Relationship between mandible morphology and leaf preference in leaf-cutting ants (Hymenoptera: Formicidae)

Relación entre la morfología de la mandíbula y la preferencia por hojas en hormigas arrieras (Hymenoptera: Formicidae)

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Abstract: Morphological differences among mandibles of grass and leaf-cutting ant species were examined. Specifically, we compared morphometric measurements from mandibles of four species (*Atta laevigata*, *A. bisphaerica*, *Acromyrmex balzani*, and *Ac. subterraneus*). It was found that these *Atta* species had similar mandible morphologies, primarily differing in distal tooth length. In contrast, the two *Acromyrmex* exhibited considerable variation in morphology: *Ac. balzani* mandibles are more robust and massive, while *Ac. subterraneus* mandibles are elongated. These results support that morphological differences in mandible shape and dentition are related to preference for either grasses or eudicot leaves.

Key words: Grasses. Eudicots leaves. Morphometry. Ant jaws.

Resumen: Este trabajo examina las diferencias morfológicas entre las mandíbulas de hormigas arrieras que cortan hojas y pastos. Especificamente, se compararon las mediciones morfométricas de mandíbulas de cuatro especies (*Atta laevigata, A. bisphaerica, Acromyrmex balzani* and *Ac. subterraneus*). Se encontró que las especies de *Atta* presentan menos diferencias en la morfología mandibular, siendo la principal diferencia la longitud del diente distal. En contraste, las mandíbulas en *Ac. balzani* son más robustas y macizas, mientras que en *Ac. subterraneus* fueron alargadas. Estos resultados permiten afirmar que las diferencias morfológicas dan lugar a una preferencia refinada por pastos u hojas de eudicotiledóneas.

Palabras clave: Pastos. Hojas Eudicots. Morfometría. Mandíbulas de la hormiga.

Introduction

Among all fungus-growing ants, leaf-cutting ants are quite diverse being represented by 81 species (*Atta*, 19 species and *Acromyrmex*, 62 species) (Brandão *et al.* 2011). All of them cut and utilize plant foliage to nourish the fungus that they culture inside their nests (Della-Lucia, 2011). While some species show preferences for grass to cultivate their fungus garden, others use eudicots leaves. Thus, many taxa can be separated into grass-cutting (*e.g. A. capiguara, Ac. balzani*) and leaf-cutting species (*e.g. A. sexdens, Ac. subterraneus*). Both grass and leaf-cutting species do a complex treatment of fresh leaves substrate from the cutting of leaves to their incorporation into the fungus garden (Forti *et al.* 2004; Lopes *et al.* 2004; Camargo *et al.* 2007).

Grass and leaf-cutting ants vary in behavior when cutting leaves, in part because grass cutting species tend to have shorter metathoracic legs than leaf-cutting species (Fowler *et al.* 1986). Leaf-cutting species use the leg as a pivot while cutting the plant material, and thus, access a compatible weight load that a forager can carry (Lutz 1929). In contrast, grass-cutting species do not do exhibit this pivot movement and the grass blades are cut linearly and transversally along their length (Lopes *et al.* 2003). This different way of cut leaves could also be a result of different mandible morphology, resulting in a different size-matching relationship.

In addition, behavioral variation in relation to leaf preference (Lopes *et al.* 2004), variation in mandible

morphology also exists among leaf cutting ant species. Grasscutting ants tend to have more massive and short mandibles. while leaf-cutting ants tend to have longer and slimmer mandibles (Fowler et al. 1986). Even though the mandibles are important tools required for many tasks (Hölldobler and Wilson 1990; Paul 2001), quantitative information on how their morphology varies among species is largely lacking. Mandible specialization is not only dependent on morphology, but also on kinematics - the speed and on the force the mandibles can generate. For example, catching elusive preys obviously requires different movement characteristics and tactics than cracking seeds (Paul 2001). In other ants a strong relationship exists between mandible morphology and foraging behavior, especially for trap-jaw ants whose mandible specialization may be driven by prey type (Brown and Wilson 1959; Moffett 1985; Hölldobler and Wilson 1990; Ehmer and Hölldobler 1995; Dejean et al. 2002; Spagna et al. 2009).

This work aims to provide a more detailed morphological differentiation of mandibles among grass and leaf-cutting species by comparing some morphometrical measurements from mandibles of two grass-cutting (*A. capiguara, Ac. balzani*) and two leaf-cutting (*A. sexdens, Ac. subterraneus*) ant species. It is predicted that the material properties and venation patterns exhibited by grasses and leaves influence mandible specialization in leaf cutting ants. Grasses have increased silica and parallel and dense veins over a small surface, while eudicots leaves have conducting vessels across

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a larger surface area, forming a network of veins (Mauseth 1988). Together, these properties should influence mandible morphology in species that harvest them.

Material and methods

To compare ant mandible morphology, 30 foraging workers of Ac. balzani at Botucatu SP. Brazil (48°26'36"W 22°54'26"S) were collected, and also 30 foraging workers of A. laevigata, A. bisphaerica and Ac. subterraneus each at Juiz de Fora MG, Brazil (21°46'49"S 43°22'24"W). All collected ants were transporting leaf fragments at the foraging trail, from one colony of each species. Although it was not possible to access the workers age, this procedure was chosen because age polyethism known in these ant species (Camargo et al. 2007). We assumed that foragers were similar in age and should exhibit similar abrasion patterns on their mandibles. In addition, the analyzed mandibular features, although subject to wear, comprise a minor fraction of the whole mandible (Patterson 1984). Collected foragers were individualized and transported to MirmecoLab, UFJF, Juiz de Fora, Brazil and confirmed as forager ants (Table 1) by head width measurements (A. bisphaerica: Hughes et al. 2001; A. laevigata: Hernandez et al. 1999; Ac. subterraneus: Camargo et al. 2007; Ac. balzani: Ichinose et al. 2007).

Dissection was performed to remove the right mandible of workers under stereoscopic microscope. The mandibles were then mounted on slides with Hoyers solution (40mL of distilled water, 30 g crystals of Arabic gum, 200 g of chloral hydrate, 20 mL of glycerol) (Flechtmann 1975). The Hoyers solution was used to clarify the mandibles and, thus allowing a better measurement. The mandibles were arranged anteroposteriorly, and put for 48 hours in an oven at 50 °C. Then, the following measurements were taken: (1) mandible length, from basal region to apical, (2) mandible width, distance between the mandible edge, (3) length of the first tooth, from basal region to apical one, (4) length of the second tooth, from basal region to apical one, (5) number of teeth, just counting the protuberance or teeth and (6) head width (mm). Measurements were made using an evepiece micrometer attached to a stereomicroscope.

Principal Component Analysis (PCA) was used to examine variation in the morphometric data among species using the free software PC-ORD 5.15 (McCune and Mefford 2011). The number of principal components (PCs) was determined considering eigenvalues higher than those generated by the broken-stick method. The morphometric measurements which most contributed to the axes, were selected by Pearson (r > 0.7).

Results and discussion

We found differences in the morphology among the mandibles from different ants, especially for *Ac. balzani* (Fig. 1). *Ac. balzani* mandibles are striking different, being shorter and massive, generally with two teeth. The second tooth is very short, too close to the first one and sometimes could be absent, which must be a result of the mandible wear. Along the entire length of the cutting boarder, we can see a concave depression, giving a sickle aspect. This kind of mandible configuration characterizes a grass-cutting ant mandible according to Fowler *et al.* (1986).

Ac. subterraneus has elongated mandibles with about 9 teeth, also creating a serrated-pattern. The first tooth, the distal, and the second create a V-like blade, which is typical of a leaf-cutting mandible (Fowler *et al.* 1986). The other teeth, as observed for *A. laevigata*, are progressively shorter in their length, but the teeth-series are not so morphologically evident like could be found in *A. laevigata* (Fig. 1).

A. laevigata and *A. bisphaerica* present larger and more elongated mandibles, with numerous teeth (6-9 teeth). The main difference between them is related to the distal tooth, which is longer in *A. bisphaerica* than in *A. laevigata* (Table 1). The other teeth are also more conspicuous in *A. bisphaerica* because the reentrance between them is deeper. These teeth present a progressive reduction in their length, creating a serrated-pattern. Differently, a serrated-pattern is observed for *A. laevigata* mandibles with two different three-teeth series. Robust and large teeth while the proximal one is formed by sharpened and thin teeth form the distal three-teeth series.

The mandible morphology could allows leaf-cutting ants to asymmetrically to slice the leaves, with the lagging one often held at a nearly fixed orientation during the cut,



Figure 1. Mandibles and head front view from species. A. Atta bisphaerica. B. Atta laevigata. C. Acromyrmex balzani. D. Acromyrmex subterraneus.

Ant species	Head width	Mandible length	Mandible width	Number of teeth	Length of first tooth	Length of second tooth
Atta bisphaerica	2.93 ± 0.44	1.51 ± 0.13	0.82 ± 0.07	7.43 ± 1.00	0.44 ± 0.08	0.20 ± 0.045
Atta laevigata	3.03 ± 1.03	1.61 ± 0.36	0.82 ± 0.20	7.20 ± 0.81	0.37 ± 0.12	0.17 ± 0.07
Acromyrmex balzani	2.19 ± 0.30	0.93 ± 0.16	0.56 ± 0.12	2.56 ± 0.97	0.13 ± 0.06	0.05 ± 0.04
Acromyrmex subterraneus	1.78 ± 0.21	1.27 ± 0.14	0.57 ± 0.09	8.93 ± 0.78	0.22 ± 0.04	0.10 ± 0.02

Table 1. Morphometric measurements (mean and standard deviation) (mm) of the mandibles and teeth of four ant species (n = 30/ species).

while the leading one opens and closes (Tautz *et al.* 1995). During the cutting process, the distal tooth of the lagging mandible penetrates beyond the leaf surface opposite from the ant. Major workers can cut the entire thickness of the leaf between the two distal teeth (Schofield *et al.* 2011) in a way that just the tip of the mandible is used. The following teeth-series must serve to hold, shred or chew the leaves during transport and incorporation. Leaf-cutting ants cut in a semi-circular shape.

In contrast, grass-cutting workers are positioned beside the grass blade surface. The legs of one side of the body are maintained over the fragment, whereas the meta- and mesothoracic legs of the other side are stretched backward. During the process, the worker moves its head from side to side of the grass blade making a complete cross-section. The cut fragment has therefore a rectangular shape (Lopes *et al.* 2004). Thus, considering that the grass blade is thin, we suspect that grass-cutting species use all teeth during the cutting process, either by pressing or slicing the grass by *A. bisphaerica* and *Ac. balzani* respectively.

In Acrididae (Orthoptera), different diets are related with different mandible configuration primarily reflected in progressive fusion and flattening of teeth into grinding plates (Patterson 1984), as observed for *Ac. balzani*. Comparing the mandible length between the species within the same genus, across insect orders may reveal that grass-cutting species have shorter mandibles while graminivorous Acrididae present a reduction in the mandible length (Patterson 1984) and



Figure 2. Analysis of Principal Components (PCA) of the ant species. Cross: *Atta bisphaerica;* Circle: *Atta laevigata;* Triangle *Acromyrmex subterraneus;* Asterisk: *Acromyrmex balzani.* multiple independent convergences to chisel shaped incisor cusps and ridged molar regions for grinding (Chapman 1964; Bernays 1991). In leaf-cutting ants different size classes of workers present different mandible sizes what reflects in their division of labor during the execution of different tasks (Camargo *et al.* 2007; Hastenreiter *et al.* 2015).

Tooth wear is increased by the presence of silica (Massey *et al.* 2006), which is known to be present in the tissues of grasses to reduce levels of grazing by invertebrates herbivores (Vicari and Bazely 1993). It is reasonable to expect that grass-cutting species have more robust mandibles than leafcutting ones to resist this wear. One way to work out with the mandibles abrasion problem is the incorporation of inorganic components in "tools" which interact with the environment, in a way that their mechanical properties are modified. For example, *A. sexdens* shows a high degree of correlation between tooth hardness and zinc content (Schofield 2002).

Principal component analysis using five mandible morphometric characteristics produced two axes that together explained 86.96% of the total variance. The first axis accounted for 69.30% (Fig. 2). The corresponding correlation coefficients were: 1st tooth length, 0.929; Mandible length, 0.915; 2nd tooth length, 0.913; and Mandible width, 0.758. The second axis explained an additional 17.65% and showed a significant relationship just with the number of teeth (R = 0.738). *Atta* species are less dissimilar due to the length of teeth being separated from *Acromyrmex* species. On the other hand, between the *Acromyrmex* species the dissimilarity is mainly due the number of teeth.

This work highlights a more detailed evaluation of the morphological differences among mandibles of grass and leaf-cutting ant species. We suspect that these differences could lead to a refined preference for grasses or eudicots leaves, but to really check this pressing issue, experiments to verify the costs and benefits of using grass and eudicots leaves must be measured for these ant species.

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