Influence of sex-specific stimuli on ovarian maturation in primary and secondary reproductives of the dampwood termite *Zootermopsis angusticollis*

C O L I N S . B R E N T and J A M E S F . A . T R A N I E L L O
Department of Biology, Boston University, Boston, MA, U.S.A.

Abstract. It was hypothesized that female primary and secondary reproductives of the termite *Zootermopsis angusticollis* Hagen require sex-specific stimuli from a reproductive male to trigger ovarian maturation. To test this hypothesis, experimental laboratory colonies were established that contained female primary or secondary reproductives nesting: alone; with a male; with a female; and with three third- to fourth-instar larvae. Following colony initiation, we measured changes in body mass, ovariole number and fecundity over 60 days. Results indicate that the reproductive maturation of female primaries was promoted by contact with a male but inhibited by the presence of another female. Secondary females were not affected by the presence of another reproductive, regardless of sex, but the development of reproductive competency of primary females appeared to depend on male-specific stimulation. Reliance on male–female interaction to induce female reproductive development may ensure that the resources of newly dealate females are not wasted on producing larvae that would have a poor chance of surviving in the absence of a male. By contrast, secondary females maturing within established colonies are likely to have a mate and immediate assistance with non-reproductive tasks, and therefore do not delay ovarian maturation and reproduction until they perceive male-specific stimuli. These results demonstrate that male-specific stimuli affect only the reproductive development of primary females, and suggest that the degree to which primary and secondary females depend on mate assistance may have shaped their physiological responses to the presence of a reproductive male.

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

Introduction

Insect ovarian development is a multistage process modulated through hormonal mechanisms triggered by various exogenous and endogenous signals. For many insects, the rate and timing of oocyte maturation and oviposition is sensitive to extrinsic factors such as temperature, humidity and the availability of nutrients (Johansson, 1964; Norris, 1964; Leather, 1995). This sensitivity has evolved in females to ensure that young are produced under conditions that promote their survival and prevent wasted parental effort. In many species of the Dictyoptera, female reproductive development is also sensitive to social conditions. In particular, stimuli supplied by a male

may be necessary to trigger reproductive maturation and behaviour (Engelmann, 1959; Roth, 1970; Gadot *et al*., 1989). The sex-specific information provided by a male, especially through copulatory stimuli (Engelmann, 1959), may indicate to the female that she will be able to produce fertilized eggs, but for many species the exact information responsible for initiating reproduction is unknown (Gadot *et al*., 1989).

Termites (Order Isoptera) are eusocial and depend on social cues to guide their highly plastic reproductive development. In several species of lower termites, the social stimuli that a female experiences following separation from her natal colony can affect both her reproductive behaviour and physiology. For example, primary females of *Hodotermes mossambicus* and *Zootermopsis angusticollis* Hagen will complete their reproductive maturation and exhibit reproductive behaviours only when isolated from their parent colony (Hewitt & Nel, 1969; Watson, 1972; Greenberg *et al*., 1978; Greenberg & Stuart,

Correspondence: Colin S. Brent, Department of Entomology, North Carolina State University, Raleigh, North Carolina 27695, U.S.A. Tel.: +1 919 515 3784; fax: +1 919 515 7746; e-mail: colin_brent@ncsu.edu

1979). After separation from the natal nest, additional social contact is usually necessary for primary females to complete their reproductive maturation, but the nature of the contact can vary between species. Females of *Kalotermes flavicollis* must be paired with a reproductive male (Vieau, 1990), whereas primary females of *Hodotermes mossambicus* (Hewitt & Nel, 1969) only require contact with another individual, independent of sex, to complete their development. Nestmates could provide a female a number of different stimuli, such as individual-specific odours, tactile contact through antennal palpation or allogrooming, trophallactic feeding (Shellman-Reeve, 1990), the transfer of promoting substances (Lüscher, 1972) and the neural stimulus accompanying copulation or insemination (Engelmann, 1959, 1970; Roth, 1970; Ehrlich & Ehrlich, 1978; Oberhauser & Hampton, 1995), to promote ovarian maturation. Any or all of these stimuli may be necessary to elicit reproductive activity in primary females of a particular species of termite.

Given the important role of brood care in colony development, social cues associated with this task may be significant for some termites. Rearing a brood of slow-maturing offspring in nutritionally poor wood is metabolically costly and may be beyond the ability of a single reproductive (Nalepa, 1988; Shellman-Reeve, 1990, 1994; Nalepa & Jones, 1991). For a species such as *Zootermopsis angusticollis*, in which both parents provide parental care (Rosengaus & Traniello, 1991), the female may delay her reproductive maturation until she is paired with a male to ensure the survival of her first brood (Shellman-Reeve, 1990, 1994; 1997). The cues that a male provides the primary female may be an assurance that she will have assistance rearing her first brood.

In contrast to the response of primaries, the stimulus of being paired with a male is by itself insufficient to elicit ovarian maturation in newly moulted *Z. angusticollis* secondary females (Greenberg *et al.*, 1978; Greenberg & Stuart, 1979; Brent & Traniello, 2001). The stimuli provided by several nestmates appear to be necessary to promote secondary female maturation (Greenberg *et al.*, 1978; Greenberg & Stuart, 1979; Greenberg & Stuart, 1982; Brent & Traniello, 2001). Although the added nestmates can promote ovarian maturation, the presence of a reproductive male may be necessary to trigger development in secondaries because they, like primary females, need a source of sperm to produce fertilized eggs.

In this paper the changes in ovarian maturation in primary and secondary female reproductives of *Z. angusticollis* nesting in isolation and under various social conditions are described. The objective was to determine whether sex-specific cues regulate reproductive maturation in females. Do primary females require the presence of a male reproductive for ovarian maturation to occur? Do primary and secondary females require male-specific stimuli, or would any social stimuli indicating the availability of assistance for brood care be sufficient to ensure ovarian maturation? In an attempt to answer these questions a series of experiments was conducted in which females were exposed to specific social stimuli from male and female reproductives.

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 randomly selected from 14 different colonies collected between 1992 and 1998. Parent colonies were kept in plastic boxes containing moist paper towels and wood in which they were originally collected, supplemented as needed. Colonies were maintained in an environmental chamber under a LD 14 : 10 h light cycle at 23 °C and were sprayed regularly with distilled water to maintain humidity.

Alates that attempted to take flight upon opening the containers housing the parent colony were used as a source of primary reproductives. Secondary reproductives were generated from groups of female and male fourth- to sixth-instar larvae isolated from parent colonies in clear covered plastic boxes (17 × 12 × 6 cm) containing moistened filter paper and wood. Individuals were collected within 1–3 days of moulting to a pigmented reproductive form. Primary and secondary reproductives were sexed, weighed and then immediately placed in experimental colonies.

Seven groups of experimental colonies were established, each with a different social condition. The first four groups contained primary females nesting with a primary male (*n* = 103), a primary female (*n* = 96), in isolation (*n* = 127), or with three third- or fourth-instar larvae (*n* = 127). The remaining groups contained secondary females nesting with three third- or fourth-instar larvae and a secondary male (*n* = 132), a secondary female (*n* = 110) or only larvae (*n* = 122). These groups were used to test whether the social stimuli provided by a reproductive male can promote ovarian maturation and oogenesis in primary and secondary females beyond levels observed in isolated females. The groups also allowed us to discriminate between the effects of being housed with another reproductive, independent of sex, and the effects of being provided with brood care assistance, independent of the caste providing it. The reproductives and larvae were paired randomly with respect to colony of origin. The larvae provided were sufficiently mature to perform most colony tasks (Rosengaus & Traniello, 1993) and helped to ensure the maturation of the secondaries (Greenberg *et al.*, 1978; Greenberg & Stuart, 1979; Brent & Traniello, 2001), but were sufficiently small to prevent the rapid depletion of the food supply, which could adversely affect female development (Lenz, 1994).

Experimental colonies nested in covered 67 mL plastic cups (Solo Cup Co., Chicago, IL) containing 2 g (dry weight) of birch sawdust. The sawdust was moistened with distilled water and compressed by hand to form a solid mass and remove excess water. 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 weight change and ovarian development

At 10, 20, 30, 45 and 60 days after colony establishment, females were sampled from each group to monitor ovarian development during the normal time period of oviposition, and to assess the timing of oviposition and insemination. An additional 22 primary and 37 secondary females were sampled on Day 0 to provide baseline data of ovarian development for all groups.

Termites were weighed on a Mettler AE-163 balance (Mettler Toledo, Switzerland) before and after each experiment. Individual termites were placed live in labelled 500 μL Eppendorf tubes containing Dietrich's Fluid (Gray, 1954) to preserve them until dissection. All dissections were carried out using a Wild M5A dissecting microscope (Wild, Heerbrugg, Switzerland). Incisions were made along both pleural intersegmental membranes between the second and third sternites to expose the abdominal cavity. The ovaries were removed and placed on a slide for microscopic examination. The spermathecae were also removed from primary females nesting with a male and secondary females nesting with a male and three larvae. An Olympus BH-2 (Wild Heerbrugg, Switzerland) stereoscopic microscope was used to record the total number of ovarioles in each ovary and to determine whether each spermatheca contained sperm. 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. Oocytes are approximately cylindrical; therefore, oocyte volume was estimated as ($*$[radius]*[-length]. Based on observed patterns of oocyte maturation, an oocyte and the ovariole in which it was developing were considered to be vitellogenic if terminal oocyte volume was greater than or equal to 0.01 mm^3 (Hewitt *et al.*, 1972).

The three primary measures of female growth and ovarian maturation were percent change in body mass, average ovariole number and fecundity. An increase in body mass may be due, in part, to increased water intake (Watson *et al.*, 1972), but should also be indicative of the increasing size and development of both the ovaries and the fat body. Assuming that the fat body is the primary source for egg vitellogenins for *Z. angusticollis* (Greenberg *et al.*, 1978), mass gain should be a correlate of a female's general nutritional state, and thus her ability to allocate energy toward rearing her first brood. The number of functional ovarioles comprising an ovary is indicative of a female's investment in her reproductive development and her potential capacity to produce eggs (Watson, 1972). The fecundity measure is the cumulative number of eggs, newly eclosed larvae and vitellogenic terminal oocytes produced by a female.

The timing of insemination and oviposition were estimated by determining for each sample day the percentage of females with spermathecae containing sperm and that had oviposited. The sample day on which 33% or more females were inseminated or had oviposited was considered to be the average time following colony establishment necessary for initiating these events. A 33% estimate was used because it permitted a clear delineation between the groups, and because there were no sample days on which 50% or more females were inseminated or had oviposited.

Statistical analysis

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

Results

Growth, ovarian maturation and egg production of primary female reproductives

All three indicators of the degree of reproductive maturation were significantly lower when female primaries nested without a primary male. Females nesting with a male increased their body mass faster than those in other treatments (Fig. 1a). By day 60 the average increase was 70.52 ± 2.55%, which was significantly greater than the increases observed in females nesting with a female (36.42 ± 7.33%; $T_{16,20} = 176.0$, $P < 0.001$), in isolation (52.46 ± 3.38%; $T_{20,25} = 622.0$, $P < 0.001$) or nesting with three larvae (54.72 ± 2.21%; $T_{20,27} = 660.0$, $P < 0.001$). Females nesting alone or with larvae gained mass at comparable rates and by day 60 their average gains were similar ($T_{25,27} = 654.0$, $P = 0.884$). Co-maturing females stopped increasing mass after day 30, and by day 60 their average mass gain was less than those females nesting with a male ($T_{16,27} = 269.0$, $P = 0.038$), with larvae ($T_{16,27} = 269.0$, $P = 0.038$) or alone ($T_{16,25} = 273.0$, $P = 0.095$), suggesting that the presence of another female has a negative effect on stored resources.

Ovariole development, like mass gain, varied in primaries by treatment (Fig. 2a). Females nesting with a male or alone developed additional active ovarioles, and by day 60 they had 34.73 ± 0.78 and 32.96 ± 0.81 ovarioles per ovary ($T_{20,25} = 525.5$, $P = 0.138$), respectively. However, females nesting alone initially experienced a decrease in the number of active ovarioles, averaging 27.25 ± 1.05 on day 30. Females nesting with another female maintained the same number of ovarioles between day 0 (30.80 ± 0.81) and day 60 (31.22 ± 1.40; $T_{16,20} = 303.0$, $P = 0.836$), suggesting that the presence of another primary female may inhibit the development of additional active ovarioles. Females nesting with larvae had a net decrease of 5.4 active ovarioles per ovary between days 0 and 60 (25.44 ± 0.82; $T_{20,27} = 657.0$, $P < 0.001$). Of these four treatments, only females nesting with larvae had ovariole development that was not strongly correlated with mass gain (Table 1).

Fecundity, which here includes both oviposited and actively maturing oocytes, changed significantly only in females
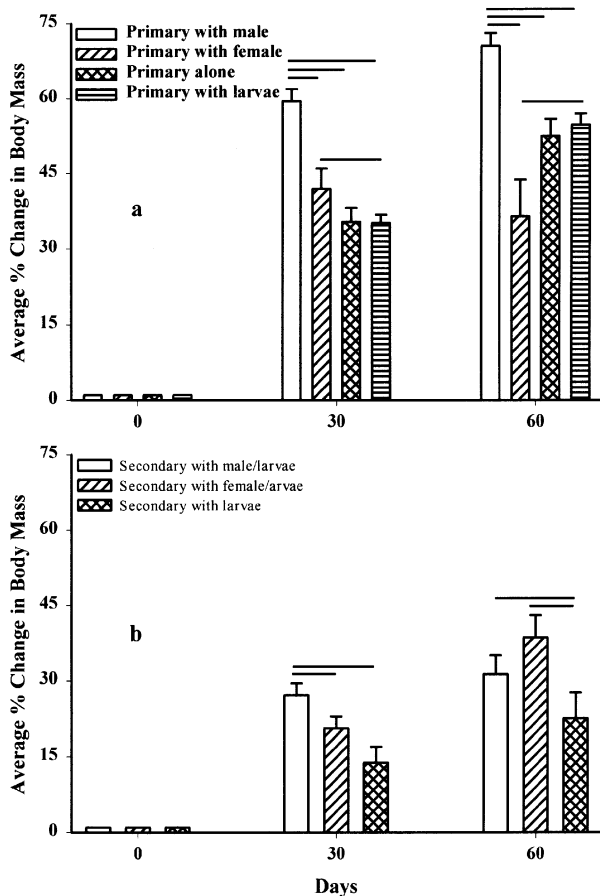
Fig. 1. Average change in body mass (mean % ± SE) of female primary (a) and secondary (b) reproductives nesting with a reproductive male, another reproductive female, in isolation, or with larvae. All secondary females nested with three larvae. Horizontal lines above bars indicate comparisons between groups that were significantly different ($P < 0.05$).

Fig. 2. Average number of ovarioles per ovary (mean ± SE) of female primary (a) and secondary (b) reproductives nesting with a reproductive male, another reproductive female, in isolation, or with larvae. Legend as in Fig. 1.

nesting with a male, increasing from 4.10 ± 0.44 to 7.75 ± 0.70 between days 0 and 60 (Fig. 3a; $T_{20,20} = 275.5$, $P < 0.001$). Females nesting with another female, alone, or with larvae maintained a similar number of vitellogenic oocytes over the sample period, having 60-day averages of 4.13 ± 0.81, 4.40 ± 0.57 and 3.85 ± 0.40, respectively. There was a significant positive correlation between fecundity and the change in body mass only for females with males (Table 1). Primary females nesting alone or with a female had not oviposited by day 60, whereas females nesting with a male or larvae began to oviposit within 31–45 days following colony initiation.

Growth, ovarian maturation and egg production of secondary female reproductives

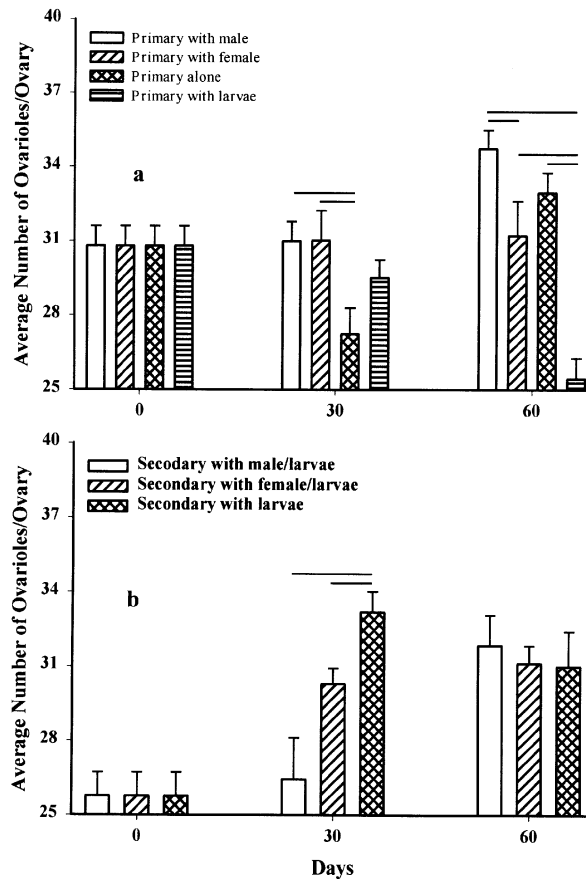The rate and extent of ovarian maturation in secondary females did not differ by treatment, but mass gain and the

timing of oviposition were affected. The average number of ovarioles that developed in each ovary did not vary by treatment (Fig. 2b; $P > 0.2$). The average number of ovarioles on day 60 for females nesting with a male, a female or without a reproductive were 31.85 ± 1.22, 31.11 ± 0.72 and 31.00 ± 1.42, respectively. Fecundity did not differ significantly between these groups (Fig. 3b; $P > 0.2$), having respective averages of 2.47 ± 0.70, 2.52 ± 0.55 and 1.73 ± 0.61 at day 60. Females nesting with either a male or a female gained body mass at about the same rate (Fig. 1b; $T_{17,42} = 529.0$, $P = 0.757$), with respective average increases of 31.26 ± 3.75 and 38.53 ± 4.46% at 60 days. Females nesting with only larvae increased body mass by 22.55 ± 5.12% after 60 days, which was significantly less than females with a male ($T_{15,17} = 186.0$, $P = 0.021$) or another female ($T_{15,42} = 322.0$, $P = 0.041$). Unlike the pattern observed in primary females, both ovariole number and fecundity were correlated significantly with percentage change in body mass for all three groups of females tested (Table 1). The average onset time of oviposition increased from 31 to

Table 1. Correlations (*r*) between change in body mass and average number of ovarioles per ovary and fecundity for female primary and secondary reproductives nesting with a reproductive male, another reproductive female, alone, or with larvae. Probabilities were adjusted for multiple comparisons using Scheffe's correction. The intercept (b) and the slope (m) of the regression line are provided

| Stimuli	Primary females				Secondary females		
	Male	Female	Isolated	Larvae	Male	Female	Isolated
Correlations between change in body mass and average number of ovarioles per ovary							
r	0.462	0.268	0.300	0.067	0.323	0.264	0.281
F	27.435	8.025	9.998	0.560	15.161	9.025	16.091
P	< 0.001	0.006	0.002	0.456	< 0.001	0.003	< 0.001
b	29.754	29.174	27.781	28.699	26.133	28.394	28.187
m	0.061	0.049	0.072	– 0.013	0.122	0.105	9.068
Correlations between changes in body mass and fecundity							
r	0.323	0.072	0.069	0.092	0.479	0.241	0.462
F	11.781	0.542	0.483	1.064	38.670	11.542	32.605
P	< 0.001	0.463	0.490	0.304	< 0.001	< 0.001	< 0.001
b	3.981	3.849	3.467	5.363	0.243	0.978	0.660
m	0.034	0.010	0.008	– 0.011	0.063	0.027	0.056
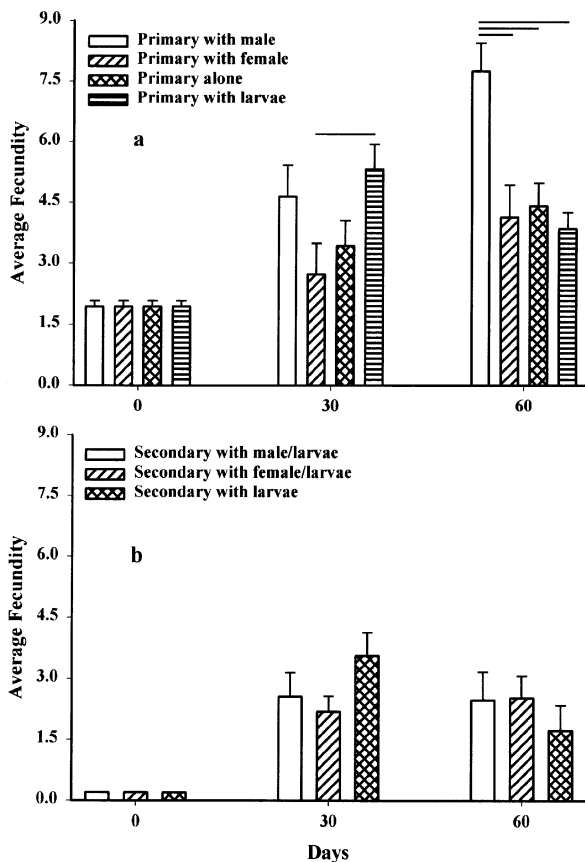
Fig. 3. Average fecundity (mean ± SE) of female primary (a) and secondary (b) reproductives nesting with a reproductive male, another reproductive female, in isolation, or with larvae. Legend as in Fig. 1.

45 days for females nesting with a male or another female to greater than 60 days for females nesting only with larvae.

Discussion

Reproductive maturation of primary females

Reproductive development in primary females of *Z. angusticollis* is influenced strongly by the social environment. Maturation and oviposition are inhibited in alates until the females have flown from their natal colony to establish their own nest. Stimuli from functional queens and other alates within the natal nest appear to inhibit ovarian development (Lüscher, 1977; Greenberg & Stuart, 1979; Noirot, 1990). Isolating a newly dealate female from other individuals is sufficient to promote both mass gain and the development of additional functional ovarioles. The departure from the natal nest may signal a female to prepare for producing her first brood (Miller, 1969; Greenberg & Stuart, 1979), but she may require contact with a male reproductive to trigger oogenesis, as has been demonstrated in *Kalotermes flavicollis* (Vieau, 1990). During the founding stage of a colony, a primary female is usually in constant association with a primary male, engaging in interactive behaviours such as allogrooming, antennal palpation, trophallaxis and copulation (Heath, 1903; Nutting, 1969; Shellman-Reeve, 1990; Rosengaus & Traniello, 1991). The present experiment indicates that these male-specific stimuli promote reproductive development and may be essential for inducing oogenesis in primary females. Maturing females nesting in heterosexual pairs gained more body mass, developed greater numbers of functional ovarioles, and had higher fecundities than females exposed to other social stimuli.

One means by which a reproductive male may influence a primary female's maturation is by enhancing her nutritional

state through dietary supplements and assisting with colony labour (Light, 1942–43; Shellman-Reeve, 1990), thereby increasing her ability to dedicate resources towards development. Resource accumulation is crucial during colony establishment, ensuring that reproductives have sufficient reserves for oogenesis, brood care and nest maintenance (LaFage & Nutting, 1978; Grandi *et al.*, 1988; Shellman-Reeve, 1996). If the change in body mass is an accurate indicator of female nutritional state, then the significant correlations between body mass, ovariole number and fecundity for females nesting with males may indicate that nutritional state determines the extent of development possible. The presence of a male reproductive appeared to stimulate an additional 18–34% increase in mass over that of females nesting in other conditions. This extra mass may have promoted the increases in fecundity and ovariole number.

Although *Zootermopsis* males transfer nutrients to females (Shellman-Reeve, 1990), it is unlikely that this was the sole reason for the additional mass gained. Larvae, which can also supplement a female's diet (McMahan, 1969; Rosengaus & Traniello, 1991; Nalepa, 1994; Brent & Traniello, 2001), failed to enhance female mass gain beyond levels observed in isolated females. A primary male may be more effective at supplementing a female's diet than three third-instar larvae, or the presence of a male may stimulate a female to increase her feeding.

Although nutritional status may be a strong determinant of female reproductive development, appropriate social stimuli may be necessary to ensure ovarian maturation. Primary males may also influence female development through sex-specific tactile and/or chemosensory stimuli that precipitate a neurohormonal cascade regulating metabolic functions and behaviour. Females in other groups maintained the same number of vitellogenic oocytes during the entire length of the experiment, despite increases in stored resources. Even the presence of larvae, which can promote oogenesis in females nesting with males (Brent & Traniello, 2001), failed to enhance female fecundity in the absence of a reproductive male. Body mass may determine the rate of egg production, but stimuli from a male appear to be necessary to trigger oogenesis.

There are a number of reasons why a female may delay reproduction until a male is available. Shellman-Reeve (1997) found that colonies headed by a single reproductive had a reduced chance of survival compared with those headed by a pair of primaries, possibly due to the higher energetic demands on the female and decreased ability to resist competitors, predators (Shellman-Reeve, 1997) and infection (Rosengaus *et al.*, 1998). Although *Z. angusticollis* females can produce parthenogenic larvae (Light, 1944), isolated primary females may not risk energetic reserves by producing offspring with a lowered probability of survival. Instead, these females increased body mass and ovariole number, and sustained several oocytes at vitellogenic levels. This response may prepare the female for future brood production in anticipation of future opportunities for finding a mate resulting from the non-synchronized swarms of *Zootermopsis* alates (Castle, 1934; Shellman-Reeve, 1994).

The assurance of a sperm supply may also be important to the female, because parthenogenic larvae have low survival rates and are poor replacement reproductives (Light, 1944). A female could utilize one generation of parthenogenic larvae to found the colony if her mate was initially unable to inseminate her, but sexual reproduction would be necessary to ensure long-term survival of the colony. Her first brood might be a mixture of asexually and sexually generated offspring, depending on whether the male was ready to copulate before she completed the first bout of oogenesis. By not waiting to be inseminated prior to maturing, a female could produce her first brood sooner, resulting in offspring that will be sufficiently old to perform non-reproductive colony tasks soon after colony establishment. This would increase a colony's chance of surviving ecological threats (Shellman-Reeve, 1997) or the loss of a reproductive (Heath, 1903), and would increase growth rate (Oster & Wilson, 1978; Tschinkel, 1987).

The male-specific cues that promote positive changes in female body mass, ovariole number and fecundity are most likely not the stimuli associated with insemination, because only 38% of ovipositing primary females nesting with males had detectable quantities of sperm in their spermatheca by day 60. In addition, there was no apparent difference between inseminated and non-inseminated female development. Although it is possible that a male may have copulated with a female without transferring detectable quantities of sperm, the males may have wholly refrained from copulation. Males may instead provide sex-specific information through odours and/or other non-sexual interactions, indicating to the female that a source for sperm and assistance with brood care is available, meeting her criteria for initiating the production of offspring.

Little evidence has been found to support a regulatory role of pheromones in *Z. angusticollis* and related species, suggesting that tactile cues are important (Light, 1943; Hewitt *et al.*, 1972; Stuart, 1979). However, our attempts to examine pheromonal effects, by separating male and female reproductives within the same colony using a mesh screen, resulted in a high mortality rate, so we cannot rule out the possibility that odour is an important male signal. The cause of mortality of these separated reproductives appeared to be disease, which is known to have a dramatic effect on the survivorship of primary reproductives in incipient colonies (Rosengaus *et al.*, 1998, 2000).

In addition to the effect of male-specific stimuli on primary female maturation, stimuli from co-maturing females and larvae also influenced development. After 60 days, primary females nesting together gained 16% less mass and had significantly fewer functional ovarioles than females nesting alone. Females did not appear to act aggressively or directly interfere with each other when feeding, so their response may be based on the perception of a female-specific cue. This response may be similar to the inhibitory effect that mature females have on the reproductive maturation of larvae (Castle, 1934; Light, 1934; Light, 1943; Lüscher, 1972; Greenberg & Stuart, 1979, 1982) or the mutual inhibition of grouped alates (Greenberg & Stuart, 1979). The females that were paired in this study may have inhibited each other, so that their final

maturation was abnormally slow. In particular, females may reduce each other's feeding behaviour, similar to what has been described in *Hodotermes mossambicus* (Hewitt *et al*., 1972).

Given the positive effects that nutritionally independent larvae could have on female nutrition (Oster & Wilson, 1978; Porter & Tschinkel, 1986; Nalepa, 1994; Brent & Traniello, 2001), we expected that primary female development would be enhanced by the presence of larvae. However, relative to females nesting in isolation, those in contact with larvae had similar fecundities and their average ovariole number per ovary declined by 18%, despite an increase in body mass. This was also the only group in which ovariole number was not significantly correlated with body mass. This decline may be an indirect effect of maturing vitellogenic oocytes. Only females nesting with a male or with larvae oviposited; these females thus expended energy that might otherwise have been allocated towards maintaining or adding to ovariole number. The availability of larvae to assist with brood care may have stimulated the females to oviposit (Brent & Traniello, 2001), but at the expense of the number of functional ovarioles which they could maintain.

Reproductive maturation of secondary females

The response of *Z. angusticollis* secondary females to isolation and different social conditions did not parallel those of primary females. Neotenics did not appear to require male-specific stimuli to initiate ovarian maturation. The only significant difference observed among test groups concerned females nesting with only larvae, which gained less mass than females nesting with larvae and a reproductive. This difference may be the result of secondary females responding to the number of individuals within the colony. We previously demonstrated that increasing colony size can positively affect secondary female body mass (Brent & Traniello, 2001), possibly by increasing the energetic reserves of a female by transferring nutrients (Shellman-Reeve, 1990; Nalepa, 1994) or by relieving the female of task performance (Rosengaus & Traniello, 1993; Nalepa, 1994). Had females nesting without reproductives been allowed to associate with four larvae so that there were an equivalent number of nestmates in all test groups, there may not have been a significant difference in mass gain between treatments.

The number of ovarioles that mature in each ovary and the fecundity of females in all three groups of secondaries were strongly correlated with changes in body mass, indicating that the availability of stored energy reserves was important in reproductive development. The decrease in mass gain for females nesting only with larvae did not have a significant effect on the other parameters measured, indicating that either the females still had sufficient energy to invest in ovarian development and oogenesis, or that other, untested factors may promote or inhibit development in addition to the female's nutritional state.

The only social requirement that secondary females seem to have before they complete their reproductive maturation is that their colony attains a threshold size (Light, 1943; Greenberg & Stuart, 1979; Brent & Traniello, 2001). Male-specific stimuli do not appear necessary for neotenic females to mature and oviposit. Secondary females resort readily to parthenogenesis when a male is not available (Light, 1944), and in contrast to the primary females, it is likely that a replacement male will arise from among siblings if a reproductive male is not already present to supply sperm (Castle, 1934). Although the presence of a neotenic female may influence the development of another neotenic, there was no discernible difference between paired females and those nesting with a male in the measures of reproductive competence recorded here. Secondary females may not be as sensitive as primary females to the presence of another reproductive female, because neotenics are siblings and would not necessarily be competing reproductively with each other for resources (Thorne, 1997). In addition, newly moulted secondary females are less mature than recently dealate primaries, and may require additional time to become capable of inhibiting the reproductive development of other females. Multiple neotenic females are common in *Z. angusticollis* colonies (Castle, 1934; Light & Illg, 1945; Myles & Nutting, 1988) and their occurrence may benefit a colony by producing more eggs than a single mature primary female (Castle, 1934). This potential for increasing colony size rapidly could enhance survival and productivity (Oster & Wilson, 1978; Porter & Tschinkel, 1986; Miramontes & DeSouza, 1996; Shellman-Reeve, 1996; Myles, 1999; Thorne *et al*., 1999).

Comparisons with dictyopteran species

The differences in the reproductive responses of *Z. angusticollis* primary and secondary reproductives to various social stimuli accentuate the importance of parental care as a significant factor in termite evolution. In dictyopteran species, biparental care is uncommon, except in some species of wood-feeding roaches (Nalepa, 1994), and the main stimuli necessary to trigger oogenesis and oviposition, aside from the nutritional state of the female (Roth & Stay, 1961a; Roth, 1970), are provided by intromission and insemination (Engelmann, 1959; Roth & Stay, 1961b; Roth, 1970; Gadot *et al*., 1989). Like the offspring of the subsocial woodroach *Cryptocercus punctulatus* (Nalepa, 1988), slowly developing termite larvae require a considerable amount of long-term parental care, including the assistance of a primary male to rear the first brood (Shellman-Reeve, 1990, 1994; Nalepa & Jones, 1991; Rosengaus & Traniello, 1991). Without such assistance a colony is more likely to fail (Shellman-Reeve, 1997). Reproductive maturation of primary females is sensitive to sex-specific stimuli provided by reproductive males. It may be advantageous for a female to delay reproduction until assured of help with brood care and a supply of sperm. Contact with a male may only be necessary during a colony's founding stage, so that a primary female could continue producing eggs even if the male is subsequently removed, as has been found in *Kalotermes* (Vieau, 1990). During the ergonomic stage, female primaries can depend on larvae to provide assistance with colony tasks,

and reproductive males are relegated to providing sperm. By contrast, secondary females, arising as replacement reproductives within established colonies, are assured of mates. Neotenic females appear to have their reproductive maturation follow the availability of assistance within the colony (Greenberg & Stuart, 1979; Brent & Traniello, 2001) and have little or no reliance on male-specific stimuli.

Acknowledgements

References

Brent, C.S. & Traniello, J.F.A. (2001) Social influence of larvae on ovarian maturation in primary and secondary reproductives of the dampwood termite *Zootermopsis angusticollis*. *Physiological Entomology*, **26**, 78–85.

Castle, G.B. (1934) The damp-wood termites of western United States, genus *Zootermopsis*. *Termites and Termite Control* (ed. by C. Kofoid), pp. 264–282. University of California Press, Berkeley.

Ehrlich, A.H. & Ehrlich, P.R. (1978) Reproductive strategies in the butterflies. I. Mating frequency, plugging and egg number. *Journal of the Kansas Entomological Society*, **5**, 323–331.

Engelmann, F. (1959) The control of reproduction in *Diploptera punctata* (Blattaria). *Biological Bulletin*, **116**, 406–419.

Engelmann, F. (1970) *The Physiology of Insect Reproduction*, pp. 41–141. Pergamon Press, New York.

Gadot, M., Burns, E. & Schal, C. (1989) Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica:* effects of grouping and mating. *Archives of Insect Biochemistry and Physiology*, **11**, 189–200.

Grandi, G., Barbieri, R. & Colombo, G. (1988) Oogenesis in *Kalotermes flavicollis* Fabr. (Isoptera, Kalotermitidae) I. Differentiation and maturation of oocytes in female supplementary reproductives. *Bolletino di Zoologia*, **55**, 279–292.

Gray, P. (1954) *The Microtomists' Formulary and Guide*. Blakiston, New York.

Greenberg, S.L.W., Kunkel, J.G. & Stuart, A.M. (1978) Vitellogenesis in a primitive termite, *Zootermopsis angusticollis* Hagen (Hodotermitidae). *Biological Bulletin*, **155**, 336–346.

Greenberg, S.L.W. & Stuart, A.M. (1979) The influence of group size on ovarian development in adult and neotenic reproductives of the termite *Zootermopsis angusticollis* Hagen (Hodotermitidae). *International Journal of Invertebrate Reproduction*, **1**, 99–108.

Greenberg, S.L.W. & Stuart, A.M. (1982) Precocious reproductive development (neoteny) by larvae of a primitive termite *Zootermopsis angusticollis* Hagen. *Insectes Sociaux*, **29**, 535–47.

Heath, H. (1903) The habits of California termites. *Biological Bulletin*, **4**, 47–63.

Hewitt, P.H. & Nel, J.J.C. (1969) The influence of group size on the sarcosomal activity and the behaviour of *Hodotermes mossambicus* alate termites. *Journal of Insect Physiology*, **15**, 2169–2177.

Hewitt, P.H., Watson, J.A.L., Nel, J.J.C. & Schoeman, I. (1972) Control of the change from group to pair behaviour by *Hodotermes mossambicus* reproductives. *Journal of Insect Physiology*, **18**, 143–150.

Johansson, A.S. (1964) Feeding and nutrition in reproductive processes in insects. *Royal Entomological Society of London Symposia*, **2**, 43–55.

LaFage, J.P. & Nutting, W.L. (1978) Nutrient dynamics of termites. *Production Ecology of Ants and Termites* (ed. by M. V. Brian), pp. 165–232. Cambridge University Press, Cambridge.

Leather, S.R. (1995) Factors affecting fecundity, fertility, oviposition and larviposition in insects. *Insect Reproduction* (ed. by S. R. Leather and J. Hardie), pp. 143–174. CRC Press, New York.

Lenz, M. (1994) Food resources, colony growth and caste development in wood-feeding termites. *Nourishment and Evolution in Insect Societies* (ed. by J. H. Hunt and C. A. Nalepa), pp. 159–210. Westview Press, San Francisco.

Light, S.F. (1934) The constitution and development of the termite colony. *Termites and Termite Control* (ed. by C. Kofoid), pp. 22–41. University of California Press, Berkeley.

Light, S.F. (1942–43) The determination of castes of social insects. *Quarterly Review of Biology*, **17**, 312–316, 18, 46–63.

Light, S.F. (1944) Parthenogenesis in termites of the genus *Zootermopsis. University of California Publications in Zoology*, **43**, 405–412.

Light, S.F. & Illg, P.I. (1945) Rate and extent of reproductive development of neotenic reproductives in groups of nymphs of the termite genus *Zootermopsis. University of California Publications in Zoology*, **53**, 1–40.

Lüscher, M. (1972) Environmental control of juvenile hormone (JH) secretion and caste differentiation in termites. *General and Comparative Endocrinology Suppl.*, **3**, 509–514.

Lüscher, M. (1977) Queen dominance in termites. *Proceedings of the International Union for the Study of Social Insects Congress, Wageningen*, **8**, 238–242.

McMahan, E.A. (1969) Feeding relationships and radioisotope techniques. *Biology of Termites*, Vol. 1 (ed. by K. Krishna and F. M. Weesner), pp. 387–406. Academic Press, New York.

Miller, E.M. (1969) Caste differentiation in the lower termites. *Biology of Termites*, Vol. 1 (ed. by K. Krishna and F. M. Weesner), pp. 283–310. Academic Press, New York.

Miramontes, O. & DeSouza, O. (1996) The nonlinear dynamics of survival and social facilitation in termites. *Journal of Theoretical Biology*, **181**, 373–380.

Myles, T.G. (1999) Review of secondary reproduction in termites (Insecta: Isoptera) with comments on its role in termite ecology and social evolution. *Sociobiology*, **33**, 1–91.

Myles, T.G. & Nutting, W.L. (1988) Termite eusocial evolution: a re-examination of Bartz's hypothesis and assumptions. *Quarterly Review of Biology*, **63**, 1–23.

Nalepa, C.A. (1988) Cost of parental care in the woodroach *Cryptocercus punctulatus* Scudder (Dictyoptera: Cryptocercidae). *Behavioral Ecology and Sociobiology*, **23**, 135–140.

Nalepa, C.A. (1994) Nourishment and the origin of termite eusociality. *Nourishment and Evolution in Insect Societies* (ed. by J. H. Hunt and C. A. Nalepa), pp. 57–104. Westview Press, San Francisco.

Nalepa, C.A. & Jones, S.C. (1991) Evolution of monogamy in termites. *Biological Review*, **66**, 83–97.

Noirot, Ch (1990) Sexual castes and reproductive strategies in termites. *Social Insects: an Evolutionary Approach to Castes and Reproduction* (ed. by W. Engels), pp. 5–35. Springer-Verlag, New York.

Norris, M.J. (1964) Environmental control of sexual maturation in insects. *Royal Entomological Society of London Symposia*, **2**, 56–65.

Nutting, W.L. (1969) Flight and colony foundation. *Biology of Termites*, Vol. 1 (ed. by K. Krishna and F. M. Weesner), pp. 233–282. Academic Press, New York.

Oberhauser, K.S. & Hampton, R. (1995) The relationship between mating and oogenesis in monarch butterflies (Lepidoptera: Danainae). *Journal of Insect Behavior*, **8**, 701–713.

Oster, G.F. & Wilson, E.O. (1978) *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton.

Porter, S.D. & Tschinkel, W.R. (1986) Adaptive value of nanitic workers in newly founded red imported ant colonies (Hymenoptera: Formicidae). *Annals of the American Entomological Association*, **79**, 723–726.

Rosengaus, R.B., Maxman, A.B., Coates, L.E. & Traniello, J.F.A. (1998) Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis*. *Behavioral Ecology and Sociobiology*, **44**, 125–134.

Rosengaus, R.B. & Traniello, J.F.A. (1991) Biparental care in incipient colonies of the dampwood termite *Zootermopsis angusticollis* Hagen. *Journal of Insect Behavior*, **4**, 633–647.

Rosengaus, R.B. & Traniello, J.F.A. (1993) Temporal polyethism in incipient colonies of the primitive termite *Zootermopsis angusticollis*: a single multistage caste. *Journal of Insect Behavior*, **6**, 237–252.

Rosengaus, R.B., Traniello, J.F.A., Lefebrve, M.L. & Carlock, D. (2000) Social transmission of disease between adult male and female reproductives of the dampwood termite *Zootermopsis angusticollis*. *Ethology, Ecology and Evolution*, **12**, 419–433.

Roth, L.M. (1970) L'influence des stimuli externes sur la gametoge-nese des insectes. *Centre National de la Recherche Scientifique*, **189**, 267–286.

Roth, L.M. & Stay, B. (1961a) Oocyte development in *Blattella germanica* and *Blattella vaga* (Blattaria). *Annals of the Entomological Society of America*, **55**, 633–642.

Roth, L.M. & Stay, B. (1961b) Oocyte development in *Diploptera punctata* (Eschscholtz) (Blattaria). *Journal of Insect Physiology*, **7**, 186–202.

Shellman-Reeve, J.S. (1990) Dynamics of biparental care in the dampwood termite, *Zootermopsis nevadensis* (Hagen): response to nitrogen availability. *Behavioral Ecology and Sociobiology*, **26**, 389–397.

Shellman-Reeve, J.S. (1994) Limited nutrients in a dampwood termite: nest preference, competition and cooperative nest defense. *Journal of Animal Ecology*, **63**, 921–932.

Shellman-Reeve, J.S. (1996) Operational sex ratios and lipid reserves in the dampwood termite *Zootermopsis nevadensis* (Hagen) (Isoptera: Termopsidae). *Journal of the Kansas Entomological Society*, **69**, 139–146.

Shellman-Reeve, J.S. (1997) Advantages of biparental care in the wood-dwelling termite, *Zootermopsis nevadensis*. *Animal Behaviour*, **54**, 163–170.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry: the Principles and Practices of Statistics in Biological Research*. W.H. Freeman, New York.

Stuart, A.M. (1979) The determination and regulation of the neotenic reproductive caste in the lower termites (Isoptera): with special reference to the genus Zootermopsis (Hagen). *Sociobiology*, **4**, 223–237.

Thorne, B.L. (1997) Evolution of eusociality in termites. *Annual Review of Ecology and Systematics*, **28**, 27–54.

Thorne, B.L., Traniello, J.F.A., Adams, E.S. & Bulmer, M. (1999) Reproductive dynamics and colony structure of subterranean termites of the genus *Reticulitermes* (Isoptera; Rhinotermitidae): a review of the evidence from behavioral, ecological and genetic studies. *Ethology, Ecology and Evolution*, **11**, 149–169.

Tschinkel, W.R. (1987) Relationship between ovariole number and spermathecal sperm count in ant queens: a new allometry. *Annals of the Entomological Society of America*, **80**, 208–211.

Vieau, F. (1990) L'inflence du male sur l'activité reproductice de la femelle dans les jeunes colonies expérimentales de *Kalotermes flavicollis* Fabr. *Insectes Sociaux*, **37**, 169–180.

Watson, J.A.L. (1972) Ovarian activity during development of incipient colonies of the termite *Hodotermes mossambicus*. *Journal of Insect Physiology*, **18**, 603–607.

Watson, J.A.L., Nel, J.C.C. & Hewitt, P.H. (1972) Behavioural changes in founding pairs of the termite, *Hodotermes mossambicus*. *Journal of Insect Physiology*, **18**, 373–387.