

# Social influence of larvae on ovarian maturation in primary and secondary reproductives of the dampwood termite *Zootermopsis angusticollis*

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**Abstract.** We tested the effect of larvae on the reproductive maturation and fecundity of female primary and secondary reproductives of the termite *Zootermopsis angusticollis* Hagen (Isoptera; Termopsidae) by varying the number of third- to fourth-instar larvae nesting with heterosexually paired reproductives. Primary females had higher fecundities and oviposited sooner when nesting with larvae than females lacking larvae, but gained less body mass and had fewer functional ovarioles per ovary. Secondary reproductives nesting with larvae also had higher fecundities and oviposited sooner, but unlike primaries, they gained more body mass and had more functional ovarioles when larvae were present. The specific response of both primary and secondary females varied according to the number of larvae present. These results suggest that larvae can enhance the fecundity of primary and secondary females. Larvae may increase the energetic reserves of reproductives by performing colony labour, reducing pathogen load and providing trophallactic secretions. Trophallaxis with larvae may significantly enhance endogenous nitrogen, which is a limiting nutrient for termites.

Primary females, which normally need to produce a first brood quickly to initiate a new colony, may expend limited nutritional resources on oogenesis rather than producing additional ovarioles. Primaries may also store fewer energetic reserves for long-term brood care, and therefore gained less mass when larvae were present to attend to non-reproductive tasks. Secondary females may exhibit a greater positive response to larvae than primaries because they begin reproductive life with fewer stored resources and thus their development and fecundity are more dependent on assistance from larvae. Both primary and secondary reproductives may become more dependent on the contributions of larvae as their rate of egg production increases with subsequent bouts of oviposition.

**Key words.** Fecundity, larvae, oocyte, ovary, reproduction, social insects, *Zootermopsis*.

## Introduction

The nutritional ecology of an animal is a major constraint on reproduction, especially when essential nutrients are limiting. In termites, the high cost associated with rearing slowly maturing offspring on a nutritionally poor diet of wood may have been a catalyst for cooperative living in

their primitive ancestors (Nalepa, 1994). The low nitrogen content of wood and the extended period of maturation of immature termites may have led to the delayed maturation and retention of larvae within the natal colony, allowing reproductives to shift the cost of rearing additional offspring to the first brood (Lenz, 1987; Myles, 1988; Nalepa, 1994; Thorne, 1997). Reproductives may then allocate resources towards increasing fecundity and thus the rate at which the colony grows and matures (Oster & Wilson, 1978; Porter & Tschinkel, 1986; Tschinkel, 1988). This hypothetical advantage assumes that larvae (*sensu*

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Thorne, 1996) can provide an ergonomic and/or nutritional benefit that females can then use to enhance their reproductive development. Despite the importance of the contribution that non-reproductive helpers make toward increasing colony productivity, little is known about the direct effects that termites have on the development and fecundity of the reproductives that they assist.

In the dampwood termite, *Zootermopsis angusticollis* Hagen (Isoptera; Termopsidae), the fecundities of both primary and secondary (neotenic) reproductives may depend upon the contributions of offspring. Primary reproductives in incipient colonies produce only a single small clutch of eggs until their first brood is sufficiently mature to perform colony tasks (Heath, 1903; Castle, 1934; Weesner, 1969). Although primary males may participate in brood care and nest maintenance (Rosengaus & Traniello, 1991; Shellman-Reeve, 1997a), the costs of producing and raising the first brood can deplete the primary female's endogenous resources (Shellman-Reeve, 1990; Nalepa & Jones, 1991) and may preclude producing additional offspring (Nalepa, 1988; Shellman-Reeve, 1990). Newly moulted neotenic appear to be even more dependent on assistance, maturing very slowly and producing little vitellogenin when in isolated pairs (Greenberg *et al.*, 1978; Greenberg & Stuart, 1979). Larvae may release reproductives from the energetic constraints of performing non-reproductive colony tasks (Heath, 1903; Castle, 1934; Nalepa, 1994) and may provide trophallactic secretions rich in nitrogen (McMahan, 1969; LaFage & Nutting, 1978; Breznak, 1982; Shellman-Reeve, 1990; Rosengaus & Traniello, 1993; Nalepa, 1994) allowing the queen to dedicate more resources towards reproduction. Larvae may also reduce the disease risk by allogrooming reproductives and reducing the number of pathogens within the colony (Rosengaus *et al.*, 1998). Heath (1903) showed that the first brood of a primary female soon contributes to colony labour, achieves a smaller than normal size and is shorter lived than subsequent offspring, indicating that larvae may free the female from energetic limitations on reproduction. If larvae are an important determinant of female fecundity, then the addition of larvae to an incipient colony should have a positive effect on the reproductive maturation of primary and secondary females. Fecundity in particular should increase with larvae number (Oster & Wilson, 1978) until the queen is saturated with help and is limited only by her physiological capacity to produce eggs (Tschinkel, 1988).

In this paper we describe the changes in ovarian maturation occurring in primary and secondary female reproductives of *Z. angusticollis* nesting with varying numbers of larvae in experimental colonies. Our objective was to determine the influence of the larvae on reproductive maturation. Does the presence of larvae result in increased ovarian maturation and oogenesis in primary and secondary reproductives? Do reproductive females respond only to the presence of larvae or is the response graded to the number of larvae in a colony? Are the physiological responses of primary and secondary females equivalent, and how would their respective responses affect colony growth?

## Materials and Methods

### *Establishment of experimental colonies*

Termites originated from stock colonies of *Z. angusticollis* collected from the Redwood East Bay Regional Park, near Oakland, California. Termites were selected randomly from 14 different colonies collected between 1992 and 1998. Parent colonies were kept in plastic boxes containing moist paper towels and pieces of the wood in which they were originally collected, supplemented with additional decayed wood. Colonies were maintained in an environmental chamber at LD 14:10 h and 23°C and were regularly sprayed with distilled water to maintain humidity.

Alates that attempted to take flight upon opening the containers enclosing the parent colony were used as a source of primary reproductives. The wings of the alates were removed by folding them back towards the head, along the dorsal suture. Secondary reproductives were generated from groups of female and male fourth- to sixth-instar larvae that were isolated from parent colonies in clear covered plastic boxes (17 × 12 × 6 cm) containing moistened filter paper and damp wood. Individuals were removed from the group within 1–3 days of moulting to a pigmented reproductive form. Primary and secondary reproductives were sexed, weighed and immediately placed in experimental colonies.

Six groups of experimental colonies were established, each representing a different social condition. Groups A, B and C contained primary reproductives, whereas Groups D, E and F contained secondary reproductives. Colonies of Groups A ( $n=87$ ) and D ( $n=104$ ) contained one female and one male. Colonies of Groups B ( $n=107$ ) and E ( $n=94$ ) contained one female, one male and three larvae. Colonies of Groups C ( $n=81$ ) and F ( $n=89$ ) contained one female, one male and six larvae. Female and male reproductives were paired randomly with respect to colony of origin. Although *Z. angusticollis* larvae can range up to eighth instar and beyond, third- and fourth-instar larvae of both sexes were used in each colony because they were sufficiently mature to perform most colony tasks (Rosengaus & Traniello, 1993), and yet were small enough that they would not significantly deplete the food supply available to the female, prior to the colony being sampled. This protocol allowed us to control for the amount of food available to the reproductives, which has been shown to affect oogenesis in some species (Lenz, 1994).

Experimental colonies were housed in covered 67 mL plastic cups (Solo Cup Co.), containing 2 g (dry weight) of birch (*Betula* spp.) sawdust. The sawdust was moistened with distilled water and compressed by hand to remove excess water and to form a solid mass. The cups were stored inside clear covered plastic boxes (30 × 23 × 10 cm), and placed in an environmental chamber with the parent colonies.

### *Quantification of development*

At 10, 20, 30, 45 and 60 days following colony establishment, females were sampled from each group to monitor

ovarian development during the normal period of oviposition. The presence of eggs or newly eclosed larvae was also noted. An additional 22 primary and 37 secondary females were sampled on Day 0, to provide baseline data of ovarian development for all experimental groups.

All termites were weighed on a Mettler AE-163 balance before and after each experiment. Individual termites were placed live in labelled, 500  $\mu$ L Eppendorf tubes containing Dietrich's fluid to preserve them until dissection. All dissections were conducted using a Wild M5A dissecting scope. Incisions were made along both pleural intersegmental membranes, between the second and third sternites, to expose the abdominal cavity. The ovaries and the genital chamber and spermatheca, were removed and placed on a slide for microscopic examination. An Olympus BH-2 stereomicroscope was used to determine whether the spermatheca contained sperm and to record the total number of functional ovarioles in each ovary. The length and width of the terminal oocytes in the first five ovarioles of each ovary were measured using a stereoscopic microscope fitted with an ocular micrometer. The shape of an oocyte was roughly cylindrical; therefore, oocyte volume was estimated as  $\pi \times [\text{width}]^2 \times [\text{length}]$ . Based on observed patterns of oocyte maturation, including yolk deposition and the visibility of the nucleus, an oocyte and the ovariole producing it were considered to be vitellogenic if the terminal oocyte volume was greater than or equal to 0.01 mm<sup>3</sup>.

The three primary measures of female maturation used were: (1) percent change in body mass; (2) average ovariole number and (3) fecundity, defined as the cumulative number of eggs, newly eclosed larvae and vitellogenic terminal oocytes produced prior to sampling. Increasing body mass, which is associated with female physogastry, is indicative of the increasing size and development of both the ovaries and fat body. The fat body stores metabolic resources used by the females for oogenesis and brood care (Nalepa & Jones, 1991; Lenz, 1994; Shellman-Reeve, 1996), and is the probable source of extraovarian egg vitellogenins (Greenberg *et al.*, 1978). Body mass therefore serves as a correlate of a female's ability to allocate resources towards reproduction. Such a relationship has been found in the Termitidae (Thorne, 1982). The number of functional ovarioles is also indicative of fecundity, because each ovariole is capable of producing oocytes once they are sufficiently mature (Watson, 1972). The ovaries of newly flown alates of *Z. angusticollis* consist of 20–40 panoistic ovarioles (Child, 1934; Weesner, 1969). The terminal oocytes of the ovarioles develop first, but maturation only occurs within the first five ovarioles prior to the initial bout of oviposition. A queen may enhance fecundity through increases in the percentage of ovarioles that are actively maturing oocytes (Weesner, 1969; Noirot, 1990). The number of ovarioles may also increase over time (Grassé, 1949), although there is currently little evidence for this in termites (Weesner, 1969). The increases may be due to the maturation of pre-existing ovarioles beyond a filamentous stage so they can be identified or they may be newly developed ovarioles. Regardless of their origin, change in observable ovariole

number represents a measure of the energy expended towards reproductive development.

The time to insemination and the onset of oviposition were estimated for females in each group by determining the percent of females for each sample day that contained sperm or had laid eggs, respectively. The first day during the 60-day sample period that the 33% benchmark was reached and then maintained for each successive sample day was considered to be the average time of initiation for both parameters. This estimate permitted a clear delineation between groups. A 50% benchmark, the norm for measures such as mortality rates, could not be used because it was never achieved in any of the groups on any sample day.

### Statistical analysis

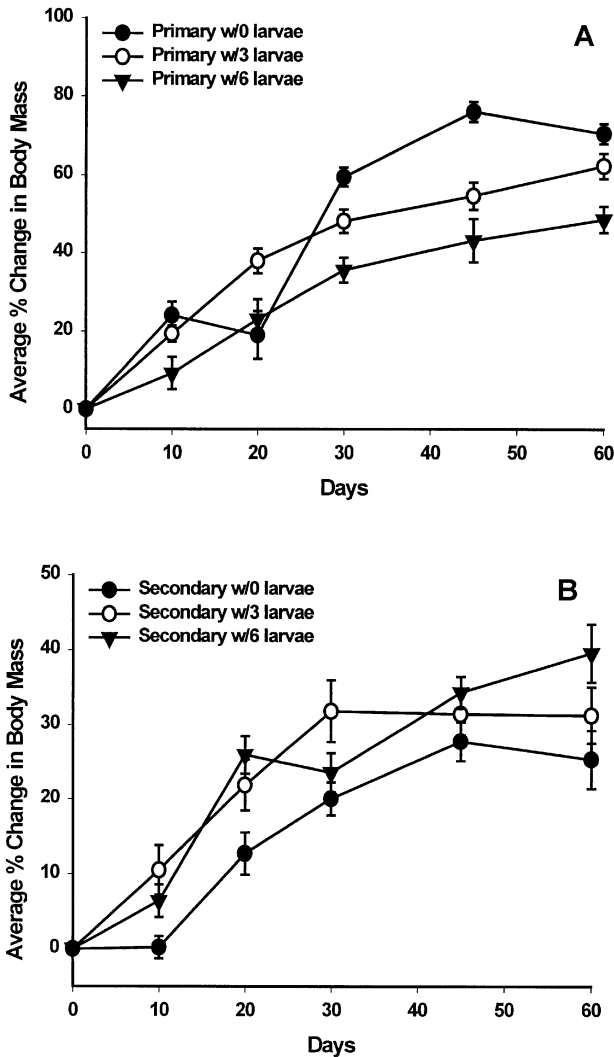
All statistical analyses were performed using Sigmasat vs. 2.03 (SPSS Inc., Chicago, IL). Significant interaction effects between the number of days prior to sampling and the social group, coupled with consistent violations of normality, necessitated using the non-parametric Mann–Whitney rank sum test (Sokal & Rohlf, 1995) to compare percent change in body mass, average ovariole number and fecundity between females within different groups on individual days. Probability values ( $\alpha=0.05$ ) were adjusted for multiple tests using Scheffe's correction (Miller, 1981).

## Results

### *Growth, ovarian maturation and egg production of primary reproductives*

All three indicators of reproductive maturation changed significantly when female primaries nested with larvae. The rate of gain of body mass of female primaries decreased as the number of larvae increased (Fig. 1a). By day 60, the change in body mass of females nesting without larvae ( $70.5 \pm 6.5\%$ ) was significantly greater than for females with three larvae ( $62.4 \pm 10.3\%$ ;  $T_{20,31} = 627.0$ ,  $P = 0.040$ ), which in turn was significantly greater ( $T_{21,31} = 408.0$ ,  $P = 0.006$ ) than that for females nesting with six larvae ( $48.7 \pm 11.5\%$ ). Observations suggest that much of the mass gained by primary and secondary reproductives could be attributed to changes in the size of the fat body and the gut contents.

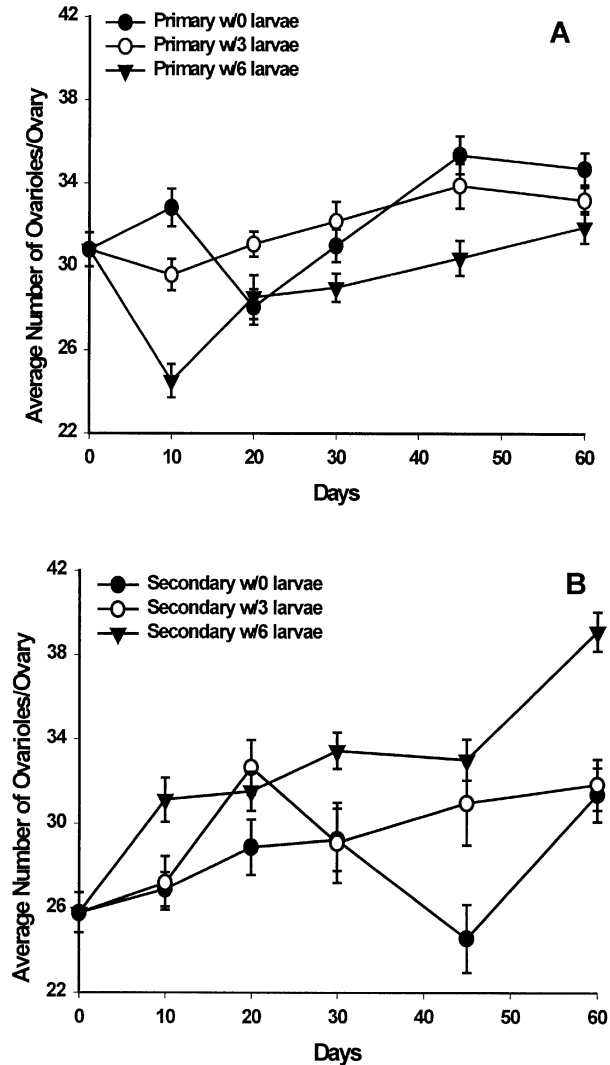
The rate at which primary females developed additional functional ovarioles in each ovary also decreased when larvae were present (Fig. 2a). By day 60, females nesting with zero, three or six larvae had averages of  $34.7 \pm 0.6$ ,  $33.2 \pm 0.4$  and  $31.0 \pm 0.8$  ovarioles per ovary, respectively. The negative impact of immatures was particularly evident when six larvae were present; ovariole number did not change between day 0 and 60 ( $T_{20,21} = 384.5$ ,  $P = 0.361$ ). The difference between females with zero and six larvae was statistically significant on day 60 ( $T_{20,21} = 518.0$ ,  $P = 0.011$ ). The number of active ovarioles that developed was significantly correlated with the



**Fig. 1.** Average percent increase in body mass ( $\pm$  SE) of female primary (A) and secondary (B) reproductives nesting with a reproductive male and zero ( $\bullet$ ), three ( $\circ$ ), or six ( $\blacktriangledown$ ) larvae. Note that (A) and (B) use different scales for mass change. Primary females housed with either three or six larvae gained significantly ( $P < 0.01$ ) less body mass, whereas secondary females housed with six larvae gained significantly more body mass, compared to reproductive females nesting without larvae.

change in body mass of primary females that were not nesting with larvae ( $r = 0.46$ ,  $F = 27.44$ ,  $P < 0.001$ ), but the correlation declined when three ( $r = 0.20$ ) or six ( $r = 0.16$ ) larvae were present.

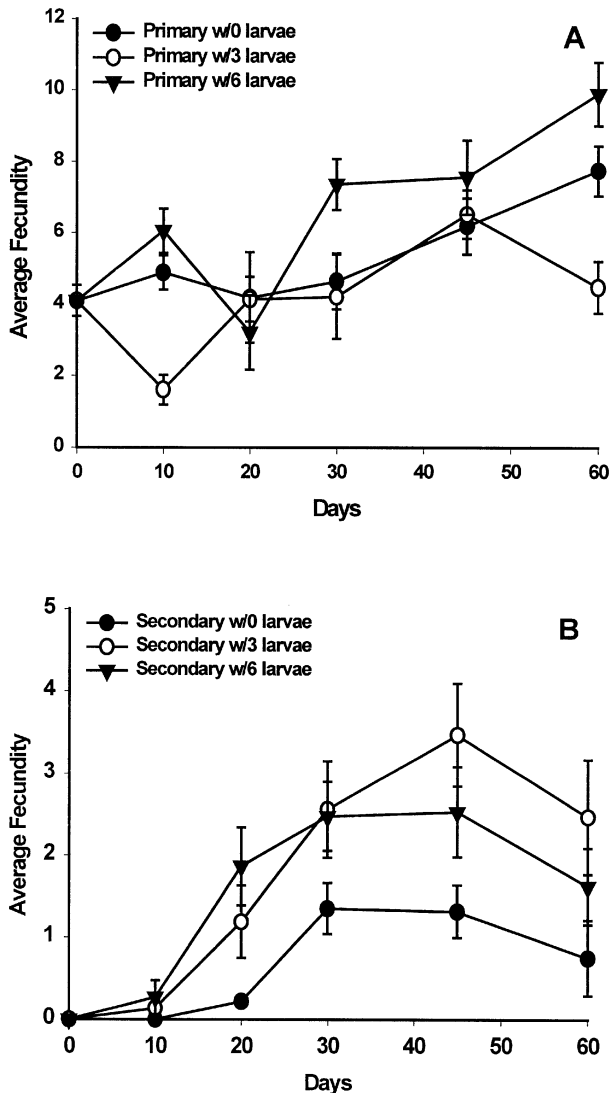
Primary female fecundity also changed with the number of larvae (Fig. 3a). Compared to females nesting without larvae, fecundity at day 60 decreased by 42%, from  $7.75 \pm 0.70$  to  $4.48 \pm 0.72$  for females with three larvae ( $T_{20,31} = 676.0$ ,  $P = 0.003$ ), but increased by 28%, to  $9.91 \pm 0.90$ , for females nesting with six larvae. Although the difference in fecundity between females nesting with zero or six larvae was not significant ( $T_{20,21} = 345.5$ ,  $P = 0.054$ ), the



**Fig. 2.** Average number of functional ovarioles per ovary ( $\pm$  SE) of female primary (A) and secondary (B) reproductives nesting with a reproductive male and zero ( $\bullet$ ), three ( $\circ$ ), or six ( $\blacktriangledown$ ) larvae. Primary females with six larvae developed significantly ( $P < 0.01$ ) fewer ovarioles, whereas secondary females with three or more larvae developed significantly more ovarioles, than those nesting without larvae.

difference between females with three and six larvae was highly significant ( $T_{21,31} = 767.5$ ,  $P < 0.001$ ). There was a significant positive correlation between fecundity and the change in body mass for primary females nesting with zero ( $r = 0.46$ ,  $F = 27.44$ ,  $P < 0.001$ ) or six ( $r = 0.38$ ,  $f = 21.61$ ,  $P < 0.001$ ) larvae, but not for females with three larvae ( $r = 0.19$ ).

The timing of oviposition also changed for primary female reproductives that were housed with larvae. The average time to the onset of oviposition decreased from 31–45 days after colony initiation for reproductives nesting without larvae to 21–30 days for those nesting with three or six larvae.



**Fig. 3.** Average fecundity ( $\pm$ SE) of female primary (A) and secondary (B) reproductives housed with a reproductive male and zero (●), three (○), or six (▼) larvae. Note that (A) and (B) use different scales for fecundity. Primary females nesting with three larvae developed significantly ( $P < 0.01$ ) fewer eggs, whereas females with six larvae produced significantly more oocytes, than primary females housed without larvae. Secondary females nesting with either three or six larvae produced significantly more eggs than secondary females nesting without larvae.

#### Growth, ovarian maturation and egg production of secondary reproductives

The rate and extent of reproductive maturation in secondary females was affected by nesting with different numbers of larvae. Females nesting with three or six larvae gained mass at a faster rate than those nesting without larvae. By day 60 there was a positive progression of average changes in body mass,

going from  $25.4 \pm 15.2$  to  $31.3 \pm 14.0$  to  $39.6 \pm 15.4\%$  for females with zero, three or six larvae, respectively. Although the difference between females with three or six larvae was not significant ( $T_{16,17} = 278.0$ ,  $P = 0.843$ ), the presence of any larvae significantly increased mass gain over females nesting without larvae ( $T_{12,33} = 183.0$ ,  $P = 0.018$ ).

The increase in the number of functional ovarioles for females nesting with zero or three larvae was very similar, but females nesting with six larvae developed additional ovarioles at a faster rate (Fig. 2b). By day 60, females with six larvae had an average of  $39.1 \pm 0.9$  ovarioles per ovary, which was significantly greater than that of females nesting with either zero ( $31.4 \pm 1.3$ ;  $T_{12,16} = 95.5$ ,  $P < 0.001$ ) or three ( $31.9 \pm 1.2$ ;  $T_{16,17} = 376.5$ ,  $P < 0.001$ ) larvae. Ovariole number was significantly correlated with the change in body mass for females nesting with three ( $r = 0.33$ ,  $f = 15.90$ ,  $P < 0.001$ ) or six ( $r = 0.56$ ,  $f = 55.47$ ,  $P < 0.001$ ) larvae, but not for females nesting without larvae ( $r = 0.053$ ,  $f = 0.34$ ,  $P = 0.056$ ).

Secondary females also produced eggs at a faster rate when nesting with larvae (Fig. 3b). The difference in fecundity between females nesting with or without larvae was most pronounced on day 45, when females without larvae produced an average of  $0.88 \pm 0.32$  eggs, whereas females with three larvae had  $3.69 \pm 0.62$  eggs ( $T_{16,17} = 371.5$ ,  $P < 0.001$ ) and those with six larvae had  $2.53 \pm 0.55$  eggs ( $T_{15,17} = 413.0$ ,  $P = 0.013$ ). The difference in fecundity between females nesting with three or six larvae was not significant ( $T_{15,16} = 195.5$ ,  $P = 0.082$ ). The fecundity of secondary females was significantly correlated with changes in body mass for secondary females with zero ( $r = 0.30$ ,  $f = 11.64$ ,  $P < 0.001$ ), three ( $r = 0.41$ ,  $f = 25.98$ ,  $P < 0.001$ ) and six ( $r = 0.47$ ,  $f = 34.31$ ,  $P < 0.001$ ) larvae. The average onset time of oviposition changed when six larvae were present, decreasing from greater than 60 days for females nesting with no or three larvae to 31–45 days for females with six larvae.

#### Reproductive maturation in primary and secondary females

There were significant differences in the way in which primary and secondary females matured and responded to the presence of larvae. Comparisons were made between groups that exhibited the highest degree of maturation for each character to control for the variation in the response to the presence of larvae. The greatest increases in body mass were observed in primaries nesting without larvae and secondaries nesting with six larvae. The average mass gain by day 60 was  $70.5 \pm 6.5\%$  for primaries, which was significantly greater than the increase of  $39.6 \pm 15.4\%$  for secondaries ( $T_{16,20} = 157.0$ ,  $P < 0.001$ ). Secondaries nesting with six larvae had the greatest number of functional ovarioles, having an average of  $39.1 \pm 0.9$  active ovarioles per ovary by day 60 compared to primaries nesting without larvae that averaged  $34.7 \pm 0.6$  ovarioles ( $T_{16,20} = 398.5$ ,  $P < 0.001$ ). Primary females housed with six larvae produced an average of  $9.91 \pm 0.90$  eggs at 60 days, which was significantly greater than the  $2.47 \pm 0.70$

eggs produced by secondary females nesting with three larvae ( $T_{17,21} = 181.5$ ,  $P < 0.0001$ ).

## Discussion

### *Reproductive maturation of primary reproductives*

*Zootermopsis angusticollis* primary females begin to produce eggs in larger numbers and for a longer duration once their first brood has matured and the colony they founded enters its ergonomic stage of development (Castle, 1934). By providing newly dealate primary females with both a mate and third- or fourth-instar larvae, we tried to simulate this early ergonomic stage to test the hypothesis that the increased fecundity of primary females during the ergonomic stage was in part a result of the contribution of larvae rather than a consequence of the additional time females had to mature. We expected that larval assistance in task performance and nutrition would allow the reproductives to allocate more energy toward ovarian growth and egg production rather than storing it for extended brood care, as might occur during the founding stage. Indeed, we observed that the larvae carried out many non-reproductive tasks, such as nest excavation and maintenance, allogrooming and trophallaxis; their presence strongly affected the development of the reproductives. Primary females nesting with three or more larvae gained significantly less body mass and began ovipositing 10–15 days earlier than females nesting with only a primary male. The effect on egg production varied, decreasing significantly for females with three larvae, but increasing significantly for females with six larvae, relative to females nesting without larvae. This variation may be the result of the females relying on separate endogenous and exogenous indicators of their readiness to produce eggs.

The endogenous indicator may be a measure of the resources stored in the reproductive female's fat body. During the founding stage, when no larvae are present, a queen's fecundity may be regulated primarily by her nutritional state (Nalepa, 1994). Assuming that our measure of changing body mass was an accurate indicator of nutritional state, the significant correlation between fecundity and change in body mass for females nesting without larvae is evidence of the association of these variables. The exogenous indicator may be the ergonomic or nutritional benefits provided by varying numbers of larvae available to perform non-reproductive tasks. When three larvae were present, reproductive females oviposited earlier but produced fewer eggs than if nutritional state alone was a determining factor, as indicated by the significant decline in fecundity and the non-significant correlation between percent increase in body mass and fecundity. Three larvae may have provided the reproductive female with an insufficient level of either assistance or social stimuli for her to produce more than a few eggs. Females nesting with six or more larvae may be sufficiently stimulated to increase the rate of egg production above levels observed during the founding stage, so that egg production again closely matched the stored resources available to the female. Oster &

Wilson (1978) predicted that adding workers to small colonies would result in exponential gains in colony productivity that could translate into additional energy available for colony growth. This non-linear relationship may explain the large increase in fecundity that occurs in primary females when the number of larvae increases from three to six. Six larvae may also have been able to supply enough nitrogen through trophallactic exchanges with the females to enhance their rate of vitellogenin production.

We also expected that the presence of larvae would stimulate the activation of additional ovarioles, which is one means for insects to achieve a higher potential fecundity (Wilson, 1971; Tschinkel, 1987). We found that functional ovariole number increased steadily over 60 days in females nesting with zero or three larvae, but development was arrested in females nesting with six larvae. The extra energy and nitrogen used by primary females nesting with six larvae to produce additional eggs may require using resources normally allocated towards maturing additional ovarioles. A greater number of larvae (6–22 in the average first brood; Castle, 1934) may be needed before the primary female is adequately supplied with resources, or is relieved of non-reproductive tasks to the extent that she can increase both ovariole number and fecundity. Females may have preferentially invested in egg production because only a subset of functional ovarioles was needed to produce oocytes at that time. The maturation of additional ovarioles may not be necessary until the colony grows large enough to support the commensurate increase in female fecundity, whereas additional offspring initially would increase the productivity and growth rate of the colony (Oster & Wilson, 1978; Porter & Tschinkel, 1986).

### *Reproductive maturation of secondary reproductives*

Female *Z. angusticollis* neotenic normally develop within colonies that have reached the ergonomic stage of development (Heath, 1903; Castle, 1934). Neotenic females fail to produce measurable quantities of vitellogenin or to oviposit when left in isolated pairs (Greenberg & Stuart, 1979). But what effect do larvae have on the rate of ovarian maturation in neotenic? In contrast to primary reproductives, which leave their natal nest with sufficient resources to initiate a new colony (Grassé, 1949; Nutting, 1969; Shellman-Reeve, 1990, 1996), newly moulted secondaries start their reproductive lives with fewer stored resources; they have the fat bodies and endogenous nitrogen reserves equivalent to larvae of the same instar as a result of becoming reproductives in a single moult (Castle, 1934; Miller, 1969). However, *Z. angusticollis* secondaries usually inherit an established nest containing galleries and numerous helpers (Myles, 1988), which may allow them to compensate quickly for any physiological deficits. Because of the energetic and nitrogen limitations on their reproductive development, we expected that the neotenic would respond strongly to the presence of larvae old enough to assist in labour and nutrition. Our results demonstrate that interactions with as few as three larvae can enhance the rate of reproductive development in neotenic females. As observed in

primary reproductives, oocyte production increased significantly and the start of oviposition occurred approximately 15 days earlier when neotenic females nested with larvae. However, unlike primary females, secondary females gained more body mass with each addition of larvae, so that females nesting with six larvae had gained the most mass by day 60. Heath (1903) found that secondaries perform little or no work within the colony and have a diet that is almost exclusively composed of proctodeal food from larvae. Therefore, increasing the number of larvae in a colony may directly enhance the nutritional state of the neotenic. Surplus nutrients could be stored in the fat body for later use in oogenesis (Greenberg *et al.*, 1978; Shellman-Reeve, 1990).

Also, in contrast to the responses of primary females, ovariole number increased significantly in neotenic nesting with larvae. Although *Z. angusticollis* secondary females start with 5–15 fewer ovarioles per ovary than newly flown alates, they usually produce eggs more rapidly and in significantly greater numbers than primary females during their first bout of oviposition (Castle, 1934; Light, 1934). Increasing the number of functional ovarioles may be necessary to achieve this higher initial fecundity. Because a secondary female matures within a colony that has already reached its ergonomic stage of development, she may make a large initial investment in producing active ovarioles to match her fecundity to a colony's ability to care for offspring.

The differences in the physiological responses of female primary and secondary reproductives of *Z. angusticollis* to colony social conditions appear to reflect adaptations to two successive stages in an evolutionary continuum. Social cues become increasingly important in determining sexual maturation and oogenesis as social complexity increases. At one end of this continuum are the cockroach-like ancestors of termites. In the more solitary forms, social contact with conspecifics and mates may have had a strong positive effect on sexual maturation and oocyte production, as occurs in *Blattella germanica* (Izutsu *et al.*, 1970; Gadot *et al.*, 1989; Schal *et al.*, 1997) and other insect species (Leather, 1995). In subsocial forms similar to *Cryptocercus punctulatus*, ovarian activity was likely regulated by contact with maturing larvae (Nalepa, 1988, 1994). In this case, immature larvae may have had an inhibitory effect on ovarian activity in the adult that may be ascribed to the costs of rearing young. Primary reproductives of *Z. angusticollis*, which represent a basal stage in termite social evolution, face similar conditions during the founding stage of a colony. The presence of dependent immature larvae may also have an inhibitory effect on oocyte production. In addition to the ability to found colonies autonomously, primary reproductives of extant termite species have also evolved the ability to respond positively to the presence of larvae sufficiently mature to perform colony tasks. Secondary reproductives appear to have lost the ability to function autonomously, and their reproductive development is dependent on assistance from nestmates. However, secondary reproductives may be more adapted to optimizing the rate of colony growth during the ergonomic stage of colony development, when reproductive output should be matched to the number of workers available for non-reproductive tasks (Oster

& Wilson, 1978). Primary females are well suited to initiating a colony, but the physiological limitations on their individual fecundity may become the primary limiting factor for colony growth once the support capacity of the colony reaches a threshold level. We have shown that secondary females of *Z. angusticollis* can utilize the workforce that they normally inherit to allow them to mature quickly, and achieve a higher individual fecundity during their first bout of oviposition than primary females (Heath, 1903; Castle, 1934; Greenberg *et al.*, 1978). Multiple secondary females also often develop simultaneously in colonies following the loss of the primary female (Miller, 1969; Myles, 1999). Secondaries therefore may be able to achieve a higher overall fecundity than a primary female would have been capable, which could enhance the colony's chances of survival and the rate at which it matures (Heath, 1903; Light, 1934; Miramontes & DeSouza, 1996; Shellman-Reeve, 1997b; Myles, 1999; Thorne *et al.*, 1999). Such rapid growth could provide direct and indirect benefits to both reproductive and non-reproductive members of the colony (Shellman-Reeve, 1997b; Myles & Nutting, 1988).

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