

Electronic supplementary material

Materials and Methods

Data collection

We prepared a database searching in the published literature and consulting experts to obtain the geographical range, taxonomic status, phylogeny and waste location of LCA. Published studies were surveyed with searches in Biological Abstracts, Current Contents, ISI Web of Science and Google Scholar databases using the key words “leaf-cutting ants”, “refuse dump”, “LCA waste” “LCA phylogeny” and “LCA distribution”. We also included information available in the Ant Web (www.antweb.org), and from our own unpublished data and personal records. LCA with controversial taxonomic status (e. g., *Atta goiana*, *Atta silvairi* and *Acromyrmex nobilis*) or lacking information about waste location or geographical range (i. e., *Atta opaciceps*, *Acromyrmex diasi* and *Acromyrmex hystrix*) were excluded from the analyses. *Acromyrmex volcanus* was also excluded because this species has arboreal nesting behaviour [1].

Phylogenetic signal and phylogenetic correction.

To test whether the phylogeny had an effect on the behaviour to locate refuse inside or outside the nest, we estimated the value of phylogenetic signal “D” [2]. D-values approaching zero indicate that the trait has strong phylogenetic signal, whereas a D-value approaching one indicates that the trait has a weak phylogenetic signal, i.e. that the phylogeny has little influence on the evolution of the trait. First, we used a cladogram of *Atta* [3] to construct a tree with the species where we have information about refuse dump location (all but *At. opaciceps*) and comprised subspecies of *Atta sexdens* as a single branch. This cladogram has one polytomy, but it does not affect our analysis or conclusions. For *Acromyrmex* we constructed a tree based on the phylogeny of Sumner et al. [4], because it includes more species than other phylogenies [5–7]. We could not include some *Acromyrmex* species in the phylogenetic correction despite having the natural history information (Table S1), because their position on the phylogenetic tree is still unknown (*Ac. ambiguous*, *Ac. asperses*, *Ac. disciger*, *Ac. fracticornis*, *Ac. hispidus*, *Ac. laticeps*, *Ac. lobicornis*, *Ac. niger* and *Ac. striatus*). We did not include *Ac. versicolor* because of incongruent placement in different phylogenies: as sister species to *Ac. echinator* and *Ac. octospinosus* [5,6], as the most basal species [7], or because it was not included in the study [4]. However, the inclusion of *Ac. versicolor* in our analysis would not change the conclusions, because either placed as sister species of *Ac. echinator* and *Ac. octospinosus* [5,6] or basal to other *Acromyrmex* [7], it also has external refuse dump and lives in xeric

environments (see results). We set branch lengths to 1, and combined the two trees placing *Atta* and *Acromyrmex* as sister genera, following [5–7]. We then recoded the trait as binary to estimate the phylogenetic signal D (1 = having internal refuse dumps, 0 = not having internal refuse dumps), and use the function `phylo.d` of the “caper” package [8] for R software [9]. We run 10000 permutations to assess whether the estimated D value was statistically different from zero (strong phylogenetic signal) or one (weak phylogenetic signal). To analyse the effect of ecological contexts, we tested whether having internal refuse dump was associated with the lower or the higher latitude of the species distribution, and with the latitudinal range, or with living on xeric habitats (desert and dry grassland were coded as xeric environment; forest, savannah, shrubland and grassland as humid environments). We run phylogenetic logistic regressions (“PLR” [10]) using the function `pglm` of the package `phylolm` [11] for R, setting the method as “logistic_IG10”, and using the tree we created to test for phylogenetic signal.

Ancestral state reconstruction

We reconstructed the ancestral state of refuse dumps using parsimony (unordered model, equal probabilities of gains and losses) in Mesquite [12]. We used parsimony because we were working with a cladogram with branch lengths set at 1. For this analysis, we added three species of *Trachymyrmex* (*T. septentrionalis*, *T. arizonensis* and *T. smithi*) as an out-group to the *Acromyrmex* and *Atta* phylogenetic tree. Two studies independently assign a clade with these three species as the sister lineage to the LCA [7,13]. All these *Trachymyrmex* species have external refuse dumps [13,14]. For the analysis, we used two states (0 = external refuse dump; 1 = internal refuse chambers), and assigned the character as polymorphic (0 and 1) to species that present both external and internal refuse dumps. The conclusions of this analysis should be interpreted carefully because several *Acromyrmex* species could not be included in the phylogeny.

Lab experiment

To determine whether the typical hot soil surface temperatures of xeric habitats may reduce the pathogenicity level of ant waste located in external piles, we heated samples of external refuse dumps of *Atta colombica* and monitored the presence of the LCA fungal parasite *Escovopsis*. We sampled refuse dumps from 8 mature nests (5 m diameter) located in a rainforest of Panamá (9° N, 79° W) in Pipeland Road (Gamboa, Soberanía National Park). A full description of this site is available in [15][63]. In the laboratory, samples from each nest (300 g) were subdivided in two samples of 150 g: one sample was assigned to the heat treatment and the other was used as unheated control. Refuse samples in the heat treatment were subjected to 4 hours at 50 °C and 20 h at room temperature (~ 28° C) during two days. This warming regime tried to simulate the temperature and period length of the hottest hours typical of soil and refuse dump surfaces at the geographical limits of LCA distribution ([16]; table S1), where LCA species often have external refuse

dumps. Controls were maintained at room temperature during the same period. After that, each sample of 150 g was subdivided in 30 subsamples of 5 g and transferred into sterile petri dishes containing potato dextrose agar (PDA, 19.5 g per 500 ml distilled water). The small Petri dishes (60 x 12 mm) were placed on a larger Petri dish of 100 x 16 mm containing 20 mL of water agar as a source of moisture. We covered the larger petri dishes with the 100 mm Petri dish cover and sealed it with PVC film. All sub-samples were incubated at room temperature for eight more days. At the end of the experiment we tested for *Escovopsis* presence in all sub-samples. *Escovopsis* identification was performed using morphological markers following the classical taxonomic keys [17] and specific literature sources [18–20]. We estimated the prevalence of *Escovopsis* on refuse per nest as the number of sub-samples with *Escovopsis* divided by all sub-samples of each nest (n = 30). We compared this proportion between heat and control treatments with a t-test, using nests as replicates (i. e., n = 8).

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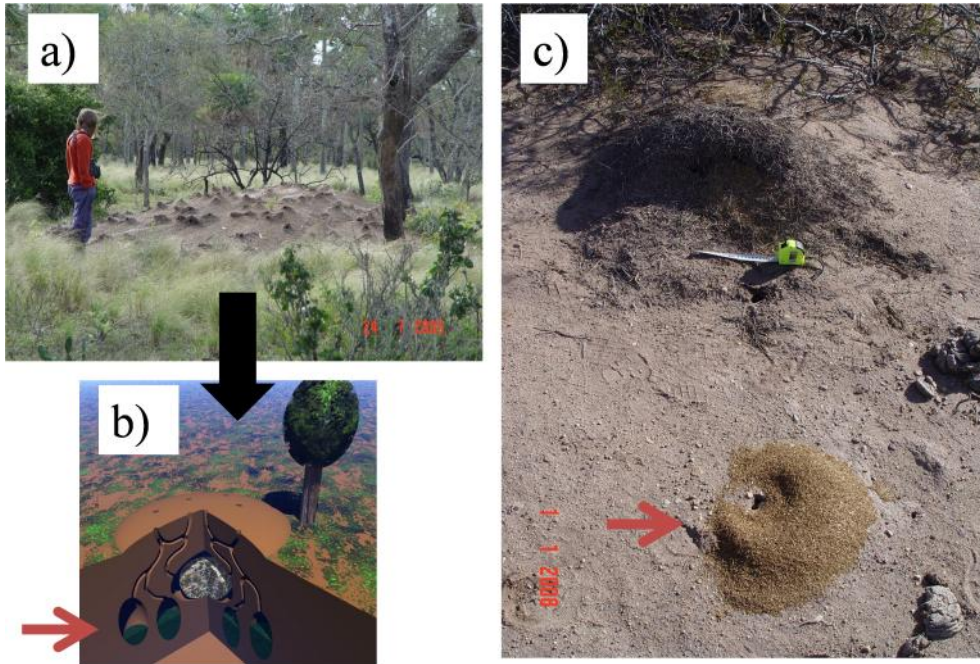


Figure S1. Leaf-cutting ant species vary in the location of waste. Some species, such as (a) *Atta wollenveideri* in subtropical savannahs, store waste in refuse chambers inside the nest, as shown in the (b) diagram. Other species, such as (c) *Acromyrmex lobicornis* in a Patagonian arid steppe drop waste on external refuse dumps. Red arrows indicate the location of waste. Photo credits: AGFB. Scheme (b) modified from <http://bioold.science.ku.dk/drnash/media/attanest.jpg>

Table S1. Waste location (inside: buried in underground chambers; outside: piled in external refuse dumps), geographical range and habitat of leaf-cutting ant species. *Habitat types*: F = tropical, subtropical and temperate wet forest and scrublands; X = Xeric habitats including dry forest, dry grasslands and deserts; G = tropical, subtropical and temperate grassland, rangeland and savannah. References in dataset.

Species	Subgenus	Waste location	Geographical range	Amplitude	Maximum range	Habitat	Reference
<i>Acromyrmex ambiguus</i>	<i>Acromyrmex</i>	inside	15°S - 35°S	20	35°S	G	3, 35, 39
<i>Acromyrmex aspersus</i>	<i>Acromyrmex</i>	outside	14°S - 32°S	18	32°S	F, G	35,39
<i>Acromyrmex balzani</i>	<i>Moellerius</i>	outside	20°S - 30°S	10	30°S	X	1,3,35
<i>Acromyrmex coronatus</i>	<i>Acromyrmex</i>	both	15°N - 28°S	43	28°S	F	3,35,36, 40,45
<i>Acromyrmex crassispinus</i>	<i>Acromyrmex</i>	both	22°S - 30°S	8	30°S	F (inside) X (outside)	35,53,54
<i>Acromyrmex disciger</i>	<i>Acromyrmex</i>	inside	22°S - 29°S	7	29°S	F	3,35,56
<i>Acromyrmex echinator</i>	<i>Acromyrmex</i>	outside	9°N - 17°N	8	17°N	X	41,45,66
<i>Acromyrmex fracticornis</i>	<i>Moellerius</i>	outside	16°S - 34°S	18	34°S	X	3,35,36
<i>Acromyrmex heyeri</i>	<i>Moellerius</i>	outside	25°S - 35°S	10	35°S	X, G	26,35,37,

							66
<i>Acromyrmex hispidus</i>	<i>Acromyrmex</i>	outside	23°S - 30°S	7	30°S	X	3,35,42,55,60
<i>Acromyrmex landolti</i>	<i>Moellerius</i>	outside	9°N - 20°S	29	20°S	G	16,33,35
<i>Acromyrmex laticeps</i>	<i>Acromyrmex</i>	inside	2°S - 34°S	32	34°S	G	3,35,42
<i>Acromyrmex lobicornis</i>	<i>Acromyrmex</i>	outside	24°S - 44°S	20	44°S	X	3,25,38,61,67
<i>Acromyrmex lundi</i>	<i>Acromyrmex</i>	both	2°S - 40°S	38	40°S	G	3,20,39,42,44,54,66
<i>Acromyrmex niger</i>	<i>Acromyrmex</i>	inside	4°S - 27°S	23	27°S	F	35,56,62,66
<i>Acromyrmex octospinosus</i>	<i>Acromyrmex</i>	outside	23°N - 2°S	25	23°N	X, F	35,42,45,65
<i>Acromyrmex rugosus</i>	<i>Acromyrmex</i>	inside	2°S - 31°S	29	31°S	F, G	3,5,15,35,42
<i>Acromyrmex striatus</i>	<i>Moellerius</i>	outside	28°S - 42°S	14	42°S	X, G	3,35,36,38,56
<i>Acromyrmex subterraneus</i>	<i>Acromyrmex</i>	both	1°S - 28°S	27	28°S	F, G	35,47,56,63
<i>Acromyrmex</i>	<i>Moellerius</i>	outside	29°N - 35°N	6	35°N	X	12,13,19,

<i>versicolor</i>						48
<i>Atta bisphaerica</i>	inside	20°S - 23°S	3	23°S	G	10,32,62, 64
<i>Atta capiguara</i>	inside	19°S - 25°S	6	25°S	G	3,30,62,64
<i>Atta cephalotes</i>	inside	18°N - 13°S	31	18°N	F	14,27,62, 69
<i>Atta colombica</i>	outside	8°N - 11°N	3	11°N	F	2,14,31,57
<i>Atta insularis</i>	inside	20°S - 23°S	3	23°S	F	14,46,66, 70
<i>Atta laevigata</i>	inside	9°N - 24°S	33	24°S	G, F	3,9,27,62, 64,69
<i>Atta mexicana</i>	outside	18°N - 32°N	14	32°N	X	6,17,34,58, 66
<i>Atta robusta</i>	inside	18°S - 23°S	5	23°S	F	28,29,50, 52,63
<i>Atta saltensis</i>	inside	21°S - 32°S	11	32°S	G, F, X	3,24
<i>Atta sexdens</i>	inside	10°N - 28°S	38	28°S	F	27,32,57,

<i>Atta texana</i>	inside	20°N - 33°N	13	33°N	F, X	8,17,18,19, 34
<i>Atta vollenweideri</i>	inside	21°S - 33°S	12	33°S	G, X	3,14,21,24

Lower Attini with external refuse dumps: *Apterostigma bruchi* (26) *Trachymyrmex arizonensis* (12,13), *Sericomyrmex amabilis* (45), *Trachymyrmex smithi* (13). See references in dataset.