

Morphometry of the sexual forms of *Acromyrmex subterraneus brunneus* Forel, 1911 (Hym., Formicidae) in queenright and queenless laboratory colonies

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Abstract: A morphometric study was performed on the sexual forms of *Acromyrmex subterraneus brunneus* in colonies kept under laboratory conditions. The males studied were obtained from queenright and queenless colonies, i.e. they were produced by either the queen or the workers. Cluster analysis revealed a wide distribution in the size of the sexual forms produced in queenless colonies compared with queenright colonies, and that some of the worker-produced males were significantly smaller than those produced in queenright colonies. However, we found no indication that the males produced in queenless colonies are unable to mate, as smaller and larger males had identical genitalia that varied only in size, and therefore probably represent an alternative route of reproduction in this species.

Key words: *Acromyrmex subterraneus brunneus*, leaf-cutting ant, sexual forms

1 Introduction

In social Hymenoptera all males are usually haploid, whereas all females are diploid and can differentiate into reproductive females or workers depending on environmental factors (WILSON, 1953; CROZIER, 1977). One widely known fact is that workers frequently, but not universally, lay unfertilized eggs which develop into males (HÖLLDOBLER and WILSON, 1990). This reproductive ability has been reported for many ant species (PEETERS and CREWE, 1985; OLIVEIRA and HÖLLDOBLER, 1990; PEETERS, 1991; WALSUN et al., 1998), including fungus-growing ants (FOWLER, 1978; FEBVAY and OGIER, 1984; VILLESEN and BOOMSMA, 2003).

Interestingly, *Acromyrmex rugosus rugosus* workers are able to produce males in orphan colonies (queenless colonies) (FOWLER, 1982). These orphan colonies generate a distinct distribution of male phenotypes, in which characteristics such as scape and gaster length are significantly larger than in those produced in queenright colonies (FOWLER, 1978). In the absence of queen-derived eggs, the workers may be able to allocate more time and, consequently, more food to their own eggs, so that more robust males are raised in a final attempt by the workers to propagate their own genes.

Sexual dimorphism of queens and males is pronounced in leaf-cutting ants (*Atta* and *Acromyrmex*),

but variation in the size of reproductive individuals of the same sex has been previously reported for queenless and queenright colonies (FOWLER, 1978; JUTSUM and CHERRETT, 1978; BUENO et al., 2002). However, little is known about the origin of this variation and the possible implications for reproductive success of the colony as a whole.

The aim of the present study was to analyze the morphometric variation observed among the sexual forms of *Ac. subterraneus brunneus* in queenright and queenless colonies kept under laboratory conditions. In addition, morphometry and description of the genitalia of males produced in queenless colonies were compared with those of males produced in queenright colonies.

2 Materials and Methods

Five 2-year-old *Ac. subterraneus brunneus* colonies kept under laboratory conditions, collected in the town of Piracicaba, São Paulo, and maintained in the Laboratory of Social Pest Insects, Faculty of Agronomical Sciences, São Paulo State University, Botucatu, São Paulo, Brazil, were studied.

The colonies were housed individually in plastic containers covered with a 1-cm layer of plaster of Paris that was kept wet to maintain humidity for fungus culture. The ambient

Table 1. Morphometric measurements (mm, mean \pm SE) obtained for the sexual forms of *Acromyrmex subterraneus brunneus* according to the groups obtained by cluster analysis

Clusters	Interocular width	Intraocular width	Transoccipital width	Head length	Length of the antennal scape	Length of the gaster	Length of the thorax, petiole and postpetiole	Length of the metathoracic femur
1 ($n = 179$)	1.09 \pm 0.06	1.65 \pm 0.09	0.97 \pm 0.06	1.96 \pm 0.11	1.54 \pm 0.09	3.11 \pm 0.25	3.92 \pm 0.22	2.11 \pm 0.12
2 ($n = 42$)	2.14 \pm 0.05	2.61 \pm 0.06	2.23 \pm 0.05	3.19 \pm 0.06	2.05 \pm 0.04	3.27 \pm 0.14	4.26 \pm 0.13	2.37 \pm 0.06
3 ($n = 21$)	0.86 \pm 0.05	1.28 \pm 0.08	0.72 \pm 0.06	1.51 \pm 0.11	1.14 \pm 0.13	2.31 \pm 0.24	3.04 \pm 0.21	1.57 \pm 0.09

Cluster 1 – males produced in queenright and queenless colonies, cluster 2 – females produced in queenright colonies, and cluster 3 – males produced in queenless colonies.

temperature was maintained at approximately $24 \pm 2^\circ\text{C}$, with a relative humidity of $70 \pm 20\%$.

The queens of four colonies were removed on 31 March 2003 so that the workers remained without a queen and consequently started to lay eggs and to produce reproductive individuals. The males reached maturity in December 2003, when they were collected and stored in 70% alcohol (FOWLER, 1978).

The four queens were reallocated together with a small amount of fungus and workers in similar plastic containers and allowed to reestablish a new colony. The fifth colony remained with its queen which produced males and females that were collected in December 2003 and stored in 70% alcohol for later analysis.

For population estimation of the *Ac. subterraneus brunneus* nests, colonies (fungus garden + eggs + larvae + pupae + workers) were individually weighed on a semi-analytical scale, and five samples weighing 5 g each were randomly removed. The number of workers was counted in the samples for calculation of the total population of the nest (PEREIRA and DELLA LUCIA, 1998). For morphometric analysis, 100 winged males derived from the four queenless colonies and 100 winged males and 42 females derived from the queenright colony were selected.

The following measurements were made using the ocular micrometer of a stereoscopic microscope: (1) head width across the eyes, (2) head width behind the eyes, (3) head width through the occiput (transoccipital width), (4) head length, i.e. from the cephalic vertex to the end of the mandible, (5) length of the antennal scape, (6) length of the gaster, (7) length of the thorax, petiole and postpetiole, and (8) length of the metathoracic femur.

The morphometric data obtained for the sexual forms were submitted to cluster analysis by Wong's hybrid method (WONG, 1982), which produces the adequate number of groups through pseudo F-statistics and pseudo t-squared statistics. The canonical components 1 and 2 explained 11 and 87% of the variation, permitting an ideal clustering of the groups by Wong's hybrid method using the SAS version 6.12 software.

The external genitalia of males were removed under a stereoscopic microscope in 0.9% NaCl. The males of queenless colonies were divided into small and large individuals according to table 1. These males were then fixed in Dietrich solution (30 ml 96% alcohol, 10 ml glacial acetic acid, and 60 ml distilled water) for 24 h and washed in 70% alcohol. After fixation and washing, the specimens were processed in the following sequence according to the method of ANDRADE (2002): (a) clearing in 10% KOH at 40°C in an oven for 10 h; (b) rapid washing in distilled water; (c) immersion in 2% glacial acetic acid for 1 to 2 min; (d) dehydration in 70% alcohol, absolute alcohol I and absolute alcohol II for 15–30 min each; (e) immersion in phenol for 12 h; (f) immersion in xylene for 5 min;

(g) mounting of the external genitalia on a slide with Canada balsam. The coverslip was held in place with a small support to avoid deformation of the material. The slides were incubated in an oven at 40°C for 10 days.

The dimensions of the volsella (fig. 1), an important structure of the genitalia, was studied by morphometry. This structure varies between species and its morphology has been investigated in some species of the genus *Acromyrmex* by ZOLESSI and ABENANTE (1973), ZOLESSI and GONZÁLES (1974) and ANDRADE (2002).

Measurements of the volsella were obtained based on the study of CLAUSEN (1937/1939) for the male copulatory organs. The following measurements were made in the ventral view

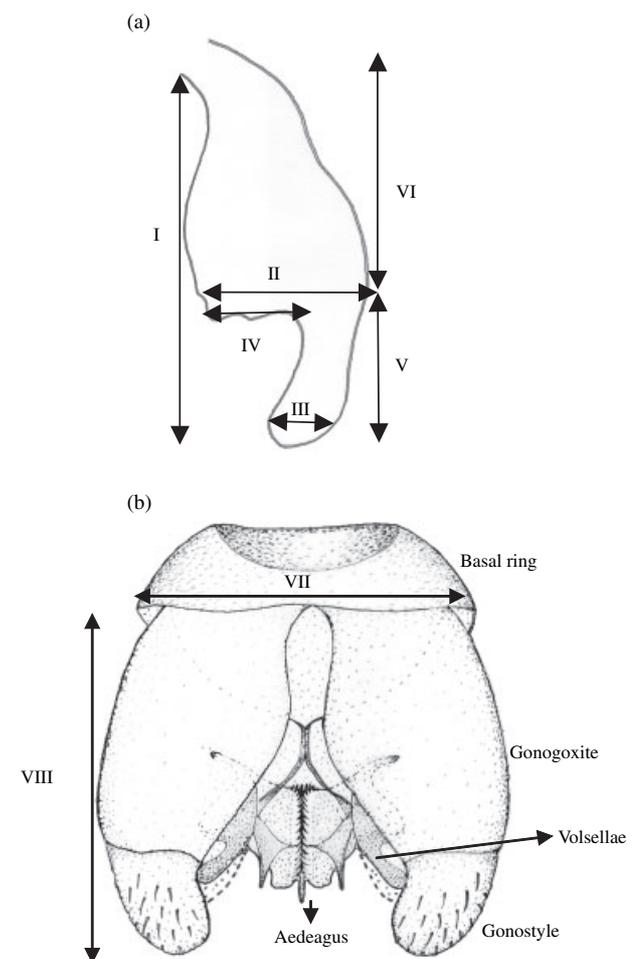


Fig. 1. Schematic figures the genitalia of *Acromyrmex subterraneus brunneus* males. (a) Volsella; (b) ventral view of the male genitalia

Table 2. Number of sexual forms and workers of *Acromyrmex subterraneus brunneus* produced in queenright and queenless colonies kept under laboratory conditions in December 2003

Colonies	Sexual Forms		Number of workers
	Males	Females	
Queenright	204	42	6782
Queenless	802	–	3089
Queenless	904	–	2411
Queenless	277	–	5942
Queenless	101	–	5607

of the male genitalia using the ocular micrometer of a stereoscopic microscope: (i) longitudinal length of the volsella, (ii) width of the volsella, (iii) width of the digitus, (iv) width of the base of the volsella, (v) longitudinal length of the digitus, and (vi) length of the volsella from its insertion in the gonocoxite to its base (fig. 1a). The width of the external genitalia (region between the insertion of the gonocoxite and basal ring) (vii) and the longitudinal length of the gonocoxite and gonostyle (viii) were also measured (fig. 1b).

3 Results and Discussion

The queenright colony with an estimated population of 6782 workers produced 204 males and 42 females, i.e. a male/female numerical sex ratio of 4.9. The estimated populations of the queenless colonies ranged from 2411 to 5942 workers and produced only males and no females (table 2). In comparison, a queenless colony of *Ac. rugosus* with about 500 workers produced 94 males, while a natural queenright colony with a population of more than 5000 workers produced 128 males at a sex ratio of 9.85 (FOWLER, 1978). These results suggest that queenright colonies produce a smaller number of males than queenless colonies, in which workers produce non-fertilized eggs.

A recent study using microsatellite genotyping demonstrated that workers of queenless colonies are able to produce males, but males produced by workers were not detected in queenright colonies of eight fungus-growing ant species (VILLESEN and BOOMSMA, 2003). This finding indicates that the queen is able to

control and monopolize the production of males in the colony, with the workers taking on this reproductive role only when the queen dies or is removed.

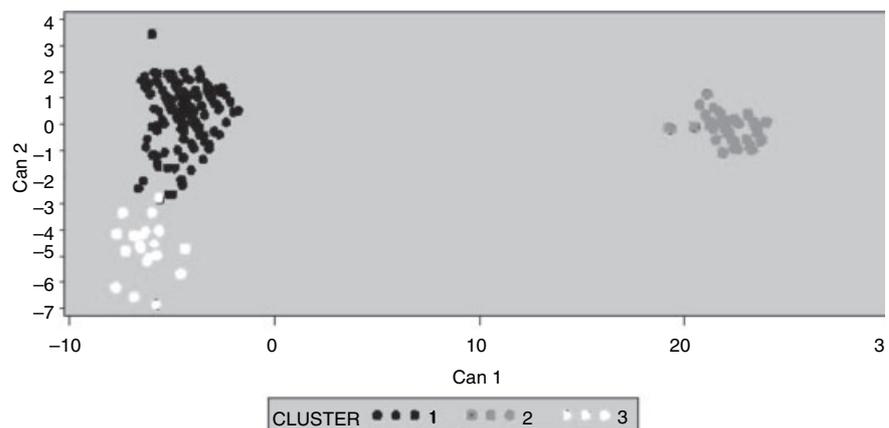
The eight morphometric variables studied (table 1) permitted grouping of the sexual forms of *Ac. subterraneus brunneus* into the following three distinct groups (fig. 2): cluster 1 – consisting of 100 males produced by the queenright colony and 79 males produced by the queenless colonies; cluster 2 – consisting of 42 females produced by the queenright colony and cluster 3 – consisting of 21 males produced by the queenless colonies. Females (cluster 2) presented greater mean head and body lengths than males (clusters 1 and 3), demonstrating the presence of sexual dimorphism in this species. Males of cluster 1 were morphologically larger than males of cluster 3 (table 1).

The wide distribution in the body size of males produced in queenless colonies compared with queenright colonies indicates that orphaned workers do produce haploid eggs, but that some of these eggs develop into smaller males than normally observed in queenright colonies. FOWLER (1978), comparing cephalic phenotypes of males from queenless and queenright colonies of *Ac. rugosus rugosus*, demonstrated that queenless colonies produce a larger number of size phenotypes than queenright colonies.

With respect to the composition of the external genitalia of males, no morphological differences were observed in males from queenright or queenless colonies (fig. 3). In *Acromyrmex hispidus* and *Acromyrmex lobicornis*, the male genitalia are robust and consist of curved and wide gonostyles, and the volsella possesses a wide digitus and a small tooth in its curvature which distinguishes these two species (ZOLESSI and ABENANTE, 1973; ZOLESSI and GONZÁLES, 1974). The same pattern was observed for the genitalia of *Ac. subterraneus brunneus*; however, the volsella had few setae and the base was characterized by an angular structure with numerous setae distributed along the inner margin of the structure (fig. 3b, d, f). This formation was observed in all slides analyzed, demonstrating that males produced in queenright and queenless colonies show the same characteristics specific for this species.

Analysis of the morphometric data showed that the external genitalia of males from cluster 3 were proportionally smaller than those of males from

Fig. 2. Clusters of the sexual forms of *Acromyrmex subterraneus brunneus*. Cluster 1 – males produced in queenright and queenless colonies; cluster 2 – females produced in queenright colonies and cluster 3 – males produced in queenless colonies



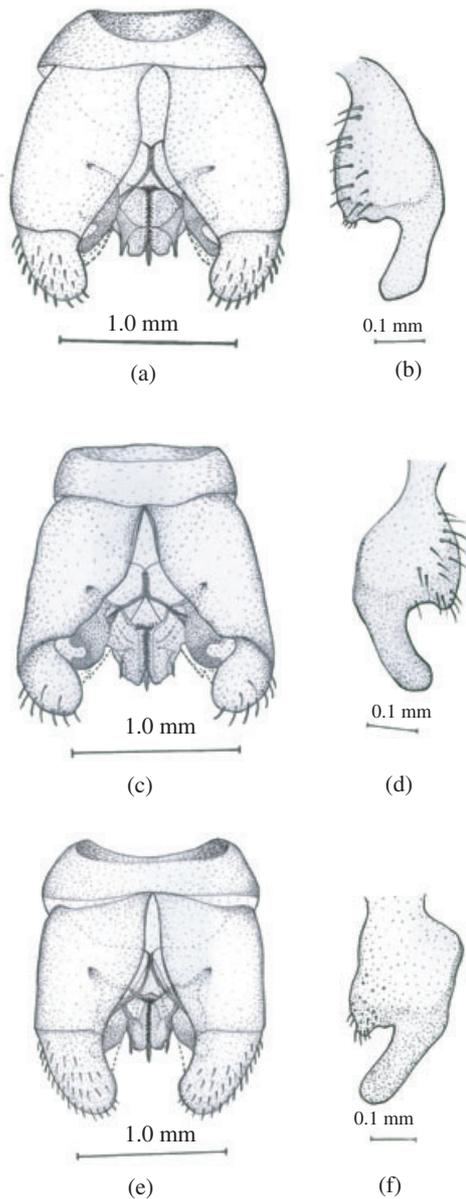


Fig. 3. Ventral view of the genitalia of *Acromyrmex subterraneus brunneus* males. (a, b) External genitalia and volsella of a male produced in a queenless colony belonging to cluster 1; (c, d) external genitalia and volsella of a male produced in a queenless colony belonging to cluster 3; (e, f) external genitalia and volsella of a male produced in a queenright colony belonging to cluster 1

cluster 1 produced in queenright and queenless colonies. However, the volsella presented similar proportions, indicating that these males are able to copulate like those produced in queenright colonies (table 3). According to CLAUSEN (1937/1939), the male and female copulatory organs fit like a key into a lock, and the integrity of the species is preserved due to this morphology. However, the author further argues that the lock-and-key hypothesis is partially rejected by the observation that the male genitalia vary widely in length and shape. The female genitalia do not consist of rigid chitinous parts but of a musculature that promotes contractions which confer a greater safety on the connection between the copulatory organs (CLAUSEN 1937/1939).

Furthermore, within the Class Insecta males are more variable than females (SHAPIRO and PORTER, 1989). In Lepidoptera, examples of interspecies, intergeneric and even interfamily crosses have been reported which demonstrate the inefficacy of the genital anatomy as a barrier for mating (SENGÜN, 1944). Thus, one may conclude that *Ac. subterraneus brunneus* males from queenright and queenless colonies are anatomically able to mate; however, there are some reservations regarding this hypothesis. It is impossible to know whether *Ac. subterraneus brunneus* males in queenless colonies receive the same care by workers as males reared in queenright colonies, thus permitting them to respond to environmental stimuli and to be capable of a good performance during the nuptial flight.

Flight and copulation capacity are important parameters for the reproductive success of leaf-cutting ants. In these insects, the nuptial flight basically depends on ideal meteorological conditions and carbohydrate reserves as a flight fuel (JUTSUM and QUINLAN, 1978). In laboratory simulations, JUTSUM and QUINLAN (1978) observed that laboratory-reared *Atta sexdens* males fly after a period of 9–10 hours 'of lights on, even in the absence of environmental clues'. Another interesting result was the use of large amounts of carbohydrates as flight fuel.

The existence of worker reproduction in natural situations is still unknown. Hypothetically, in natural situations when the queen dies, workers become capable of producing unfertilized eggs. The production of these males needs to be synchronized with other nests that have a queen and are in the reproductive period, i.e. these nests must have virgin females for mating, to perpetuate their descendants.

The biological meaning of worker reproduction in leaf-cutting ants remains an open question, permitting

Table 3. Morphometric measurements (mm, mean \pm SE) of the external genitalia obtained for the sexual forms of *Acromyrmex subterraneus brunneus* according to the groups obtained by cluster analysis

	I	II	III	IV	V	VI	VII	VIII
A ($n = 15$)	0.48 \pm 0.03	0.21 \pm 0.01	0.08 \pm 0.01	0.11 \pm 0.01	0.16 \pm 0.01	0.33 \pm 0.02	1.20 \pm 0.04	1.45 \pm 0.03
B ($n = 15$)	0.45 \pm 0.02	0.18 \pm 0.01	0.06 \pm 0.01	0.09 \pm 0.01	0.15 \pm 0.01	0.29 \pm 0.02	1.04 \pm 0.05	1.34 \pm 0.03
C ($n = 15$)	0.50 \pm 0.02	0.22 \pm 0.01	0.08 \pm 0.01	0.10 \pm 0.01	0.16 \pm 0.01	0.33 \pm 0.02	1.39 \pm 0.04	1.42 \pm 0.03

A: males produced in queenless colonies belonging to cluster 1; B: males produced in queenless colonies belonging to cluster 3; C: males produced in queenright colonies belonging to cluster 1.

only to suggest that these males are able to mate, probably representing an alternative route of reproduction in this species.

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