SCIENTIFIC NOTE

Occurrence of Winged Forms of *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae) in Laboratory Colonies

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Ocorrência de Formas Aladas de *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae) em Colônias de Laboratório

RESUMO - Foi observada, pela primeira vez no Brasil, a produção de centenas de formas aladas em formigueiros de *Atta sexdens rubropilosa* Forel, mantidos em laboratório. Larvas de último instar de machos foram encontradas em 5 de agosto de 2000 em um formigueiro com quase seis anos de fundação, contendo aproximadamente 110 L de esponja de fungo distribuída em 21 panelas. Entre 30 e 40 dias mais tarde apareceram larvas de fêmeas aladas. Os adultos sexuados apresentaram tamanhos compatíveis com os encontrados em formigueiros de campo. Foram observadas duas aparentes tentativas frustradas de revoada na última semana de outubro, após o que começaram a aparecer machos e fêmeas mortos no lixo do formigueiro.

PALAVRAS-CHAVE: Saúvas, formigas cortadeiras, reprodução, formas aladas, revoada.

ABSTRACT - The production of hundreds of reproductive forms of *Atta sexdens rubropilosa* Forel in nests maintained in laboratory was observed for the first time in Brazil. Last instar male larvae were found on August 5, 2000 in a six-year-old colony with approximately 110 L of fungus garden distributed in 21 pots. Thirty to forty days later, queen larvae started to be seen. The adult sexual forms were comparable in size with those found in the field. Two apparent failed attempts of a nuptial flight were observed during the last week of October, followed by the appearance of dead males and females in the garbage piles of the colony.

KEY WORDS: Leaf-cutting ants, reproductive forms, winged males, winged females, nuptial flight.

The occurrence of nuptial flight in *Atta* colonies is characterized by the appearance of a large number of winged forms (males or "bitús" and females or "içás") which mate in the air after leaving the nest. This phenomenon usually takes place in mature field colonies from September to December in the state of São Paulo, Brazil, (Autuori 1941, Mariconi 1970).

The factors which trigger the nuptial flight are mostly unknown, but Della Lucia & Bento (1993) suggested that high humidity could be a favorable climatic condition for the founding of a new colony by the young queen. Moser (1967) stated that at least 7 mm of rain must soak the soil before *Atta texana* Buckley sexuals engage on mating swarms. The strategy of producing thousands of winged forms ensures the reproductive success of the colony, since the great majority of them dies in the first hours after leaving the nest (Hölldobler & Wilson 1990).

Reports of maintenance of leaf-cutting ant colonies in laboratory conditions are frequent in places of natural occurrence (American Continent - Neotropics and south of the Nearctic region) as well as in the United States and Europe (United Kingdom, France, Holland and Germany). In Brazil, several research institutions have maintained *Atta* nests in laboratory conditions for many years, but there are no reports of the occurrence of winged forms in these colonies to our knowledge.

Autuori (1947) reported that colonies of *Atta* kept in laboratory for many years (including a 14-year-old colony) never attempted nuptial flight, nor was the appearance of larvae or pupae of reproductive forms ever observed. On the other hand, *Acromyrmex* colonies kept in the same laboratory performed nuptial flights annually. The author carried out, with no success, many attempts to discover the possible causes preventing alate production, such as varying food quality and quantity and maintaining the nests under constant temperature and humidity levels comparable to those of natural conditions.

The few references about the appearance of reproductive forms in laboratory conditions report the production of a small brood, unlike the production of thousands of sexuals in field colonies. Jutsum & Cherrett (1978) reported the production

of 3, 7 and 11 sexuals in *Atta cephalotes* Linnaeus nests and one miniature female in each of two other nests. Jutsum & Quinlan (1978) mentioned the production of one female and some males by an *Atta sexdens* Linnaeus nest collected in Guyana in 1969. All of these nests were maintained in the Department of Applied Zoology in the University College of North Wales, U.K. Gösswald (1985) reported the occurrence of sexuals in *Atta* colonies "collected in South America in 1965" and maintained in laboratory conditions in the University of Würzburg, Germany. Bass (1994) describes the appearance of a small alate brood in a ten-year-old *A. cephalotes* laboratory colony collected in Trinidad and also maintained in the University College of North Wales, U.K.

Since 1984, many *Atta sexdens rubropilosa* Forel colonies have been kept in the Instituto de Biociências of Unesp - Rio Claro, São Paulo, Brazil. The first colony lasted 12 years, while another (collected later) lived for nine years without ever producing sexual forms. In December 1996, a colony collected in the field in April 1995 (about six months after its founding) was transferred to a climate-controlled room in the Centro de Estudos de Insetos Sociais of this Institute. Having ample space to forage and increase the number of fungus chambers, this colony grew rapidly in size. By July 2000, this 5-year-old colony occupied 21 fungus chambers of varying sizes (1.2, 2.6, 4.0 and 7.0 L) totaling approximately 110 L of fungus garden (Fig. 1A).

A

B

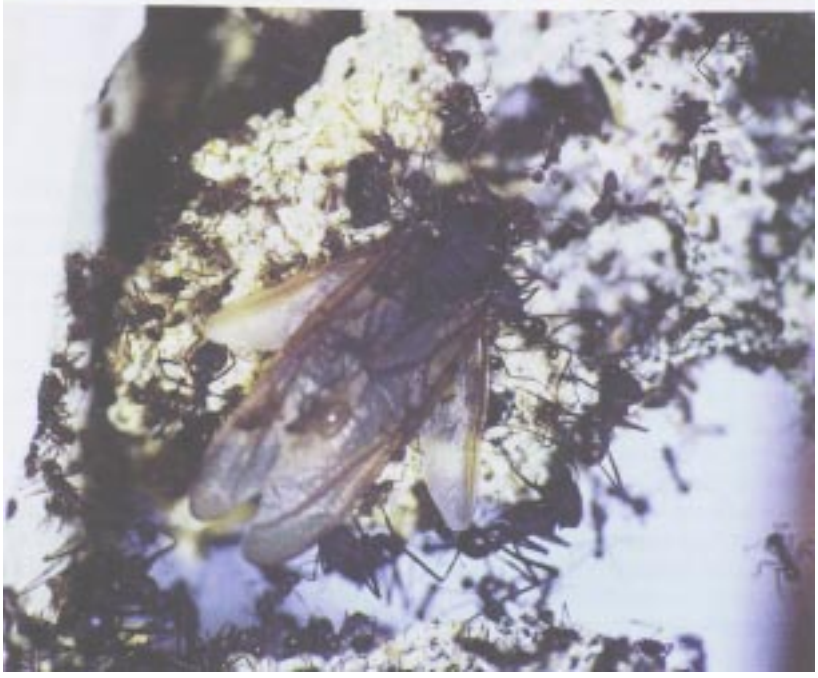

Figure 1. Laboratory colony during production of reproductive forms. (A) Overall view of fungus chambers. (B) Winged female being groomed by workers on the fungus garden.

On August 5 of the same year, unusually large larvae were first seen. These larvae were about three times larger in length than those of the largest worker castes. They were in fact last instar male larvae since male pupae were observed in the nest a few days later. Even larger larvae were seen 30 to 40 days later, followed by the appearance of female pupae. At this time the colony was being maintained in 24ºC average room temperature and 70% relative humidity. The nests maintained in the University College of North Wales were kept in slightly higher temperatures (25-30ºC and 26-28ºC) and higher relative humidity (90-100% and 80%, for Jutsum & Quinlan 1978 and Bass 1994, respectively).

It is worth noting that in early July of the same year, a fungus chamber of this nest was opened in the laboratory for a routine collection of material and no large worker larva was found. The small larvae present were unusually turgid and plump and their heads seemed to be located more apically on the body than the typical worker larvae. It is possible that these larvae were initial instars of reproductive castes.

During the production of immature stages of reproductive forms, the fungus garden went through changes in its architecture. Unusually large spaces were opened in the fungus sponge in order to accommodate the large larvae. In late August, hundreds of adult males could be seen in the fungus garden and hundreds of adult winged females could also be seen by early September (Fig. 1B). These winged forms were compatible in size with ones collected in the field (Fig. 2). The number of workers and the foraging activity of the colony seemed to be greatly increased. During this period, the fungus garden developed considerably, taking up all space available for it. Workers apparently also became more prone to displaying defensive behavior. Small disturbances on the nest that would normally not affect the workers now caused them to aggressively move around with their mandibles open.

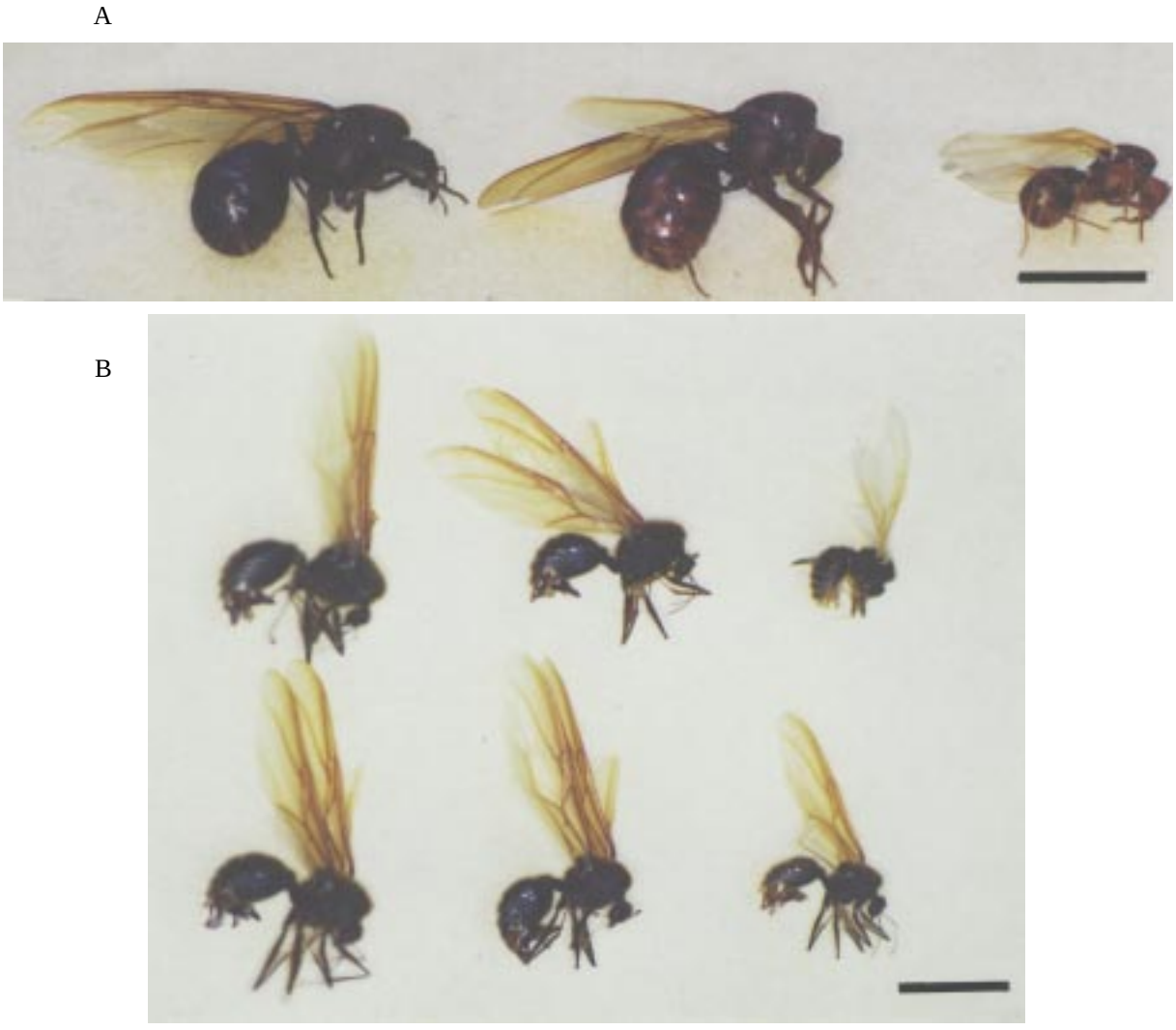

Figure 2. Winged forms produced during the second semester of 2000. (A) Females and (B) males. Left column: reproductive forms from nest excavated in the field; center column: normal-sized individuals from the first laboratory nest to produce winged forms; right column: miniature reproductive forms from a second laboratory colony. Bars = 1 cm

Brood care activities as well as caring for the adult winged forms (specially females) were filmed in VHS with mini and microcameras. Posterior analysis showed that larvae, pupae and adult males and females seemed to be cared for with special interest by the workers in the colony. Dozens of workers were constantly grooming them. Small pieces of fungus sponge were frequently given to the larvae and adult females by the workers.

In an attempt to verify if it would be possible for the reproductive forms to take part in a nuptial flight, a new arena was placed outside the building and connected to the nest by a plastic hose so that winged adults would have access to outside the building. On two occasions while nuptial flights were taking place on the nests in the field, many males of the laboratory colony attempted to leave the nest by flying inside the room. Later, the males started to gather in the outside arena but apparently did not fly. In the last week of October, body parts of winged forms started to accumulate in the nest's garbage, while pieces of their tegument were seen being carried by the workers. Winged females were seen in the nest until the end of December. The alates mentioned by Bass (1994) did not engage in a nuptial flight either.

The observed increase in worker contingent, the unceasing work with brood care and the impressive uptake of symbiotic fungus by the reproductive forms seemed to require a great amount of energy from the colony and the fungus garden went through a short decline period in which there was a significant decline on the amount of fungus garden in the colony. By February of the following year the nest had already returned to its normal size and level of activity.

A second nest, with 103 L of fungus garden, collected in 1994 (a year before the first nest) and maintained in another climate-controlled room under the same conditions, also initiated the production of reproductive forms in the same year. The first larvae were seen on September 27, 2000 (52 days after the first colony). This colony also produced hundreds of winged males and females but unlike the first colony, which only produced normal-sized alates, this colony also produced smaller reproductive forms (Fig. 2). These miniature males and females seem to be similar to the microgyne reported by Jutsum and Cherrett (1978).

As it happened to the first colony, the fungus garden of the second nest to produce males and females also went through a decline. The fungus garden became progressively smaller, the number of workers decreased and no brood could be seen. About the same time the larvae of reproductive forms were first noticed, the functional queen was seen walking outside of the fungus chambers on two separate occasions. This fact led us to believe that the queen was not in perfect health. Two attempts were made to substitute the original queen, with no success. Newly-mated queens returning from nuptial flights were put in "queen cages" and placed in the dwindling colony in an attempt to have the workers get accustomed to her and adopt the new queen as it is done in *Apis mellifera* Linnaeus hives. Similar frustrated attempts to replace the queen of a large colony in the process of decline after being kept for 15 years were reported by Autuori (1950) and Weber (1972). After months of decline, the colony was sacrificed. Fifteen larvae and one male pupa of markedly reduced proportions, measuring less than 1 cm, were found in the two remaining fungus chambers.

In September 2000, an adult *A. sexdens rubropilosa* nest was excavated in the field. While the first laboratory colony was replete with brood of winged forms and small workers, no brood was found in the fungus garden collected from the field colony. The field nest, however, already presented adult reproductive forms, suggesting that their production in the laboratory colonies was delayed. On the other hand, the nuptial flight only took place on October 27 and 28 (warm days immediately after a period of heavy rain) in the nest dug in the field as well as in other field nests in the area.

The causes that led these two colonies to produce the reproductive individuals are not clear. Since these were the largest colonies in our facilities, colony size seems to be a very important factor for alate production.

Acknowledgments

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Literature Cited

Autuori, M. 1941. Contribuição para o conhecimento da saúva (*Atta* spp. – Hymenoptera: Formicidae). I. Evolução do sauveiro (*Atta sexdens rubropilosa* Forel, 1908). Arq. Inst. Biol. 12: 197-228.

Autuori, M. 1947. Contribuição para o conhecimento da saúva (*Atta* spp. – Hymenoptera: Formicidae). IV. O sauveiro depois da 1ª revoada (*Atta sexdens rubropilosa* Forel, 1908). Arq. Inst. Biol. 18: 40-70.

Autuori, M. 1950. Longevidade de uma colônia de saúva (*Atta sexdens rubropilosa* Forel, 1908) em condições de laboratório. Ciên. Cult. 2: 285-286.

Bass, M. 1994. Behaviour of leafcutting ant sexuals (Hym.) in the parental nest. Entomol. Mon. Mag. 130: 141-148.

Della Lúcia, T.M.C. & J.M.S. Bento. 1993. Vôo nupcial ou revoada, p. 54-59. In T.M.C. Della Lúcia (ed.), As formigas cortadeiras. Editora Folha de Viçosa, Viçosa, 262p.

Gösswald, K. 1985. Organisation und Leben der Ameisen Wissenschaftliche. Verlagsgesellschaft MBH Stuttgart, 355p.

Hölldobler, B. & E.O. Wilson, 1990. The ants. Harvard University Press, Cambridge, Massachusetts, 732p.

Jutsum, A.R. & J.M. Cherrett. 1978. Sexuals and a

microgyne of *Atta cephalotes* (L.) (Hymenoptera, Formicidae) from laboratory cultures. Entomol. Mon. Mag. 113: 97.

Jutsum, A.R. & R.J. Quinlan. 1978. Flight utilisation in laboratory-reared males of *Atta sexdens*. J. Insect Physiol. 24: 821-825.

Mariconi, F.A.M. 1970. As saúvas. Editora Agronômica Ceres Ltda, São Paulo, 167p.

Moser, J.C. 1967. Mating activities of *Atta texana* (Hymenoptera, Formicidae). Insectes Soc. 14: 295-312.

Weber, N.A. 1972. Gardening ants, the Attines. The American Philosophical Society, Philadelphia, 146p.

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