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RESEARCH ARTICLE

# The production of soldiers and the maintenance of caste proportions delay the growth of termite incipient colonies

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**Abstract** In a termite colony, the incipient phase is the most critical part of the life of the colony. The quality of the investment in the first offspring by the primary reproductives may determine the rate of success of the colony to survive the first year and its growth rate in the following years. However, termite colonies possess a physiological constraint, forcing the group to maintain a relatively fixed caste proportion. During the development of the incipient colony, there is therefore a conflict for the group on the developmental pathways of larvae into workers or soldiers. On the one hand, the more workers produced, the more work forces would be available to provide for the primary reproductives, the brood and the nest maintenance (overall nurturing capacity). On the other hand, some larvae must develop into soldiers to maintain the caste proportion, reducing the potential number of workers. Using incipient colonies of *Coptotermes gestroi* (Wasmann), we investigated the cost of maintaining the soldier proportion over the growth of the colony within the first year. Our results showed that an incipient colony maintains a stable soldier proportion regardless of the stress imposed. The resources redirected into the replacement of soldiers not only reduced the total number of workers, but it also reduced the overall growth of the colony by delaying the development of the remaining eggs. Our observations suggest that in termite incipient colonies, because of physiological constraints, the maintenance of the soldier proportion overrides the development of the colony.

**Keywords** Nanitic soldiers · Reduced colony growth · *Coptotermes gestroi* · Asian subterranean termite

## Introduction

Termite soldiers depend on their siblings as they cannot feed on their own, and their main role is to defend the colony from predators or competitors (Grassé, 1939; Noirot, 1989). Most termite species possess a specific soldier proportion (Haverty, 1977, 1979; Haverty and Howard, 1981) and while environmental factors can influence such soldier proportion (Waller and La Fage, 1988; Liu et al., 2005a, b), Korb et al. (2003) recognized two levels of internal regulation of the soldier proportion in a termite colony: (1) the individual endocrinal regulation, where hormonal changes trigger the differentiation into a particular caste; juvenile hormones have a role in the regulation of soldier development (Hrdý et al., 1979; Mao et al., 2005; Park and Raina, 2004) and (2) the social regulation, where the existing soldier proportion serves as a mechanism to balance the production of new soldiers (Miller, 1942; Springhetti, 1973; Bordereau and Han, 1986; Su and La Fage, 1986; Park and Raina, 2005; Mao and Henderson, 2010). The self-regulatory ability of the colony results in a system that can react and adapt to sudden environmental changes by maintaining homeostatic conditions within the group and hence improve the rate of success of the colony (Emerson, 1952; Grassé, 1952; Passera et al., 1996).

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In incipient termite colonies, the nurturing of the brood is initiated by the primary reproductives and then performed by a small number of young workers, and the colony mortality can be high during this phase (Nutting, 1969). The first soldiers produced are nanitic (Noirot, 1985; Barsotti and Costa-Leonardo, 2005) and it was suggested that in some species their role is primarily to maintain a soldier proportion in the incipient colonies without having to pay the cost of producing fully developed soldiers (Chouvenc and Su, 2014). Thorne et al. (2003) proposed that in primitive termites, the soldier caste evolved in incipient colonies as a way to survive the intense intra-species competition. However, we here hypothesize that in some derived termite species, this ecological constraint may be different and the production of a large number of soldiers early in the life of the colony may have a significant cost on its growth.

The observations from King and Spink (1974) and Higa (1981) in *Coptotermes formosanus* Shiraki suggested that after the incipient phase of the colony, the colony goes through an exponential growth phase before reaching maturity (Su and Tamashiro, 1987). Such a growth model implies that the initial number of surviving workers after the first few years within a young colony determines how fast it can grow to reach maturity (Oster and Wilson, 1978). Light (1943) suggested that the inhibition of alate production was in place in young colonies because they do not have the resources to invest in reproduction yet, while soldiers would still be produced despite their inherent cost. This observation reveals a conflict for the colony and it raises the question as to why incipient colonies would spend energy on the production of a large number of nanitic soldiers, poorly functional and dependent, in a time when the investment of the group should be focused on worker production to maximize the initial colony growth.

Castle (1934) and Light and Weesner (1955) investigated the nanitic soldier replacement in two species with relatively low soldier proportion, usually a single soldier within the first year. Therefore, the potential cost for the replacement of a single soldier may not have been detected and was not investigated further. We here propose to investigate the ability to replace soldiers in *Coptotermes gestroi* (Wasmann) incipient colony, a termite species which possess a higher soldier proportion (≈10 % Costa-Leonardo and Barsotti, 1998), and for which nanitic soldier replacement would come with a significant cost to the growth of incipient colonies.

## Materials and methods

### *Coptotermes gestroi* colony foundation in laboratory

Alates from dispersal flights of *C. gestroi* were collected in Broward County (Florida, USA) during evenings in March 2013, using a light trap modified from Higa and Tamashiro (1983). Species identification of the alates was done using the description given by Su et al. (1997) and Li et al. (2010). One male and one female were introduced into individual rearing units (Higa, 1981, modified by Chouvenc and Su, 2014). A rearing unit was composed of a transparent plastic cylindrical vial (8 cm × 2.5 cm diameter, internal volume = 37 $cm^3$) with 6 g of moistened organic soil at the bottom. Four blocks of *Picea* sp. (5 cm × 0.5 cm × 0.5 cm) were positioned vertically and an additional *Picea* block (10 cm × 0.5 cm × 0.5 cm) was placed inside the vial, along the vertical side. A 3 % agar solution was poured, leaving a 2-cm space at the top of the vial. When the agar was solidified, the long *Picea* block was removed from the vial to leave a hole in the agar, providing direct access to the soil on the bottom and to the wood. A perforated plastic cap was placed on the top to allow for aeration, but to limit desiccation and prevent escape. Forty rearing units were used in this experiment and were stored at 28 °C for 230 days. A small amount of water was added to vials that showed signs of dryness. The initial development of the incipient colony could be observed within the first 100 days through the transparent plastic vial and observations were made on the occurrence of the different castes.

### Soldier removal and incipient colony transfer

At 230 days, all 40 units were opened for a first count. The agar plug was gently pulled off the vial to remove the entire content without harming the group of termites. The agar plugs were carefully disassembled and all individuals were counted and assigned to a developmental category as follows: primary reproductives (to make sure that both individuals survived), eggs, larvae, workers and soldiers. Because of the inherent rate of failure for the primary reproductives to establish the incipient colony (Costa-Leonardo and Barsotti, 1998), only 24 out of the 40 vials were successfully established at 230 days (both male and female survived with the brood). Therefore, 12 units were randomly assigned as the control group, while the 12 remaining units were assigned as the treatment group. For the control units, no action was performed on the termite colonies besides counting and categorizing all individuals. In the treatment units, all soldiers were removed from the colonies after counting and categorizing all individuals.

Because agar plugs were dismantled during the count, all individuals from each colony were reintroduced into a new rearing unit. Units were prepared as described above, however, due to the incipient colony growth and the need of space for the termite groups (Costa-Leonardo and Barsotti, 2001), larger vials were used, with the same proportion of soil, wood and agar, but with double the volume

(6 cm × 4 cm diameter, internal volume = 75 cm$^3$). Units were maintained in the same conditions as described above, and were reopened at 275, 320 days and finally at 365 days. Each time, all individuals were counted and assigned to a developmental category, and all soldiers were removed from the treatment units at 275 and 320 days, but not from the control units. After counting all individuals, all groups were reintroduced into new 75 cm$^3$ rearing units, as the colony growth was marginal in this time frame. The rationale behind removing soldiers every 45 days is that it takes less than 45 days for second instar larvae to develop into nanitic soldiers in incipient colonies of *Coptotermes* ($L_2 \rightarrow PS_1 \rightarrow S_1$, see Chouvenc and Su, 2014). Therefore, the treatment consisted of preventing soldiers from accumulating, which forced the colony to "continuously" replace the missing soldiers. The experiment was terminated at 365 days, and no soldiers were removed in the final count. In this study, for ease of description, pre-soldiers were included in the "soldier" developmental category for the analysis.

### Statistical analysis

We used R (R Core Team, 2014) and *lme4* package (Bates et al., 2012) to perform a linear mixed-effect model to analyze the effect of treatment and time on the abundance of each developmental category (eggs, larvae, workers, soldiers). As fixed effects, we entered treatment (control or removal) and day, with their interaction term into the model. The temporal variable (day) was centered around 230 days, i.e. the first day of the experiment, to limit multicollinearity and ease the interpretation (the temporal origin being now the beginning of the experiment). As random effects, we had intercepts for colonies to take into account the serial dependence introduced by the repeated measurements (at 230, 275, 320 and 365 days). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity, normality or independence of the residuals. Parametric bootstrapping over 1,000 simulations was performed to estimate 95 % confidence intervals (CIs) for the fixed coefficients, and thus assess the significance of the fixed effects (CIs overlapping with zero indicating no significant effect).

A similar approach was used to evaluate the effect of treatment and time on the soldier proportion, determined as $r = S/(S + W)$ ($S$ = number of soldiers, $W$ = number of workers). The soldier proportion was used as a binomial distribution in a generalized linear mixed-effect model with a logit link, with the same fixed and random effects as above. Again, visual inspection of the residuals did not reveal any problem, and 95 % confidence intervals for the fixed coefficients were also obtained by the parametric bootstrapping over 1,000 simulations.

## Results

In the first 100 days, we were able to observe the colony development through the transparent walls of the vials without opening the units. The first eggs were laid at 20–25 days in most units and the first instar larvae developed at 36–42 days. First instar workers were observed at 60–65 days, and the first nanitic soldier was observed at 72 days. As more workers were produced, they initiated the plastering of the walls, which prevented further direct observations. At 230 days, when vials were opened for the first count and assigned to the control or the treatment group (before the first removal of soldiers), the two treatment groups had no difference in the number of eggs, larvae, workers and soldier proportions: all $\beta_{\text{treatment}}$ had confidence intervals largely overlapping with zero, indicating no difference at the beginning of the experiment (Table 1). For the number of soldiers, the linear mixed models provided a 95 % CI not overlapping with zero (Table 1), suggesting a significant difference between treatments at 230 days; however, this effect is to be neglected for two reasons: (1) the lower bound of the CI is very close to zero (0.057). As a

**Table 1** Coefficients and 95 % confidence intervals for the linear mixed models of each developmental category abundance over time

| Developmental category | Variable | $\beta$[a] | IC 2.5 % | IC 97.5 % |
|---|---|---|---|---|
| Eggs | *Intercept* | *10.000* | *7.200* | *12.738* |
| | Treatment | −2.017 | −5.896 | 1.945 |
| | *Day* | *−0.048* | *−0.079* | *−0.016* |
| | *Treatment × Day* | *0.115* | *0.069* | *0.160* |
| Larvae | *Intercept* | *6.583* | *5.024* | *8.152* |
| | Treatment | −1.158 | −3.396 | 1.083 |
| | Day | 0.002 | −0.015 | 0.019 |
| | Treatment × Day | 0.012 | −0.013 | 0.037 |
| Workers | *Intercept* | *39.942* | *35.303* | *44.691* |
| | Treatment | 1.258 | −5.352 | 7.735 |
| | *Day* | *0.117* | *0.093* | *0.140* |
| | *Treatment × Day* | *−0.154* | *−0.185* | *−0.121* |
| Soldiers | *Intercept* | *3.583* | *2.886* | *4.317* |
| | *Treatment*[b] | *1.125* | *0.057* | *2.102* |
| | *Day* | *0.019* | *0.012* | *0.025* |
| | *Treatment × Day* | *−0.028* | *−0.037* | *−0.019* |

[a] 95 % confidence intervals were computed using a parametric bootstrapping over 1,000 simulations. Variables included in the model are the treatment type (*Control* or *Removal*, with *Control* as a reference), the number of days of the colony (centered around 230 days), and their interaction. Variables with 95 % confidence intervals not overlapping with zero, indicating significant effects, are marked/highlighted in italics

[b] For soldiers, a post hoc $t$ test on the number of soldiers revealed no significant difference between the two treatments at 230 days ($t = -0.873$, $df = 20.757$, $p = 0.393$)

**Table 2** Coefficients and 95 % confidence intervals for the generalized linear mixed model (logit link) of the soldier proportion over time

| Variable | $\beta$[a] | IC 2.5 % | IC 97.5 % |
|---|---|---|---|
| *Intercept* | *−2.400* | *−2.646* | *−2.143* |
| Treatment | 0.237 | −0.092 | 0.563 |
| Day | 0.001 | −0.001 | 0.004 |
| Treatment × Day | −0.003 | −0.007 | 0.001 |

[a] 95 % confidence intervals were computed using a parametric bootstrapping over 1,000 simulations. Variables included in the model are the treatment type (*Control* or *Removal*, with *Control* as a reference), the number of days of the colony (centered around 230 days), and their interaction. Variables with 95 % confidence intervals not overlapping with zero, indicating significant effects, are marked/highlighted in italics

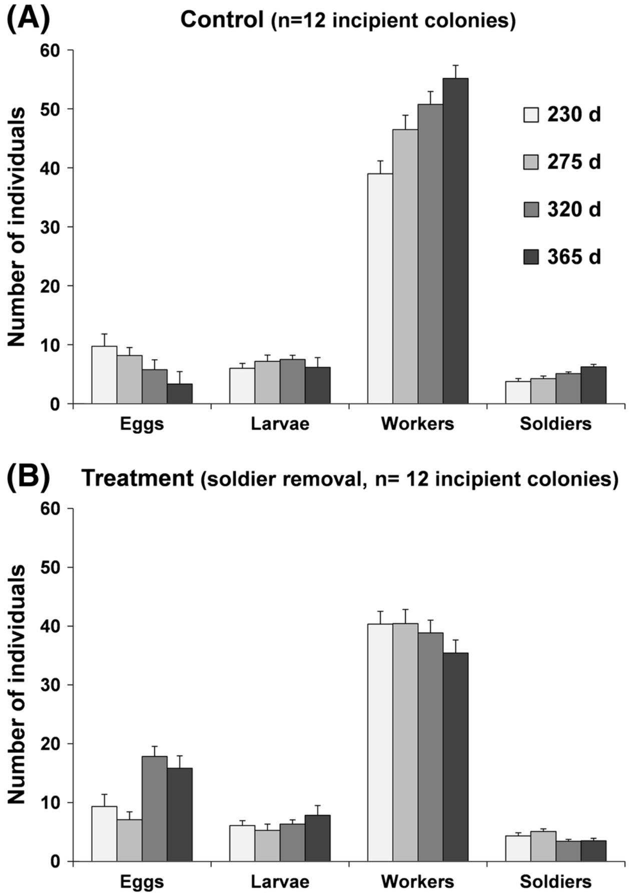

**Fig. 1** Number of individuals (average ± SE) from each developmental category at 230, 275, 320, and 365 days. **a** Control colonies (no soldier removal), and **b** treatment colonies (soldier removal)

matter of fact, 97 % CIs overlapped with 0, revealing a weak effect, and (2) due to slightly non-linear temporal trends, the model overestimated the number of soldiers for the treatment group at 230 days, and underestimated the number of soldiers for the treatment group at 230 days, which resulted in predicted difference more than twice as large as the observed difference ($\Delta_{\text{pred}} = 1.125$ vs. $\Delta_{\text{obs}} = 0.583$). In addition, a post hoc $t$ test on the number of soldiers revealed no significant difference between the two treatments at 230 days ($t = -0.873$, $df = 20.757$, $p = 0.393$). Therefore, at the beginning of the experiment (230 days), all developmental categories had the same number of individuals between treatments.

In the colonies from the soldier removal group, the average numbers of soldiers removed were 4.3 at 230 days, 5.1 at 275 days, and 3.4 at 320 days. This resulted in removing an average of 12.8 individuals from treated colonies over three removal events between 230 and 365 days. The number of individuals in each developmental category showed differences among treatments and over sampling times (Fig. 1). The changes in abundance of developmental categories between treatment groups in interaction with the effect of time were first analyzed per category using the linear mixed-effect model (Table 1).

*Eggs* Over time, the number of eggs in the control group significantly decreased (−0.048 per day, i.e. −2.2 per experimental step of 45 days) while the number of eggs in the soldier removal group significantly increased (+0.067 per day, i.e. +3.0 per experimental step of 45 days) with a difference of 12.5 eggs between the two treatment groups at the end of the experiment (365 days).

*Larvae* Temporal trends were not significant for both the control group and the removal group indicating that the number of larvae remained relatively constant in both groups and over time, with an average of 6.5 larvae per colony.

*Workers* Over time, the number of workers in the control group significantly increased (+0.117 per day, i.e. +5.3 per experimental step of 45 days), while the number of workers in the soldier removal group significantly decreased (−0.036 per day, i.e. −1.6 per experimental step of 45 days), with a difference of 19.8 workers between the two treatment groups at the end of the experiment.

*Soldiers* Over time, the number of soldiers in the control group significantly increased (+0.019 per day, i.e. +0.8 per experimental step of 45 days, Table 1, Fig. 1), while the number of soldiers in the soldier removal group significantly decreased (−0.009 per day, i.e. −0.4 per experimental step of 45 days, Table 1, Fig. 1), with a difference of 2.8 soldiers between the two treatment groups at the end of the experiment.

*Soldier proportions* Throughout the experiment, a total of 12.8 soldiers were removed from the treated colonies. Despite this continual removal, treated colonies were able to replace such soldiers within each 45 days time frame, as the generalized linear mixed-effect model showed that there was no significant difference in soldier proportion at the beginning of the experiment, and no temporal trend for both groups afterwards (Fig. 2; Table 2). Therefore, the soldier proportion was not significantly different from the average

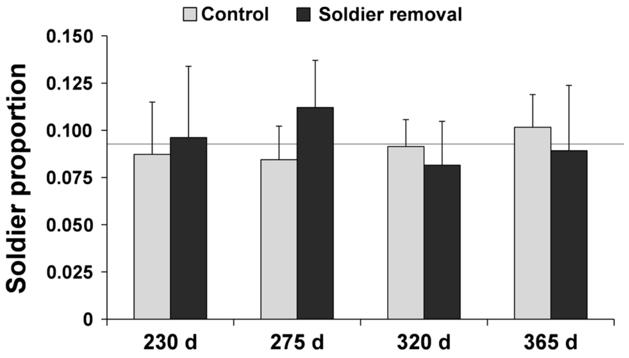

**Fig. 2** Soldier proportions in both treatments (control and soldier removal, average ± SE) over time (the line represent the overall average soldier proportion $r = 0.093$). A generalized linear mixed-effect model showed that the soldier proportions were constant in all treatments and at all times (see Table 2)

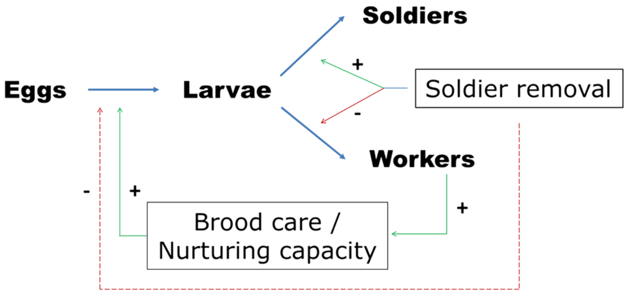

**Fig. 3** Interpretation on how the removal of soldier directly reduces the production of workers by prioritizing the replacement of soldiers, hence reducing the nurturing capacity of the colony, further delaying the development of the brood

value of 0.093 in all treatments and at all time, and the number of produced soldiers depends directly on the number of workers.

## Discussion

Our result showed that incipient colonies of *C. gestroi* can maintain their soldier proportion to ≈9.3 % independently of the internal stress imposed on the colony. This confirms that the physiological constraint on the colony (Park and Raina, 2003) maintains a soldier proportion characteristic of its own species (as described in mature groups of termites, Haverty, 1977; Su and La Fage, 1986), remains valid in incipient colonies. The removal of soldiers from incipient colonies had the direct effect of reducing the total number of individuals from the termite group and, to compensate for the loss of soldiers, some larvae initiated their development into the nanitic soldier pathway instead of developing into workers. Therefore, colonies for which the soldiers were systematically removed as they were produced had fewer workers than control colonies. However, while on average a total of 12.8 soldiers were removed in the treated colonies, the change in developmental pathways of larvae from workers to soldier should have resulted in a drop of the number of workers by an approximately equal value. Instead, the treated colonies had in average 19.8 fewer workers than the control colonies. There was therefore an average of seven workers that were unaccounted for in treated colonies. While the maintenance of existing soldiers may have a low intrinsic cost to a termite colony (Roux and Korb, 2004) as long as the soldier proportion is within the range of the species' nurturing capacity (Su and La Fage, 1987), our results imply that the production of new soldiers and the active replacement of soldiers come with an additional cost to the incipient colony.

Another remarkable observation was the relatively large number of eggs in treated colonies in comparison to control colonies at 365 days. There are two possible explanations for such result: (1) the queen could lay more eggs to "compensate" for the loss of individuals. However, during the incipient phase of the colony, the queen ovaries are limited in size (Higa, 1981), which implies that she can only produce a finite number of eggs in a given period. Therefore, in a resource-limited environment with low nurturing capacity, it is unlikely that the queen would lay more eggs than she does in control condition (Roisin and Pasteels, 1986). (2) Alternatively, the nurturing capacity of the workers is an internal limiting factor for the colony growth (Su and La Fage, 1987), where the number of helpers determines the rate of success of the brood to develop as fully functional individuals (Oster and Wilson, 1978). This process may explain why individual mortality can be extremely high in incipient colonies of *Coptotermes* (Higa, 1981; King and Spink, 1974) as a fraction of the eggs laid by the queen do not fully develop in functional workers or soldiers and die prematurely. Taking into account the developmental mortality in incipient colonies (difficult to monitor owing to the cannibalism of eggs and larvae that fail to fully develop, Morales-Ramos and Rojas, 2003), it is reasonable to assume that out of 12.5 eggs, only 7 would fully develop in workers or soldiers. Hence, we here suggest that the reduced care of the brood may delay the developmental rate of individuals and that the "surplus" of 12.5 eggs actually corresponds to the lack of 7 workers in treated colonies, where such workers have not developed yet and remain at an early developmental stage. This interpretation implies that the removal of soldiers resulted in resources being reallocated to produce a soldier instead of a worker, and this shift of resources directly reduced the nurturing capacity of the incipient colony, delaying the development of the brood into functional individuals (Fig. 3).

It was suggested that the origin of soldiers in a proto-termite emerged from the pressure of a confluence of various ecological factors in its lifestyle, including the direct intra-species conflict between colonies in a resource-limited environment, as described in *Zootermopsis* (Thorne et al., 2003). The pressure to maintain a stable soldier proportion in incipient colonies of primitive termites (Castle, 1934; Itano and Maekawa, 2008) is therefore strong to ensure colony survival in a situation of intense competition. The maintenance of the soldier proportion in most termite species could be a mechanism inherited from such prototermite and in derived termites with a relatively high soldier proportion, the production of soldiers in the incipient colony may be an atavistic trait expressed by a conserved soldier determination mechanism. Our study showed that in incipient colonies of *C. gestroi*, the mechanism that triggers the maintenance of the soldier proportion overrides the need for the colony to develop. It shows that the mechanisms that regulate the maintenance of soldier proportion are rather rigid, even in incipient colonies where the need for a large number of soldiers may be unnecessary. If the presence of soldiers was beneficial in a prototermite incipient colony, as demonstrated by Thorne et al. (2003), the soldier production in incipient colonies of derived species with a high soldier proportion can become a burden in this critical phase. In incipient colonies, the survival in the first few months relies on the ability for the first functional workers to take over the care of their siblings (Nutting, 1969; Oster and Wilson, 1978). Nanitic soldiers are produced directly from second instar larvae, instead of older workers as described in mature colonies, and this would confirm the production of nanitic soldiers as a way to inhibit further soldier production from older instars so as to maintain the soldier proportion at a minimal cost (Chouvenc and Su, 2014), and alleviate the burden to the incipient colony.

To conclude, optimizing the cost of individuals in incipient colonies, which is a bottleneck phase, was necessary for survival through evolutionary times and here we can consider the nanitic soldier as a product of the pressure on incipient colonies, in the form of the compromise: maintain the caste proportion imposed by physiological mechanisms (Su and La Fage, 1986; Park and Raina, 2005; Mao and Henderson, 2010) on the colony through its lifetime, but limit its inherent cost by producing cheap soldiers during the incipient phase. Although the cost to produce a nanitic soldier may be lower than a mature soldier, our study shows that this cost remains important, as the replacement of 12.8 nanitic soldiers resulted in the net loss of 7 additional functional workers, presumably by delaying the development of the eggs within the first year of development. Further research will investigate if a worker is as expensive as a soldier for an incipient colony to replace and will attempt to describe the physiological mechanisms behind the delay of the development of the eggs (reduced nurturing activity vs. direct interference from the primary reproductives).

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