

The Allometry of Brain Miniaturization in Ants

Marc A. Seid^a Armando Castillo^a William T. Wcislo^a

Smithsonian Tropical Research Institute, Panama, Republic of Panama

Key Words

Allometry · Ants · Brain evolution · Miniaturization · Polymorphism

Abstract

Extensive studies of vertebrates have shown that brain size scales to body size following power law functions. Most animals are substantially smaller than vertebrates, and extremely small animals face significant challenges relating to nervous system design and function, yet little is known about their brain allometry. Within a well-defined monophyletic taxon, Formicidae (ants), we analyzed how brain size scales to body size. An analysis of brain allometry for individuals of a highly polymorphic leaf-cutter ant, *Atta colombica*, shows that allometric coefficients differ significantly for small (<1.4 mg body mass) versus large individuals ($b = 0.6003$ and 0.2919 , respectively). Interspecifically, allometric patterns differ for small (<0.9 mg body mass) versus large species ($n = 70$ species). Using mean values for species, the allometric coefficient for smaller species ($b = 0.7961$) is significantly greater than that for larger ones ($b = 0.669$). The smallest ants had brains that constitute ~15% of their body mass, yet their brains were relatively smaller than predicted by an overall allometric coefficient of brain to body size. Our comparative and intraspecific studies show the extent to which nervous systems can be miniaturized in taxa exhibiting behavior that is apparently comparable to that of larger species or individuals.

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Introduction

Haller's Rule holds that the brains of smaller animals are proportionally larger than those of large-bodied forms [see Rensch, 1948]. This allometric relationship between body and brain size has been documented extensively for vertebrates [Cuvier, 1845; Harvey and Krebs, 1990; Hanken and Wake, 1993; Striedter, 2005; Gonzalez-Voyer et al., 2009]. In contrast, relatively little is known about brain allometry for the numerous invertebrate taxa with extremely small body sizes, such as tardigrades [Zantke et al., 2008] or Arthropoda (insects, mites and spiders) [see also Rensch, 1948; Cole, 1985]. This dearth of information is surprising because such small animals dominate earth's biodiversity [Grimaldi and Engel, 2004], and long ago Darwin [1871, p. 145] called attention to the ant brain as being 'marvellous', because such an 'extremely small' mass of nervous tissue could generate 'extraordinary mental activity'. Kern [1985] presented data on brain and body mass for 36 species in 8 orders of insects, but did not statistically analyze the allometric relationships. The most extensive allometric study on invertebrate brains presented data from 10 ant species, albeit with limited taxon sampling: 5 species were in the genus *Cataglyphis* and 5 were from 2 other genera (all from 1 subfamily, Formicinae) [Wehner et al., 2007]. That study demonstrated that the allometric scaling component for ants ($b = 0.57$) was similar to that for birds ($b = 0.58$) and reptiles ($b = 0.54$) but significantly different from that of

mammals ($b = 0.77$). Yet the smallest species reported in that study was ~ 2.5 mg, which is relatively large in comparison to many arthropods. In contrast, there are detailed volumetric brain studies of particular beetle (Coleoptera) or Strepsiptera species with extremely small body sizes [e.g. Beutel et al., 2005; Grebennikov, 2008; Polilov, 2008; Polilov and Beutel, 2010], but there are no data for closely related large-bodied forms.

Here we ask whether the scaling relationships observed in other taxa hold for animals with very small body sizes compared with large-bodied relatives? We studied intraspecific brain scaling in the leaf-cutter ant, *Atta colombica*, in which body mass spans 3 orders of magnitude, and polymorphic workers differ in behavior and physiology [Weber, 1972; Hölldobler and Wilson, 1990]. Furthermore, *Atta* ants have a diphasic cephalic allometry [Wilson, 1953], so we also tested for diphasic allometry in brain volume. Interspecifically, we extended an earlier study of brain scaling in ants [Wehner et al., 2007] by substantially increasing the number of species ($n = 70$ vs. 10); the taxonomic coverage (31 genera from 5 subfamilies and 2 informal groupings vs. 3 genera from 1 subfamily); and we included species that are over 60 times smaller (0.039 vs. 2.5 mg body mass), spanning approximately 4 orders of magnitude among species.

Materials and Methods

Ant Collections

For the intraspecific study, specimens were collected from a single nest of *Atta colombica* in Gamboa, Colon Province, Republic of Panama. There is extensive continuous size variation among *Atta* workers [Weber, 1972], and to sample the full range of size variation we dug into various fungus chambers and collected workers in or near the gardens, along with soldiers; newly emerged callow workers were excluded, but otherwise the ages of individuals were unknown. Individuals of 70 ant species were collected either from queen-right laboratory colonies or as individual foragers, either in the vicinity of Gamboa, or near Gainesville, Fla., USA (table 1). Voucher specimens are deposited in the Dry Reference Collection of the Smithsonian Tropical Research Institute, and the Museo de Invertebrados 'Graham Fairchild' de la Universidad de Panamá.

Interspecific Body and Brain Measurements

In most ant species there is continuous variation in worker size [Hölldobler and Wilson, 1990], but some species are monomorphic (i.e. a single mode in worker body size distribution), while others are polymorphic (i.e. multiple modes in the distribution of worker body size). It may be problematic to compare workers having different social roles among different species (e.g. large soldiers vs. small workers), or monomorphic and polymorphic spe-

cies. Furthermore, the sampling among taxa was uneven because we included multiple individuals for the polymorphic species in order to capture the full size range in polymorphic species (table 1). To address these 2 problems, we conducted 1 set of analyses using the full data set ($n = 261$ ants from 70 species), and a second set of analyses using mean values for each species ($n = 70$); we refer to these as full and reduced data sets, respectively.

*Histological Brain Sectioning and Volumetric Reconstructions for *Atta colombica**

Ants were weighed using an AND® GR-202 microbalance (accuracy to 0.01 mg). The brain of each ant was quickly removed from the head capsule and immediately placed in fixative (6% glutaraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer) in preparation for standard histological sectioning. After fixation for 12–24 h, the brains were rinsed in cacodylate buffers and post-fixed in 1–1.5% osmium tetroxide for 2–3 h. The brains were then rinsed in buffer followed by H₂O and dehydrated in DMP and acetone in preparation for embedding in Epon®. Brains were infiltrated in Epon, by first placing them in a 50/50 mixture of Epon/acetone and then transferring them to 100% Epon. They were then embedded in Epon in Beem® capsules and cured at 60°C overnight. Embedded brains were sectioned in a microtome (Microm® HM 355s) at 5- μ m sections using stainless steel disposable knives. Serial sections were kept in order and placed individually on glass slides and then stained with toluidine blue. Coverslips were then placed over the sections using Permamont® and the sections were photographed using a Nikon® 8700 camera attached to a Nikon® Eclipse E600 compound microscope. Serial digital sections were then imported into a computer, and were traced, aligned and stacked using the program Reconstruct [Fiala, 2005] to calculate the 3-D volume of each brain.

Measurements of Brain Mass for Interspecific Comparisons

For the interspecific study we used brain mass as a measure of size. Collected ants were weighed using a Sartorius® CPA2P microbalance after their removal from laboratory colonies or usually within 24 h after collection from the field. Collected ants were dissected under cold Ringer's solution (150 mM NaCl, 24 mM KCl, 7.0 mM CaCl₂, 4.0 mM MgCl₂, 5.0 mM HEPES buffer, and 131 mM sucrose, pH = 7.0). The brain, including both the supra- and sub-oesophageal ganglion and all sensory lobes, was quickly removed from the head capsule, usually in less than 1 min, and then cleaned of all tracheae and fat bodies. Each brain was then placed on a small piece of tared Parafilm® within a small droplet of Ringer's solution. The Ringer's solution was wicked away using finely twisted pieces of Kimwipes® and the brain was weighed within 4 s. To assess weight loss due to water evaporation from exposed brains, we measured weight loss through time for 5 ants of different body sizes. The steepest rate of water loss occurred within the first 20 s following removal from Ringer's solution (data not shown). We used data points for the first 20 s starting at the time we could detect weight loss, given the 1- μ g resolution of the balance, to calculate the slope of the rate of weight loss from a linear regression, and we took this to be the maximum rate loss. We used this worst-case slope to calculate the expected loss of weight over the interval needed to prepare and weigh the specimen, and then expressed this weight loss as a percentage of total brain mass (fig. 1).

Table 1. List of taxa included in allometric analyses, with ranges for body and brain sizes, and mean brain size

Genus	Morph category	Body size range ^a , mg	Brain size range ^a , mg	Mean brain size ^a , mg	n
Myrmicinae					
<i>Acromyrmex echinator</i>	P	0.658–22.244	0.006–0.255	0.127	20
<i>Apterostigma</i> sp. 1	M	0.510–0.737	0.041–0.042	0.042	3
<i>Apterostigma</i> sp. 2	M	0.724–0.750	0.048–0.054	0.051	3
<i>Apterostigma</i> sp. 3	M	2.280–2.482	0.080–0.084	0.082	3
<i>Atta colombica</i>	P	0.410–49.546	0.033–0.238	0.115	17
<i>Cyphomyrmex cornutus</i>	M	0.347–0.433	0.025–0.032	0.029	3
<i>Cyphomyrmex longiscapus</i>	M	0.347–0.429	0.023–0.035	0.028	3
<i>Cyphomyrmex muelleri</i>	M	0.457–0.607	0.032–0.035	0.033	3
<i>Cyphomyrmex</i> sp. 1	M	0.385–0.435	0.027–0.035	0.030	3
<i>Mycetophylax</i> sp.	M	1.399–1.574	0.044–0.050	0.047	3
<i>Mycrocepurus smithii</i>	M	0.302–0.338	0.021–0.024	0.022	3
<i>Myrmicocrypta</i> cf. <i>ednaella</i>	M	0.310–0.329	0.020–0.029	0.023	3
<i>Sericomyrmex</i> sp.	M	1.127–1.514	0.048–0.058	0.053	3
<i>Trachymyrmex coniktzi</i> (1)	M	0.863–0.939	0.045–0.050	0.047	3
<i>Trachymyrmex cornetzi</i> (2)	M	1.115–1.356	0.051–0.074	0.060	3
<i>Trachymyrmex</i> sp. 1	M	1.117–1.248	0.048–0.052	0.050	3
<i>Trachymyrmex</i> sp. 2	M	2.149–2.373	0.062–0.074	0.070	3
<i>Trachymyrmex zeteki</i>	M	1.971–2.282	0.061–0.066	0.064	3
<i>Cephalotes atratus</i>	P	21.025–42.506	0.341–0.428	0.417	5
<i>Cephalotes</i> sp. 1	P	3.160–11.560	0.100–0.190	0.140	6
<i>Cephalotes umbraculatus</i>	M	7.98	0.160	0.160	1
<i>Crematogaster</i> sp.	M	0.795	0.044	0.044	1
<i>Megalomyrmex</i> sp. 1	M	0.137	0.010	0.010	1
<i>Megalomyrmex</i> sp. 2	M	1.147–1.231	0.044–0.051	0.057	2
<i>Monomorium floricola</i>	M	0.065	0.006	0.006	1
<i>Monomorium trageri</i>	M	0.098–0.104	0.009–0.011	0.010	3
<i>Pheidole obscurithorax</i>	D	0.539–0.569	0.029–0.031	0.030	3
<i>Pheidole</i> sp. 1	D	0.093	0.008	0.008	1
<i>Pheidole</i> sp. 2	D	0.234	0.016	0.016	1
<i>Pheidole</i> sp. 3	D	0.069	0.008	0.008	1
<i>Pheidole</i> sp. 4	D	0.102	0.011	0.011	1
<i>Pheidole</i> sp. 5	D	0.254	0.020	0.020	1
<i>Pheidole</i> sp. 6	D	0.122	0.013	0.013	1
<i>Pheidole</i> sp. 7	D	0.873–1.181	0.047–0.055	0.049	3
<i>Pheidole</i> sp. 8	D	0.964–1.365	0.042–0.051	0.047	3
<i>Pheidole</i> sp. 9	D	0.471–0.587	0.028–0.029	0.028	3
<i>Pogonomyrmex badius</i>	P	3.503–40.379	0.112–0.240	0.139	20
<i>Solenopsis</i> sp. 1	M	0.086–0.111	0.010–0.010	0.010	2
<i>Solenopsis</i> sp. 2	M	0.473	0.031	0.031	1
<i>Wasmannia auropunctata</i>	M	0.109	0.007	0.007	1
Ectatomminae					
<i>Ectatomma ruidum</i>	M	8.184–12.878	0.208–0.232	0.220	2
<i>Ectatomma tuberculatum</i>	M	15.950–21.090	0.380–0.400	0.387	3
<i>Gnamptogenys</i> sp. 1	M	0.576	0.028	0.028	1
<i>Gnamptogenys</i> sp. 2	M	9.873	0.244	0.244	1
Dolichoderinae					
<i>Azteca</i> sp. 1	M	0.717	0.051	0.051	1
<i>Azteca</i> sp. 2	M	1.743	0.072	0.072	1
<i>Dolichoderus</i> sp.	M	3.963–5.202	0.135–0.140	0.138	3
<i>Tampinoma melanocephalum</i>	M	0.132	0.011	0.011	1
<i>Brachymyrmex</i> sp. 1	M	0.039–0.049	0.005–0.007	0.006	3
<i>Brachymyrmex</i> sp. 2	M	0.064–0.085	0.006–0.007	0.006	3

Table 1 (continued)

Genus	Morph category	Body size range ^a , mg	Brain size range ^a , mg	Mean brain size ^a , mg	n
Formicinae					
<i>Camponotus sericeiventri</i>	P	37.39	0.440	0.440	1
<i>Camponotus</i> sp. 1	M	5.0525	0.184	0.184	1
<i>Camponotus</i> sp. 2	P	7.720–36.540	0.190–410	0.295	13
<i>Camponotus</i> sp. 3	P	13.940–37.390	0.310–0.360	0.304	9
<i>Paratrechina longicornis</i>	M	0.342	0.028	0.028	1
Dorylomorphs					
<i>Eciton burchellii</i>	P	1.503–33.395	0.079–0.305	0.202	31
<i>Nomamyrmex</i> sp.	P	6.555–17.234	0.169–0.238	0.204	4
Poneromorphs					
<i>Odontomachus bauri</i>	M	15.626–15.939	0.292–0.332	0.312	2
<i>Odontomachus brunneus</i>	M	5.828–6.557	0.190–0.193	0.192	2
<i>Odontomachus hastatus</i>	M	22.970–27.680	0.410–0.460	0.430	3
<i>Pachycondyla apicalis</i>	M	39.685–41.749	0.606–0.711	0.659	2
<i>Pachycondyla obscuricornis</i>	M	15.129–15.226	0.369–0.424	0.397	2
<i>Pachycondyla</i> sp. 1	M	9.7	0.190	0.190	1
<i>Pachycondyla</i> sp. 2	M	4.358–4.599	0.148–0.162	0.155	2
<i>Pachycondyla</i> sp. 3	M	2.723	0.075	0.075	1
<i>Pachycondyla villosa</i>	M	41.480–51.390	0.470–0.521	0.503	3
<i>Paraponera clavata</i>	M	153.89–183.68	1.620–1.750	1.730	3
Pseudomyrmecinae					
<i>Pseudomyrmex</i> sp. 1	M	2.889	0.135	0.135	1
<i>Pseudomyrmex</i> sp. 2	M	0.460–0.537	0.038–0.050	0.045	3
<i>Pseudomyrmex</i> sp. 3	M	4.850–5.606	0.259–0.281	0.277	3

P = Polymorphic; M = monomorphic; D = dimorphic, but only the minor subcaste was used.

^a Range and mean not given for these taxa represented by singletons.

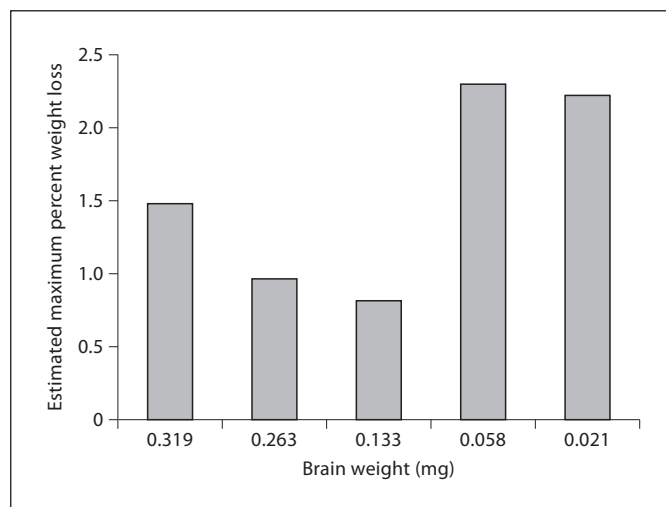


Fig. 1. Estimated maximum weight loss due to water evaporation as a function of brain weight during the time needed to prepare a specimen.

Statistical Methods

We used the statistical package R for the piecewise regression analyses, as well as for the comparisons of slopes by utilizing the ‘smatr’ library [Crawley, 2007]. Piecewise regression is a statistical method to split a single linear regression to assess whether a 2-slope model, or one with more slopes, would better fit the data than a 1-slope model [McGee and Carleton, 1970], and is commonly used in identifying different growth trajectories for dimorphic or polymorphic phenotypes in insects [e.g. Eberhard and Gutierrez, 1991; Eberhard et al., 2000]. The breakpoint is the point at which the trajectory of 1 morphotype changes to another. Breakpoints are identified by analyzing multiple models and selecting the one with the lowest residual standard errors (RSE) as providing the best fit. To access the location of the breakpoint, we fitted models for different breaks in the data using R [Crawley, 2007], and then visually inspected the RSE. To assess the robustness of the breakpoint, we repeated the regression analyses manually at each of 4 additional breakpoints, at 0.1- and 0.2-mg increments above our statistically identified breakpoint, and at 0.1 and 0.2 mg below this initial breakpoint. For each of the 4 new breakpoints we recalculated R² values and RSE. In each comparison the original breakpoint had the highest R² and lowest RSE values. We also tested whether a

model of 3 slopes would significantly improve the fit, because of the well-known observation that more complex models better fit the data than less complex ones [see discussion in Striedter, 2005].

We used major axis regression to account for error in both the x-axis and y-axis, and comparisons of slopes were performed as outlined by Warton et al. [2006], using the sample correlation between residuals and fitted values. We calculated phylogenetically independent contrasts to account for an expected lack of independence associated with phylogenetic structure [Felsenstein, 1985; Harvey and Pagel, 1991; Ricklefs and Starck, 1996], using log-transformed data analyzed with the PDAP module [Midford et al., 2005] in MESQUITE v. 2.72 [Maddison and Maddison, 2007]. The phylogeny from Brady et al. [2006] was used to create a tree at the generic level for the taxa in our analysis. Ants within a single genus were designated in the tree as an unresolved polytomy for this analysis. We set branch lengths to 1, and subtracted 1 degree of freedom for each polytomy in our analysis to yield our phylogenetic independent contrast slope for our data [Harvey and Pagel, 1991]. In additional analyses, we removed all polymorphic species [*sensu* Hölldobler and Wilson, 1990], and in cases where there were multiple individuals per species, we calculated a mean value for each species' brain and body mass, and repeated the analyses.

Results

The allometric relationship between brain volume and body mass was significant for individuals of the highly polymorphic species *Atta colombica* ($n = 48$; $F_{1,46} = 612.2$, $p < 0.0001$) (fig. 2a). A piecewise regression model with 2 regression equations fitted the data significantly better than a single-slope model, with a breakpoint at 1.4 mg body mass (ANOVA, $F_{1,2} = 20.024$, $p < 0.0001$; 2-slope model – $R^2 = 0.9634$ and $RSE = 0.09517$; 1-slope model – $R^2 = 0.9301$ and $RSE = 0.1286$); a model with 3 slopes was not significantly different from the 2-slope model ($F_{1,2} = 1.3937$, $p = 0.2594$). The RSE for the ± 0.1 and ± 0.2 incremental steps from this breakpoint (i.e. body masses of 1.2, 1.3, 1.5, and 1.6 mg) were 0.1149, 0.1012, 0.1009, 0.1009, respectively, and all were greater than the RSE at the 1.4 mg breakpoint. The allometric coefficient (the slope of the model) from the 2-slope model was significantly greater for the set of small *A. colombica* (< 1.4 mg) than the coefficient for larger individuals, or from the single-slope model (BLR = 7.8269; $p = 0.0052$; BLR = 5.659, $p = 0.0017$, respectively; fig. 2a). The coefficient for the larger *A. colombica* was significantly different from that of the single-slope model (BLR = 6.6981, $p = 0.0096$).

Similar patterns of brain scaling were observed among 70 species of ants ($n = 261$ individuals) using mass as a measure of brain size (fig. 2b). The allometric relationship was significantly different for ant species above and below a breakpoint of 0.9 mg body mass (fig. 2b). A piece-

wise regression model with 2 equations fit the data significantly better than a single-slope model (2-slope model: $R^2 = 0.9475$ and $RSE = 0.2568$; single-slope model – $R^2 = 0.9389$ and $RSE = 0.276$; $F_{1,2} = 21.07$, $p < 0.0001$, fig. 2b), but a model with 3 slopes did not improve the fit ($F_{1,2} = 1.5871$, $p = 0.2065$). The RSE for the ± 0.1 and ± 0.2 incremental steps from the 0.9 mg breakpoint (i.e. body masses of 0.7, 0.8, 1.0, and 1.1 mg) were 0.2572, 0.2570, 0.2574, and 0.2580, respectively, and all were greater than the RSE at the 0.9-mg breakpoint. As with the intraspecific analysis, the allometric coefficient for smaller ants (< 0.9 mg) from the 2-slope model was greater than that from the piecewise slope for the larger ants, or from the single-slope model [Bartlett-corrected likelihood ratio (BLR) = 54.67, $p < 0.0001$; BLR = 51.01, $p < 0.0001$, respectively; fig. 2b].

Using a reduced data set based on mean values for species, again a 2-slope model with a breakpoint at 0.9 mg body weight fitted the data significantly better than a single-slope model (2-slope model – $R^2 = 0.9762$ and $RSE = 0.2071$; single-slope model – $R^2 = 0.972$ and $RSE = 0.2173$; $F_{1,2} = 4.443$, $p = 0.016$; fig. 2c), but a 3-slope model did not ($F_{1,2} = 0.289$, $p = 0.7433$). The RSE for the ± 0.1 and ± 0.2 incremental steps (i.e. body masses of 0.7, 0.8, 1.0, and 1.1 mg) were 0.2113, 0.2072, 0.2074, 0.2088, respectively, and all were greater than the RSE at the 0.9-mg breakpoint. The slope for the smaller ants (< 0.9 mg) is again significantly steeper than for larger ants or from the single-slope model (BLR = 5.7094, $p = 0.016$; BLR = 11.9467, $p = 0.21$, $p = 0.0005$, respectively; fig. 2c). Taking phylogenetic structure into account, the slope was not significantly different (sample correlation between residuals and fitted values = -0.016 , $p = 0.8979$). Plotting the ratio of brain:body mass against body mass yields a steep exponential decay function ($y = 0.0473e^{-0.3876x}$, $R^2 = 0.8347$) such that the proportion of body mass comprised of brain is substantially greater for the smallest ants; the smallest ants (*Brachymyrmex* sp.) had brains that accounted for $> 15\%$ of their body mass (fig. 2d). Small ants (< 0.9 mg) occur in all the major taxa that we sampled, except for those in the informal groups dorylomorphs and poneromorphs (fig. 3), so there is no basis to suspect that the differences are taxon dependent rather than size dependent.

Discussion

Even though the smallest ants in our study had relatively massive brains, constituting $\sim 15\%$ of their body mass, we observed no morphological modifications of

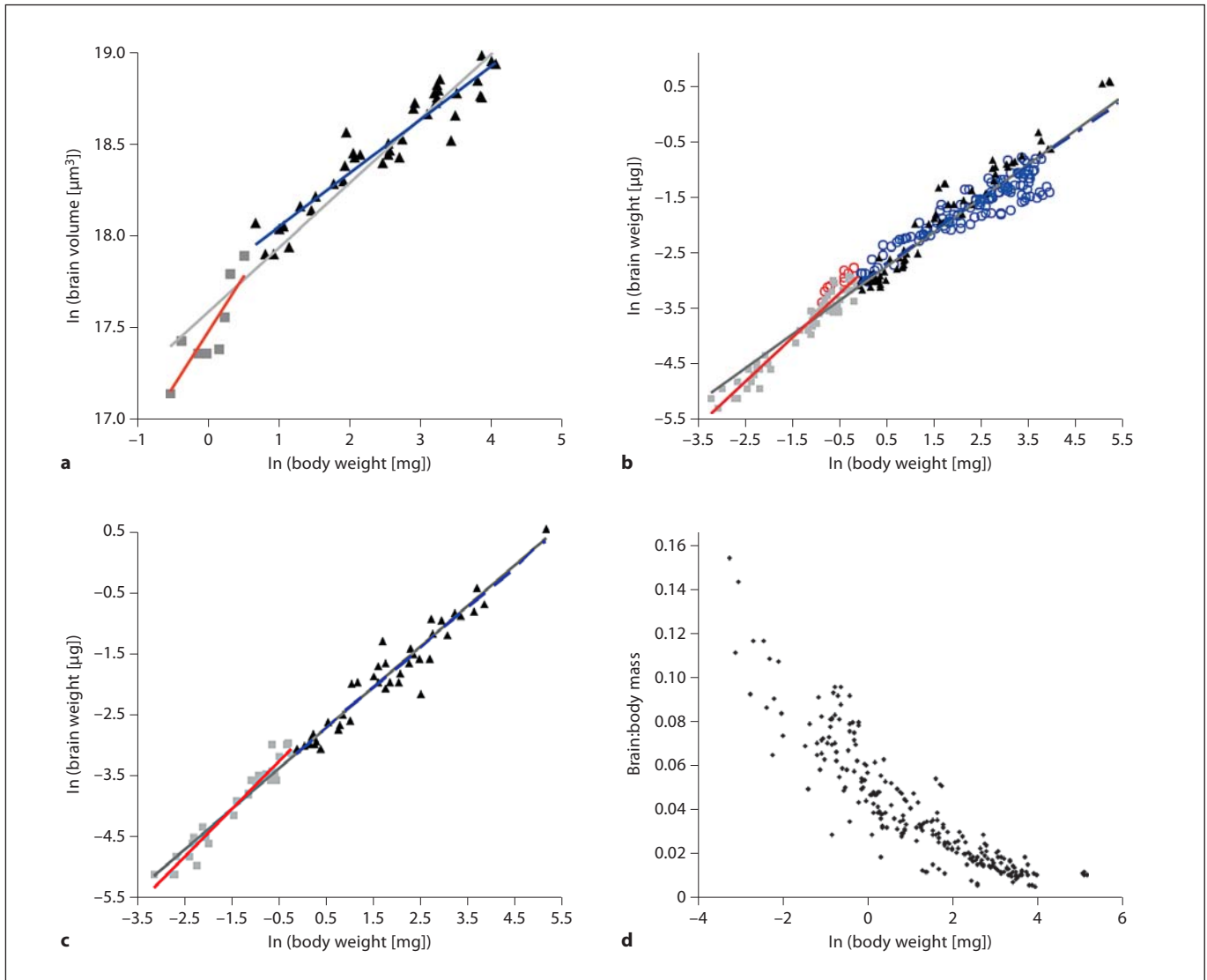


Fig. 2. Scatterplots showing how brain size in ants scales to body size. **a** Allometry for individuals of a leaf-cutter ant, *Atta colombica*, based on volumetric brain reconstructions ($n = 48$), with an overall regression of $y = 0.3503x + 17.583$ ($R^2 = 0.9301$) (solid gray line). A piecewise regression analysis with a breakpoint at 1.4 mg body mass yields 2 significantly different regressions: allometry for larger ants (black symbols) is described by $y = 0.2919x + 17.753$ ($R^2 = 0.9122$) (blue line), and that for the smaller ones (gray symbols) by $y = 0.6003x + 17.469$ ($R^2 = 0.7354$) (red line). **b** Allometric relationships for 70 ant species ($n = 261$ ants), with an overall regression model of $y = 0.5972x - 3.0419$ ($R^2 = 0.9389$) (gray line). A piecewise regression with a breakpoint at 0.9 mg body mass yields 2 significantly different regressions: allometry for larger ants (black symbols, and open blue circles) is described by $y =$

$0.5506x - 2.9446$ ($R^2 = 0.8477$) (dashed blue line), and that for the smaller ones (gray symbols, and open red circles) by $y = 0.802x - 2.8089$ ($R^2 = 0.9359$) (red line). Open circles indicate polymorphic species (see table 1). **c** Interspecific allometry for ants using a mean value for each species for those taxa represented by more than 1 individual, with an overall regression model of $y = 0.671x - 3.0582$ ($R^2 = 0.9731$) (gray line). A piecewise regression with a breakpoint at 0.9 mg body mass yields 2 significantly different regressions: allometry for larger ants (black symbols) is described by $y = 0.6692x - 3.0681$ ($R^2 = 0.9258$) (dashed blue line), and that for the smaller ones (gray symbols) by $y = 0.7961x - 2.8451$ ($R^2 = 0.9557$) (red line). **d** Scatterplot of the ratio of brain:body mass against body mass for 70 ant species.

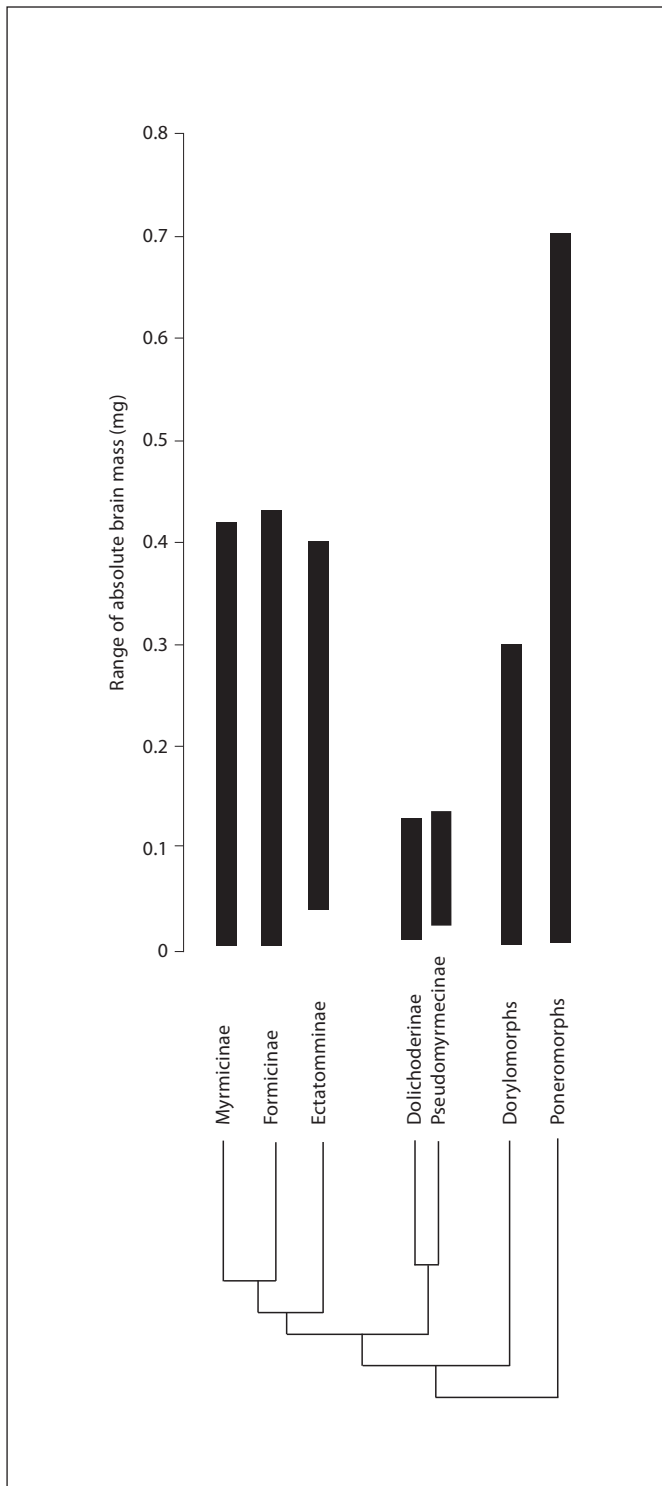


Fig. 3. Range of absolute brain mass for major subfamilies and taxonomic groupings used in this study. The phylogeny is taken from Brady et al. [2006].

head shape or size to accommodate these proportionally large brains. Other small animals facing similar challenges from brain miniaturization have morphological adaptations in which the brain invades other body parts, such as the prothorax in larval insects [Beutel et al., 2005; Grebennikov, 2008], and the coxae in legs of spiders [Quesada et al., in prep.]. Although their brains are disproportionately large as expected by Haller's Rule, extremely small ants, both intra- and interspecifically, have brains that are smaller than would be expected if they followed the allometric slope of larger ants. We do not know whether this shift represents a compensatory mechanism to cap energetic costs, or is related to constraints on head morphology. The latter alternative seems unlikely, however, given that macrocephaly has evolved repeatedly in ants [Hölldobler and Wilson, 1990], suggesting that brain size is not necessarily limited by head size.

Diphasic allometries have been demonstrated for other traits in insects [Niven and Scharlemann, 2005; Eberhard et al., 2000], including studies of cephalic allometry in ants [Wilson, 1953], but were unknown for brain scaling. Wilson [1953] hypothesized that diphasic allometry reflects a mechanism that helps to stabilize the head size of very small workers, while enabling the production of very different workers with only small differences in body size. We speculate that for polymorphic fungus-growing ants, such as *Atta*, it may be advantageous to have a smaller-than-expected head size at the small end of the size spectrum because of the need for tiny workers to move about within the interstices of the fungus garden [Weber, 1972], while maintaining the neural capabilities to process information relating to the health of the fungal cultivar, detecting the presence of pathogens in the fungal gardens, and implementing disease-control measures [e.g. Fernández-Marín et al., 2009]. Small versus large workers of 2 species of *Atta* differ in the relative size of brain components [Kleineidam et al., 2005], and these differences may be related to differential behavioral responses in the 2 size classes [Kleineidam et al., 2007]. A detailed volumetric analysis of brain region size relative to head size will elucidate how brain regions influence total brain size [Seid, Elizondo and Wcislo, in prep.].

In light of known behavioral differences among subcastes of *Atta* workers [Weber, 1972; Hölldobler and Wilson, 1990], and a report of diphasic cephalic allometry in *A. texana* [Wilson, 1953], we expected an allometric shift in brain allometry for *A. colombica*. A similar allometric shift in the interspecific study was surprising and may point to a general rule governing how ant brains are constructed beyond a critical size threshold. Our finding that

the allometric rules governing ant brain size change at extremely small body sizes, both within and among species, was derived from 2 methods of measuring brain size, and therefore it is unlikely to be an artifact of the techniques used. An allometric coefficient as high as ~ 0.8 for small ants differs significantly from that reported previously for a survey of 10 ant species [Wehner et al., 2007], which gave a scaling coefficient of 0.567. Our study and that of Wehner et al. [2007] included the same substructures in calculating total brain mass, so it is unlikely that any methodological differences account for the discordant findings. Indeed, for the larger ants in our study (>0.9 mg), the scaling coefficient from the full data set ($b = 0.5506$; fig. 2b) was nearly identical to that of Wehner et al. [2007]. Notably, the smallest ants used by Wehner et al. [2007] were substantially larger than the breakpoint (0.9 mg) in our study, which could account for the discrepancy between these studies. To our knowledge, extremely small ants (<0.9 mg body mass) are the only animals known to have a brain allometric coefficient comparable to that of mammals [White et al., 2009].

It is difficult to interpret the biological significance of diphasic allometry in the interspecific comparison because so little is known about how brain size relates to behavior in ants, but we speculate that it may be associated with energetics. The disproportionate investment in brain mass by small animals implies disproportionately high energetic costs, given that neuronal tissue is expensive to maintain [Niven and Laughlin, 2008]. Diphasic brain allometry should limit these energetic costs at very small body sizes because it produces smaller brains than expected from a monophasic allometry. An additional mechanism to reduce costs or the size of the nervous system involves relative investment in glia and neurons; limited data show that ants from 1 large species have more glia processes than ants from a smaller species [pers. obs.].

An extremely small animal may pay a severe energetic cost to maintain a disproportionately large CNS in order

to have information processing capabilities equivalent to large-bodied species [Niven and Laughlin, 2008; Niven et al., 2007]. Alternatively they may have evolved life history or behavioral traits, or more elaborate peripheral sensory systems, to reduce the need for relatively large and sophisticated information processing systems, and hence minimize energetic costs. Unfortunately, the functional consequences of brain miniaturization are not well understood and there are few data to distinguish between these 2 alternatives. Both Snell-Rood et al. [2009] and Mares et al. [2005] hypothesize that brain size may limit learning abilities in insects [also Rensch, 1956, 1959]. Limited empirical studies on the behavior of extremely small animals indicate that they do not suffer from inferior behavioral capabilities [Eberhard, 2007; Hesselberg, 2010], and we are not aware of any behavioral deficiencies in small ants. Many animals with tiny brains express behavior comparable to large-brained animals [Miklos, 1998; Chittka and Niven, 2009] and ants are no exception, but more studies are needed to understand compensatory mechanisms for adapting to small body sizes and the energetic costs for maintaining a relatively large brain in small-bodied animals.

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