroditic form of the nematode *C. elegans* (Altun and Hall, 2008), approximately 254 in the nematode *Ascaris lumbricoides* (Bullock and Horridge, 1965), and perhaps 300–400 and certainly less than 1000 in the nauplius larva of the copepod *Dactylopusia* (T. Lacalli, personal communication).

6.2 BEHAVIOUR

6.2.1 *Insects*

What is known about the behavioural capacities of tiny insects? Cole (1985) argued persuasively that several previous studies that attempted to relate behavioural complexity and brain size (Eisenberg, 1981; Eisenberg and Wilson, 1978; Harvey *et al*., 1980; Howse, 1974) suffered from a lack of objective criteria for quantifying behavioural ''complexity.'' Although it may be intuitively obvious that some animals have more complex behaviour than others (e.g. a limpet vs. a monkey), we lack methods to objectively characterize behavioural complexity for different taxa, or even the different developmental stages of a single species, in ways that are biologically meaningful (see Tononi *et al*., 1994 for methods to describe brain complexity). This problem of crafting an operational definition of behavioural complexity resembles that faced by U.S. Supreme Court Justice Potter Stewart when trying to develop objective criteria to identify ''hard-core pornography''; Stewart (1964) admitted that he could perhaps never objectively establish such criteria, but claimed that ''I know it

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[pornography] when I see it.'' Indeed, the concept of ''behavioural complexity'' is so intuitively appealing it continues to be invoked, even though it is vague and unquantifiable in a biologically useful way. We have no confidence we can objectively distinguish pornography, and likewise believe that ''behavioural complexity'' is best abandoned due to lack of objective criteria.

''Behavioural complexity'' was the focus of studies of ants that concerned inter-specific or inter-caste differences to assess the behavioural consequences of miniaturization, but these studies yielded unconvincing and inconsistent results (Cole, 1985; Wilson, 1984). In the first place, they relied on a questionable method of quantifying behavioural complexity—the size of the behavioural repertoire (number of tasks) of a species. Cole (1985) found a positive correlation between head size and behavioural repertoire in nine species of as many genera, and concluded that the behaviour of smaller species of ants is less complex. This conclusion suffered from problems in quantifying ''complexity,'' including the uncritical acceptance of several untested assumptions: all tasks are equally demanding with respect to neural capabilities; tasks that are assigned the same name in different species (e.g. ''feed the larvae'') are equally demanding; the rate of errors in the performance of a given task does not vary between species; head size correlates with brain size in the same way in different ant species; all species respond to laboratory environments in the same way *viz-à-viz* expression of behaviour; and different investigators categorized behaviour in the same way, with no differences in lumping or splitting among different observers. In addition, appropriate controls for phylogenetic inertia (Harvey and Pagel, 1991) were lacking. A number of more recent studies have followed Cole (1985) and used size of behavioural repertoire as a metric of behavioural complexity, but suffer from the same problems (e.g. Anderson and McShea, 2001; Changizi, 2003; Chittka and Niven, 2009).

Other studies appeared to show the opposite correlation between small size and behavioural complexity.'' The brains of termite species were said to show the opposite correlation—species with greater behavioural ''complexity'' (in terms of nest architecture) have smaller brains with fewer neurons (Howse, 1974). Comparative studies of 10 species of *Pheidole* ants showed no correlation between repertoire size and body size in minor workers, and a weak (non-significant) negative correlation in majors (Wilson, 1984). These studies suffered from the problems in quantifying complexity described above.

Some authors have argued against the size-limitation hypothesis in insects on the basis of only negligible behavioural data. Beutel *et al.* (2005) noted that tiny strepsipteran larvae have several types of sense organs, can jump, and are able to locate hosts; they speculated that these larvae may have no behavioural impairments compared with larger insects, but provided no further data. Polilov (2005) cited unspecified indications that the behaviour of very small mites (0.08 mm long) is simpler than that of larger ones. Polivov and Beutel (2009) wrote that the tiny first instar larva of *Mikado* sp. possesses ''... a complex behavior pattern ...,'' but gave no supporting citations or data.

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In general, detailed studies of the behaviour of tiny insects are lacking, and it is presently not possible to assess whether the behavioural capabilities of tiny animals are generally affected by brain-related scaling problems, such as increased error rates, slower execution, greater bias toward computationally less demanding movements such as jumping (as opposed, for example, to reaching for objects in different directions), or other possible manifestations of reduced performance. In sum, there are far too few data to evaluate the behavioural limitation hypothesis in insects.

6.2.2 *Spiders*

Orb-weaving spiders are especially attractive subjects for studies that address questions related to size in behavioural and neural system trade-offs. In the first place, they vary widely in size, spanning more than five orders of magnitude in weight, from < 0.005 mg in newly emerged mysmenid and anapid nymphs to > 2000 mg in mature female nephilids (Quesada *et al.*, in review). Secondly, fine details of their web-building behaviour are especially easy to study, because orb construction produces a detailed and exquisitely precise record of hundreds of behavioural decisions and the contexts in which they occurred (Witt *et al.*, 1968). Even the webs of tiny individuals can be easily recorded and analysed in photographs (Fig. 7; Eberhard, 2007). Many features of an orb are readily quantifiable, allowing unusually detailed analyses of behavioural decisions. Some behaviours are repeated over and over during the construction of each web, thus allowing analyses of both the responses to particular stimuli, and of the precision or repeatability of these responses. This helps solve the difficult

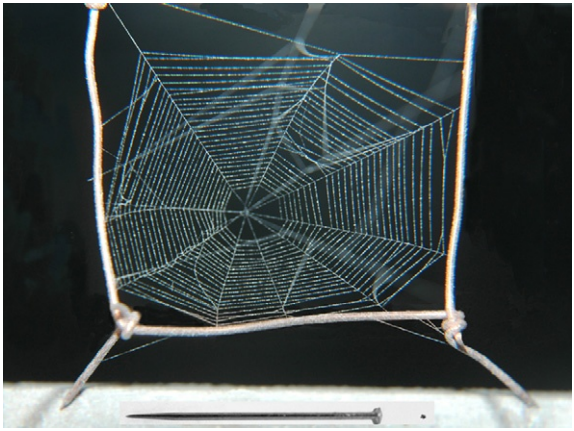

FIG. 7 Montage of photos (all to the same scale) of the web of an early instar *Anapisona simoni* built in captivity, a sewing pin, and the spider that built the web (the dark spot to the right of the pin).

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problem of devising meaningful metrics to compare behavioural capabilities among diverse groups of animals (Healy and Rowe, 2007; Tinbergen, 1951). The other side of this coin is, of course, that only certain types of behaviour are included: web construction represents only a sample of a spider's behavioural capabilities.

In addition, details of many behaviour patterns used to build orbs are phylogenetically quite conservative (Eberhard, 1982; Scharff and Coddington, 1997), and are very similar in species with widely different body sizes. Ontogenetic changes in external morphology and predatory behaviour are typically small in spiders, so especially powerful intra-specific comparisons can be made that span large size ranges, thus obviating possible biases associated with phylogenetic inertia that can affect inter-specific comparisons (Felsenstein, 1985; Harvey and Pagel, 1991). The higher level phylogeny of spiders, especially in the group Orbiculariae, is relatively well studied (Griswold *et al*., 1998; Kuntner *et al*., 2008; Scharff and Coddington, 1997), and it is possible to identify cases in which miniature body size is secondarily derived in groups that have comparable web designs.

These advantages of orb weavers have begun to be exploited (Eberhard, 2007, 2011; Hesselberg, 2010), providing the most extensive and detailed comparisons of the behaviour of tiny and moderately sized arthropods currently available. These results are discussed below, and summarized in Table 1.

6.2.2.1. Behavioural precision Due to the problems in quantifying ''behavioural complexity'' (see Section 6.2.1), we focus mostly on two other variables that could reflect possible behavioural limitations: behavioural precision—the animal's ability to repeat the same behaviour precisely; and flexibility—the animal's ability to adjust its behaviour appropriately to different environmental circumstances. Both traits have been hypothesized to be less developed in animals with smaller brains (Misunami *et al*., 2004), because they would presumably require sensors and processors to perform consistent behaviour in different circumstances, to assess environmental conditions, and to appropriately trigger and adjust expression of alternative behaviours.

The logic of using behavioural precision is the following. There are several reasons to suspect that motor behaviour, coordination, and orientation may be less precise in an animal with a reduced nervous system, particularly when the numbers of neurons are reduced (Grebennikov, 2008). Smaller animals have fewer sense organs, and thus have less complete sensory input (see Section 1). They may also perform less thorough or precise analyses of sensory inputs, due to lower numbers of inter-neurons, fewer dendrites, or fewer or more noisy connections between them (Chittka and Niven, 2009; Faisal *et al*., 2005; Wittenberg and Wang, 2007). If they have less extensive internal feedback mechanisms, the behavioural imprecision that results from intrinsic noise in the nervous system may also be greater (Calvin and Stephens, 1967, 1968, Eberhard, 1990a, 2000). The smaller numbers of motor axons and reduced

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TABLE 1
Tests of the size-limitation hypothesis using the behaviour and morphology of orb-weaving spiders, utilizing behavioural data from (in order of increasing size) second instar nymphs and adults of *A. simoni*, second instar nymphs and adults of *Allocyclosa bifurca*, and adults of *Leucauge mariana* (from Eberhard, 2007, 2011), and morphological data from these and other species (from Quesada *et al*., submitted; Vargas, unpublished)

Predictions of the size-limitation hypothesis for smaller spiders	Finding	Support hypothesis? (Comments)
Behaviour		
Reduced adaptive flexibility		
A. Lack of correlation between number of radii and number of sticky spiral loops	Correlations occur in spiders of all sizes	No
B. Lack of correlation between angles between adjacent radii and their lengths	Correlations occur in spiders of all sizes	No
C. Lack compensatory reduction in sticky spiral spacing following an over-sized space	Lack compensatory spacing lack in smallest species; no lack in small nymphs of another species[a]	Yes, but Weber-Fechner Law offers alternative explanation for lack of compensatory space in the smallest species
Reduced behavioural precision		
D. Greater imprecision in spacing of sticky spiral	No correlation between spider size and imprecision	No
E. Weaker correlation between number of radii and number of sticky spiral loops	Weaker correlation in smaller species but no difference between conspecific nymphs and adults[b]	Mixed[b]
F. Weaker correlation of the lengths of adjacent radii and the angle between them	No intra-specific differences between large and small spiders[c]	No[c]
G. Larger differences between adjacent spaces of sticky spiral	No trend with body size between or within species	No
H. Weaker correlation between numbers of upper radii and sticky lines above the orb	No difference between nymphs and adults of *A. simoni*	No

(*continues*)

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TABLE 1 (*Continued*)

Predictions of the size-limitation hypothesis for smaller spiders	Finding	Support hypothesis? (Comments)
Reduced ''complexity''		
I. Simpler design of orb	Smallest species has lost temporary spiral	Yes[d] (but the selective advantage of losing the temporary spiral offers an alternative explanation[d])
J. Single web design	Only smallest species has alternative web design	No (opposite)
K. No modification of design according to available space	Only smallest species modifies orb design (angle of cone) to available space	No[e] (opposite) (larger species have only two-dimensional webs, so such adjustments are not feasible)
L. No correlation between radial and sticky lines above the orb	Only smallest species modifies orb on basis of number of lines above the orb	No[e] (opposite) (larger species do not have sticky lines above the orb)
Morphology		
M. Lack of distortion of body design to house ''overly large'' brain	tiny spiders have bulging sterna and sub-esophageal ganglion extending into coxae; tiny insects have brains extending into prothorax; tiny mites have brain extending into coxae	No

[a] Also occur in webs of the moderately large araneid *M. duodecimspinosa*

[b] Scatter around the regression line was significantly greater in the tiny species *A. simoni* than in larger species, and in small nymphs than in adults of *A. bifurca*. This support for the size-limitation hypothesis was inconsistent, however, because the scatter in nymphs of *A. simoni* was not greater than that in conspecific adults.

[c] Because the behaviour thought to be responsible for producing this correlation occurs during the production of ''secondary'' but not that of ''primary'' radii (Eberhard, 1990b; Hingston, 1920; LeGuelte, 1967), and because the webs of different species in this study have different mixes of primary and secondary radii (below), it is appropriate to test for size effects by making intra-specific rather than inter-specific comparisons. Comparisons in both *A. bifurca* and *A. simoni* showed nearly identical amounts of scatter around regression lines for nymphs and adults (Eberhard, 2011).

[d] Loss of the temporary spiral may be an adaptation to allow construction of sticky lines above the plane of the orb as part of sticky spiral construction (Eberhard, 2011).

[e] Direct comparison between present-day larger and smaller spiders is not possible, but evolutionary prediction of reduction in behavioural abilities when evolve small size is contradicted.

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feedback could also result in less subtle control of body movements. In an animal like a web-building spider, which uses the movements and positions of its own legs to provide sensory input (Barth, 2002), limitations in the precision of leg movements and in the proprioreceptive analysis of these movements could significantly reduce the accuracy of its sensory perception of the world.

Construction of the sticky spiral in an orb is well-suited to analyses of behavioural precision. The spider starts near the periphery of the orb and works inward, repeating some processes such as those illustrated in Fig. 8 at each radius it crosses; the space between loops of sticky spiral is determined each time the spiral is attached to a radius. During the construction of an orb the spider repeatedly (often hundreds of times) senses the location of the inner loop of sticky spiral, measures the distance along the radius at which the current loop should be attached, and then attaches it.

Simulation studies have shown that a relatively simple set of instructions can produce an at least superficially spider-like sticky spiral pattern (Eberhard, 1969; Krink and Vollrath, 1999). Nevertheless, control of the behaviour that determines the spacing between sticky spiral loops is affected by at least six different factors: the length of the exploratory leg I (Reed *et al.*, 1965; Vollrath, 1987); the angle of the radius with gravity (LeGuelte, 1967; Vollrath, 1992; Witt *et al.*, 1968); the distance of the attachment from the hub (LeGuelte, 1967; Vollrath, 1992); the age of the spider (Reed *et al.*, 1969); an internally determined ''set point'' that is influenced by silk supplies (Eberhard, 1988a); and the distance of the previous sticky spiral attachment from the outer loop of temporary spiral (Eberhard, 2011; Eberhard and Hesselberg, in review).

Sticky spiral construction behaviour is highly conserved taxonomically (Eberhard, 1982; Griswold *et al.*, 1998). Sticky spiral placement behaviour involves only relatively simple behavioural actions, and perhaps also relatively low level analyses of stimuli by the spider, so it can only test a relatively strong version of the size-limitation hypothesis: that even relatively undemanding behaviour show limitations in tiny animals (more demanding behaviour might be more likely to be limited by size—see below).

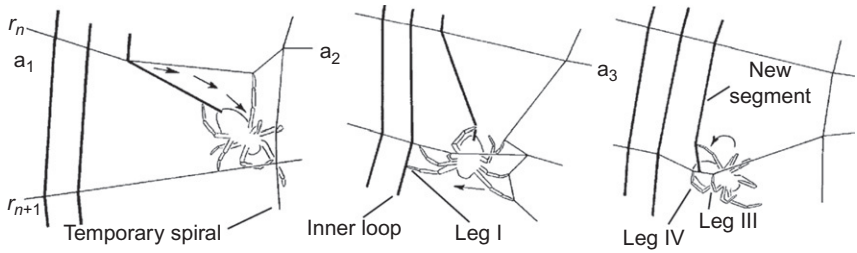

FIG. 8 Processes that are repeated up to hundreds of times during the placement of the sticky spiral in an orb web.

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The expectation of the size-limitation hypothesis is that very small spiders will show greater imprecision in sticky spiral construction behaviour. This would result in greater differences in the adjacent spaces between loops of sticky spiral. The highly regular spaces between successive loops of sticky spiral in orb webs imply that, at least in a given area of the orb, a particular spacing is advantageous (Eberhard, 1986; Witt, 1965); thus variation between neighbouring spaces probably result from mistakes. The validity of using behavioural comparisons between young and old spiders of the same species to test for possible handicaps of miniaturization depends on the assumption that learning does not reduce variation. This assumption is supported by the repeated finding that juvenile experience does not influence major aspects of orb construction (Eberhard, 2007; Mayer, 1952; Reed *et al*., 1970; Vollrath, 1992). An additional possible consequence of size limitation might be that the speed of execution of given behaviour patterns would be reduced in smaller individuals. The expectation of the size-limitation hypothesis is thus that to achieve a similar level of precision, tiny spiders might work more slowly.

Comparing first instar nymphs and adults from three different spider species that spanned a range from 0.6 mm body length and < 0.005 mg wet weight (first instar nymphs of the anapid *Anapisona simoni*) to body length about 7 mm and wet weight about 50–80 mg (adult females of the tetragnathid *L. mariana*), the smallest individuals showed no more imprecision than did the larger spiders (Eberhard, 2007; Table 1) (the precision of sticky spiral spacing was defined as a measure of intra-individual imprecision in the spacing of successive loops of sticky spiral). The imprecision values of the tiniest spiders were in fact significantly smaller than those for the larger second instar nymphs of *Allocyclosa bifurca*, and were not significantly different from those of the much larger adults of *A. bifurca* and *L. mariana* (Eberhard, 2007).

The importance of these data as evidence against the size-limitation hypothesis is emphasized by two further considerations. Anapids must travel much larger distances from one attachment of sticky spiral to the next during sticky spiral construction (in relation to their body size) than do most orb weavers (Fig. 9). The radial distances from the hub inward and back out are used as cues to direct sticky spiral spacing (Eberhard and Hesselberg, in review; Krink and Vollrath, 1999), and temporary spiral placement (Eberhard, 1988b). Although the psychophysics of spider perception is not well understood, general psychophysical principles should hold for them as well. The Weber-Fechner Law (in the sense used by Cheng *et al*., 1999) holds that an animal's ability to detect a just noticeable difference (JND) for a given stimulus is a function of the magnitude of the stimulus, and the JND is a constant proportion across a large range of magnitudes (Shettleworth, 2010; Stevens, 1975). A consequence of this law is that small things are assessed more precisely in absolute terms than larger things. Thus, a measurement made by a spider walking a longer distance along a line is

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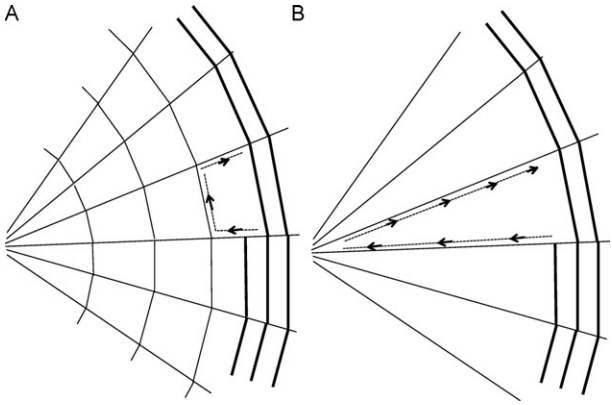

FIG. 9 Paths taken by the spider in moving from one attachment of the sticky spiral to the next during sticky spiral construction by a typical orb weaver (left) and by an anapid, whose web lacks a temporary spiral (right).

expected to be less precise than one measured over a shorter distance. This implies that *A. simoni* is less likely to be able to sense small differences between the relatively long distances it must travel inward and outward during sticky spiral construction (Eberhard, 2011). In short, despite the probable greater difficulty for the anapids to sense important cues, they showed comparable or better precision in sticky spiral spacing.

A second extenuating fact is that the webs of anapids have fewer loops of sticky spiral. In many species the sticky spiral spacing is different at different distances from the hub. In comparing the space between two loops of sticky spiral with the spaces between adjacent loops of sticky spiral, this distance from the hub is more likely to introduce variation in anapid webs and cause their precision values to be higher.

Another size-limitation prediction—that smaller spiders move relatively more slowly—was also not met. The mean times between successive attachments of the sticky spiral during the early phase of web weaving were 3.0 s in a small individual of the smallest species, *A. simoni*; 1.9 and 3.4 s in a young nymph (1.4 mg) and an adult female (30 mg) of *A. bifurca*, respectively, and 1.5 and 7.3 s in mature females of *L. mariana* (50 mg) and the araneid *Gasteracantha cancriformis* (100 gm), respectively. The smallest spiders had to move much farther between attachments with respect to their body size (Fig. 9, Eberhard, 2011), and they were thus moving much *more* rapidly in terms of their body size.

Imprecision may arise from three different sources: imprecision in what the spider *intended* to do (e.g. due to conflicting motivations or variable conditions); imprecision in the control of the behavioural movements that were involved in executing these plans; and imprecision in executing other,

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previous behaviour patterns that, as a secondary consequence, influence execution of the behaviour in question. Data are generally lacking to assess the relative importance of these possibilities. A possible example of the first type of effect (variation in intention) would be the association recently found in sticky spiral spacing by *Micrathena duodecimspinosa* between over-sized sticky spiral spaces and the distance to the nearest temporary spiral scar (Eberhard and Hesselberg, in review). In this species, imprecision in sticky spiral spacing may be associated with inconsistency in the influence of occasional sharp changes in the distance from the outer loop of temporary spiral that ''distract'' the spider, causing it to pay less attention to other cues. This species also provides a possible example of the third source whereby imprecision arises as a consequence of imprecision in other behaviour. Sticky spiral spacing also seems to be influenced by the presence of intact temporary spiral lines which the spider failed to break during sticky spiral construction (W. Eberhard, unpublished data). In this case, the apparent cause of some of the imprecision in sticky spiral spacing is the occasional previous failure to cut the temporary spiral during sticky spiral construction.

6.2.2.2. Behavioural flexibility and the precision of adjustments among web variables It may be that the precision of small spiders just discussed involves, at least on the motor side, behaviours that are relatively undemanding in terms of nervous system capacities, and that it thus constitutes an overly-stringent test of the size-limitation hypothesis. Placement of a new segment of sticky spiral will depend on where legs III and IV grasp the radius relative to the site where exploratory taps with leg I contacted the inner loop, as the site where the spinnerets attach the sticky spiral line to the radius is between these legs (Fig. 8). Consistency in sticky spiral spacing is thus likely to be, at least to some extent, a direct result of the size of the spider's own body (Vollrath, 1987). Perhaps the behavioural limitations of tiny spiders are only manifest in the execution of more neurobiologically challenging tasks. Study of more challenging behaviour patterns might thus give more sensitive tests of the size-limitation hypothesis. Such tests could involve the abilities of spiders to adjust one web variable to another, or of the precision with which such adjustments are made. The results of several such tests are summarized in Table 1, and discussed below.

Some tests involved the degree to which spiders of different sizes flexibly adjusted one web variable to another, as indicated by the existence of a significant correlation between the two variables, and the precision of these adjustments. Precision was measured by the tightness of this relationship, in terms of the residuals around a regression of one variable on the other. The general patterns in both the existence and the precision of these adjustments failed to fit size-limitation predictions (Table 1). Several of the correlations are probably selectively advantageous (summary in Eberhard, 2011).

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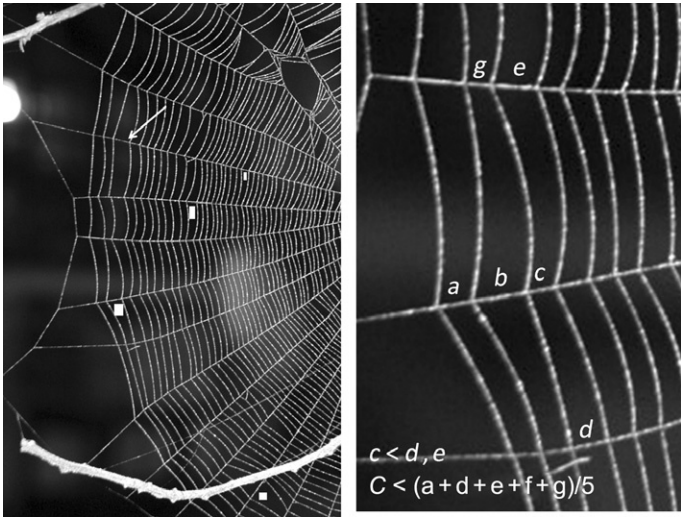

FIG. 10 An over-sized space between loops of sticky spiral (*b*) that was followed by a reduced space (*c*) in the web of an adult female *Micrathena duodecimspinosa*. The two inequalities indicate ways to calculate whether or not a spider reduced the space (*c*) that followed an over-sized space.

The trend to produce compensatory reductions in sticky spiral spacing following over-sized spaces in orb webs (Fig. 10; Table 1C) has only recently been recognized (Eberhard, 2011), but it may also be a general pattern in orb webs (Eberhard, unpublished data). Compensatory adjustments may function to reduce irregularities in the path of the inner loop of sticky spiral during sticky spiral construction, and help re-establish the uniformity of the spacing of sticky spiral loops after a discontinuity. They are probably triggered when the spider senses differences in the distances travelled inward and outward along radii as it builds the sticky spiral (Eberhard and Hesselberg, in review). This adjustment was lacking in the smallest species (Table 1).

6.2.2.3. Qualitative differences in behavioural flexibility: adding and subtracting abilities Although quantitative metrics for behavioural complexity are problematic (see Section 1), there are several qualitative differences in which the behavioural capacities of different-sized spiders can be compared.

The number of upper radii in the webs of the smallest species, *A. simoni* (Fig. 11), showed a positive correlation with the number of sticky lines attached to any single upper radius in the webs of both adults and small nymphs (Table 1 L). None of the other species built sticky lines out of the plane of the orb, and the plesiomorphic state for orb weavers is planar orbs (Griswold *et al.*, 1998), so the ability of *A. simoni* to adjust the sticky lines to upper radii is

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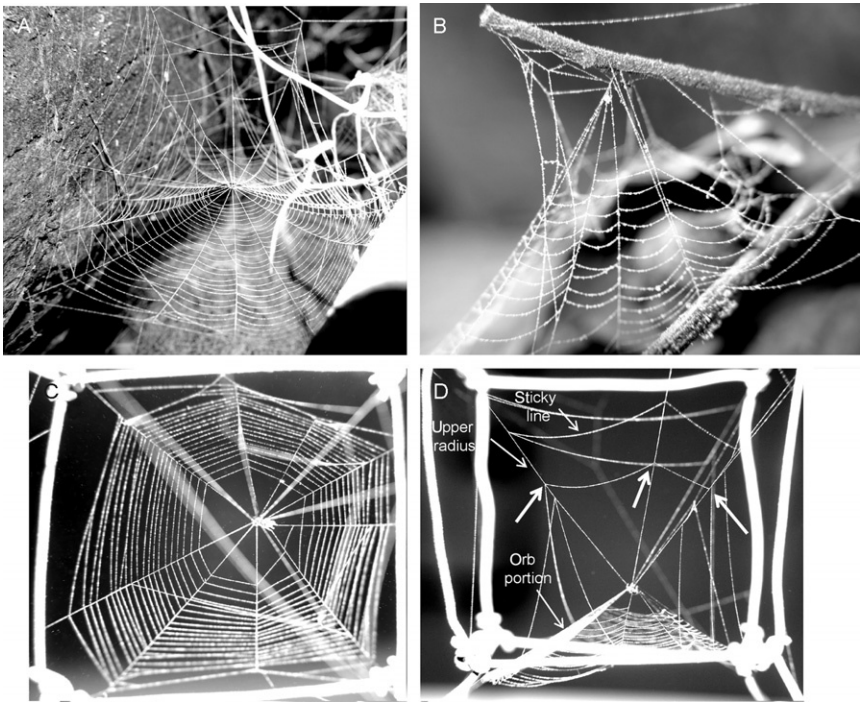

FIG. 11 Lateral views of webs of *Anapisona simony* (A, D), showing radii above the plane of the orb and sticky spiral lines attached to these radii (dorsal view in C). The alternate web design in *A. simony* is shown in B. The radii of an incomplete orb converge in an area near the underside of a twig where the spider rests, but do not form a hub.

derived. Similarly, when adult *A. simoni* were offered two different sizes of wire cubes in which to build their webs, the hub of the orb portion of the web was drawn upward more sharply when the spider built in a smaller cube (Table 1K). This modification of the orbs in smaller cubes increased the area covered by the catching spiral over the area it would have had if the orb had been more nearly planar. No such adjustments occurred in the webs of the other, larger species, which built only planar or nearly planar orbs.

Still another additional ability in *A. simoni* was for the same spider to build either a normal orb or an alternative web form lacking a discrete hub and with the radii converging on the underside of a twig or other large object under which the spider rested (Fig. 11B; Table 1J). Construction of this alternative, ''sector web'' design probably involves alterations in frame, radius and hub construction behaviour (Eberhard, 2011). A similar sector web design occurs, along with complete orbs, in another, unidentified tiny anapid (Madrigal and WGE, unpublished data). Alternate forms of prey capture webs were never seen in samples of hundreds of orbs in the field in species with which we have extensive experience

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(the tetragnathids *L. mariana*, *Leucauge argyra*, and *Dolichognatha* sp.; the araneids *A. bifurca*, *M. duodecimspinosa*, *Micrathena sexspinosa*, *Metazygia gregalis* and *Araneus expletus*; and the uloborid *Uloborus diversus*), and are not mentioned in general reviews of orb webs (Vollrath, 1992; Witt *et al*., 1968). We know of only four other cases, all of which are clearly independent (Eberhard, 1969, 1990c; Edmunds, 1978; Sandoval, 1994). Lack of alternative webs is plesiomorphic in orb weavers (Griswold *et al*., 1998), so construction of alternative web forms in anapids is derived, suggesting these tiny spiders have additional behaviours relative to those of many larger spiders.

One final qualitative difference involves web features that are common in larger species yet lacking in minute ones: the orbs of the smallest species, *A. simoni,* lack temporary spirals, which are standard in the orbs of most other orb weavers (Table 1I). This support for the size-limitation hypothesis is weakened, however, by the fact that there is a likely alternative explanation. Omission of the temporary spiral permits the spider to extend its sticky spiral lines into the space above the orb, where they can aid in trapping prey passing above the plane of the orb (Eberhard, 2011).

6.2.2.4. General conclusions and summary of orb construction behaviour data In general, the behavioural data just reviewed contradict rather than support the size-limitation hypothesis that smaller animals are more limited in their behaviour (Table 1). They also contradict the more general-hypothesis of behavioural inferiority for species with brains of smaller absolute sizes (Byrne, 1997; Striedter, 2005). Both intra- and inter-specific comparisons lead to the same conclusions, so phylogenetic bias is probably not a problem in these analyses. To our knowledge, these are the most detailed comparative behavioural data ever assembled for animals with very small body sizes.

A simple tally of the numbers of contradictions and confirmations in Table 1 (nine against; one in favour; two with mixed support) is inappropriate, however, because these behavioural capabilities are not necessarily comparable. There are reasons to expect that some of these capabilities may be more costly or cheaper in terms of nervous tissue than others. It is possible to make educated guesses regarding what neuronal capacities are needed to confer the ability to adjust construction behaviour appropriately to different environmental conditions. Some adjustments may be relatively simple for a spider to accomplish. For instance, the radius angle could be adjusted to vary inversely with the length of the radius simply by always walking a fixed distance from the exit radius along the frame line before attaching the new radius (Hingston, 1920; though in fact this distance is not constant even in a given orb—Eberhard, 1972, unpublished data on *M. duodecimspinosa*). In contrast, it would seem much more difficult for a spider to assess the area available in which to build its orb, as it probably involves measurements of distances and directions travelled, and memories of both (Vollrath, 1992). This ability may be ancestral (Vollrath, 1992; Eberhard and Barrantes, in preparation). The ability of *A. simoni* to build

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more steeply sloping orbs to take better advantage of limited spaces (Table 1K) may also be ancestral, as it occurs in the larger, distantly related *Philoponella vicina* (Barrantes and Eberhard, unpublished data). The ability of *A. simoni* to correlate the number of upper radii and the number of sticky lines attached to them (Table 1L) also seems likely to be relatively demanding, and probably requires memory (Eberhard, 1987, 2011). The equal precision in sticky spiral spacing in *A. simoni* (Table 1D) weighs especially heavily against the size limitation option, because of the Weber-Fechner Law and the lack of temporary spirals in their orbs imply that this task is especially difficult for these spiders (Eberhard, 2011).

In addition, some of the evidence that supports the size limitation option is weak. The lack of compensatory adjustment of sticky spiral spacing following over-sized spaces in the anapid is likely a result of the loss of temporary spiral lines in the webs of this family.

As noted above, comparisons between conspecific nymphs and adults constitute a valid test of the effects of body size on imprecision only if learning has minimal or no effect on behavioural imprecision. This was indeed the case, as there were no significant differences between first instar nymphs and adult females in *A. simoni* or *A. bifurca*, and the insignificant trends that did occur were in opposite directions in both species. In sum, the evidence against the size limitation option is strong in the comparisons involving possibly more ''difficult'' tasks, strengthening the overall trend in the data against this hypothesis.

Rejection of the size limitation option would leave the ''over-sized brain'' and the ''economy of design'' options as possibilities for orb weavers. The behavioural data do not allow a choice between these options, which in any case are not mutually exclusive. The morphology of the brains of some of the smallest orb-weaving spiders, and that of the structures that house them (see Section 6.1.2), support the ''over-sized'' brain hypothesis. But until a broader survey of spiders is examined to determine the allometric line for the entire group, it is not known whether some of these spiders are relatively highly encephalized compared with others (see Section 7.1 below)

7 Discussion

7.1 MORPHOLOGY

''Haller's Rule'' is now well documented for both vertebrates and invertebrates (Fig. 1): within a grade smaller animals have relatively larger brains. Why this rule? We do not have an answer, but note that the wide taxonomic scope of the present discussion yields evidence against two of the hypotheses previously inspired by vertebrates. The idea that the rule results from scaling between body surface area and volume (Jerison, 1973) is not able to deal with the multiple between-grade differences, in which similar-sized animals of different taxa show sharply different nervous system allometries. Similarly, the hypothesis

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that metabolic constraints could explain the grade differences between poikilotherm reptiles and their homeotherm descendents, birds and mammals, does not explain differences between other poikilotherm groups such as ants, spiders and salamanders (Wehner *et al.*, 2007; Fig. 1).

A second way to visualize grade changes is to extrapolate the brain–body allometry slopes of different taxonomic groups. Figure 12 uses the summary graph of vertebrate brain–body allometries of Striedter (2005; Fig. 4.3B) as a base, and assumes that 20% of the body weight dedicated to the CNS is an absolute upper limit for a functional animal (the highest direct measures of these values that we know of are approximately 16–17% in ants and a beetle larva—see Section 6; also Fig. 1). Even with this relatively permissive assumption, the minimum feasible body size for a bird would be about 200 mg, for a typical mammal about 10 mg, and for a typical amphibian about 2 mg (Fig. 12). Obviously, many invertebrates are much smaller than these ''limits.'' Similarly, nematodes and kinorhynchs have body sizes substantially smaller than would be feasible for animals with the body designs of insects and spiders. These grade changes involved solving scaling problems that were apparently insuperable for otherwise successful and hyperdiverse groups such as spiders and insects.

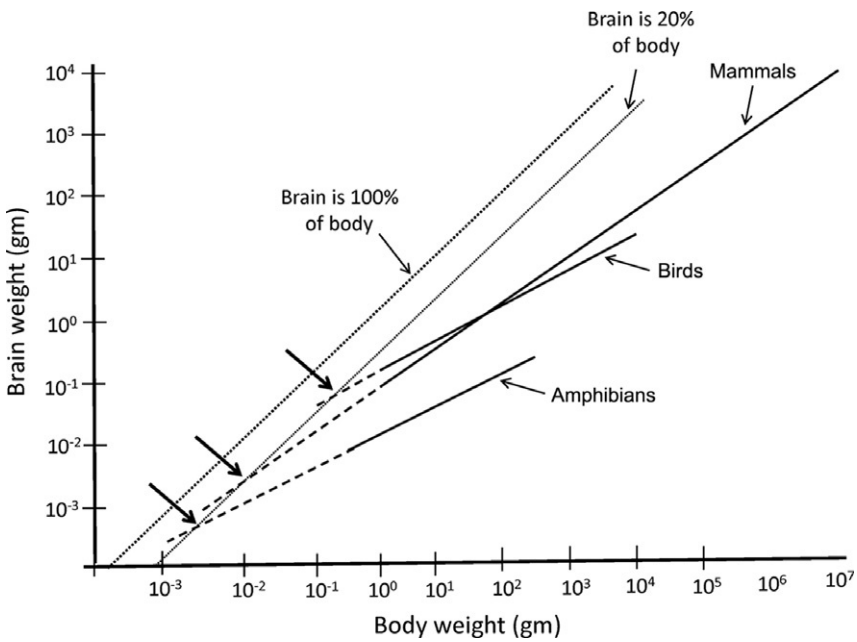

FIG. 12 Extrapolations (dotted lines) of allometric lines for mammals, birds and amphibians in Fig. 4.3B of Striedter (2005) (solid lines) show the expected inferior limits of body size in these groups (thick arrows) if one assumes that 20% is the maximum feasible amount of the body that can be dedicated to brain tissue.

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Striking alterations of morphological design often occur in the smallest species in a taxon (grade). The brains of miniature salamanders are so large relative to their heads that some skull bones have been lost, apparently to make room for the brain (Hanken, 1983; Roth *et al*., 1990). Similarly, the brains and structures housing them and their normal contents are deformed in very tiny insects, spiders and mites to accommodate the relatively large CNS (Beutel *et al*., 2005; Grebennikov and Beutel, 2002; Quesada *et al*., submitted; G. Vargas and R. Madrigal, unpublished; see Figs. 5, 6). The morphology of very tiny species of vertebrates, insects and spiders thus suggests that the smallest individuals in each group are near absolute group-specific lower limits in size. For instance, a study of the brains of miniature plethodeontid salamanders concluded that ''visual system design may represent a primary limit to ... body size decrease,'' and that ''further reduction of body size in these lineages, without an accompanying reduction in genome and cell size, may be possible only by impairment or even loss of visual function'' (Roth *et al*., 1990, pp 187, 188). Striedter (2005) and Grebennikov (2008) speculated that the vertebrate body plan, with the brain enclosed tightly in the cranial cavity, has imposed lower size limits due to limited abilities to accommodate larger brains by deforming the brain or the structures that house it. These limits do not seem to apply for other smaller taxa, in which even smaller animals have only ''reasonably'' sized brains (e.g. Fig. 4). Importantly, the external and internal deformations in tiny insects and spiders do not fit the prediction of unaltered form made by the size limitation and economy of design options.

At first glance it might appear that data supporting Haller's Rule (Fig. 1) imply that the smaller members of a given taxon have adopted the over-sized brain option. In fact, however, the log–log scales and the huge absolute ranges represented in Fig. 1 hide very substantial differences in brain–body allometries in different species within a grade. Many studies of vertebrates have correlated differences of this sort (e.g. the so-called encephalization quotients) with different behavioural capabilities (e.g. Striedter, 2005), and these differences may also be correlated with differences in economy of design. Similar variation in allometry between taxonomic groups is already apparent in insects (compare ants with weevils in Fig. 1), and it seems likely that future studies will find correlations with differences in their behavioural capabilities and economies of design within insects, spiders and other invertebrates.

It will be interesting to make further comparative surveys of scaling relationships within invertebrate taxa as has been done in vertebrates (Striedter, 2005). A study of 70 species of ants showed that there is a statistically significant break point in the allometric relationships relating brain volume to body mass near the lower end of the range of ant body size (Seid *et al*., 2011). The slope for species below 0.9 mg body mass is steeper than that for larger species, and consequently the smallest species have brains that are *smaller* than expected if they followed the same slope as the larger species. This change in the statistical trend effectively dampens the strength of Haller's Rule, and may help reduce costs that

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would otherwise result from especially large brain sizes. Intraspecifically, a fungus-growing ant, *Atta colombica,* also showed a similar breakpoint, though at a higher body mass (1.4 mg); the smallest individuals again have brains that are smaller than would be expected if they followed the scaling relationship for larger sizes (Seid *et al*., 2011). The behavioural significance of these break points is not known, and it is not clear whether the tiny ants with smaller than expected brains have reduced behavioural capabilities.

7.2 BEHAVIOUR

Efforts to understand the significance of Haller's Rule are impeded by the dearth of relevant behavioural data. The most detailed data come from only a single taxonomic group (orb-weaving spiders) and a single type of behaviour (web construction). It is clear, however, that the lack of size-imposed limitations in orb construction behaviour in the tiny *A. simoni* is probably not limited to this particular species. Other highly organized, three-dimensional derivatives of orbs also occur in species in other genera of Anapidae (Fig. 13), and also in the even smaller species in the related symphytognathoid families, Symphytognathidae and Mysmenidae (Coddington, 1986a; Eberhard, 1987; Lopardo *et al*., 2010; Platnick and Shadab, 1979). Symphytognathoids include the smallest adult spiders of any species known, and their generally minute size is a derived trait with respect to other orb weavers (Griswold *et al*., 1998). Orbs with sticky and non-sticky lines in the third dimension are widespread in symphytognathoids, where they apparently evolved once basally (Lopardo *et al*., 2010). Some species even combine multiple orbs in the same web (Fig. 13). Superficial examination of photos of webs of other species in these families (Coddington, 1983, 1986a,b; Eberhard, 1987; Lopardo *et al*., 2010; Platnick and Shadab, 1978, 1979) does not reveal obviously greater imprecision in the spacing of sticky spiral loops. In sum, the evolution of additional, complex web forms was associated with tiny body size, the opposite of expectations under the size-limitation hypothesis. Building a planar orb with additional lines that are oriented in consistent ways in the third dimension requires additional behavioural routines, which could be considered more ''difficult.'' The association of small body size with three-dimensional additions to orbs that are behaviourally challenging is in the opposite direction from that predicted by the size-limitation hypothesis (Fig. 12).

Even very tiny orb-weaving spiders have not made detectable sacrifices in their behavioural capabilities, at least with respect to web construction, despite the likelihood that they are under intense selection to do so because of the relatively greater metabolic costs imposed by their relatively large brains (Quesada *et al*., in review). Thus they pay the cost of these capacities by having proportionally gigantic brains (the ''over-sized brain'' option). Less complete data from other groups (ants, termites, vertebrates) do not show consistent

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FIG. 13 The geometric complexity of the orbs of some tiny spiders is illustrated by this ''quilt'' web of a penultimate male of the tiny *Tasmanapis strahan* (approximately 1 mm long; like other tiny spiders, its sternum bulges ventrally, where part of its brain is housed); multiple orbs are apparently joined along their frame lines to form a single more or less horizontal planar structure, a composite design unknown in the many hundreds of species of larger spiders in the families Araneidae, Tetragnathidae and Uloboridae whose web forms are known (photo by L. Lopardo).

trends (Bonner, 2006; Coddington, 1986a,b; Howse, 1974; Rensch, 1960; Wilson, 1984).

It is not clear whether or not the evolutionary ''refusal'' of tiny orb-weaving spiders to sacrifice web construction behaviour represents a general trend in other groups of very small animals. It is possible that orb weavers are unusual in that they rely more heavily on behaviour to acquire resources than, say, a larval beetle that simply eats its way through the fungus where it lives. A preliminary, weak test did not support this idea: the brain scaling of three secondarily kleptoparasitic spider species was similar to that of their orb-weaving relatives (Quesada *et al*., in review; of course, kleptoparasitism has its own behavioural requirements).

There are, however, very tiny insects that obviously also accomplish the same general tasks, such as finding food, avoiding predators and reproducing as those of larger insects. Some tiny insects make behavioural decisions that are apparently based on analyses involving multiple factors, such as the flexible searching and patch-leaving decisions of some parasitoid wasps that are influenced by both memory and internal physiology (reviews in Burger *et al*., 2006; Hanson and Gauld, 2006; Outreman *et al*., 2005; Vos *et al*., 1998). It has not been tested, however, whether they perform their tasks less effectively (e.g. learn more slowly, with more mistakes, make fewer or less precise or subtle adjustments to environmental stimuli). The behavioural capacities of even tinier metazoans, such as mites, kinorhynchs, tardigrades, etc. are even less studied. Some

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polyphagous predatory mites are able to make relatively complex analyses of chemical cues from plants attacked by their prey, and to learn associations with different odours (summary in van Wijk *et al*., 2006), thus providing a preliminary suggestion that the possible grade change in this secondarily miniaturized group (Fig. 1B) was not due to reductions in behavioural abilities.

The general failure of the predictions of the size limitation option for spiders does not mean that this option is not utilized by other groups. Nor does it mean that the logic of the arguments used to derive it is incorrect. The size limitation option is based on two suppositions, both of which are likely to be correct: (1) a smaller animal probably needs a relatively larger brain compared with its body size to maintain the same behavioural capabilities; and (2) a relatively large nervous system is especially expensive to build and maintain. The basic question is empirical, not theoretical: do very small animals tend to sacrifice their behavioural capacities due to allometric constraints on the relative sizes of their nervous systems? The answer from orb weavers seems to be ''No,'' but other groups will need to be studied to determine the generality of this answer.

7.3 THE MYSTERY OF ''GRADE CHANGES'' IN BRAIN ALLOMETRY

Figure 1 shows both that invertebrates follow Haller's Rule, and that there are clear grade differences between invertebrate taxa. Thus ants and web-building spiders appear to have similar lines that are to the left of the lines of reptiles, amphibians and teleost fish (Wehner *et al*., 2007; Seid *et al*., 2011, Fig. 1B), but to the right of the line for weevils. In insects, just as in vertebrates, different taxa have quite different brain–body allometries (Fig. 1B). In addition, the points for individual species of very small animals, including an immature crustacean and non-arthropod invertebrates such as kinorhynchs and nematodes suggest further grade changes, as they are substantially to the left of the lines of insects and spiders and their brains occupy only moderately small fractions of their bodies (Figs. 1B and 4).

How are some animals able to be so much smaller than others? Although it may be tempting to adopt a line of reasoning along the lines of the size limitation option, and suppose that animals in the ''lower'' grade are behaviourally inferior, such reasoning is unlikely to be consistently sustained by facts. Mammals and birds are indeed probably capable of more elaborate, diverse and adaptively flexible behaviour than are reptiles and salamanders. If such a general size-limitation type of idea is correct, however, one would expect that a honeybee should be behaviourally inferior to a miniature salamander, as it is in a grade whose line lies substantially to the left of that of the salamander (Fig. 1B). Although we lack a meaningful metric to quantify behavioural comparisons, we strongly doubt that this simple size-limitation prediction is true. Among other things, a honeybee can fly rapidly and manoeuvre accurately in the air, orient precisely over long distances, communicate complex messages to nestmates about the direction and distance of floral resources, make

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consensus-based decisions regarding new nest sites, master concepts such as sameness versus different and above versus below (Avargués-Weber *et al.*, 2011), build complex nests, learn diverse spatial and temporal patterns in its environment, and flexibly adjust foraging tactics to the array of resources that are available and to the needs of her colony (Seeley, 1985, 2010; von Frisch, 1967; see summary in Chittka and Niven, 2009).

A kinorhynch or a gastrotrich might be expected to be behaviourally inferior to a tiny beetle larva or a spider. At least some aspects of gastrotrich locomotory behaviour do seem relatively simple (though more complex than that of unicellular ciliates; Banchetti and Ricci, 1998) when compared, for example, with that of an orb-weaving spider; but their behaviour may not necessarily be simple when compared with that of a tiny ptiliid beetle larva, which has a comparatively gigantic brain (Fig. 4A). The larva probably feeds only on fungal spores it encounters while moving on or within the fruiting body of a fungus, where it lives from the time it hatches from an egg, and defends itself simply by crawling into a spore tube (W. Gene Hall, personal communication). The list of behaviours that a *C. elegans* nematode can perform with its tiny array of 302 structurally simple neurons is striking: it includes context-dependent release of neurosecretory products; sensitivity to various chemicals, O_2 concentration, gentle and harsh contact with other objects, osmolarity, pH, temperature, light and the density and sex of nearby conspecifics via pheromones; and motor functions that include different movements for swimming as well as for crawling on a surface; the ability to turn and to reverse these movements; pacemaker functions to impose rhythms on behaviours; orientation and movement toward and away from various stimuli; foraging for food; using rhythmic movements to engulf and swallow food; grinding ingested food; social feeding; defecation; copulation; oviposition; and learning to modify several of these motor behaviours on the basis of previous experience (Altun and Hall, 2008). Responses to a given stimulus can be complex; for instance, gentle contact can elicit avoidance, reset the defecation cycle, and suppress both pharyngeal movements and oviposition (Altun and Hall, 2008). One is reminded that studies of artificial nerve networks have shown that relatively few neurons are sufficient to produce surprisingly refined behaviour (Smith *et al.*, 2008); perhaps the most pertinent question is not how small brains produce complex behaviour, but rather what is the advantage of having large brains (Chittka and Niven, 2009).

Just as our understanding of the sensory and nervous systems of tiny marine and freshwater animals is based on very limited sampling, our understanding of their behaviour is even more fragmentary, especially for even smaller immature stages. Despite their relatively simple nervous systems (García-Arrarás *et al.*, 2001), larval echinoderms gather numerous particles with their cilia, but when these potential food items arrive at the mouth they may be either swallowed or rejected (Strathmann, 1975). In general, the larvae of many marine invertebrates express behaviour that is more sophisticated than generally appreciated, and, depending on the taxa, may involve sensors that enable functional responses to variation in water

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chemistry from biotic and abiotic sources; water-borne sounds; white light gradients and polarized light; water current movement and water pressure; and magnetism (reviewed in Kingsford *et al*., 2002). For presettlement stages of the larvae of 11 marine taxa, there is a positive association between an index of sensory capabilities and swimming speed, such that faster (and presumably stronger) swimmers have more sensory inputs (Kingsford *et al*., 2002).

In some taxonomic groups the properties of neurons and their connections may be specially designed in especially small individuals so as to allow maximal behavioural capabilities from minimal amounts of nervous tissue (the economy of design option). We give a speculative list of traits of possible interest in this context. The CNS of *C. elegans* displays several apparent economical traits, including muscle plate synapses that allow several muscles to be innervated by a single neuron (Fig. 2; also seen in a copepod nauplius—Lacalli, 2009), and widespread multifunctionality for particular neurons, including various combinations of sensory, motor and inter-neuron functions in single neurons, as well as diversity in a single modality such as sensitivity to several types of stimuli (Altun and Hall, 2008). Neurons with multiple functions also occur in tiny male polychaete worms (Windhoffer and Westheide, 1988).

One set of traits that may be associated with a grade change is the anatomy of individual neurons. In the ''neuron-profligate'' vertebrates, the CNS is dominated by one type of neuron morphology (heteropolar multipolar) that is found only here and there among invertebrates (especially in the visceral plexuses that resemble the diffuse ancestral coelenterate organization; Bullock and Horridge, 1965). Also in contrast with typical invertebrate neurons, many vertebrate synapses are on the cell body, rather than on the dendrites (Bullock and Horridge, 1965). Still another difference was recognized long ago by the pioneering neuroanatomist Ramon y Cajal (see the quote at the head of chapter): the neurons of insects are much more elaborate morphologically than those of vertebrates (see also Strausfeld, 1976). The functional consequences of these different morphologies are not known, so it is not clear whether any of these differences contribute to grade changes in brain allometry.

Morphological exhuberance in neuron morphology does not extend to some even smaller animals such nematodes like *C. elegans* and others, as their only modest numbers of neurons generally have simple processes that are mostly unbranched (Bullock and Horridge, 1965). Even the diameters of different fibres of a given neuron are relatively fixed (Bullock and Horridge, 1965), and the neurons make only very modest numbers of synaptic connections (*C. elegans* has a total of 6400 chemical and 900 gap junction synapses for 302 neurons, giving a mean of 24 synapses/cell; Altun and Hall, 2008). Some individual insect neurons have more branches than the total number of neurons in the entire nervous system of a nematode like *Ascaris* and *C. elegans* (Bullock and Horridge, 1965; Strausfeld, 1976). A less complete inventory of neurons in the nauplius larva of the tiny copepod *Dactylopusia* sp. suggests similarly small numbers and morphological simplicity. For instance, each retinular cell has a

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total of only ''one-several'' synaptic bars with the visual processing cells (Lacalli, 2009). Further studies are needed to understand whether other anatomical diversity within cells, such as the mysterious radial striations seen in many nematode neurons, their tight packing, lack of glial sheaths, and their apparently anastomosing processes (Bullock and Horridge, 1965), also have economy of design consequences.

Morphological differences associated with grade changes are also apparent at another level of organization. The internal division of the brains of very small insects into distinct subregions is less clear than in their larger relatives (Grebennikov, 2008); similarly, the brain of a copepod nauplius also lacks clear segmentation and connectives, although it does have distinct ''cartridges'' that receive visual inputs (their vision functions to trigger escape responses to shadows; Lacalli, 2009). In other extremely small marine invertebrate larvae ($\leq$ 150 microns long), the nervous system is generally diffuse rather than condensed (Lacalli, 2009), suggesting still another possible miniaturization tactic. The fact that vertebrates rely almost exclusively on heteropolar multipolar neuron form suggests that their brain anatomy and function are organized quite differently, with cell bodies and neuropil mixed intimately together (Bullock and Horridge, 1965). In addition, they have a greater morphological variety of glial cells than invertebrates (Bullock and Horridge, 1965). Perhaps the relative numbers of neurons dedicated to sensory (incoming information), motor (outgoing information) and inter-neuron functions (analysis and coordination) also affects economy of design. Sensory and motor neurons constitute the vast majority of the nervous system of *C. elegans* (Altun and Hall, 2008); only 14.2% of the neurons in the CNS of *Ascaris lumbricoides* do not have direct connections with either sense organs or muscles.

Still another suite of traits that might be associated with grade changes and miniaturization concern intra- and inter-cellular transfer of information. Insects appear to more often utilize graded depolarizations (as opposed to the more energetically expensive action potentials) to transmit impulses (Chittka and Niven, 2009). Very tiny marine invertebrates typically lack synapses completely (Lacalli, 2009). In the nauplius of a copepod, three of the nine retinular cells of the eye, as well as some neural processes that end on muscles, lack synapses. The use of analogue transmission is energetically more efficient (see Section 5.3), but the functional significance of the latter two differences is unclear.

In sum, there are many possible kinds of economies of design, but at the moment their possible importance in grade changes is still highly speculative.

7.4 GENERAL IMPORTANCE AND CONSEQUENCES OF BRAIN SCALING

Problems of CNS size and design associated with miniature size probably have a variety of ecological and evolutionary consequences. We briefly discuss a few, many of which are only speculative, to illustrate the wide range of possibilities, and to highlight questions that are rarely asked and are in need of study.

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Due to the higher metabolic demands of nerve tissue, the over-sized brain option to solve miniaturization problems should increase the probability that smaller animals will fall into unfavourable energy balances, unless they have compensatory traits. Simply maintaining a CNS that constitutes $>75\%$ of the entire cephalothorax likely results in sizeable energy costs for small anapid spiders. The energy budget of a newly emerged spiderling of *Nephila clavipes* (0.7 mg) and its susceptibility to shortages of resources must differ substantially from those of a mature female (2000 mg). In addition, relatively large brains take up space that would otherwise be profitably utilized by other structures. For instance, the digestive cecae that are abundant in the cephalothorax of an adult *Phidippus clarus* are nearly completely missing from the cephalothorax of a second instar nymph (Hill, 1975), presumably reducing the speed or efficiency of digestion of food.

Behaviour could also be affected in subtle ways. For instance, there are several types of learning in insects, and long-term memory storage can be more costly energetically (Hoedjes *et al*., 2011). In small individuals, in which energy balance problems are likely to be more acute, animals might be more likely to avoid using long-term memory. Both forming memories and erasing them consume energy (Burns *et al*., 2011), and smaller individuals might opt to minimize the use of memory or learning in general. The organization of behaviour could also be modified by efficiency of design adjustments, using sub-routines to render a given task less demanding. This tactic is employed by designers of robots (Clark, 1998), and by predators such as salticid spiders, which first turn to face directly toward the prey before they attempt to leap onto it.

Another phenomenon possibly related to brain miniaturization involves possible gains and losses of chromosomes. Recent studies have shown that neuronal cells of humans and mice can lose (or gain) a chromosome during normal ontogeny, so that the CNS is a genetic mosaic (Rehen *et al*., 2001; Yurov *et al*., 2005). These aneuploid neurons are not confined to a specific brain region (Kingsbury *et al*., 2005), but are integrated into brain circuitry, and can alter gene expression profiles (Kaushal *et al*., 2003). To our knowledge, nothing is known about the frequency of aneuploid neurons in arthropods, or if they are more frequent in small animals in general. We speculate that a facultative reduction in genome size could be a design economy to reduce the relative nucleus size, and in turn reduce overall cell size. Limited evidence suggests that evolutionary lineages with relatively small genomes do not seem more prone to evolve miniature species, as miniaturization is especially common in frogs and salamanders (Hanken and Wake, 1993), which have unusually large genomes (Gregory, 2002). Nevertheless, reduction in chromosome numbers would be particularly advantageous in just this type of situation. It would be worth searching for facultative genome reductions in the neurons of especially small animals, for more compact packing of their chromatin (Grebennikov, 2008), or for evidence that genes needed for neural function are concentrated on certain chromosomes, thus allowing other chromosomes to be discarded from neurons.

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Differences in general morphological designs within a grade may also be illuminated by understanding brain–body scaling problems. We have already mentioned that the ventrally bulging sterna that are characteristic of small spiders (at least in some groups—R. Madrigal, in preparation) apparently represent adjustments to house their relatively large brains. A search for similar body design changes that correlate with relatively large brain volumes in especially small individuals of other groups might be rewarding. To illustrate possible consequences with one possible example, the brains of especially small ants constitute a very large proportion (16%) of their body weight (Seid *et al.*, 2011). Does the need to house this large brain in the head capsule impose a limit on the sizes of the mandibular muscles, and thus on the possible life styles of especially small ant species?

Possible ecological and life-history changes that may be associated with the energetic consequences of the over-sized brain and the size-limited behaviour options are summarized, respectively, in Table 2A and B. For example, endowing young nymphs with behavioural capabilities comparable to those of adults presumably improves their abilities to forage successfully. The relatively larger size of the eggs of smaller spiders, in terms of the female's body size (Head, 1995), could thus be due at least in part to brain scaling problems, because the

TABLE 2

A sampler of possible ecological consequences of adopting different options to reduce brain scaling problems in small animals (young individuals, or adults of small species)

A. Adopt over-sized brain option
- Reduced capacity to survive in sites where energy resources are less predictable or more scarce (could be due to differences in seasonality, rainfall, fluctuations in prey populations, etc.)
- Reduced foraging efficiency due to reduced use of long-term memory (more energetically expensive)
- Reduced optimality of body design for ecological tasks (agility, rapidity, strength, coordination) due to structural modifications needed to house the relatively large brain
- Reduced clutch sizes, resulting from increased relative size of offspring (due to lower limit of size of ecologically viable offspring)

B. Adopt size-limited behaviour option
- Reduced ability to adaptive behavioural flexibility to respond to changes in biotic and abiotic environment
- Reduced effectiveness of foraging, due to
  - Reduced sensory information
  - Reduced ability to integrate sensory information
  - Reduced ability to learn
  - Inferior motor coordination

The objective here is to call attention to the wide range of possibilities, not to demonstrate proven correlations.

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costs of supporting proportionally larger amounts of nervous tissue in newly emerged nymphs may impose a lower limit on egg size. Smaller species of mites also have smaller clutches of relatively larger eggs (Walter and Proctor, 1999). Grebennikov (2008) notes that many hymenopteran egg parasitoids may escape such a limit because their larvae hatch into a host environment that provides necessary nutrients for them to develop, allowing them to evolve smaller body sizes than non-parasitic species.

For tiny predators such as orb-building spiders, these costs of relatively large brains may be exacerbated if prey that are small enough to be captured are rare. Several types of prey were found, however, being fed upon by adults of the small anapid *A. simoni* in the field; most were small nematocerous flies (56%), and others included ants, collembolans and mites. So at least at this body size (about 0.8 mg) a variety of prey are potentially available (no data are available on the prey of small nymphs of this species).

Similar considerations regarding lower size limits on ecologically functional offspring are probably important in many other groups, because spiders are probably the norm rather than the exception in generating very small offspring from medium sized individuals and in having lower feasible size limits for their offspring. The lower size limits of different taxa are very different. The deformation of brains and the structures that house them in the forms near the lower end of each spectrum (tiny salamanders, tiny beetles, spiders, etc.) suggest that brain allometry problems may be important in establishing these lower body size limits. Presumably the CNS design traits that determine the brain–body allometry line for a given grade or taxon originally evolved in intermediate or large-sized ancestors (this is the case for spiders, judging by recent phylogenies—Wheeler and Hayashi, 1998; Coddington, 2005). The evolution of warm-bloodedness may have helped moderately sized birds and mammals move independently onto allometric lines above that of their reptilian ancestors. Within such a derived group, forms with reduced body sizes may then evolve later, but only down to the limits imposed by the new basic design. At this body size limit, CNS sizes begin to become unsustainably large. As the species of an evolutionary line approach such lower body size limits, selection for compensatory traits, such as reductions in behavioural abilities, or increased efficiency in the amount of nerve tissue needed to generate behavioural abilities is likely to become more intense. The fragmentary data summarized above on orb-weaving spiders indicate that reductions in behavioural abilities have not occurred, despite the relatively great burden imposed by their relatively large CNS. On the other hand, reductions in the relative sizes of portions of the nervous system dedicated to functions other than vision suggest that behavioural losses (which have not been detected) may have occurred in salamanders (Hanken and Wake, 1993).

The likely high intensity of natural selection favouring design economy in such species means that their neurophysiology and neuroanatomy are likely to be especially interesting, and worthy of study. Possibly the reduced internal

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differentiation into separate substructures of the brain (central body, corpora pedunculata, commissures, chiasmata) in the tiny larvae of strepsipterans and ptiliid beetles (Beutel *et al.*, 2005) results from economy of design, using neurons for multiple tasks (Chittka and Niven, 2009). Similarly a single neuronal pathway in a rotifer appears to connect both the lateral horns and dorsolateral apical receptors, suggesting that convergence of synaptic connections may be common in the afferent sensory systems of rotifers (Hochberg, 2009). Some other modifications also seem plausible. The tendency toward greater condensation or fusion of the CNS in especially tiny insects (Grebennikov, 2008) may reflect a more favourable balance between transmission distances for intraganglion stimuli as opposed to sensory and motor stimuli that enter and leave the ganglion. A similar explanation may account for the recurrent fusion of neighbouring ganglia in the ventral nerve cord of insects (Niven *et al.*, 2008; Wille, 1961). On the other hand, why is it that the nervous systems of tiny larvae of marine invertebrates are dispersed rather than centralized (Lacalli, 2009)?

Many of the ideas here are only speculations, but it seems likely that exciting discoveries are waiting to be made from studies of the nervous systems of miniature animals.

7.5 LIMITATIONS OF CURRENT DATA AND QUESTIONS FOR THE FUTURE

Discussing the evolution of ''the'' brain as a single entity can be seriously misleading. A brain is a mosaic of many interacting but to some extent independent or modular regions that can evolve semi-independently. Wide-ranging comparisons like those presented here can be helpful in understanding brain evolution, but are they complicated by the fact that different functions may be performed in different portions of the CNS in different taxa (Table 3). For

TABLE 3
Divisions of the CNS in different taxa that correspond roughly to each other with respect to function

Vertebrates	
Brain	Spinal cord
Insects	
Brain (supra- + sub-esophageal ganglia)	Ventral nerve cord (thoracic and abdominal ganglia)
Spiders	
Supra-esophageal ganglion	Sub-esophageal ganglion
Tiny invertebrates	
Cephalic ganglion	Other ganglia

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instance, the ''brain'' of an insect corresponds to some extent to the supra-esophageal ganglion of a spider, but there is no certainty that the functions that are carried out in the brain of an insect are necessarily performed in the supra-esophageal rather than the sub-esophageal ganglion of a spider. Comparing brains or their equivalents in different groups in order to understand behavioural capabilities may to some extent involve comparing apples and oranges. One hopes that improved knowledge of the functions of different portions of the nervous systems of different animals will permit a more precise focus in future comparisons. The question of whether some functions are performed at different sites (brain vs. spinal cord, head ganglia vs. ventral nerve cord) in different groups can be difficult to untangle. For example, learning typically is thought to occur in the brain, but simple lessons (e.g. altering leg position to avoid noxious, repeated stimuli) can be learned by the ventral nerve cord (VNC) of a headless cockroach (Horridge, 1962; learning was faster and retention was longer, however, when insects had an intact head/brain—Chen *et al*., 1970). Similar spinal cord learning is also known in mice and other vertebrates (e.g. Jindrich *et al*., 2009). The question is open as to whether or not miniature animals shunt control of some functions from the brain to ganglia in the VNC or vice versa. Comparisons involving the entire central nervous system (CNS) would thus be preferable, especially when widely different taxa are involved. But even here interpretation can be complicated, as the sense organs of some groups can perform functions performed by the CNS in other groups (Franzl and Barth, 2009; Wehner, 1987).

Unfortunately, most published data on insects and vertebrates regarding the allometry of nervous systems concern measurements of the ''brains'' rather than of the entire CNS, so we have been obliged to use these data rather than CNS data in most places. Even within vertebrates, the percentage of the CNS dedicated to the spinal cord (rather than the brain) varies widely; in 31 species it ranged from 2.3% in a porpoise to 37.1% in a domestic chicken (MacLarnon, 1996). The allometric slope of spinal cord mass on body mass is lower than that for brain on body mass, and reconstruction of a large dinosaur suggests its spinal cord mass was even greater than that of its brain (Striedter, 2005). MacLarnon (1996, p. 81) concluded that ''. . . there are significant differences in the size of the cord relative to body size'' between vertebrate classes. In insects there is a dearth of data to assess how the total nervous system is distributed between the brain and the ventral nerve cord (VNC; Niven *et al*., 2008; Wille, 1961). In a subterranean termite, *Reticulitermes flavipes* (Rhinotermitidae), the cross-sectional areas for the brain, and each ventral nerve cord ganglion (three in the thorax and nine in the abdomen) differed with respect to the total nervous system between for female alates (57%), soldiers (48%) and workers (42%) (Nuss *et al*., 2008). As noted in the caption of Fig. 1, values for ''brains'' of spiders and mites, which include ganglia corresponding to those of the VNC of insects, are higher than would be appropriate for comparisons with most other groups. Extreme miniaturization, as well as fusion of different ganglia, may alter these relative proportions (Beutel *et al*., 2005; Grebennikov, 2008; Niven, 2010).

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As noted above, behavioural data on tiny species are extremely limited in both number and degree of subtlety. Our current ignorance is probably due largely to the theoretical difficulty of quantifying behavioural traits, the practical difficulty of studying the behaviour of tiny animals, and the biased idea that larger animals (e.g. adults as opposed to immatures) are more interesting or important. An especially large hole in current knowledge of the behaviour of tiny species is a nearly complete lack of data on their abilities to learn. Tiny parasitoid wasps are able to learn relatively simple lessons such as changes in attraction to different hosts on the basis of the host plants on which their host larva developed (Hanson and Gauld, 2006; Papaj and Lewis, 1993), and the nematode *C. elegans* can learn lessons associated with several different types of stimuli (Altun and Hall, 2008); but the comparative ease with which large and small species learn the same lessons remains to be investigated. Much more work will be needed to obtain a reasonable evaluation of how frequently the size-limited option has been used.

Another significant limitation to understanding brain-behaviour relations is the dearth of comparative data resulting from the collective blinders imposed by the ''model system'' approach to studies of both behaviour and morphology; approximately 75% of research efforts by neuroscientists involve brains of mice, rats and humans, an infinitessimal fraction of the nervous systems on the planet (Manger *et al.*, 2008, p. 1). Another limitation is that we have been consistently typological throughout this discussion because of the lack of data on intra-specific variation, even though we know that the brains of vertebrates and insects (and probably other groups) show both genetic variation and experience-dependent plasticity in size and cell number. There are differences of up to 100% in the numbers of neurons for at least some functions in different races of the same species, as well as substantial variation even within members of the same population (Burns *et al.*, 2009; Heisenberg *et al.*, 1995; Miklos, 1998). We have used a single value for the weight of each species, when obviously weights vary substantially even for a single individual. We used the gross weight, without discriminating lean body mass, which correlates more strongly with brain weight in some species (Schoenemann, 2004). Hopefully future studies will be more sophisticated in this respect and assess the functional significance of variation in such traits. We have interpreted graphs without attempting to correct for phylogenetic inertia, but believe that the relatively tight groupings of the members of most taxa (except insects—see Fig. 1) around the same line for that taxon indicates that such correlations would not likely change our conclusions.

A significant deficiency in our understanding of the neuroanatomy of miniature invertebrates is data on how neuronal cell size and neuron number (and hence neuronal density) scale with body size (for vertebrates, see e.g. Herculano-Houzel, 2010; Sarko *et al.*, 2009). The limited studies that present data on cell size or neuron number (e.g. Babu, 1975; Babu and Barth, 1984 on spiders) do not always specify the stereological methods used, so it is not clear if appropriate

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sampling methods were used to infer three-dimensional properties from two-dimensional samples (see Howard and Reed, 1998).

The short-comings sketched here can serve as a roadmap to outstanding questions for the future, which will provide the data needed to more fully understand the causes and consequences of brain miniaturization, and its limitations. The length of the list emphasizes the speculative nature of much of our discussion. In many cases we lack data to answer these questions because they are rarely asked. We need to understand the causal mechanism(s) underlying Haller's Rule (our only progress in the present paper has been to argue against some previous hypotheses). The extent to which these design challenges shape animal evolution is not clear, nor is it clear to what extent different solutions have been adopted by different lineages. Detailed neurophysiological, neuroanatomical and behavioural data are needed for a wide taxonomic range of animals and life-history stages. Several taxonomic groups might be especially promising for future studies. Especially tiny invertebrates such as kinorhynchs and planktonic crustaceans are of obvious interest. Ants are also interesting, because they seem to lie on an allometric line below those of some other insects (Fig. 1B). Even relatively large ant species (which should presumably be easier to study) could be interesting, as they lie on the same line. In addition, detailed behavioural studies of leaf-cutter ants, *Atta*, in which a huge range of sizes is spanned in a single species, checking tiny workers for signs of size limitation such as reduced ability to learn, greater clumsiness, higher error rates, or reduced behaviour repertoires could be interesting. Another interesting group is the mites. The fragmentary data currently available suggest that the allometric line of mites lies below those of insects and spiders (Fig. 1B). In accord with this, mites have evolved adult body sizes substantially below those of the smallest insects and spiders (Grebennikov, 2008). Some mites are nevertheless capable of analyses and learning, using a brain with only approximately 10,000 neurons (van Wijk *et al*., 2006). Both the behaviour and the nervous systems of this poorly studied group would repay further study.

As we noted above, many brain-behaviour associations may be obscured by too much lumping. We do not know the extent to which the same behaviours in different lineages are governed by the same brain regions, or whether different lineages rely to different extents on mechanisms such as learning. For most taxa of small invertebrates we need stereologically valid (Howard and Reed, 1998) data on neuronal cell size, number and density to assess whether animals with extremely small body sizes maintain information-processing capabilities comparable to those of large-bodied species via a reduction in neuron size. Similarly, we do not know how taxa of strikingly different body sizes have adjusted the relative numbers of glia and non-glia cells, or more generally whether different taxa follow different cellular scaling rules (e.g. Herculano-Houzel *et al*., 2006; Sarko *et al*., 2009). Understanding behavioural variation requires a way to replicate in other taxa the kinds of data that have been collected with

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orb-weaving spiders, which in turn requires methods to make quantitative behavioural comparisons among disparate taxa.

7.6 CONCLUSIONS

The evolution of small body sizes poses a number of challenges to animals, because smaller individuals tend to have larger and disproportionately costly brains, which reach, in small insects and spiders, up to 15–16% of the animal's body mass. To accommodate these relatively large brains, some species use other portions of the body to house the CNS, deform structures to increase internal space, or displace internal tissues. This would imply that extremely small animals sacrifice some morphological aspects of body design to accommodate their relatively large brains. Their relatively large brains are also probably costly, because nervous tissue is metabolically expensive to maintain. These problems of diminutive size are of very general importance, because they occur not only in species with miniature adults (and even smaller immatures), but also in many other species with large adults and with very small, free-living early developmental stages.

Increased intensity of natural selection favouring reduced costs in small animals could favour traits that are less demanding in terms of behavioural capabilities. To date, however, the only detailed behavioural studies that are available, concerning the construction behaviour of orb web spiders, suggest that very small animals are not behaviourally impaired compared with larger individuals.

The brain scaling in insects and spiders is quantitatively different from that of vertebrates, and probably also from that of very small invertebrates such as nematodes, tiny crustaceans, gastrotrichs and kinorhynchs. Such ''grade changes'' in brain allometry are probably not always linked in simple ways to differences in behavioural capabilities, and may instead (or in addition) be due to differences in the anatomy of their neurons and other basic aspects of how their nervous systems operate. We have provided largely speculative lists of possible economies in design. The idea that there are profound differences in mechanisms of brain function in different taxa seems to fly in the face of some basic similarities in the anatomical designs of sets of neurons subserving similar sensory processes in widely different taxa (e.g. structures analysing olfactory stimuli in insects, crustaceans and vertebrates; visual pathways in cephalopods, vertebrates and arthropods—Strausfeld, 1976; Chittka and Niven, 2009). Nevertheless, the contrast between the few hundred structurally simple neurons whose modest numbers of processes are connected computer-like, only in certain, consistent ways in the brains of nematodes (Fig. 14; Altun and Hall, 2008; Bullock and Horridge, 1965), as opposed to the astronomical numbers of neurons in a human brain, with huge numbers of connections for each cell (a typical pyramidal cell in the human cortex can have 10,000 connections—Edelman, 1998), whose numbers, properties and connections

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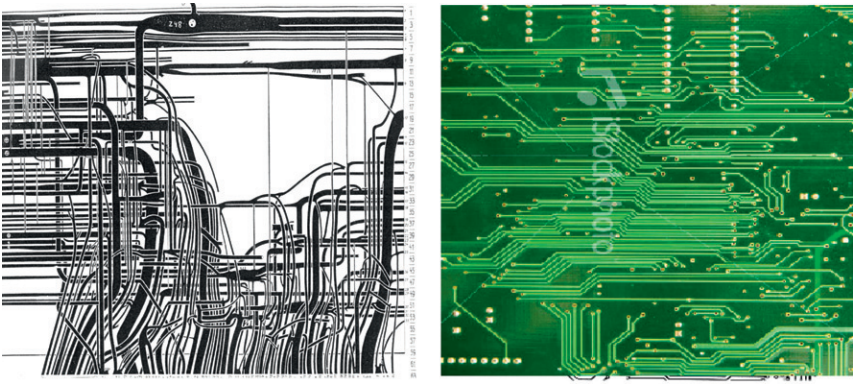

Ascaris megalocephala Chip computer

Goldschmidt 1909 Google Images 2011

FIG. 14 Diagrammatic representation (left) of the connections of 70 of the 134 fibres in the circumenteric ring near the origin of the ventral nerve cord of the nematode *Ascaris megalocephala* (from Bullock and Horridge, 1965, after Goldschmidt, 1909). The uncanny visual resemblance to a computer chip (right) emphasizes the computer-like traits of nematode nervous systems (fixed numbers of elements, invariant connections between them) that are exactly the computer-like aspects that do *not* occur in vertebrate nervous systems (Edelman, 1998). This emphasizes the likely profound differences in how the brains of taxa in different grades function.

are continually shifting with use and disuse, whose patterns of behaviour are based only on population trends rather than individual consistency, and which show a highly degenerate organization that provides multiple physical representations of something like a memory (Edelman, 1998), suggests quite different modes of operation. We believe that grade changes, which we have emphasized in this paper, have probably involved the evolution of such basic differences.

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