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Morphometric Analysis of Characters of the Polymorphic Leaf-Cutting Ant Workers *Acromyrmex rugosus rugosus*

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Abstract – **The leaf-cutting ant present a polymorphism accentuated with a coordinate division of labor among castes of workers. However, there are controversies regarding the number of castes in *Acromyrmex* species. In this way, the objective of this work was to determine the number of size classes of *Acromyrmex rugosus rugosus* workers and the morphometric variations of characters of the different size categories. 540 workers from three colonies were visually separated into three size classes: small, medium and large. After this procedure the following measures were performed: total worker length, cephalic capsule length, cephalic capsule width, body length, posterior tibia length, interocular space, lateral pronotal spine length and anterior mesonotal spine length. For the determination of significant differences of the morphometric variables among the size classes, it was decided to perform the Nemenyi means comparison test, which is appropriate for data that do not present a normalized distribution. The test of correlation of Pearson also was accomplished, in order to if to obtain the correlation index among all the variables for the three classes of workers. The variables of morphometric characters evaluated presented significant differences among the size categories allowing to confirm the grouping of the three determined size groups. Moreover, when the variables were correlated in function of the class of the workers' size, it was verified that all the characters are directly correlated. Thus, based on these data, we suggest the existence of 3 castes of workers in this subspecies.**

Keywords **– Caste Determination, Morphology, Physical Castes, Social Insects.**

I. Introduction

Workers of any ant genus vary in size and activity, but certain species present more than one size of worker (polymorphism) with a coordinate division of labor among the castes (Wilson 1980; Wetterer 1999). According to Hölldobler & Wilson (1990), the term caste is used to describe the physical or behavioral phenotype of an eusocial individual, or both. Some authors, for example, Buschinger & Winter (1976, 1978), define castes according to function alone, whereas others such as Peeters & Crozier (1988) advocate a strictly morphological definition. Already according to Oster & Wilson (1978) caste is a set of individuals from a colony specializing in particular activities that exert for extended periods.

Oster & Wilson (1978) mention that the most fundamental division within the colony is between the reproductive caste and the caste of workers. Within the caste of workers, we can classify one or more classes of size and/or age, which are called castes or subcastes. Therefore, there are temporal castes whose behavior is related to age and physical castes whose behavior is related to size being this phenomenon known as polyethism.

In the case of age polyethism, younger workers tend to perform tasks within the nest, while older workers are committed to external tasks (Wilson 1971; Hölldobler & Wilson 1990; Camargo *et al.* 2007). Already in size-related polyethism in most leaf-cutting ant colonies, the small and medium workers are responsible for a large number of tasks performed within the nest, while large workers are specialized for defense or foraging (Litttledyke & Cherrett 1976; Wilson 1980; Andrade *et al.* 2002; Lopes 2004; Verza 2007).

In *Acromyrmex* the workers present varying sizes and perform different functions, but the number of castes is still controversial since Wetterer (1999) suggested that some species of this genus have only two: small and large workers, the medium would not be a distinct caste, but rather an extension of size distribution of smaller workers. However, other authors suggest the existence of at least three (small, medium and large) or more (Gonçalves 1961; Andrade 2002; Lopes 2004; Forti *et al.* 2004). Being that within each caste there are specimens with variations in the size and position of the spines of the head and trunk, and that do not present bilateral symmetry (Gonçalves 1961; Ribeiro & Queiroz 1993).

Face of the displayed one above, this work aimed to determine the number of worker size classes of *Acromyrmex rugosus rugosus* F. Smith, 1858 (Hymenoptera, Formicidae) and the morphometric variations of the characters of the different size categories.

II. Materials and Methods

Were used three nests of *A. rugosus rugosus* collected in Botucatu, SP, Brazil (22º53’09’’S; 48º26’42’’W). The nests 1, 2 and 3 had the following loose soil area size 0.33, 0.26 e 1.00 m², respectively. In nest 3 were found winged male and female demonstrating that this nest was adult; in the others two we could not determine its age.

The identification of this subspecies was done under stereomicroscope, using the identification key developed by Gonçalves (1961) and Forti *et al.* (2006). Based on the technique used by Andrade (2002) and Simas *et al.* (2003), the workers of three colonies were visually separated in three size classes.

The classes were thus denominated: (S) small, (M) medium (L) and large. 60 workers of each size category were measured in each nest. For all specimens studied, the adopted variables were selected from the measurements used by Ribeiro & Queiroz (1993), Andrade (2002) and Simas *et al.* (2003).

Measurements used: TBl- total body length (including the head), CCl- cephalic capsule length, CCw- cephalic capsule width, Bl- body length (excluding the head) PTl- posterior tibia length and IS- interocular space. In addition to these measures, for the specimens of the larger workers the LPSl - length of the lateral pronotal spine and the AMSl - length of the anterior mesonotal spine were also measured (Figures 1 and 2), these measurements were performed in frontal and lateral view of the worker using the micrometer eyepiece of the stereoscope microscope.

For the determination of significant differences of the morphometric variables between the size classes and between the nests, an exploratory data analysis was performed (Univariate procedure, SAS® 2001). This analysis aimed to verify the occurrence or not of a normalized distribution of the data obtained, which was not found. In this case, the Nemenyi means comparison test (Zar 1996) was chosen at the 5% probability level that is appropriate for data that do not show normalized distribution. The Pearson correlation test ($\alpha = 0.05$) was performed among all analyzed morphometric variables (Corr procedure, SAS® 2001) in order to obtain the correlation index of these variables for the 3 worker size classes.

III. Results

The mean width values of the cephalic capsule of the *A. rugosus rugosus* workers found in the small category were 0.67 - 1.07mm; in the medium category were 1.08 - 1.47; and in the large category were 1.48 - 2.01. The Nemenyi test indicated significant differences among the size categories for the six variables of morphological characters evaluated, making it possible to determine the grouping of 3 size groups (Table 1). When the morphometric variables were correlated according to the size classes of the workers, it was verified that all the characters are directly correlated (Tables 2, 3 and 4). Thus, it is entirely justified to use head width as representative of the morphometric characters to determine the castes of this subspecies.

When comparing the mean values of the morphometric characters among the three nests, no significant difference was found for the variables: total worker length, cephalic capsule width and posterior tibia length (Table 5). However, the data from the cephalic capsule length, body length and interocular space were significantly different among colonies (Table 5).

Regarding the variables LPSl and AMSl of workers classified as large, the analyzes did not indicate significant differences among the nests (Table 6).

IV. Discussion

Unlike the one proposed by Oster & Wilson (1978) and Wetterer (1999), which suggested the existence of a bimodal distribution, corresponding to two castes of workers for *A. octospinosus* and *A. volcanus*, our results showed the grouping of three size groups. These three castes resulting from morphometric data are in agreement with data obtained from behavioral studies by Lopes (2004) who studied how the tasks performed during the incorporation of vegetables in the fungus garden are distributed among the size classes of *A. rugosus*. This author established five size classes: very small (cephalic capsule: 0.60 - 0.75 mm), small (0.75 - 0.90), mean I (0.90 - 1.00), mean II (1.00 - 1.30) and large (1.30 - 1.60), and according to the observation made, suggested the existence of at least three castes. Still in the same research, it was added that the performance of the medium size classes is indisputably essential during the process of incorporation vegetable substrates into the fungus garden, since they are responsible for most tasks, such as licking the leaves, chewing, incorporating, depositing the fecal fluid and cleaning the colony. While workers of the very small class (category inserted within the small class in the present work) are preferably responsible for performing the task of inserting tufts of hyphae on the substrate.

It should be noted here that the small and medium workers are predominant in a colony of *A. rugosus rugosus*, representing around 80% of the workers population (Verza 2007). The work inside the nest is generally safer. Therefore, the work outside the nest (or the exploration of food items) is at high risk due to the presence of predators, diseases and competitors. In polymorphic species, forage workers are usually the largest because need to have more behavioral flexibility to survive outside the comfort of the nest. In this way, the size differences among the workers in a nest are considered as an important component of the ergonomics of the work division, so that all the tasks necessary for its growth and reproduction are efficiently fulfilled (Oster & Wilson 1978; Fowler *et al.* 1991).

The morphological variations of the workers among the studied nests have already been reported for the workers of *Acromyrmex* spp. and vary both intra and inter colony (Gonçalves 1961; Ribeiro & Queiroz 1993; Andrade 2002). And although there are variations, according to Ribeiro & Queiroz (1993), these do not prevent that specific diagnoses based on statistical analyzes are elaborated.

The spines are important characters for this species, since they serve to identify and to differentiate *A. rugosus rugosus* and *Acromyrmex rugosus rochai*, the first presenting the well-developed lateral pronotal spines and approximately the same length of the anterior mesonotal tubercles, besides presenting the tubercles of the gaster more developed in the larger workers. Thus, the larger workers have the most defined characters and therefore are the most used in taxonomy (Gonçalves 1961).

The size of the workers varies both intra and inter speci-

-fically (Weber 1972; Hölldobler & Wilson 1990), and in a colony, the natural selection does not act directly on the worker, which is mostly sterile, but throughout the colony, which functions as a single reproductive unit (Wetterer 1999). Thus, the size and age of the colony influence the size distribution of the workers (Wilson 1983; Simas *et al.* 2003; Tschinkel 2005). In addition, according to Wetterer (1999), differences in size can be determined by the way colony workers feed their larvae. However, it is already known that the destination of the castes (Fraser *et al.* 2000; Hughes *et al.* 2003; Rheindt *et al.* 2005; Schwander *et al.* 2005) and behavior within castes (Waddington *et al.* 2010) have genetic influence. Furthermore, it can also be determined by environmental factors, such as the temperature at which the larvae are reared and the food they receive from the workers (Weber 1972; Bollazi & Roces 2002; Hughes *et al.* 2003; Lopes *et al.* 2005; Schwander *et al.* 2005).

In *Atta*, the size variation of a species is continuous covering very distinct castes morphologically (Oster & Wilson 1978). In this way, the physical caste of workers is represented by the gardener-nurses, generalists, foragers and defenders (soldiers), the latter being a characteristic that differentiates the genus *Acromyrmex* (Weber 1972; Wilson 1980). According to Oster & Wilson (1978), the castes of the genus *Acromyrmex*, are in an intermediate category between *Trachymymex* and *Atta*, having at least one polymorphic species possessing two castes of workers.

V. Conclusion

Morphometry data of the present work demonstrated the grouping of three workers castes (small, medium and large) for *A. rugosus rugosus*.

Acknowledgment

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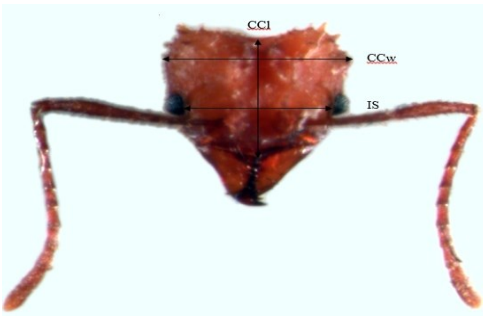

Fig. 1. Frontal view of a worker of *Acromyrmex rugosus rugosus* with the positioning of the morphometric measurements performed: cephalic capsule width (CCw), cephalic capsule length (CCl) and interocular space (IS).

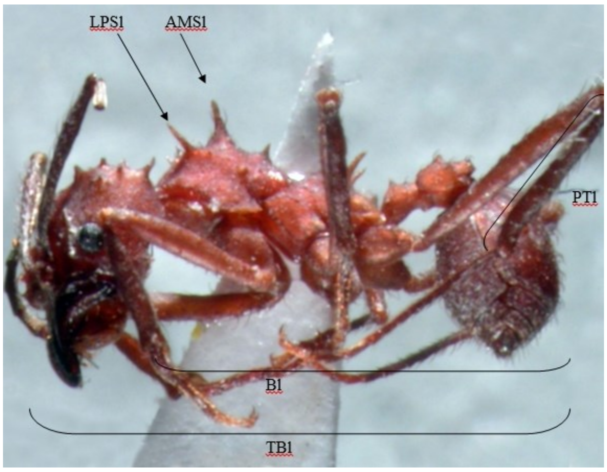

Fig. 2. Side view of the body of a worker of *Acromyrmex rugosus rugosus* with the positioning of the morphometric measurements performed: total worker length (TBl), body length (Bl), posterior tibia length (PTl), lateral pronotal spine length (LPSl) and anterior mesonotal spine length (AMSl).

Table 1. Mean total length (TBl), mean length of the cephalic capsule (CCl), mean width of the cephalic capsule (CCw), mean body length (Bl), mean length of the posterior tibia (PTl) and mean interocular space (IS), measured in workers of *Acromyrmex rugosus rugosus* of different size classes.

Classes	Measure (mm)[1] (± Standard error)					
	TBl	**CCl**	**CCw**	**Bl**	**PTl**	**IS**
Small	3.06 ± 0.03 c	0.813 ± 0.009 c	0.881 ± 0.009 c	2.262 ± 0.024 c	0.885 ± 0.015 c	0.718 ± 0.009 c
Medium	4.37 ± 0.03 b	1.192 ± 0.009 b	1.344 ± 0.009 b	3.432 ± 0.028 b	1.459 ± 0.017 b	1.159 ± 0.010 b
Large	5.60 ± 0.02 a	1.417 ± 0.006 a	1.754 ± 0.006 a	4.357 ± 0.023 a	1.917 ± 0.008 a	1.414 ± 0.005 a
CV (%)	25.09	23.35	27.71	26.97	31.61	27.44

[1]Averages followed by the same letter in the column did not differ significantly by the non-parametric Nemenyi test at 5% probability.

Table 2. Correlation index among the morphometric variables: cephalic capsule length (CCl), cephalic capsule width (CCw), body length (Bl), posterior tibia length (PTl), interocular space (IS) and total length of worker (TBl), measured in workers of *Acromyrmex rugosus rugosus* of the small size class collected in different colonies.

	Correlation index					
	CCl	**CCw**	**Bl**	**PTl**	**IS**	**TBl**
CCl	---	0.7955*	0.7503*	0.7503*	0.7777*	0.6992*
CCw	---	---	0.7464*	0.8185*	0.8815*	0.7582*
Bl	---	---	---	0.7498*	0.7933*	0.8891*
PTl	---	---	---	---	0.8258*	0.7568*
IS	---	---	---	---	---	0.7919*

*Pearson correlation coefficient significant at the 5% probability level.

Table 3. Correlation index among the morphometric variables: cephalic capsule length (CCl), cephalic capsule width (CCw), body length (Bl), posterior tibia length (PTl), interocular space (IS) and total length of worker (TBl), measured in workers of *Acromyrmex rugosus rugosus* of the medium size class collected in different colonies.

	Correlation Index					
	CCl	CCw	Bl	PTl	IS	TBl
CCl	---	0.8378*	0.7892*	0.7410*	0.8287*	0.8201*
CCw	---	---	0.8306*	0.8040*	0.8980*	0.8594*
Bl	---	---	---	0.7433*	0.7874*	0.9180*
PTl	---	---	---	---	0.7868*	0.7433*
IS	---	---	---	---	---	0.8157*

*Pearson correlation coefficient significant at the 5% probability level.

Table 4. Correlation index among the morphometric variables: cephalic capsule length (CCl), cephalic capsule width (CCw), body length (Bl), posterior tibia length (PTl), interocular space (IS), length of the lateral pronotal spine (LPSl), length of the anterior mesonotal spine (AMSl) and total length of worker (TBl), measured in workers of *Acromyrmex rugosus rugosus* of the large size class collected in different colonies.

Correlation Index								
	CCl	CCw	Bl	PTl	IS	LPSl	AMSl	TBl
CCl	---	0.6153*	0.6348*	0.5534*	0.6210*	0.3777*	0.3479*	0.6337*
CCw	---	---	0.6544*	0.7076*	0.7666*	0.3247*	0.3417*	0.6555*
Bl	---	---	---	0.5242*	0.6619*	0.2845*	0.2814*	0.9517*
PTl	---	---	---	---	0.6435*	0.2464*	0.2670*	0.5198*
IS	---	---	---	---	---	0.2369*	0.2193*	0.6553*
LPSl	---	---	---	---	---	---	0.9049*	0.2855*
AMSl	---	---	---	---	---	---	---	0.2886*

*Pearson correlation coefficient significant at the 5% probability level.

Table 5. Mean total length (TBl), mean length of the cephalic capsule (CCl), mean width of the cephalic capsule (CCw), mean body length (Bl), mean length of the posterior tibia (PTl) and mean interocular space (IS), measured in workers of *Acromyrmex rugosus rugosus* collected in different colonies.

Colony	Measure (mm)[1] (+ Standard error)					
	TBl	CCl	CCw	Bl	PTl	IS
C1	4.529 ± 0.091 a	1.192 ± 0.021 a	1.374 ± 0.029 a	3.505 ± 0.075 a	1.489 ± 0.034 a	1.144 ± 0.024 a
C2	4.283 ± 0.076 a	1.122 ± 0.020 b	1.314 ± 0.029 a	3.273 ± 0.064 b	1.412 ± 0.035 a	1.078 ± 0.023 b
C3	4.454 ± 0.094 a	1.160 ± 0.022 ab	1.371 ± 0.031 a	3.461 ± 0.077 ab	1.454 ± 0.039 a	1.129 ± 0.026 ab
CV (%)	25.09	23.35	27.71	26.97	31.61	27.44

[1]Averages followed by the same letter in the column did not differ significantly by the non-parametric Nemenyi test at 5% probability.

Table 6. Length of lateral pronotal spine (LPSl) and anterior mesonotal spine length (AMSl) of workers classified as large, of *Acromyrmex rugosus rugosus* collected in different colonies.

Colony	Measure (mm)[1] (+ Standard error)	
	LPSl	AMSl
C1	0.477 ± 0.002 a	0.477 ± 0.002 a
C2	0.467 ± 0.004 a	0.470 ± 0.003 a
C3	0.475 ± 0.003 a	0.476 ± 0.003 a
CV (%)	4.57	4.38

[1]Averages followed by the same letter in the column did not differ significantly by the non-parametric Nemenyi test at 5% probability.