

Colony size predicts division of labour in Attine ants: Electronic supplementary material

Henry Ferguson-Gow<sup>1,2</sup>\*†, Seirian Sumner<sup>2,3</sup>, Andrew F. G. Bourke<sup>1</sup> and Kate E. Jones<sup>2,4\*</sup>

<sup>1</sup> School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR4 7TJ, United Kingdom

<sup>2</sup> Institute of Zoology, Zoological Society of London, Regent's Park, London, NW1 4RY, United Kingdom

<sup>3</sup> Current address: School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, United Kingdom

<sup>4</sup> Centre for Biodiversity and Environment Research, Department of Genetics, Evolution and Environment, University College London, Gower Street, London, WC1E 6BT, United Kingdom

† Current address: Centre for Biodiversity and Environment Research, Department of Genetics, Evolution and Environment, University College London, Gower Street, London, WC1E 6BT, United Kingdom

\* Corresponding author: [h.ferguson-gow@ucl.ac.uk](mailto:h.ferguson-gow@ucl.ac.uk) and [kate.e.jones@ucl.ac.uk](mailto:kate.e.jones@ucl.ac.uk)

**Table S1.** Life history and environmental traits and data sources for 57 species of Attini. W and Q represent Worker and Queen, respectively. Values for colony size, worker and queen head-widths represent per-species means (in mm) calculated by averaging the mean value from each observation weighted by its sample size;  $\Sigma ns$  is the sum of all the sample sizes of the observations contributing to the per-species mean for each trait (colony size, worker and queen head width) (assumed to be 1 where observation sample sizes were not given); worker size variation is the coefficient of variation in worker head-widths; queen-worker dimorphism is percentage change between mean queen and worker head-width; and mean diurnal temperature range, isothermality, temperature seasonality and precipitation seasonality are taken from BioClim (www.worldclim.org/bioclim). Taxonomy follows Bolton World Catalogue (www.antweb.org), and - denotes missing data.

Species	Mean	$\Sigma ns$	Mean W	$\Sigma ns$ W	Mean Q	$\Sigma ns$ Q	Head-Width	Head-Width	Head-Width	Head-Width	W Size	Q-W Variation	Dimorphism	Polyandrous	Mean Diurnal Temp.	Isotherma lity	Temp. Range	Seasonality	Precipitation Seasonality	N Geolocated Points	Data Source
	Colony Size	Colony Size	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width	Head-Width
<i>Acromyrmex ameliae</i>	-	-	1.1	30	1.4	21	27.27	24	-	-	130	69	1681	85	6	[1]					
<i>Acromyrmex echinatior</i>	137500	5	1.87571	826	2.58	11	32.05	31.61	Yes	100.02	74.32	745.85	80.9	42	[2-7]						
<i>Acromyrmex insinuator</i>	100	1	-	-	2.325	27	-	-	Yes	72	72	724	60	13	[3, 4, 7, 9, 10]						
<i>Acromyrmex octospinosus</i>	13375	5	1.80769	581	2.9	5	43.33	46.41	Yes	84	75.6	717.48	39.54	71	[2-4, 6, 11-14]						
<i>Acromyrmex subterraneus</i>	4766.2	5	1.58767	16	2.15	17	27.71	30.09	-	130	69	1681	85	4	[1, 15, 16]						
<i>Acromyrmex versicolor</i>	-	-	2.0692	5	-	-	33.36	-	Yes	162.56	46.22	6971.11	53.67	18	[1, 2, 15, 17]						
<i>Apterostigma collare</i>	23.98	128	0.8695	3	0.91	2	7.56	4.55	No	90.13	77.19	688.05	32.93	165	[3, 15, 19-21]						
<i>Apterostigma dentigerum</i>	25	1	1.069	3	1.14	1	9.53	6.43	-	89.3	76.99	715.96	30.42	93	[3, 15]						
<i>Apterostigma mayri</i>	20	25	-	-	-	-	-	-	-	70	72.5	706	58.5	10	[12]						
<i>Apterostigma</i> sp. 2	25	1	-	-	1.09	1	-	-	-	72	72	724	60	2	[3]						
<i>Apterostigma urichii</i>	23	1	-	-	-	-	-	-	-	95	83.33	470.67	46.33	3	[22]						
<i>Atta cephalotes</i>	3000000	1	1.98714	949	4.88	5	64.37	84.25	Yes	90.11	77.19	649.9	30.43	214	[3, 6, 15, 23, 24]						
<i>Atta colombica</i>	2266666.67	5	-	-	4.31	5	-	-	-	76.58	74.21	680.68	56.68	30	[3, 12, 25, 26]						
<i>Atta laevigata</i>	3500000	1	2.87929	7	-	-	33.5	-	Yes	108.2	74.6	1095.2	62	5	[15, 25, 27, 28]						
<i>Atta sexdens</i>	6000000	1	2.46363	1016	3.62	4	50.93	38.16	Yes	84.67	78	667.33	79	6	[2, 3, 15, 25]						
<i>Atta texana</i>	-	-	2.304	3	-	-	48.98	-	Yes	132	39	7287	16	7	[15, 29]						
<i>Atta vollenweideri</i>	5500000	1	2.99467	3	4.91	2	53.96	48.61	-	122.33	54.67	3465	46.33	5	[15, 25, 26]						
<i>Cyphomyrmex cornutus</i>	2021.75	4	-	-	-	-	-	-	-	90.18	77.01	671.58	29.58	73	[15, 30]						
<i>Cyphomyrmex costatus</i>	96.5	53	-	-	0.58	5	-	-	-	85.96	76.29	645.87	45.09	48	[3, 12, 15, 31]						
<i>Cyphomyrmex faunulus</i>	16	1	-	-	-	-	-	-	-	92	86	338	20	2	[15, 22]						
<i>Cyphomyrmex longiscapus</i>	54.13	203	0.65778	93	0.746667	25	10.53	12.66	No	85.24	75.36	637.48	49.14	42	[15, 21, 32, 33]						
<i>Cyphomyrmex muelleri</i>	-	-	0.63833	78	0.7	23	6.68	9.22	-	-	-	-	-	-	-	-	-	-	[33]		
<i>Cyphomyrmex rimosus</i>	136.75	69	0.632	4	0.646333	3	15.58	2.24	-	93.68	74.44	1005.29	45.61	259	[3, 12, 15, 34]						
<i>Kalathomyrmex emeryi</i>	100	1	0.82467	10	0.8375	2	10.69	1.54	-	97.75	65.75	1826	61.75	5	[3, 15]						
<i>Mycetagricus cerradensis</i>	373	2	0.8705	3	-	-	3.98	-	-	126	69	671.73	44.92	5	[15, 3]						
<i>Mycetagricus inflatus</i>	-	-	0.715	2	-	-	0.99	-	-	-	-	-	-	-	-	-	-	-	[36]		
<i>Mycetarotes carinatus</i>	-	-	-	-	0.88	1	-	-	-	-	-	-	-	-	-	-	-	-	[58]		
<i>Mycetarotes parallelus</i>	110.57	13	0.9175	4	0.95	3	0.23	3.48	-	126.91	56.91	3284.68	20.41	21	[3, 15, 37]						
<i>Mycetophylax conformis</i>	72.25	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	[15, 38]		
<i>Mycetophylax morschi</i>	72.25	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	[15, 38]		
<i>Mycetophylax simplex</i>	191.94	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	[15, 38, 39]		
<i>Mycetosoritis clorindae</i>	70.5	2	-	-	-	-	-	-	-	92	48	3306	9	3	[40]						
<i>Mycetosoritis explicate</i>	-	-	0.8	2	0.93	1	14.52	15.03	-	-	-	-	-	-	-	-	-	-	[41, 15]		
<i>Mycetosoritis hartmanni</i>	50	2	0.6375	4	0.77	3	4.53	18.83	-	110.4	45.5	5210.9	36.8	10	[3, 15]						
<i>Mycocoepurus castrator</i>	1	2	-	-	0.6	16	-	-	-	116	63	2405	71	6	[42]						
<i>Mycocoepurus goeldii</i>	678.86	7	-	-	0.78	2	-	-	-	91.63	76.88	945.63	50.44	16	[15, 42-44, 47]						
<i>Mycocoepurus obsoletus</i>	-	-	-	-	-	-	-	-	-	87	76	594	71	2	[15, 43]						
<i>Mycocoepurus smithii</i>	62.63	162	0.6275	4	0.614	1	6.65	2.17	-	89.83	78.07	779.41	27	46	[15, 39, 44-47]						
<i>Myrmicocrypta bucki</i>	-	-	0.64333	4	-	-	3.91	-	-	105	73	1014	45	3	[48]						

<i>Myrmicocrypta camargoii</i>	-	-	0.75333	4	1.09	2	5.53	36.53	-	117	63	2333	60	5	[48]
<i>Myrmicocrypta ednaella</i>	86.78	35	-	-	-	-	-	-	No	88.3	78.06	642.76	46.81	67	[12, 21, 34]
<i>Myrmicocrypta erectapilosa</i>	-	-	0.59333	4	0.71	1	5.42	17.9	-	83	81	446	47	5	[48]
<i>Sericomyrmex amabilis</i>	972.67	12	0.9335	10	1.255	6	6.74	29.38	No	88.26	76.74	708.61	32.36	334	[2, 3, 12, 15, 20, 49]
<i>Trachymyrmex arizonensis</i>	1000	1	1.15333	6	1.285	2	14.11	10.8	-	172.5	49.44	6419.72	68.72	17	[15, 50, 52]
<i>Trachymyrmex carinatus</i>	100	1	0.94767	5	1.246	2	13.63	27.2	-	166	51	5626	113	3	[15, 52]
<i>Trachymyrmex cornetzi</i>	161.83	62	0.975	5	1.06	2	12.46	8.35	No	87.96	76.58	721.07	33.04	147	[2, 3, 15, 49, 57]
<i>Trachymyrmex desertorum</i>	-	-	1.0584	5	1.375	2	16.71	26.02	-	-	-	-	-	-	[15, 52]
<i>Trachymyrmex isthmicus</i>	100.67	43	-	-	-	-	-	-	-	81.5	75.29	722.14	39.86	28	[52]
<i>Trachymyrmex jamaicensis</i>	525	2	1.37033	3	1.65	2	5.23	18.52	-	91.5	54.75	2696.25	48.25	7	[15, 52]
<i>Trachymyrmex nogalensis</i>	-	-	1.34225	4	1.425	2	16.57	5.98	-	-	-	-	-	-	[52]
<i>Trachymyrmex pomona</i>	183	1	0.865	2	1.066667	3	13.9	20.88	-	178	50	6516	70	3	[52]
<i>Trachymyrmex septentrionalis</i>	474.7	93	0.967	552	1.160667	2	7.87	18.2	-	122.93	48	4974.6	44.27	17	[15, 52-55]
<i>Trachymyrmex smithi</i>	652.5	4	1.37329	6	1	2	10.76	31.46	-	167.5	55.5	4822	88.5	5	[15, 52]
<i>Trachymyrmex</i> sp. 3	1000	1	-	-	1.22	5	-	-	-	72	72	724	60	3	[3]
<i>Trachymyrmex turrifex</i>	300	1	0.9535	4	1.15	2	11.72	18.68	-	173	51	5626	113	4	[15, 52]
<i>Trachymyrmex urichi</i>	-	-	-	-	-	-	-	-	-	109.5	78	586.5	57	3	[15, 56]
<i>Trachymyrmex zeteki</i>	146.78	136	-	-	1.29	5	-	-	No	80.55	74.62	708.28	46.76	30	[2, 3, 15, 20, 49, 51]

Social Complexity in Attine Ants: ESM – Ferguson-Gow *et al.*

Table S1 Data Sources.

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Social Complexity in Attine Ants: ESM – Ferguson-Gow *et al.*

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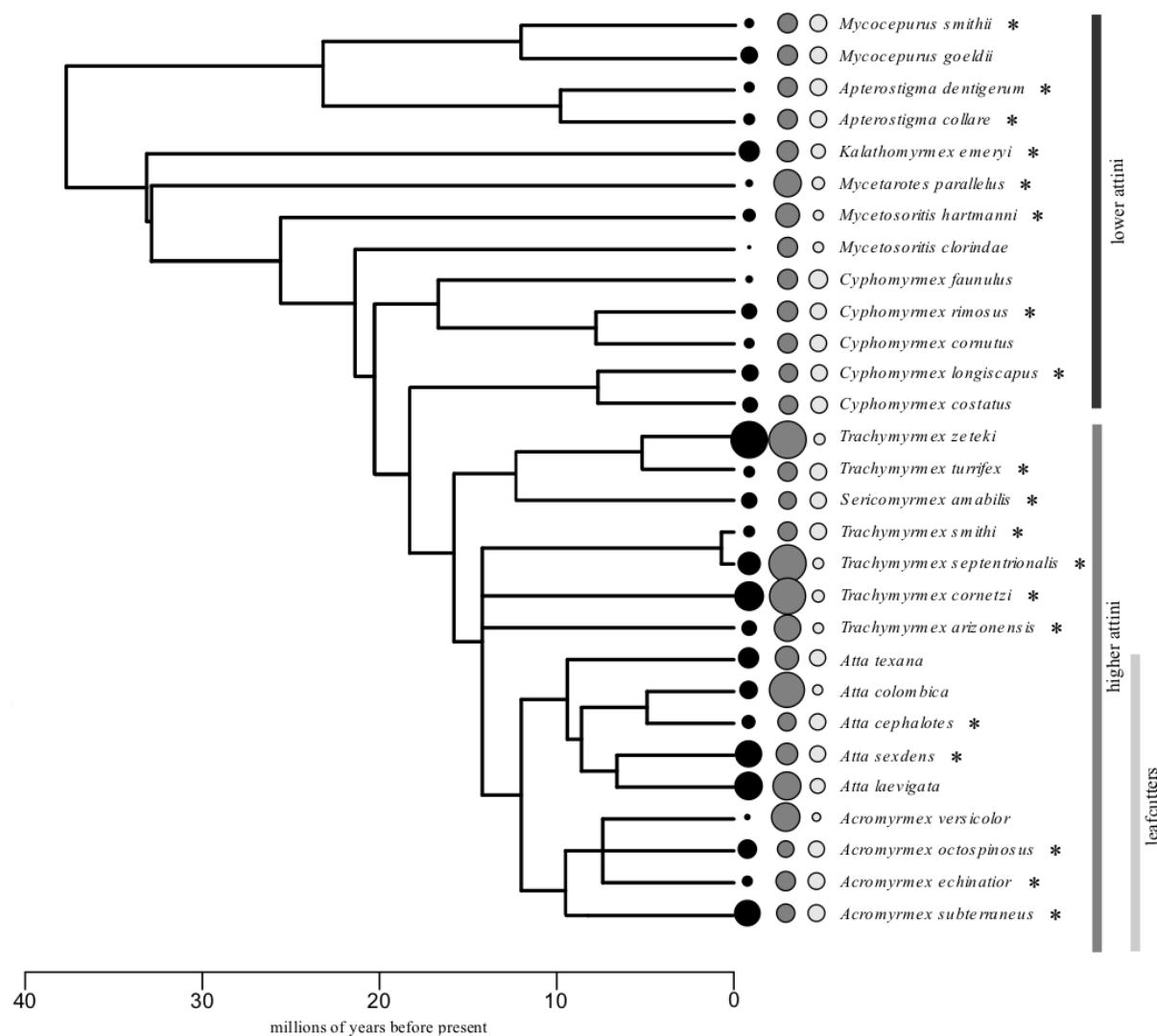


Figure S1. Distribution of precipitation seasonality, mean temperature fluctuation and isothermality on a phylogenetic supertree of the Attini (29 species). The full tree (electronic supplementary material, figure S2) was pruned to include only the species for which there were data on at least one trait and appeared in the phylogeny. Black, grey and white circles are proportional to precipitation seasonality, mean temperature fluctuation and isothermality, respectively. \* denotes species used in the final analysis. Branch lengths are proportional to time (millions of years).

Table S2. Phylogenetic sources for the Attini supertree.

Reference	Figure number	Data type	No. Attini species
Bacci Jr et al 2009	1	mtDNA + tRNA	13
	2	nDNA	13
	3	mtDNA + nDNA	13
Brandao and Mayhé-Nunes 2007	2	Morphology	32
Moreau et al 2006	1	nDNA + mtDNA	4
Schultz and Brady 2008	1	nDNA + mtDNA	65
Shultz and Meier 1995	3	Larval morphology	51
Sumner et al 2004	1	mtDNA	19
Villesen et al 2004	6	mtDNA	12
Wetterer et al 1998	1	mtDNA + tRNA	14
	2	Amino acid sequence + morphology	14

Table S2 references.

- Bacci Jr M, Solomon SE, Mueller UG, Martins VG, Carvalho AO, Vieira LG, Silva-Pinhati, ACO. 2009 Phylogeny of leafcutter ants in the genus *Atta* Fabricius (Formicidae: Attini) based on mitochondrial and nuclear DNA sequences. *Mol. Phylogenet. Evol.* **51**, 427-437.
- Brandao CRF, Mayhé-Nunes AJ. 2007 A phylogenetic hypothesis for the Trachymyrmex species groups, and the transition from fungus-growing to leaf-cutting in the Attini. *Mem. Am. Entomol. Inst.* **80**, 72-88.
- Moreau CS, Bell CD, Vila R, Archibald SB, Pierce NE. 2006 Phylogeny of the ants: diversification in the age of angiosperms. *Science* **312**, 101-104.
- Schultz TR, Brady SG. 2008 Major evolutionary transitions in ant agriculture. *Proc. Natl. Acad. Sci. USA* **105**, 5435-5440.
- Schultz TR, Meier R. 1995 A phylogenetic analysis of the fungus-growing ants (Hymenoptera: Formicidae: Attini) based on morphological characters of the larvae. *Syst. Entomol.* **20**, 337-370.
- Sumner S, Aanen DK, Delabie J, Boomsma JJ. 2004 The evolution of social parasitism in Acromyrmex leaf-cutting ants: a test of Emery's rule. *Insectes Soc.* **51**, 37-42.
- Villesen P, Mueller UG, Schultz TR, Adams RM, Bouck AC. 2004 Evolution of ant-cultivar specialization and cultivar switching in Apterostigma fungus-growing ants. *Evolution* **58**, 2252-2265.
- Wetterer JK, Schultz TR, Meier R. 1998 Phylogeny of fungus-growing ants (Tribe Attini) based on mtDNA sequence and morphology. *Mol. Phylogenet. Evol.* **9**, 42-47.

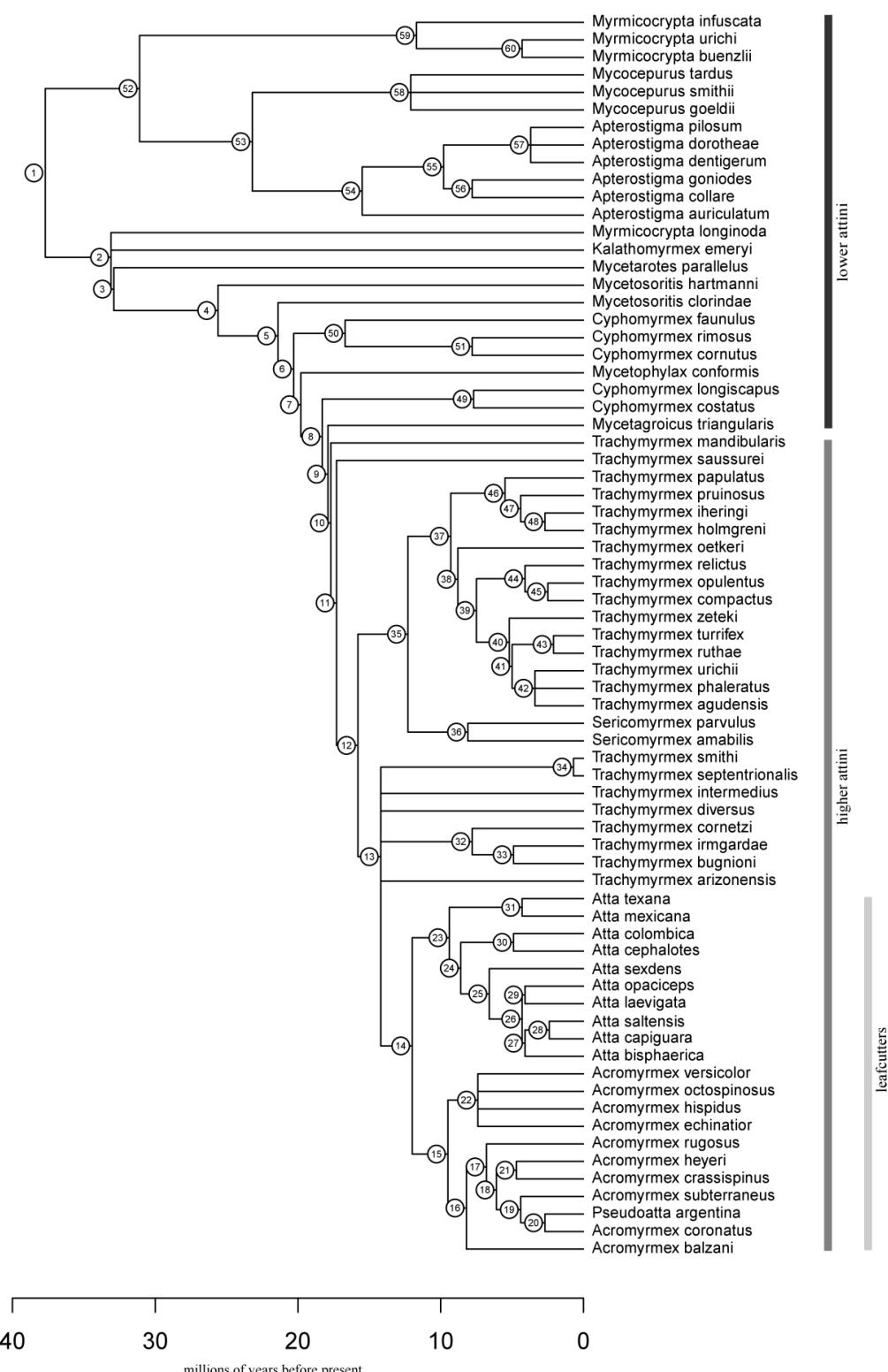


Figure S2. A phylogenetic supertree for 71 species of the Attini. Numbers on nodes are arbitrary numbers. Branch lengths are proportional to time (millions of years). See table S3 for nodal support values.

Table S3. Reduced qualitative support (rQS) scores for the Attini supertree (figure S1). rQS values range between 1 and -1 where 1 indicates full agreement for a node in the source trees, and -1 full disagreement.

Node #	rQS value	Node #	rQS value	Node #	rQS value
1	0.83	21	0.08	41	0.00
2	0.75	22	0.42	42	0.00
3	0.75	23	0.50	43	0.00
4	0.67	24	0.50	44	0.00
5	0.67	25	0.00	45	0.00
6	0.67	26	0.00	46	0.00
7	0.67	27	0.08	47	0.00
8	0.58	28	0.08	48	0.00
9	0.58	29	0.00	49	0.00
10	0.58	30	0.33	50	0.33
11	0.58	31	0.08	51	0.33
12	0.58	32	0.17	52	-0.83
13	0.33	33	0.00	53	0.25
14	0.50	34	0.08	54	0.42
15	0.33	35	0.17	55	0.25
16	0.83	36	0.33	56	0.25
17	0.42	37	0.00	57	0.01
18	0.17	38	0.00	58	0.25
19	0.17	39	0.00	59	-0.17
20	0.17	40	0.00	60	-0.17

Table S4. All models with  $\Delta\text{AICc} < 7$  describing the predictors of a) worker size variation, b) queen-worker dimorphism and c) colony size in the Attini. Regression slope estimates from phylogenetic least squares (PGLS) models are reported.

Table S4a.

	Precipitation seasonality	Mean diurnal temperature fluctuation	log Colony size	Queen-worker dimorphism	Degrees of freedom	Log likelihood	AICc	$\Delta\text{AICc}$	AICc weight
Intercept									
2.342		-0.011	0.394		3	-23.97	55.7	0.68	0.19
1.027			0.401		2	-25.83	56.4	1.43	0.13
2.563	0.020	-0.020	0.347		4	-22.73	56.5	1.57	0.12
2.253	0.018	-0.020	0.353	0.152	5	-20.70	56.9	1.88	0.12
0.627	0.003		0.430	0.028	4	-22.79	56.9	1.93	0.10
0.832	0.005		0.391		3	-24.85	57.4	2.43	0.08

Table S4b.

	Precipitation seasonality	Isothermality	log Colony size	Degrees of freedom	Log likelihood	AICc	$\Delta\text{AICc}$	AICc weight
Intercept								
0.912			0.154	2	-20.45	45.7	0	0.44
1.358		-0.007	0.168	3	-19.79	47.3	1.64	0.20
0.911	-0.001		0.159	3	-19.87	47.5	1.8	0.18
1.637				1	-23.84	49.9	4.26	0.05
1.291	0.008			2	-22.75	50.3	4.64	0.04
1.536	-0.002	-0.009	0.174	4	-19.76	50.6	4.93	0.04
0.681		0.014		2	-22.94	50.7	5.02	0.04
0.253	0.007	0.016		3	-22.34	52.4	6.74	0.02

Table S4c.

Intercept	Precipitation seasonality	Mean diurnal temperature fluctuation	Isothermality	Latitude	Precipitation seasonality * isothermality	Degrees of freedom	Log Likelihood	AICc	ΔAICc	AICc weight
1.040			0.056			2	-61.93	128.4	0	0.17
6.658	0.044	-0.035				3	-60.73	128.6	0.18	0.16
4.245	0.015					2	-62.04	128.6	0.23	0.15
7.291	-0.173		-0.055		0.003	4	-59.81	129.5	1.14	0.10
4.976		-0.002				2	-62.57	129.7	1.29	0.09
1.437	0.011		0.044			3	-61.67	130.4	2.05	0.06
1.249		-0.002	0.056			3	-61.91	130.9	2.54	0.05
0.926			0.057	0.006		3	-61.92	130.9	2.55	0.05
4.240	0.015			0.002		3	-62.04	131.2	2.8	0.04
5.520	0.040	-0.032	0.015			4	-60.69	131.3	2.91	0.04
6.663	0.045	-0.035		0.007		4	-60.71	131.3	2.96	0.04
7.581	-0.202		-0.062	0.037	0.003	5	-59.36	131.7	3.34	0.03
4.992		-0.002		-0.005		3	-62.56	132.2	3.84	0.03

### Multinomial Model Analysis

We categorised species by their agricultural system as follows: lower Attines (lower agriculture), higher Attines, excluding leafcutters (higher agriculture), and leafcutter ants (leafcutting agriculture). These classifications can be seen on figure 1, supplementary material figures S1 and S2. Using the fungal-agricultural system as the dependent variable and ln mean colony size as the independent variable, we fitted a univariate, multinomial logistic regression model using the R package MCMCglmm [1]. We used non-informative priors with a low degree of belief across all parameters, and ran the model for 6,000,000 generations, sampling every 1000<sup>th</sup> generation and discarding the first 25% of samples as burnin. We visually inspected the trace output to ensure model convergence and proper mixing, and made sure effective sample sizes were large enough to ensure robust parameter estimates.

We found strong evidence that the degree of fungal-agricultural system is predicted by colony size. Specifically, larger colony sizes are associated with a higher probability of higher agriculture versus lower agriculture (expected log-odds change per unit increase in ln colony size=1.21, CI<sub>95</sub>=2.34, pMCMC=0.02), and leafcutter agriculture versus lower agriculture (expected log-odds change per unit increase in ln colony size=4.71, CI<sub>95</sub>=7.19, pMCMC<<0.001).

1. Hadfield J.D. 2010 MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software* **33**(2), 1-22.