Metabolism and the Rise of Fungus Cultivation by Ants

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abstract: Most ant colonies are comprised of workers that cooperate to harvest resources and feed developing larvae. Around 50 million years ago (MYA), ants of the attine lineage adopted an alternative strategy, harvesting resources used as compost to produce fungal gardens. While fungus cultivation is considered a major breakthrough in ant evolution, the associated ecological consequences remain poorly understood. Here, we compare the energetics of attine colony-farms and ancestral hunter-gatherer colonies using metabolic scaling principles within a phylogenetic context. We find two major energetic transitions. First, the earliest lower-attine farmers transitioned to lower mass-specific metabolic rates while shifting significant fractions of biomass from ant tissue to fungus gardens. Second, a transition 20 MYA to specialized cultivars in the higher-attine clade was associated with increased colony metabolism (without changes in garden fungal content) and with metabolic scaling nearly identical to hypometry observed in hunter-gatherer ants, although only the hunter-gatherer slope was distinguishable from isometry. Based on these evolutionary transitions, we propose that shifting living-tissue storage from ants to fungal mutualists provided energetic storage advantages contributing to attine diversification and outline critical assumptions that, when tested, will help link metabolism, farming efficiency, and colony fitness.

Keywords: colony size, tribe Attini, hunter-gatherer, metabolic scaling, evolutionary transition.

Introduction

Living organisms have generally become increasingly large, complex, and diverse over nearly 4 billion years of evolutionary history (Maynard Smith and Szathmáry 1995; Bonner 2004; DeLong et al. 2010). At critical transitions, these increases occurred when existing entities formed collectives—genes into genomes, cells into metazoans, individuals into eusocial societies, and species into mutualisms (Bourke 2011). The ants (Hymenoptera: Formicidae) epitomize this trend, with more than 14,000 species whose eusocial hunter-gatherer colonies have anywhere from tens to millions of workers that cooperate to harvest resources and provision developing larvae inside the nest (Hölldobler and Wilson 2008). A further transition occurred 50 million years ago (MYA), when ants in the attine lineage adopted fungus cultivation, diversifying over time into more than 230 species common across the New World tropics and subtropics (Weber 1972; Mueller et al. 2005, 2011; Schultz and Brady 2008). While fungus cultivation is considered a major breakthrough in ant evolution (Mueller and Rabeling 2008; Hölldobler and Wilson 2010), there has been little exploration of the ecological costs and benefits that enabled the transition from hunting and gathering.

An attine farming system (hereafter, colony-farm) consists of ants and their fungi within a nest and a foraging territory from which ants collect the plant material and detritus they use as compost to sustain the growth of their fungal gardens (Leal and Oliveira 2000; Wirth et al. 2003). Benefits of fungus cultivation include the extraction and synthesis of resources from this compost that are unavailable to most other ants (Martin and Weber 1969; Mueller et al. 2005; Schultz and Brady 2008). The costs include the extra labor needed to prepare compost (Burd and Howard 2005), protect cultivated gardens from pests (Bass and Cherrett 1994; Poulsen et al. 2002; Currie et al. 2003; Rodrigues et al. 2008; Fernández-Marín et al. 2009; Yek et al. 2012), and maintain microclimates that promote fungal growth (Mueller et al. 2005; Bollazzi and Roces 2007, 2010). The earliest attines likely also incurred nutritional costs because switching from insect prey to fungi increased nutritional differences between consumer and food (Sterner and Elser 2002), paralleling the general de-

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cline in health when humans transitioned to agriculture some 10,000 years ago (Mummert et al. 2011). We explore the energetic and demographic consequences of the farming transition in the descendants of these early ant farmers, a clade of lower attines that inhabit small colony-farms with around 100 workers and rely on insect biomass (carcasses and frass) for garden compost, measuring CO_2 emission rates (hereafter, metabolic rate) and colony composition (e.g., relative mass of adult workers).

A critical transition occurred 20 MYA when the higher attines adopted a narrow clade of fungi derived from a lower-attine ancestor (Schultz and Brady 2008). Lower attines cultivate leucocoprinaceous fungi (Basidiomycota: Agaricales: Agaricaceae) that retain enzymes for degrading plant cell walls typical for litter-decomposing, free-living fungi (de Fine Licht et al. 2010), possibly because they have been repeatedly acquired over millions of years (Mueller 2002; Vo et al. 2009). Higher-attine cultivars, in contrast, appear to lack free-living populations, possibly because they have evolved traits that would not be suitable unless tended by ants, such as swollen, nutrient-rich hyphal tips called gongylidia (Mueller 2002; Mikheyev et al. 2010) and enzyme profiles suited for extracting nutrients and detoxifying noxious chemicals from composted substrate (Van Bael et al. 2009; de Fine Licht et al. 2010, 2012). This cultivar domestication by higher attines coincided with a dramatic increase in the size and complexity of colony-farms, culminating with the most derived leaf-cutter genus *Atta*, whose colonies can have several million workers with specialized castes of different sizes (Hölldobler and Wilson 2010).

We explore the energetic correlates of this transition to increased cultivar domestication using a metabolic scaling approach. Specifically, one of the best-known physiological patterns—the trend toward lower mass-specific energy use with increasing body mass (Kleiber's mouse to elephant curve)—has recently been extended to whole ant colonies (Hou et al. 2010; Shik 2010; Waters et al. 2010; Shik et al. 2012). We test whether the extended phenotypes of attine colony-farms exhibit a similar size dependence of metabolic rate, using phylogenetically informed analyses spanning 39 ant species (21 genera, five subfamilies) spread across >100 million years of evolutionary diversification to compare scaling coefficients between ant clades and test for isometry. We also test for phylogenetic signal in metabolic regressions using phylogenetic generalized least squares (Pagel 1997) and identify major transitions in colony metabolism using phylogenetic independent contrasts (Nunn and Barton 2000). These analyses explore, for the first time, the energetic consequences of (1) the rise of lower attine fungus cultivation from a hunter-gatherer ancestor and (2) the adoption of a coevolved cultivar lineage by higher attines.

Methods

Harvesting and Culturing Ants

We harvested ants from habitats across the southern United States and from Barro Colorado Island (9°09′N, 79°51′W), a lowland tropical forest managed by the Smithsonian Tropical Research Institute in Panama. Attine colony-farms were established in respirometry chambers designed for long-term ant habitation and connected by plastic tubing to foraging arenas (fig. A1*a*; figs. A1, A2 available online). We maintained these colonies in the lab, allowing them to regrow and restructure their fungal gardens over periods ranging from 3 weeks to more than 2 months. Hunter-gatherer colonies were harvested as part of a larger project on colony energetics (Shik et al. 2012). See appendix, available online, for information about colony collection and culturing.

Respirometry Methods

We used CO_2 emission rates to estimate the standard metabolic rates (MRs) of individual ants, hunter-gatherer colonies, and attine colony-farms. We performed constant volume respirometry (e.g., stop-flow respirometry) with equipment from Sable Systems International (Las Vegas, NV; fig. A1*b*), recording MR (μL CO_2 h^{-1}) averaged over five hourly measurements following a 1-h acclimation period. We then standardized each colony measurement by measuring the empty chamber for CO_2 after ants had been removed from chambers. While ant species in this study likely vary in their respiratory exchange ratios, from oxidation of mostly carbohydrates to mostly fat, such measurements were outside the goals of the present study, which involved quantifying differences in total energy use across a broad range of species.

After experimental measurements, colony components (e.g., workers, queens, brood, and fungal gardens) were frozen, dried at 60°C for 24 h, and weighed to the nearest 10^{-3} mg. For single-ant MR, individual workers were always used for only one trial. We partitioned MR between adult ants and cultivars by removing fungal gardens following colony-farm trials and measuring MR for all remaining workers and queens. Independent measures of brood mass and metabolism were not attempted for attine colony-farms for two reasons. First, larvae provide enzymes critical for fungal production (Abril and Bucher 2002; Erthal et al. 2007) and are functionally integral parts of garden metabolism. Second, eggs, larvae, and pupae are completely embedded in fungal matrices (Armitage et al. 2012), and the stress of extraction would likely have influenced their metabolic activity. We discuss the implications of this functional integration briefly in the "Discussion" section.

We used SSI ExpeData software to subtract the empty chamber CO_2 from each experimental measurement, correct for small variations in flow rate (± 0.1 mL min^{-1}), transform CO_2 measurements from ppm to μL h^{-1}, and integrate these values for trial intervals. We standardized each data point to 25°C, assuming a Q_{10} temperature coefficient of 2 (per Lighton 2008), although minimal temperature corrections were needed because the mean (± 1 SD) of all 435 hourly temperature measurements recorded during this study was 22.53° ± 1.16°C. See appendix for detailed respirometry methods.

Estimating Fungal Garden Composition

Attine gardens are complex matrices that include cultivated fungi and associated microbes (Pinto-Tomas et al. 2009; Scott et al. 2010), brood, and undigested substrate. Hypometric ($b < 1$, where b is the exponent in the scaling equation $\log_{10}(\text{MR}) = \log_{10}(a) + (b)\log_{10}(\text{Mass})$) or hypermetric ($b > 1$) scaling of MR with colony-farm mass might reflect allometric scaling in the relative amount of fungus cultivar present in gardens. We thus tested for allometric variation in garden mass and composition for all colony-farms examined in this study, using chitin assays to generate an index of relative garden fungal content (appendix). We also used two-sided nonparametric Mann-Whitney tests to compare species averages of percent chitin content in gardens and percent garden mass in colony-farms between higher and lower attines.

Statistical Analyses and Phylogenetic Inference

We determined scaling relationships using standard and phylogenetic approaches. For the standard analysis, we used ordinary least square (OLS) regression to estimate the intercept (a) and slope (b) in the scaling equation $\log_{10} y = \log_{10} a + b\log_{10}\text{Mass}$. Scaling characterized the dependence of MR (y) on the dry mass (mg) of hunter-gatherer colonies and/or attine colony-farms (Mass). Scaling relationships were considered isometric if the 95% confidence interval (CI) of the observed slope value contained the value 1. Where necessary, we used ANCOVA to detect variation in scaling relationships. All mass values used in this study are dry (mg), and all data provided in text are followed with ± 1 SD.

For phylogenetic inference, we first estimated a chronogram of 39 ant species using a topologically constrained tree derived from published ant phylogenies (Brady et al. 2006; Moreau et al. 2006; Schultz and Brady 2008; see appendix for details about tree construction). We then used phylogenetic independent contrasts (PICs) to identify evolutionary changes in the scaling of ant-colony metabolism (i.e., metabolic transitions; Nunn and Barton 2000). To do this, we first estimated PICs of the $\log_{10}$-transformed MR and mass data with the pic function of the ape package in R, version 3.0.3 (Paradis et al. 2004). Second, we regressed the positivized $\log_{10}$MR-PICs against $\log_{10}$Mass-PICs by enforcing the intercept to be zero, as required for PIC interpretation (Garland et al. 1992). Third, we identified metabolic transitions as outlier PICs that are above or below the 95% CI of the PIC regression line. These outliers represent significant changes in allometric coefficients between the contrasted lineages that retained the same allometric exponent (Nunn and Barton 2000).

We estimated phylogenetically informed scaling regressions using phylogenetic generalized least squares (PGLS; Pagel 1997). The PGLS analyses used the pgls function and ML method of the caper package in R, version 1.0 (Orme 2011). This approach addresses phylogenetic signal by accounting for phylogenetic nonindependence of the input data when estimating regression coefficients. This analysis estimates Pagel's λ for each regression as a tree-transformation parameter that gradually excludes underlying phylogenetic structure (Pagel 1993, 1999). Significant phylogenetic signal using λ is determined against a null hypothesis of no phylogenetic signal (H_0: $\lambda = 0$) by contrasting ML scores using likelihood ratio test (LRT), which is $-2[\ln(\text{likelihood for one-rate model}) - \ln(\text{likelihood for two-rate model})]$, with a significance approximate by a χ^2 with 1 df. Phylogenetic signal ($\lambda \to 1$, $P < .05$) can be defined as greater similarity between closely related species (e.g., sister taxa) than a pair of species randomly selected from the phylogeny. Standard OLS analyses are preferred in the absence of phylogenetic signal (e.g., $\lambda \to 0$, $P > .05$). Separate PGLS regressions for MR of lower and higher attine species were not calculated due to low sample sizes. Data underlying all figures, tables, and analyses are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.sc574 (Shik et al. 2014).

Results

Transitions in Colony Metabolism

Hypometric ($b < 1$) scaling of colony-level metabolic rate across all ant species in this study is supported by both standard analysis of species means (MR ~0.38(colony mass)$^{0.79 \pm 0.07}$) and phylogenetic analysis (MR ~0.29(colony mass)$^{0.85 \pm 0.08}$; table 1; fig. 1). However, significant phylogenetic signal ($\lambda = 1.00$, $P < .05$) indicates that PGLS should be used to interpret this overall pattern (table 1). Moreover, this phylogenetic signal is driven by the attine clade ($\lambda = 1.00$, $P < .05$), not the hunter-gatherer lineage ($\lambda = 0.46$, $P > .05$; table 1). Scaling of PICs indicates that this result is due to contrasts between the nodes separating (1) lower attines from hunter-gatherer ancestors and (2)

Table 1: Ordinary least square regressions for scaling of $\log_{10}$-transformed traits for attine colony-farms and hunter-gatherer colonies

Trait, method	N	λ	F	R^2	Intercept	Slope	95% CI of b
Attine garden mass:							
Standard	12	...	25.68	.72	1.08 (.28)	1.05 (.21)	.46
PGLS	12	.00	25.68	.69	1.08 (.28)	1.05 (.21)	.41
Attine fungus mass:							
Standard	12	...	42.28	.81	−1.87 (.37)	.98 (.15)	.33
PGLS	12	.00	42.28	.81	−1.87 (.37)	.98 (.15)	.29
MR for all ant species:							
Standard	39	...	495.65	.93	.38 (.06)	.79 (.04)	.07
PGLS	39	1.00*	392.90	.91	.30 (.18)	.85 (.04)	.08
PIC	38	...	392.90	.91	0	.85 (.04)	.08
MR for hunter-gatherer species:							
Standard	27	...	461.79	.95	.38 (.05)	.86 (.04)	.08
PGLS	27	.46	523.50	.95	.32 (.07)	.89 (.04)	.08
MR for all attine species:							
Standard	12	...	68.65	.87	−.25 (.30)	1.00 (.12)	.27
PGLS	12	1.00*	97.15	.90	−.02 (.27)	.84 (.09)	.17
MR for higher attine species:							
Standard	8	...	99.63	.94	.27 (.23)	.85 (.09)	.21
PGLS	...	...	...	...	...	...	...
MR for lower attine species:							
Standard	4	...	43.86	.96	.29 (.17)	.58 (.09)	.38
PGLS	...	...	...	...	...	...	...

Note: Standard and phylogenetic (phylogenetic generalized least squares [PGLS] and phylogenetic independent contrasts [PIC]) regressions were used for scaling analyses. To compute metabolic scaling relationships, colony biomass for hunter-gatherer species includes ants and brood, and colony-farm biomass for attines includes ants, brood, and fungus gardens. PGLS regressions are estimated by including λ, which provides information about the magnitude of phylogenetic signal (i.e., $\lambda \to 1$ indicates high signal and $\lambda \to 0$ is no signal). PIC regressions are forced through the origin, as required for their interpretation. N indicates number of species (or PICs) in an analysis. MR is metabolic rate (μL CO_2 h^{-1}). Garden fungus mass is estimated as (% chitin × total garden mass). The coefficient values for the intercept and slope are provided with standard errors in parentheses. An asterisk indicates that $\lambda = 0$ (i.e., no phylogenetic signal) was rejected at $P < .05$. CI = confidence interval.

higher attines from the clade containing lower attines. These two contrasts represent metabolic transitions that fall outside the 95% CI of the regression line scaling MR across all ant species (fig. 1) and indicate a substantial decline in mass-specific MR at the initial farming transition, followed by a subsequent increase at the higher-attine transition (fig. 2).

Across only the higher attines, MR scaled as 0.27(colony-farm mass)$^{0.85 \pm 0.21}$ with a slope (ANCOVA: $F_{1, 31} = 0.02$, $P = .89$) and intercept (ANCOVA: $F_{1, 31} = 0.14$, $P = .71$) that did not differ from the scaling for hunter-gatherers (MR ~0.38(colony mass)$^{0.86 \pm 0.08}$; table 1; fig. 2). Metabolic rate scaled hypometrically across the lower attine colony-farms, but this relationship must be interpreted cautiously given that this regression includes only four species (table 1; fig. 2).

Transitions in Colony Composition

The transition to farming was accompanied by a shift in biomass from adult workers (as in hunter-gatherers) to fungus gardens (fig. 1). Adult workers made up 81.94% ± 18.04% of the total biomass of hunter-gatherer colonies but were only 7.87% ± 5.68% of the total biomass in attine colony-farms (fig. 1). Within the attine clade, garden mass increased isometrically with the mass of the workforce (~1.08(worker mass)$^{1.05 \pm 0.46}$), and fungus mass increased isometrically with garden mass (~−1.87(garden mass)$^{0.98 \pm 0.33}$; table 1; fig. 3). This isometric scaling implies that MR hypometry across attine species is not simply due to systematic changes in the relative mass or composition of gardens with increasing colony-farm size. In addition, lower and higher attines did not differ in relative garden mass within colony-farms ($Z_{8, 4} = 0.76$, $P = .44$) or relative fungus mass within

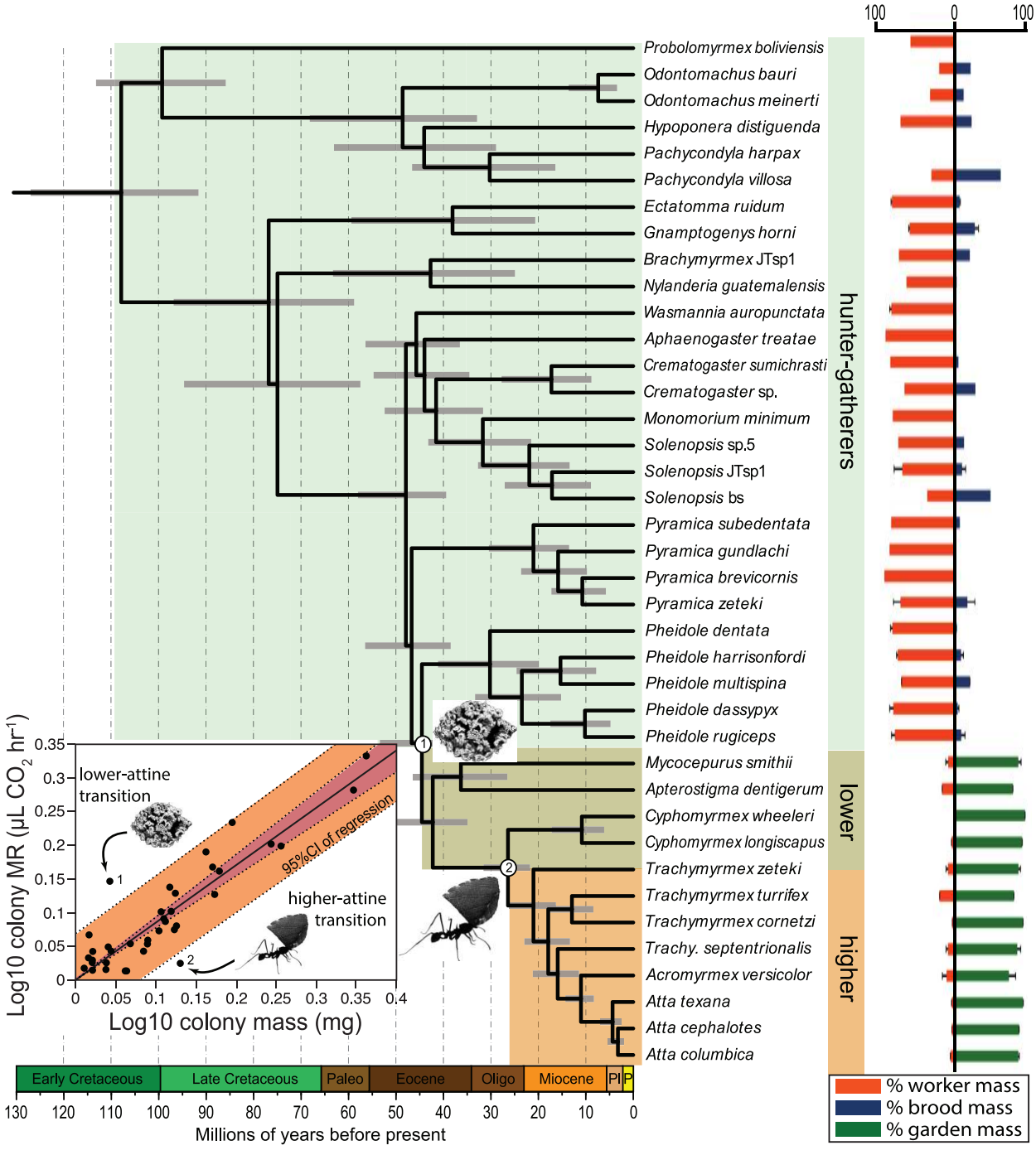

Figure 1: Transitions in metabolic rate (MR) and colony structure across the ant phylogeny. Scaling of phylogenetic independent contrasts (PICs) indicated an overall MR allometry across all ant species in this study (MR ~$M^{0.85}$; table 1) and two metabolic transitions: (1) the adoption of fungus cultivation by the lower-attine ants and (2) the rise of fungus domestication by the higher-attine ants. PICs for these two nodes were outliers, beyond the 95% confidence interval (CI) of the overall scaling relationship. As indicated in the bar graph, the lower-attine transition also coincided with a shift in colony structure, from colonies where most of the biomass is worker tissue to colony-farms where most of the biomass is in fungus gardens (a complex matrix including fungal cultivars, associated microbial consortia, and brood). To compute scaling relationships, colony biomass for hunter-gatherer species includes ants and brood, colony-farm biomass for attines includes ants, brood, and fungus gardens. Data have been positivized in the regression plot, such that PICs have been given positive signs with respect to the *X*-axis.

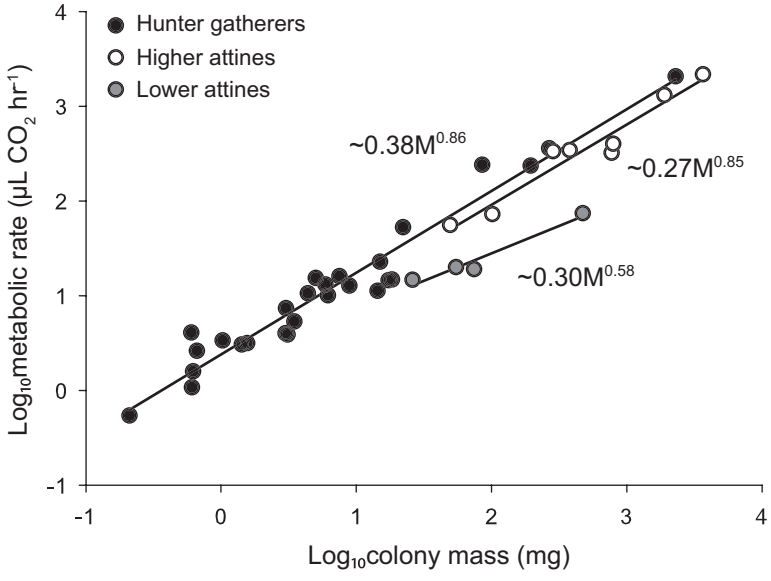

Figure 2: Metabolic scaling across hunter-gatherer colonies (ants and brood) and attine colony-farms (ants, brood, and fungus gardens). Each data point represents a species average. All mass measurements are dry mass (mg). Higher attines include the genera *Trachymyrmex*, *Acromyrmex*, and *Atta*. Lower attines include the genera *Mycocepurus*, *Apterostigma*, and *Cyphomyrmex*.

gardens ($Z_{8,4} = -0.42$, $P = .67$), suggesting that these factors are not sufficient to explain the MR transition observed within the attine clade.

An energetic consequence of the shift from using harvested resources to directly produce ant tissue to the production of fungi in attine colony-farms is that fungus gardens generate 72.1% ± 13.1% of a colony-farm's energy demands, even though gardens have lower mass-specific MR than worker tissue (fig. A2). Thus, high CO_2 concentrations observed in underground nests of some attine species (Kleineidam and Roces 2000; Bollazzi and Roces 2012) may be to be due to the sheer mass of fungus under cultivation rather than increased per-gram fungal respiration.

Discussion

This study seeks to understand the ecological consequences for ant societies that transitioned from hunting and gathering to farming. With the adoption of farming came a shift in colony biomass from ant tissue to fungus gardens and a transition to colony-farms with lower mass-specific metabolism. The reduction in garden metabolism was apparently lost at the transition to specialized higher-attine cultivars, without any corresponding changes in the relative fungal biomass content of gardens. Higher attines further exhibit scaling that is nearly identical to the hypometry observed across colonies of hunter-gatherer species, although only the hunter-gatherer regression can be distinguished from isometry. Our analyses thus identify two major metabolic transitions across the clade: (1) a decrease at the initial adoption of fungus cultivation by ancestral lower attines and (2) an increase associated with the evolution of a coevolved crop by higher attines.

It is possible that shifting living-tissue storage from the ants themselves to fungal mutualists provided energetic storage advantages that facilitated attine diversification. However, we emphasize that linking colony-farm metabolism with farming-efficiency benefits will ultimately involve linking cultivar metabolism with the production and provisioning of sterile workers (colony growth) and winged sexual ants (colony reproduction). Establishing these links is difficult because basic details of colony-farm life history (e.g., size at reproductive maturity) are unknown for most attine species—especially the largest leaf-cutter colony-farms that likely receive the greatest fitness benefits from cultivar domestication. And, while leaf-cutter queens in *Atta* and *Acromyrmex* have exceptionally high capacity for high sperm storage (Tschinkel 1987*a*), further study will be needed to determine whether this increased capacity for egg production was facilitated by the transition to faster metabolism in the higher attine cultivar.

One possibility is that elevated MR in higher-attine cultivars reflects intrinsically elevated production value—the energetic costs of chemical work performed as gongylidia concentrate nutrients (Schiøtt et al. 2008). Alternatively, higher-attine cultivars may simply consume more resources, with colony-farms relegating large amounts of

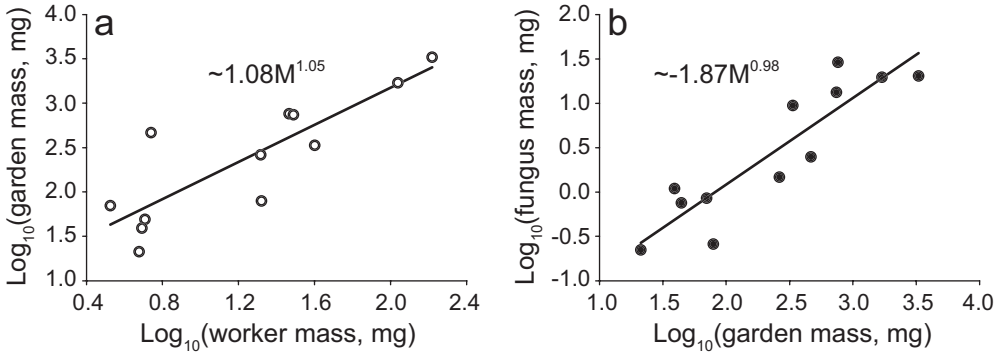

Figure 3: Scaling of garden mass with the mass of attine workforce (*a*) and fungus mass (garden mass × % chitin) with garden mass (*b*). Data are species averages.

harvested resources to trash heaps due to inefficient digestion of plant cellulose (Abril and Bucher 2002; Wirth et al. 2003; de Fine Licht et al. 2010). Such inefficiencies may incur additional foraging costs (e.g., leaf cutting is extremely energetically costly; Roces and Lighton 1995), while the potentially toxic CO_2 respired by fungi can accumulate within the nest (Kleineidam and Roces 2000; Bollazzi and Roces 2002; Bollazzi et al. 2012). With these caveats in mind, we outline a new storage-benefits hypothesis regarding the eco-evolutionary costs and benefits of farming and describe how it could be tested with additional data.

A central benefit of eusociality is cooperative nest defense that enhances queen and offspring protection and increases food-storage capacity (Oster and Wilson 1978). Harvested resources within ant nests are stored within adult ants (Rissing 1984; Tschinkel 1987*b*; Roma et al. 2009) and developing brood (Nonacs 1991) and externally as raw materials (e.g., insect parts and seeds; Smith 2007; Gayahan and Tschinkel 2008) or fungal tissue (in attine colony-farms). We propose that living-tissue storage is especially valuable in the Neotropics, where attines originated (Schultz and Brady 2008), because microbial activity constrains raw-material storage in these humid, ever-warm forests (Janzen 1977; Wagner et al. 1997). Moreover, the low mass-specific fungal metabolism (relative to ant tissue) observed in this study suggests additional energetic benefits.

To test for storage benefits, it will be necessary to distinguish edible fractions of gardens (e.g., edible hyphae packed in clusters of gongylidia called staphylae) from structural and nonnutritive parts. Storage benefits would then be supported if the rate at which farms produce the edible fraction increases faster than the storage costs. For instance, worker-fat content in the harvester ant *Pogonomyrmex badius* increases with colony size at a faster rate ($b > 1$) than the mass of its seed stores ($b \approx 1$; Tschinkel 1999). However, stored seeds do not necessarily support *P. badius* colony productivity under experimental food restriction (Smith 2007), and manipulative feeding experiments will be critical for testing whether fungus gardens actually buffer colony-farms during periods of resource shortage. Gardens do appear to help attine farmers icebox available environmental productivity, given that the attine *Trachymyrmex septentrionalis* used experimental food surplus to rapidly expand gardens (Seal and Tschinkel 2008).

We also note that farming benefits undoubtedly extend beyond food storage, given that fungi appear to benefit ants by detoxifying substrate (Van Bael et al. 2009; de Fine Licht et al. 2012). Moreover, while pupal cocoons are absent from the clade that contains the attines (subfamily Myrmicinae), many attine species use fungal mycelia to cover their brood. The resulting cocoons, which appear to have evolved soon after the farming transition, likely protect brood from disease (Armitage et al. 2012). Such additional services provided by cultivars have enabled attines to exploit new habitats and niches (Martin and Weber 1969; Mueller et al. 2011) and have metabolic costs and benefits that remain to be explored.

Decades of research have sought to understand metabolic hypometry—why larger organisms tend to consume less energy on a mass-specific basis (reviewed by Brown et al. 2004; Glazier 2005). Fascinatingly, metabolic hypometry also appears to extend across whole ant colonies (Hou et al. 2010; Shik 2010; Waters et al. 2010; Shik et al. 2012) and, as shown for the first time in the present study, may also apply to the extended phenotypes of attine colony-farms. However, although the hunter-gatherer slope ($b = 0.86$) was nearly identical to the slope for higher attines ($b = 0.85$), the two were distinguishable when compared to isometry because the upper limit of the 95% CI for the hunter-gatherer slope ($b = 0.94$) did not contain $b = 1.0$, but it did for the higher attines ($b = 1.06$). This result might stem from sample-size differences and

highlights the value and need of extending measurements of metabolic scaling to even more higher-attine species.

Similar scaling across such diverse organisms, as well as the general evolutionary trend toward ever-larger metazoan body size (Maynard Smith and Szathmáry 1995), ant-colony size (Hölldobler and Wilson 2010), and attine colony-farm size (Schultz and Brady 2008), suggests that forming higher-level collectives has consistent energetic benefits. And yet, fungus gardens—like all metazoans—are also consortia of diverse microbes (Pinto-Tomas et al. 2009; Scott et al. 2010) and larvae whose enzymes may mediate fungal production (Abril and Bucher 2002; Erthal et al. 2007). Moving forward, it will be important to partition MR across these components, detail the contributions of each to scaling exponents, and work to translate a metabolic currency to one of these fitness benefits.

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Trachymyrmex cornetzi with fungus. Photo credit: Jonathan Z. Shik.