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Phylogenetic signal in leaf-cutting ant diet in the fragmented Atlantic rain forest

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Abstract

Leaf-cutting ants are dominant herbivores in Neotropical rain forests, and their colony densities increase in disturbed habitats such as forest edges. However, while it is well-established that leaf-cutting ants profit from changes to the food-plant community, the phylogenetic dimension of this ant–plant interaction remains poorly understood in fragmented forests. We studied diet composition of *Atta cephalotes* in the edge and interior of Atlantic forest in north-east Brazil (8°30′S, 35°50′W). We applied phylogenetic signal analysis to investigate the diet across plant lineages and performed phylogenetic generalized linear models to analyse the diet in both habitats. We found a phylogenetic signal in diet and in leaf mechanical resistance, which means that *A. cephalotes* selects closely related food plants with less resistant leaves. Most preferred species belong to Malpighiales, Rubiaceae and Melastomataceae. We also found that irrespective of phylogeny, ants select food plants with less resistant leaves, both in edge and interior. However, ants choose more abundant plants only in edges. High abundance of optimal diet facilitates foraging in forest edges and explains why colony densities increase in disturbed habitats. Finally, by favouring or disfavouring specific clades, leaf-cutting ants contribute to changes in the phylogenetic structure of tropical rain forests, e.g. phylogenetic impoverishment.

Leaf-cutting ants (LCA) are dominant herbivores in the Neotropical rain and dry forests and savannas, removing up to 30% of green biomass in the foraging area of a colony (Siqueira *et al.* 2018, Urbas *et al.* 2007, Wirth *et al.* 2003). In human-modified landscapes their impact is amplified by the fact that LCA colonies proliferate in disturbed habitats such as early-successional forests (Vasconcelos & Cherrett 1995), forest remnants (Terborgh *et al.* 2001) and forest edges (Wirth *et al.* 2007). Understanding LCA diet in disturbed forests could help us to predict their success and impacts on the ecosystem.

Although LCA are largely polyphagous and able to harvest up to 50% of local plant species (Wirth *et al.* 2003), they do exhibit a pronounced food-plant selection. Laboratory experiments have demonstrated that LCA prefer younger and softer leaves over harder and older ones (Howard 1988, Nichols-Orians & Schultz 1989). In field studies, LCA were shown to prefer particular plant groups such as the light-demanding pioneer species, presumably due to softer leaves (i.e. reduced morphological defences) and fewer chemical defences associated with a fast-growth strategy (Falcão *et al.* 2011, Farji-Brener 2001, Wirth *et al.* 2003).

In this perspective, research in human-modified tropical landscapes, particularly in fragmented forests, can provide insights into the patterns of LCA diet because pioneer plant species become more abundant in edge-affected habitats (Laurance *et al.* 2006, Santos *et al.* 2008), leading to reduced species diversity (Tabarelli *et al.* 2008). Although not all fragmented forests lose tree phylogenetic diversity (Arroyo-Rodríguez *et al.* 2012, Matos *et al.* 2017), pioneer species tend to be more related than late-successional species and drive the phylogenetic impoverishment of the forest (Norden *et al.* 2012, Santos *et al.* 2010, 2014). While phylogenetic distance between neighbouring plant species is likely to interfere with the ability of herbivores to exploit their food plants (Yguel *et al.* 2011), the effect of such human-induced floristic shifts on LCA diet remains entirely unstudied.

Here we examine LCA diet composition in a fragmented Atlantic forest. We hypothesize that: (1) There is a phylogenetic signal in LCA diet following the preference for pioneer species; (2) LCA diet consists of more abundant plant species; (3) LCA diet consists of species with less physical resistance such as softer leaves; and (4) LCA diet depends on the forest habitat (interior vs. edge).

We conducted the study in the Coimbra forest (8°30′S, 35°50′W), the largest (3500 ha) and best-preserved fragment of Atlantic rain forest in north-east Brazil (Santos *et al.* 2008). Coimbra is located on a low-altitude plateau (300–400 m asl). Annual rainfall is ~2000 mm, with a 3-mo

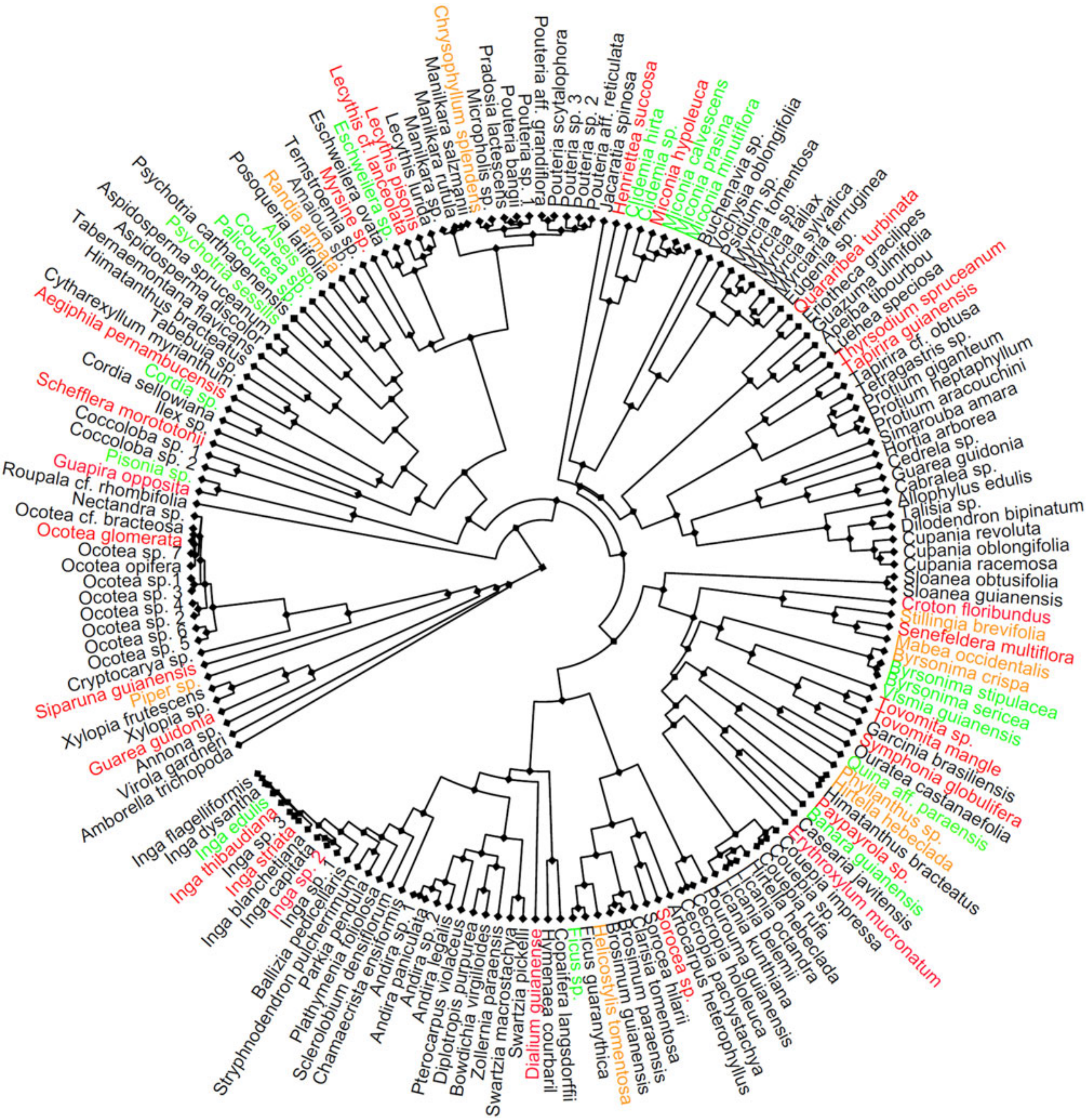

Figure 1. Phylogenetic tree of plant species that occur at the edge and in the interior of Atlantic forest in north-east Brazil (8°30′S, 35°50′W). Coloured names indicate species present in leaf-cutting ant (*Atta cephalotes*) diet, with phylogenetic signal (D = 0.52; P(random) < 0.0001, P(Brownian) < 0.0001). Orange = species in ant diet in forest interior, green = species in ant diet at forest edge, red = species in ant diet in the two habitats.

dry season (< 60 mm mo^{-1}) from November to January. The most species-rich families in the region are Fabaceae, Lauraceae, Sapotaceae, Chrysobalanaceae and Lecythidaceae (Santos *et al.* 2008, 2010).

We studied food plant selection, i.e. presence/absence of plant species in the diet (hereafter: diet) of five adult colonies of *Atta cephalotes* (L.) at the edge (<100 m from forest border, sensu Laurance *et al.* 1998) and five in the interior (>200 m from forest border) of Coimbra forest. The inter-colony distances were 2.0 ± 1.4 km (mean ± SD) in forest edge and 1.1 ± 0.4 km in the interior. The colonies were studied in every second month over a period of 1 y, and lists of plant species attacked by each colony were compiled (Falcão *et al.* 2011). The data from individual colonies were used as independent data points in each habitat.

We used full woody-plant species lists of the edge and interior habitats of Coimbra forest (Santos *et al.* 2010) as a reference species pool to infer the diet of *A. cephalotes*, and to obtain data on the relative abundance of plant species in the two habitats. To test if ant diet consists of softer leaves, we gathered data from the TRY Plant Trait database on leaf mechanical resistance, reflected by leaf tensile strength in N/m (Kattge *et al.* 2011). When species-level data were not available, we used the genus-level means. When genus-level data were not available ($N = 48$ of 175 species) we used the mean value of our species list (the results remained highly significant when species with missing data were excluded).

We constructed a time-calibrated phylogeny for the full plant species list of Coimbra forest. For every species we searched for four DNA regions (matK, 5.8S, rbcL, trnL-trnF) from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). For most species not all sequences were available, or individual species were identified to the genus level only ($N = 50$), thus we used sequences of congeneric species. We aligned the sequences separately and concatenated them in a supermatrix in Geneious 9.1.3 (www.geneious.com). We used the program BEAST v1.8.0 (Drummond *et al.* 2012) for the Bayesian analysis and to include time calibration for the tree. To time calibrate the tree we adopted four calibration points (Myrtales, Malpighiales, Sapindales and Eudicotyledoneae; Magallón *et al.* 2015).

We analysed phylogenetic signal in the diet of *A. cephalotes* with *D*-value for discrete traits (Fritz & Purvis 2010). We also tested for phylogenetic signal in leaf mechanical resistance and in plant species abundance in forest habitats with Pagel's λ-value for continuous traits (Pagel 1999). These analyses were done using the R packages 'caper' and 'phytools'. We performed phylogenetic generalized linear models (phyloglm) to test the effect of leaf mechanical resistance and plant species abundance at forest edge and interior on ant diet in these habitats, taking into account plant phylogeny to control for the effect of species' relatedness on trait values. These analyses were conducted in R using the packages 'car' and 'phylolm'.

There was a phylogenetic signal in the diet of *A. cephalotes* ($D = 0.52$, $P(\text{random}) < 0.0001$, $P(\text{Brownian}) < 0.0001$; Figure 1). The ants selected both ancient (e.g. Piperaceae, Lauraceae) and recent (e.g. Malpighiaceae, Euphorbiaceae) plant groups, with most species belonging to Malpighiales, Rubiaceae and Melastomataceae (Figure 1). We also found a phylogenetic signal in leaf mechanical resistance ($\lambda = 0.25$, $P(\text{random}) = 0.0006$, $P(\text{Brownian}) < 0.0001$), but species abundance in the habitats did not show a phylogenetic signal. The results of the phylogenetic models show that irrespective of phylogeny, ant food species have less resistant leaves at both forest edges and interior (edge: $z = -3.2$, $P = 0.0342$; interior: $z = -3.6$, $P = 0.0004$). Moreover, more abundant species in the forest were only part of the diet of *A. cephalotes* at the forest edge (edge: $z = 2.1$, $P = 00342$; interior: $z = 1.2$, $P = \text{ns}$).

Our study confirms that LCA diet is clearly not random. Firstly, our results suggest that the diet is concentrated on some phylogenetic groups, i.e. there is indeed a phylogenetic signal in the diet. Secondly, we found that irrespective of phylogeny, *A. cephalotes* strongly select food plants with mechanically less resistant leaves both at forest edge and interior. Additionally, our results revealed phylogenetic signal in leaf resistance, which means that less resistant leaves are more frequent across particular plant clades. Thus, leaf mechanical resistance represents an explanation for why the ants are specialized to specific clades. However, while not addressed in this study, this does not exclude the potential influence of other leaf traits, such as plant secondary compounds for LCA diet selection (Wirth *et al.* 2003). Moreover, using abundance data on LCA food plant selection (rather than the presence/absence data available to us) may provide additional insights into the nature of LCA diet preference.

The phylogenetic signal in the diet of *A. cephalotes* could be caused by their selection of pioneer species as food plants. Although it is not uniformly established whether pioneer species belong to phylogenetically closely related clades in neotropical forests (Norden *et al.* 2012, Santos *et al.* 2010, 2014), our results suggest this relationship due to the phylogenetic signal in ant diet, and because in the studied forest pioneer species account for more than a half of both tree species and individuals in edge-affected habitats (Oliveira *et al.* 2004, Santos *et al.* 2008). We also found that *A. cephalotes* uses plant species that are more abundant in forest edges but not in forest interiors. The ability of herbivores to exploit their food plants has been found to increase with the phylogenetic proximity between neighbouring plant species (Yguel *et al.* 2011). Our results confirm these findings because of the phylogenetic signal in ant diet and because of the abundance of pioneer species in forest edges. In order to understand the possible reciprocal influence of diet abundance and LCA colony establishment, we encourage future studies to investigate the abundance of plant species inside the foraging territories of LCA.

Phylogenetic signal in LCA diet has multifarious implications for biodiversity persistence in human-modified forests. The existence or lack of phylogenetic signal in pioneer species results in various effects on vegetation. When pioneer species are more related than late-successional species, their proliferation drives phylogenetic impoverishment of the forest (Norden *et al.* 2012, Santos *et al.* 2010, 2014). However, this is not always the case because not all fragmented forests lose tree phylogenetic diversity (Santo-Silva *et al.* 2018). We conclude that by favouring or disfavouring specific phylogenetic clades via herbivory, LCA are key ecological players in the conversion of Neotropical old-growth forests into human-modified secondary forests.

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