Sociobiology 63(1): 720-723 (March, 2016) DOI: 10.13102/sociobiology.v63i1.868

Sociobiology
An international journal on social insects

SHORT NOTE

Initial development and production of CO_2 in colonies of the leaf-cutting ant *Atta sexdens* during the claustral foundation

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Article History

Edited by
Evandro Nascimento Silva, UEFS, Brazil
Received 22 July 2015
Initial acceptance 27 January 2016
Final acceptance 21 February 2015
Publication date 29 April 2016

Keywords
Egg, larvae, nests, pupae, social insects.

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Abstract

Queens in the genus *Atta* are solely responsible for fungus cultivation and for care of herself and her offspring. Only few studies have investigated their nests in the claustral foundation and it is unknown the production rate of expelled carbon dioxide and/or of oxygen consumption in the initial colonies of leaf-cutting ants. Thus, we have studied the development of 50 initial colonies of *Atta sexdens*, and production of expelled carbon dioxide under laboratory conditions. The number of eggs was counted one week after nest foundation on the seventh day, the larvae counted on day 28, and the pupae between days 42 and 49. The workers emerged on the 63rd and 70th day. The CO_2 concentration increased steeply in the 42nd days (20.60 ± 8.36%) and 49th days (15.37 ± 13.11 %), at 42nd days, and subsequently returned to lower values, for example, 3.35 ± 2.84% at week seven. It is the first record of CO_2 emission data in initial nests, in their claustral foundation under laboratory conditions.

Annually, mature colonies of *Atta* produce male and female winged ants that leave the colony to form new colonies. Nuptial flights always occur after rains, which provide favorable conditions for the initial survival of initial colonies, facilitating the initial excavation of a vertical tunnel by the queen (Autuori 1942). After the nuptial flight, a female of the *Atta sexdens* (Linneus, 1758) digs a vertical tunnel of about 15 cm and a chamber within which she is enclosed, caring for fungus culture and for brood (Autuori, 1942; Ribeiro, 1995, Camargo et al., 2011; Fröhle & Roces, 2012). The process of constructing a new nest is the most crucial period in the life of an ant colony, whereas the mechanisms that optimize survival during this phase are of great value (Wilson, 1971).

The claustral foundation of *Atta* species always involves great effort by the queen, who is solely responsible for cultivation of fungus and caring for herself and her offspring. In this period consumption of large portion of the queen's reserves takes place (Della Lucia et al., 1995). Mintzer (1990) stated that the foundation of these nests demands the highest investment already observed among the queens of several ant species. Thus, we can explain their large body size, due to the accumulation of energy reserves prior to the nuptial flight, so they can spend them during the formation of their colonies (Fujihara et al., 2012; Camargo & Forti, 2013). Considering that there are few studies investigating nests of *Atta* in the claustral foundation, we have studied the development of 50 initial colonies of *Atta sexdens*, as well as the production of carbon dioxide expelled under laboratory conditions.

The study was conducted in the city of Botucatu, São Paulo state, Brazil with *A. sexdens rubropilosa* nests being selected in an area of Eucalyptus plantations belonging to FCA/UNESP, Botucatu, SP (22º 50.833' S and 48º 26.476' W). The area presents the Oxisol soil type (Dark Red Latosol). The 50 queens were randomly selected at the moment of nest foundation on the 5th of October, 2013.The newly collected queens were stored in plastic containers, 11 cm in diameter

Open access journal: http://periodicos.uefs.br/ojs/index.php/sociobiology
ISSN: 0361-6525

and 8 cm in height, containing 1 cm of plaster at the bottom to maintain humidity. The queens were transported to the Laboratory of Social-Pest Insects FCA/UNESP – Botucatu.

The initial development was measured through the production of brood (number of eggs, larvae, pupae and adults) until the first workers emerged. The brood of 50 colonies was counted weekly, by careful examination under a stereoscopic microscope. The mortality of colonies was measured for a period of 70 days after nest foundation.

Production of CO_2 by the colonies

The colonies were enclosed in hermetic chambers for one week, simulating a claustral foundation found in a natural environment. CO_2 production was measured by respirometry. Bacharach CO_2 sensor was employed, with a fixed probe (Bacharach model 2800; more details at http://www.bacharach-inc.com), maintained at 25°C in the laboratory. CO_2 concentration in the surrounding environment was monitored during 10 weeks, resulting in a mean of 285.7 ± 63.9 ppm (n=70).

Consecutive readings (n=7) of the CO_2 concentration from a respirometric receptacle were carried out to estimate a mean value. The results were expressed in ppm and subsequently converted to ml or mg, through the known volume of the acrylic B respirometric receptacle. Each colony had its own respirometric receptacle (mean volume = 455.9 ml). This environment was retro-fed by a peristaltic pump, thereby ensuring no loss of modified chamber atmosphere of the colonies. The number of eggs, larvae, pupae and adults was submitted to linear mixed-effects model. The simplified model was used: % CO_2 = eggs + larvae + pupae + adults + days / colonies + error. The variable number of eggs, larvae, pupae and adults during the period were categorized into three stages based on population growth (lack of population, population growth and population decrease), and it was submitted to linear mixed-effects model. The CO_2 percentage was the response variable, random effects were days given colonies, and fixed effects were eggs, larvae, pupae and adults. Statistical analyses and graphs were processed by R 2.9.0 for Windows.

The queens of *Atta sexdens* began to oviposit on the second and third day after the nuptial flight, but the eggs were here counted on the seventh day. The larvae were counted on the evaluation at 28th day, and the pupae between 42nd and 49th days. The workers emerged between the 63rd and 70th day (Fig 1).

The linear mixed-effects model showed a remarkable correlation among eggs, larvae, pupae, adults and concentration of CO_2 (Fig 1). First, the eggs phase was verified two stages, increasing and decreasing stage. The CO_2 production was 4.56 times lower in decreasing than increasing stage (t=-3.12, d.f =378; p=0.0019). In the larvae phase was verified three stages: no larvae, increasing and decreasing stage of larvae number. The CO_2 production was 6.08 times bigger in increasing stage than without larvae (t=4.59, d.f.=378; p<0.001). Although 3.80 times bigger in decreasing stage than no larvae (t=1.88, d.f.=378; p=0.0607). In the pupae phase was verified 3 stage: no pupae, increasing and decreasing stage of pupae number. The CO_2 production was 13.10 times lower in increasing stage than without pupae (t=-9.72, d.f.=378; p<0.001) and 14.51times lower in decreasing stage than without pupae (t=-8.44, d.f.=378; p<0.001).Finally, adult phase was verified 2 stage: without and increasing of adults number. The CO_2 production was 11.36 times lower in increasing stage than without adults (t=-3.17, d.f.=378; p=0.0016). The mortality of colonies was high, with 24 (48 %) dead colonies at 70 days. The CO_2 concentration within the nest increased with time, with a peak between the 42nd and 49th days (Fig 1), but decreased at following weeks. The linear mixed-effects model showed a remarkable correlation between brood and concentration of CO_2. It may be concluded the larvae were responsible for increasing of CO_2 concentration inside the colony, because they have a greater food consumption, and consequently, greater body mass (Chapman, 1998). All this growth causes greater aerobic respiration, and of course, the production of CO_2 increases. In our study, the CO_2 volume emitted weekly varied from 20.60 ± 8.36% at 42nd days to15,37 ± 13.11 % at 49th days (Fig 1).

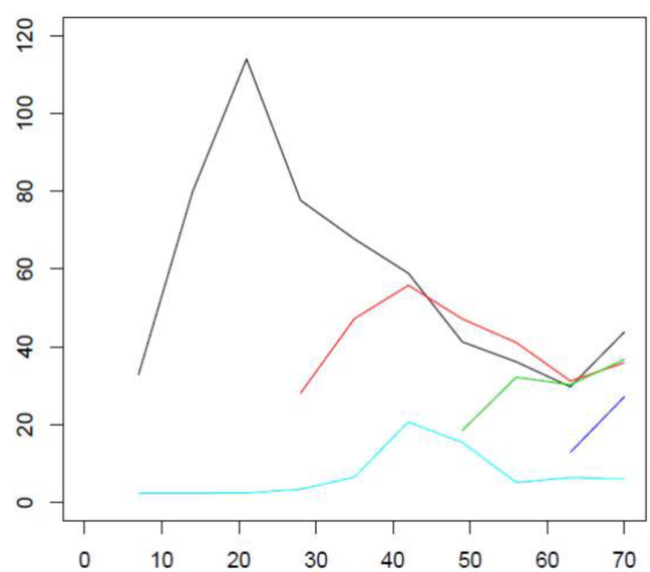

Fig 1 .Number of eggs (black line), larvae (red line), pupae (green line), adults (dark blue line) and concentration of CO_2(%) (blue line), during a period of 10 weeks, within of a claustral foundation of *Atta sexdens*.

Although this value of CO_2 concentration is high, it has been shown that in adult nests the values are much higher and that leaf-cutting ant workers are able to tolerate elevated CO_2 levels, especially the genus *Atta*, which can withstand severe hypoxia (Hebling et al., 1992), and also are capable of behaving normally at elevated CO_2concentrations, such as those of about 1.5 to 4.5% found in nests of *A. capiguara* and *A. laevigata* (Bollazzi et al., 2012). Another interesting example is found in ants that inhabit mangroves, whose nest interiors present carbon dioxide levels that become elevated during nest closure, reaching 11% (Nielsen et al., 2003). Under these hypercapnic and hypoxic conditions, the ants are able to sustain aerobic respiration even at CO_2 concentrations of up to 15% (Nielsen et al., 2006; Nielsen & Christian, 2007).

Comparing the development of initial colonies of *Atta* with data from the literature (Autuori, 1942; Pereira-da-Silva, 1979), similarity was verified in the incubation, larval and pupal periods. For *Atta sexdens*, the incubation period lasted 25 days, the larval period, 22 days and pupal period, 10 days (Autuori 1942). Camargo et al. (2011) obtained similar results from the same leaf-cutting ant species, even when measuring the excavation effort. In *Atta capiguara* Gonçalves, 1944, in the sampled periods, the eggs were most prevalent in the interval of 1-18 days, larvae at 21-38 days, pupae at 39-55 and adults at 58-67 days (Pereira-da-Silva, 1979). The incubation period is related to suitable temperature, and consequently, larva is fully formed and hatched by specific environmental stimuli (Chapman, 1998). Our study was carried out at 25°C in the laboratory, similar to temperature found on initial chamber in natural condition (25-28°C for *Atta sexdens* (Stahel & Geijskes, 1940; Eidmann, 1935), 27.5 °C for *Atta vollenweideri* (Kleineidam & Roces, 2000), and around 27°C for *A. heyeri* (Bollazzi & Roces, 2002)).

When comparing the quantity of brood produced with the findings of Autuori (1942) and Pereira-da-Silva (1979), a pattern is verified in the production of eggs, larvae, pupae and adults, in other words, generally concise numbers with high variability (Figure 1). This is due to the fact that the production of eggs is controlled by their endocrine system. According to Fowler et al. (1986), egg production by the queen during the 3 or 4 months of the colony lifespan is correlated with the activity cycle of the corpora allata. The corpora allata are responsible for the synthesis of juvenile hormone, which acts during oviposition of founder queens, as verified in females that suffer allatectomy (Barker, 1978). The juvenile hormone acts on the fatty body, initiating the synthesis of vitellogenins. Thus, the production of brood depends on body reserves (lipids from the fatty body and protein from the wing muscles), since the queen does not feed during the claustral foundation.

The queens with a claustral foundation present a greater quantity of lipids in their bodily reserves (Keller & Passera, 1989). In smaller queens of lower Attini, it was verified that *Trachymyrmex septentrionalis* McCook (1881), presents 25% fat in its dry weight while *Cyphomyrmex rimosus* Spinola (1853), has 11% (Seal & Tschinkel, 2007). In higher Attini, *A. sexdens rubropilosa* queens have a great amount of lipids in their body reserves, about 30% (Fujihara et al., 2012). During the foundational period the queen loses about 40 % of her weight, presenting the lowest body mass 4 months after the nuptial flight (*Atta sexdens* and *Atta laevigata* Smith, 1858) (Della Lucia et al., 1995). Probably, the entirety of this loss of body mass is due to the utilization of the resources in the queen's activities, including oviposition. The mortality of colonies was high in 70th averaging 48 % of colonies. However, this value is lower than found by Camargo et al. (2011), averaging 60% at 63rd days after nuptial flight due to digging effort during nest foundation. Our study is the first to present CO_2 emission data in initial nests, in their claustral foundation under laboratory conditions.

Acknowledgements

We are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support and stipends to the authors (grants 2007/04010-0 and 2007/07091-0). R.S. Camargo thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the Postdoctoral fellowship. L CF thanks CNPq for the research assistance (301917/2009-4).

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