Lipid and energy contents in the bodies of queens of *Atta sexdens rubropilosa* Forel (Hymenoptera, Formicidae): pre-and post-nuptial flight

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ABSTRACT. Lipid and energy contents in the bodies of queens of *Atta sexdens rubropilosa* Forel (Hymenoptera, Formicidae): pre- and post-nuptial flight. The nuptial flight allows males and females to meet and copulate and both need energy to perform this activity. Before leaving the nest, males and females are well nourished and ready to mate. However, little is known about the lipid and energy contents in females before the nuptial flight (virgins) and after it (mated females). In this work we measured lipid concentrations in relation to body weight in these individuals. Our results showed that 16.82% of the bodies of young virgin females one month before mating flight are composed of lipids, contrasting with the 32.62% lipid content in mated females who had not excavated their nest yet, and 32.88% in those who had. The energy content measured for virgin females was 2942.63 J, contrasting with 6110.01 J for queens before excavating the nest and 5677.51 J after excavation. Based on our results, we conclude that the body mass, and therefore the lipid and energy contents in the bodies of *Atta sexdens rubropilosa* queens double during the last month before the nuptial flight. This energy resource is fundamental to the activities required during the nuptial flight, digging the nest and the founding of the colony.

KEYWORDS. Energy content; lipid content; nest founding; nuptial flight.

RESUMO. Conteúdo de lipídeos e energético das rainhas de *Atta sexdens rubropilosa* Forel (Hymenoptera, Formicidae): pré e pós-vôo nupcial. O vôo nupcial permite que machos e fêmeas se encontrem e copulem. Para isso, tanto o macho quanto a fêmea precisam de energia para efetuar tal atividade. Antes de partir do ninho de origem, machos e fêmeas estão bem nutridos e prontos para o acasalamento. Mas pouco se conhece sobre o teor de lipídeos e conteúdo energético pré-vôo nupcial (fêmeas virgens) e pós-vôo nupcial (fêmeas acasaladas). Nossos resultados indicam que as fêmeas virgens apresentaram 16,82% de lipídeos em seu corpo, enquanto as rainhas (fêmeas acasaladas) que não escavaram 32,62%, e as que escavaram 32,88%. O conteúdo energético foi de 2942,63 J para as fêmeas virgens, 6110,01 J para rainhas sem escavação e 5677,51 J para aquelas que escavaram. Com base nos resultados obtidos, concluí-se que as rainhas de *Atta sexdens rubropilosa* dobram a sua massa corporal, e consequentemente, o teor de lipídeos e conteúdo energético, um mês antes da revoada. Esse recurso energético é fundamental para as atividades exigidas durante o vôo nupcial, escavação do ninho e a fundação da colônia.

PALAVRAS-CHAVE. Conteúdo energético; fundação do ninho; teor de lipídeos; vôo nupcial.

Nuptial flights of *Atta sexdens rubropilosa* occur in the afternoon, during the months of October – November. They are triggered by the first rains breaking a period of drought. During these flights, males form "clusters" with a diameter of 200 m, which can be found 150 m above ground level in the case of *Atta capiguara* Gonçalves, 1944 (Amante 1972 *apud* Fowler *et al.* 1984). The distances traveled during nuptial flights vary from species to species, and also depend on the air speeds achieved. *Atta texana* Buckley, 1960 can fly at speeds of 5.33 ms^{-1}, suggesting that a distance 10.4 km can be covered, and to *Atta sexdens*, speeds of 1.57 ms^{-1} suggest a travel distance of 11.1 km (Jutsum & Quinlan 1978). Field observations have shown that queens of *Atta sexdens* can travel 9.6 km (Cherrett 1968).

Both sexes need energy to engage in nuptial flights. Before leaving the nest of origin, males and females are well fed and ready for mating. Jutsum & Quinlan (1978) found that 21% of the dry weight of winged forms is composed of carbohydrates, which are completely consumed during the nuptial flight. Similarly, carbohydrates (stored as glycogen) have been shown to represent the main source of energy for the nuptial flight in a study involving *Formica lugubris* Zetterstedt, 1838 (Passera *et al.* 1989). In male *Cataglyphis cursor* Fonscolombe, 1846 and *Linepithema humile* (Mayr) (formerly *Iridomyrmex humilis*) which fly, exhibit a much higher carbohydrate content than do the non-flying females of these species (Passera & Keller 1990). From these studies it can be safely assumed that the first energy resource to be exhausted in the nuptial flight is the carbohydrate reserve in the body. Lipids and proteins are additionally more difficult to break down than carbohydrates, and spared for colony-founding activities which follow the nuptial flight.

After the nuptial flight, the queens land on the ground, shed their wings, and start excavating the soil. After 6 to 10 hours (Autuori 1942) of digging, a tunnel and a chamber result. During the excavation, the queens perform 300 trips on average for piling excavated soil outside, each trip lasting from 1 to 2 minutes, resulting in an average speed of 3 cm/hour (Ribeiro 1995). Because this activity is very intense, it is assumed that the energy costs of the excavation are high. How-

ever, there are no data available in the literature of the amount of energy required for nest excavation, and of the substrate used to fuel it. It has been postulated that the required energy is drawn from lipid reserves, because the carbohydrates are mostly depleted during the nuptial flight (Jutsum & Quinlan 1978; Passera *et al.* 1989). In order to make claustral colony founding possible, the queen must have a large reserve of fats (lipids), which is consumed at this stage of the colony life. However, *A. sexdens* queens feed on the fungus garden when founding new colonies, indicating that, although not exogenous, fungal staphylae, together with trophic eggs, supply the founding queens with a ready energy source they need during the founding stage (Augustin *et al.* 2011).

The foundress queen loses about 40% of its weight during this phase, achieving her lightest body mass four months after the nuptial flight (*Atta sexdens* and *Atta laevigata* F. Smith, 1858) (Della Lucia *et al.* 1995). Queens belonging to smaller, socially and morphologically more basal species of Attini, such as *Trachymyrmex septentrionalis* McCook, 1881, have up to 25% of their dry weight composed of fat (11% in the case of *Cyphomyrmex rimosus* Spinola, 1851) (Seal 2009). These species, however, employ a semi- or partially claustral founding strategy, which requires less energy than the fully claustral colony founding of *Atta* spp. and other, more derived ants. However, there are no studies on the reserves of *Atta* species.

The goal of this work was to determine the lipid and energy contents in the bodies of virgin and mated females of *Atta sexdens rubropilosa*. In order to ascertain the costs of excavating, we allowed a group of queens to build their nests and then measured.

MATERIAL AND METHODS

Collecting *Atta sexdens rubropilosa* females before and after the nuptial flight. We collected virgin females (pre-nuptial flight) from a nest of *A. sexdens rubropilosa* one month before the nuptial flight. In order to have access to the insects, we dug a trench beside the nest, and gradually expanded nest chambers by digging laterally. The virgin females (N = 28) were taken to the laboratory, immediately killed and analyzed for lipid content.

The mated females (post-nuptial flight) were collected after they lost their wings and were beginning to found their colonies. After collection, queens (60) were immediately stored in plastic containers with 11 cm diameter, 8 cm high, containing 1 cm of plaster at the bottom to keep the air moist. Queens were transported to the Laboratório de Insetos Sociais-Praga – FCA/UNESP – Botucatu, SP, Brazil.

Excavation by the queen. In the laboratory, 30 mated queens, from 60 collected queens, were placed in tubes filled with soil taken from a depth of 60 cm and density of 1.6 g/cm^3, according to the methodology established by Camargo *et al.* (2010). Each tube had the following dimensions: 25 cm length and 10 cm diameter.

Determining total lipid content. Virgin and mated females were euthanized by freezing and then subjected to the experimental procedure used by Peakin (1972), Keller & Passera (1989), Johnson (2002) and Seal (2009). The procedure was as follows: 1) Fresh weight was determined individually; 2) Queens were dried for 24 hours at 70°C and their dry weight was determined; and 3) The lipid content was extracted with petroleum ether (bp 40–60°C) for 24 hours and then weighed.

The percentage of total fat content was calculated using the formula: 100x (DM – FFDM)/DM, where DM: is the dry mass and FFDM is dry, fat free mass, according to Johnson (2002).

The energy content of the ants was obtained by multiplying their lean weight by 18.87 J mg^{-1} and their fat weight by 39.33 J mg^{-1} (Peakin 1972).

Statistical analysis. The weight was subjected to analysis of variance (ANOVA) ($\pm$ = 0.05), and later to a Tukey post-hoc test. The lipid content data were submitted to Kruskal-Wallis test ($\pm$ = 0.05).

RESULTS

The average weight of virgin females of *A. sexdens rubropilosa* was 369.33 ± 54.14 mg, whereas the weight of mated females (queens) who had performed excavation was 672 ± 35.59 mg and the weight of females who had not excavated was 669.5 ± 53.7 mg (Table I). The weights of virgin and mated females differed significantly ($F_{2, 85}$ = 364.39, p < 0.0 01). By contrast, the weights of the queens who had not excavated and those who had were not significantly different (Tukey test, p > 0.05).

Lipid determination showed that 16.82% of the body weight of virgin females was due to lipids. This percentage jumped to 32.62% in mated females who had not excavated and to 32.88% in those who had (Table I). As expected, significant differences were detected in the percentage of lipids when virgin and mated females were compared (H = 37.04, df = 2, p < 0.001). Presumably, no differences were detected between queens who excavated and those who had not (Dunn's Method, p > 0.05). The energy content was 2942.63 J for virgin females, J 6110.01 for queens who had not excavated and to 5677.51 for those who had (Table I).

DISCUSSION

Our results show that gynes of *A. sexdens rubropilosa* rapidly gain weight and lipid content (on average 50%) one month prior to the nuptial flight (Table I). During this time they are constantly fed by worker ants inside their nest (Bass 1994; Bueno *et al.* 2002; Camargo *et al.* 2006). The weight gained by the queens in our research is compatible with the findings of Mintzer (1990) for the genus *Atta*, which ranges from 400–800 mg. The resulting weight of the queen is mostly due to fat tissue (body fat).

From an evolutionary point of view, the bodies of more basal species have 12–15% fat, as in *Cyphomyrmex*, whereas more derived ants such as *Atta* have about 40% fat in their

bodies (Seal 2009). These numbers reflect the transition from semi-claustral to claustral colony foundations. In this study we have found that the queens who build claustral founding, like *A. sexdens rubropilosa*, have a great amount of lipids in their body reserves, about 30%. These reserves come primarily from the fat body located in the queen's abdomen (gaster), where they are stored within a mass of cells called trophocytes and oenocytes (Roma *et al.* 2006), which firmly hold the ovaries and other organs through tracheal branches (Staurengo da Cunha & Cruz Landim 1983).

The excavation by the queens did not affect the percentages of lipids and consequently the energy content, in their bodies (Table I). Probably, energy resources for the excavation do not originate from lipid reserves, but from other energy sources, perhaps carbohydrates.

Table I. Average weights (mg), percentage of fat and energy content (J) in the bodies of queens of *Atta sexdens rubropilosa* in different experimental situations.

	Virgin females	Without excavation	Normal excavation
Weight (alive) (mg)	369.33	669.50	672.00
Dry weight (mg)	131.85	239.19	221.80
Lean weight (mg)	109.63	161.16	148.87
Lipid content (mg)	22.21	78.03	72.93
Lipid percentage (%)	16.82	32.62	32.88
Energy content (J)	2942.63	6110.01	5677.51

Jutsum & Quinlan (1978) found that 21% of the dry weight of winged forms is composed of carbohydrates which are totally consumed after the nuptial flight. Similarly, in study on *F. lugubris*, carbohydrates (stored as glycogen) were shown to be the main source of energy for the nuptial flight (Passera *et al.* 1989). Most likely, the energy used for the nest founding also comes from carbohydrates.

Based on our results we conclude that the queens of *A. sexdens rubropilosa* have their body mass and therefore their body lipid and energy content doubled one month before the nuptial flight. This energy resource is fundamental to the activities required during the nuptial flight, digging the nest and the nest founding.

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