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Research article

Social regulation of testicular development in primary and secondary males of the dampwood termite *Zootermopsis angusticollis* Hagen

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Summary. The effect of the presence of female reproductives, larvae and other reproductive males on growth and testicular development was studied in males of the dampwood termite, *Zootermopsis angusticollis*. Compared to primary males, secondary males developed testes that were significantly larger under all social conditions tested. Testis diameter was strongly correlated with sperm number in both primary and secondary males, but was negatively correlated with mass gain in primaries and positively correlated with mass gain and secondaries. The presence of female reproductives had no effect on primary male development but promoted mass gain and testicular growth in secondary males. The presence of larvae had a negative effect on primary male body mass, but promoted testicular development. Secondary males nesting with larvae gained more mass and developed larger testes than those nesting without larvae. Co-maturing secondary males did not exhibit differences in testicular development or intrasexual aggression, nor was any one male preferentially allogroomed or fed by nestmates. Co-maturing secondaries are typically closely related and are not likely to engage in reproductive competition. The dissimilar physiological responses of primary and secondary males to social stimuli may reflect adaptations to the distinct social environments in which each reproductive form normally matures.

Key words: Reproductive development, social signals, sperm production, termites, testes.

Introduction

Termites have a flexible system of caste differentiation and an individual's development can be strongly influenced by changes in its environment. The social stimuli experienced by developing termites are a particularly important source of regulatory cues, although the responses elicited vary with an individual's caste and physiological state (Miller, 1969; Noirot, 1985, 1990; reviewed in Thorne, 1997). Research on the social regulation of termite reproductive development has principally focused on female maturation (Light and Weesner, 1951; Greenberg et al., 1978; Greenberg and Stuart, 1979; Shellman-Reeve, 1990; Vieau, 1990; Nalepa and Jones, 1991; Brent and Traniello, 2001). Current knowledge of male reproductive development is primarily limited to anatomical descriptions (Imms, 1919; Child, 1934; Weesner, 1955, 1969; Grandi, 1992 a, b; 1994), and the influence of social stimuli on the course of male development is poorly understood. The present study investigates how social stimuli from nestmates may affect the maturation of reproductive males of the dampwood termite *Zootermopsis angusticollis* Hagen.

Z. angusticollis, like other lower termites, has two developmentally distinct reproductive forms: primaries, which are derived from alates and found new colonies; and secondaries (neotenics), which arise directly from immature larvae as replacement reproductives (Castle, 1934; Light, 1943; Miller, 1969; Noirot, 1985, 1990). Because these two reproductive forms have different developmental origins and roles within the colony, their maturation may be influenced differently by the same social stimuli. For example, primary females mature quickly when isolated from the social stimuli of the natal colony and paired with a mate (Greenberg et al., 1978; Greenberg and Stuart, 1979), but secondaries fail to mature when paired with just a reproductive male (Greenberg et al., 1978; Greenberg and Stuart, 1979; Greenberg and Stuart, 1982). In addition, the presence of non-reproductive nestmates promotes fecundity in both primary and secondary females, but has different effects on their ovarian development and body mass (Brent and Traniello, 2001).

Stimuli from nestmates of different castes may have a variety of developmental effects on primary and secondary male maturation. Males may delay maturation until a mate is

present, thereby avoiding unnecessary energetic expenditures on gonadal development and gamete production. Males may also respond positively to larvae, which can relieve reproductives from performing colony tasks and can supplement a reproductive's diet through trophallaxis (McMahan, 1969; Miller, 1969; Sieber and Leuthold, 1982; Shellman-Reeve, 1990; Nalepa, 1994; Brent and Traniello, 2001). Maturing secondaries might also be influenced by intrasexual interactions, because multiple neotenics often differentiate simultaneously and coexist together after the loss of a functional reproductive (Heath, 1903; Castle, 1934; Light, 1934; reviewed in Miller, 1969; Noirot, 1985, 1990; Myles, 1999). Co-maturing neotenics may exert mutual effects on development directly through agonistic interactions (Ratcliffe et al., 1952; Buchli, 1961; Grassé, 1984; Thorne and Haverty, 1991) and inhibitory pheromones (Light and Weesner, 1951; Miller, 1969; Lüscher, 1972, 1974, 1976; Greenberg and Stuart, 1982; Bordereau, 1985), or indirectly through competition for limited colony resources such as nitrogen (Cowling and Merrill, 1966; LaFage and Nutting, 1978). The extent of a neotenic male's testicular development may depend upon response to the risk of reproductive competition, which is a contingent strategy used by insects that are known to practice sperm competition (Parker, 1970; Svärd and Wiklund, 1989; Gage, 1991; Gage and Baker, 1991).

The objective of the present study was to identify how environmental cues may regulate reproduction in primary and secondary male termites. We describe changes in the reproductive development of *Z. angusticollis* males in response to the presence of a reproductive female, larvae, and co-maturing males. In addition, the development of primary males is compared to that of secondaries under these different social conditions, and the inter-relationship between body mass, testis size and sperm production is examined.

Materials and methods

Establishment of experimental colonies

Termites originated from 14 stock colonies of *Z. angusticollis* collected between 1992 and 1998 from the Redwood East Bay Regional Park, near Oakland, California. 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 under a 14L:10D light cycle at 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 colonies 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 $4^{th}-6^{th}$ instar larvae that were isolated from parent colonies in clear covered plastic boxes (17 × 12 × 6 cm) containing moist filter paper and wood. Secondaries were placed in experimental colonies within three days of their adult molt. In all colonies primary males were placed with primary females and secondary males with secondary females.

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 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.

Relationship between body mass, testis diameter and sperm number

To determine the relationship between body mass, testis diameter and sperm number, primary (n = 65) and secondary males (n = 166) were sampled from colonies containing a reproductive female and three larvae at 0, 10, 20, 30, 45 or 60 days following establishment. The mass of each reproductive was recorded on a Mettler AE-163 balance before and after each experiment, and from these measures the percent change in body mass was also calculated. Male reproductives were stored individually in Dietrich's Fluid for a minimum of two weeks prior to dissection. The testes of each male were dissected intact and placed on a microscope slide for examination. The diameter of each testis at its widest point was measured using an Olympus BH-2 stereoscopic microscope with an ocular micrometer (100 X). To determine sperm count, individual testes were separately homogenized in a 15 µl aliquot of distilled water. A 10 µl aliquot of the homogenized solution was then transferred to a Spencer Brightline hemacytometer. Visible spermatozoa were counted on a stereomicroscope and the concentration of sperm/ml of homogenate was calculated for each testis. The testes of 8 older secondary males, allowed to mature in lab colonies for at least six months, were also examined using these techniques. For all treatments, female reproductives were also preserved, and the genital chamber and spermatheca were examined for the presence of sperm.

Effect of reproductive females on growth and testicular development

To determine the effect that females have on male maturation, we established colonies containing either a primary (1°) or a secondary (2°) male with three $3^{rd}-4^{th}$-instar larvae and either one ($n_1 = 93$, $n_2 = 46$) or no reproductive female ($n_1 = 79$, $n_2 = 67$). Following colony establishment, males from each social condition were sampled at 0, 30 or 60 days. Except for sperm number, measures of reproductive development were collected from each male as described above.

Effect of larvae on growth and testicular development

To determine the effect that larvae have on testicular development, we established pairs of primary and secondary reproductives nesting with either zero ($n_1 = 84$, $n_2 = 40$), three ($n_1 = 93$, $n_2 = 46$) or six larvae ($n_1 = 88$, $n_2 = 36$). These are relatively few larvae compared to the number normally produced in the first brood (Castle, 1934; Greenberg et al., 1978). However, the presence of three or more larvae has been shown to significantly influence female reproductive development in this species (Brent and Traniello, 2001). Third and fourth instar larvae of both sexes were used because they were sufficiently mature to perform most colony tasks (Rosengaus and Traniello, 1993), yet their small size and limited numbers minimized their impact on the food supply. Food availability has been shown to affect reproductive development in some species (Lenz, 1994). Males were sampled as described above at 0, 30 and 60 days following colony establishment.

Effect of co-maturing males on growth and testicular development, and behavioral interactions in multi-male colonies

To determine the effect that co-maturing secondary males have on each other's development, we established colonies containing a neotenic female, three $3^{rd}-4^{th}$ instar larvae, and either one (n = 46) or three sibling neotenic males (n = 62). Males were sampled from colonies on 0, 30 and 60 days following colony establishment, as described above. Behavioral interactions between nestmates were also observed in 10 monogamous and 10 multi-male colonies. All interactions between the male(s), female, and larvae were recorded during ten-minute sample periods, conducted every three days for the first 20 days following colony establishment. To distinguish individuals during behavioral

observations, males were marked on the dorsal abdomen with different colors of enamel paint. The marking technique had no apparent effect on the behavior or physiology of the termites. Prior to behavioral observations, each colony was left undisturbed for five minutes, which was sufficient for them to acclimate to observation conditions. All-occurrence sampling was used to score the following interactive behaviors: allogrooming, antennae palpations, proctodeal and stomodeal feeding, lunging, biting, and mating. All behaviors have been previously described for *Z. angusticollis* (Rosengaus and Traniello, 1991).

Statistical analysis

Statistical analysis of data was performed using Sigmastat v. 2.03 (SPSS Inc., 444 North Michigan Ave., Chicago, IL 60611). Consistent violations of normality and strong interaction effects between experimental conditions and sample days necessitated the use of non-parametric analyses for both physiological and behavioral data. Spearman rank order correlations were used to determine the relationship between body mass, testis diameter and sperm number. Mann-Whitney rank sum tests were used to determine if there were significant ($\alpha_{critical}$ = 0.05) differences in mass change and testis size between groups on individual days. The Mann-Whitney analysis was also used to compare the frequency of interactions of each male with the female, larvae and the other males. Probability values were adjusted for multiple tests using Scheffe's correction (Sokal and Rohlf, 1995).

To determine if significant developmental differences occurred between co-maturing secondary males, a Mann-Whitney rank sum test was performed comparing the male with the largest testis diameter from each colony to individuals from the remaining population. To determine whether any observed difference in body mass or testis diameter represented a developmental effect or normal variation in development, a null distribution for the Mann-Whitney test was established by randomizing measures of body mass and testes diameter into 100 new groups and calculating the rank sums for each. Probabilities for the rank sum results of the original data were then determined from the null distribution.

Results

Relationship between body mass, testis diameter and sperm number

In primary males, testis diameter was positively correlated with body mass ($r = 0.25$, $p = 0.005$) and sperm number (Fig. 1; $r = 0.39$, $p < 0.001$). However, from day 10 to day 60, primary male sperm counts decreased non-significantly from 9.19×10^6 to 8.10×10^6 sperm/ml ($T_{22,22} = 553.5$, $p = 0.173$). This decline, coupled with a rapid increase in body mass during this period, resulted in a negative correlation between sperm number and mass gain ($r = -0.29$, $p < 0.001$) for primaries. It was found that 84% primary females had been inseminated by day 60.

In secondary males, there were significant positive correlations between testis diameter and body mass ($r = 0.43$, $p < 0.001$), testis diameter and sperm number (Fig. 1; $r = 0.80$, $p < 0.001$) and body mass and sperm number ($r = 0.55$, $p < 0.001$). Between day 10 and day 60, secondary male sperm counts more than doubled from 6.02×10^6 to 14.64×10^6 sperm/ml ($T_{24,54} = 1410.0$, $p < 0.001$). By day 60, 77% of secondary females had been inseminated. Male neotenics that had completed the adult molt at least 6 months prior to sampling had an average testis diameter of 1.31 ± 0.03 mm, which is 89% wider than the testes of neotenics sampled after 60 days of development. These older males had an average sperm count of 10.12×10^7 sperm/ml.

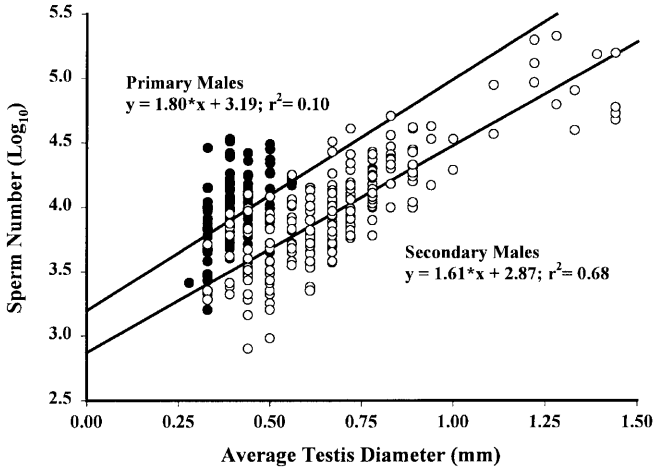

Figure 1. The relationship of sperm number (log_{10}) to testis diameter for primary (●; n = 130) and secondary (○; n = 232) male reproductives

Effect of reproductive females on growth and testicular development

The growth and reproductive maturation of primary males was not affected by the presence of a female. After 60 days, there was no significant difference in either the change in body mass (Fig. 2a; $T_{17,31} = 418.0$, $p = 0.514$) or average testis diameter (Fig. 2b; $T_{34,61} = 1761.0$, $p = 0.318$) between males nesting with or without a female. Between day 0 and 60 body mass increased 55.5 ± 4.2% in males nesting with a female, and 55.6 ± 2.7% for males nesting without a female, while the average testis diameter increased 6.8 ± 2.4% ($T_{61,100} = 5624.5$, $p = 0.017$) and 11.4 ± 2.4% ($T_{34,100} = 2893.5$, $p = 0.002$), respectively.

The development of secondaries, unlike that of primaries, was significantly affected by the presence of a neotenic female. By day 60, males nesting with a female gained 21.0 ± 4.8% more body mass than males nesting without a female (Fig. 2a; $T_{15,25} = 453.0$, $p < 0.001$), and had testes that were 5.7 ± 2.4 % wider (Fig. 2b; $T_{30,50} = 1448.5$, $p = 0.021$). Secondary males consistently gained less body mass but developed larger testes than primary males with or without females present. Comparing the development of primary and secondary males that had nested with females for 60 days, there are significant differences in both body mass ($T_{13,19} = 91.0$, $p < 0.001$) and average testis diameter ($T_{18,36} = 743.0$, $p < 0.001$). The difference in testis diameter is due in part to a disparity in initial diameter, which for primaries is 0.41 ± 0.01 mm and for secondaries is 0.48 ± 0.02 mm ($T_{40,100} = 3700.0$, $p < 0.001$). We also noted that the testes of secondary males consisted of a greater number of lobes than those of primary males, and neotenics had larger accessory glands, consisting of numerous long tubules projecting from a basal trunk. The accessory glands of primaries were smaller and had fewer tubules.

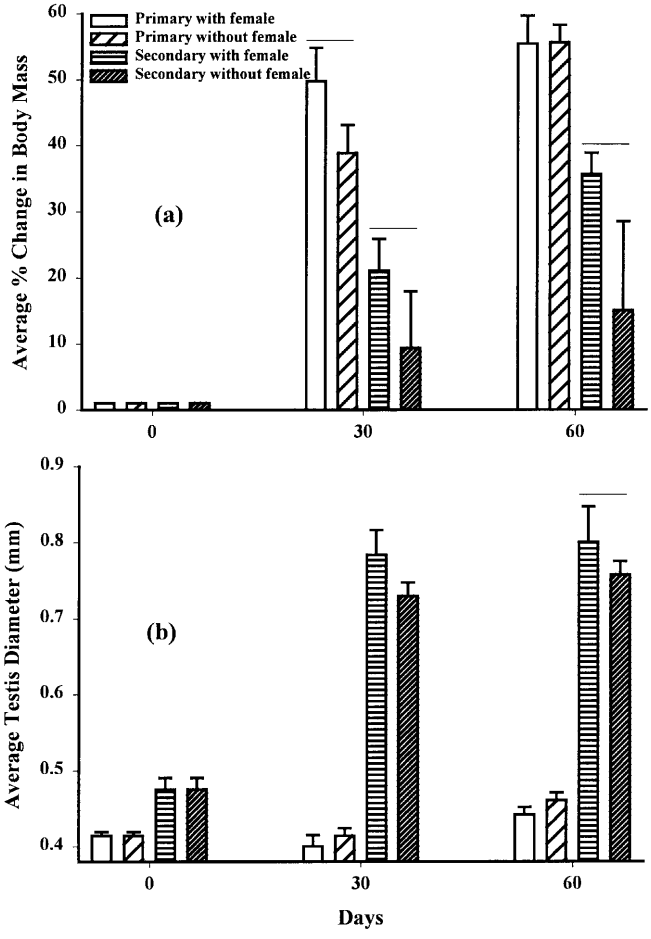

Figure 2. (a) Average change in body mass (% ± SE) and (b) testis diameter (mm ± SE) in male primary and secondary reproductives nesting with or without a female reproductive. Horizontal lines above bars indicate comparisons between treatments that were significantly different ($p < 0.05$). Values for primary and secondary males for the same day were always significantly different, except where indicated (n.s.)

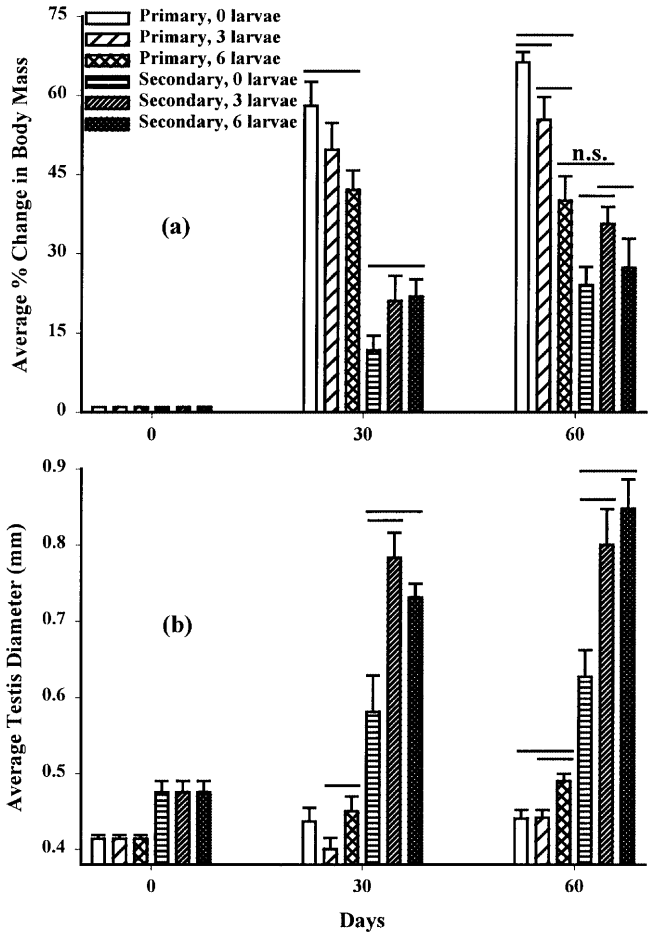

Figure 3. Average (a) change in body mass (% ± SE) and (b) testis diameter (mm ± SE) in maturing male primary and secondary male reproductives nesting with a female reproductive and zero, three, or six larvae. Legend as in Figure 2

Effect of larvae on growth and testicular development

Larvae had a significant effect on primary male growth and testicular development. After 60 days, males nesting without larvae had an average change in body mass of 66.3 ± 1.9%, but this decreased significantly to 55.5 ± 4.2% with three larvae (Fig. 3a; $T_{19,31} = 590.0$, $p < 0.001$) and to 40.1 ± 4.6% with six larvae ($T_{19,19} = 338.0$, $p = 0.004$). Average testis diameter, in contrast, did not change with the addition of three larvae ($T_{36,61} = 1766.0$, $p = 0.991$), but males nesting with six larvae developed testes that were 11.1 ± 2.3% larger ($T_{36,38} = 1090.0$, $p = 0.005$) than those of males nesting without larvae (Fig. 3b).

The presence of larvae also promoted the growth and testicular development of neotenics. Males nesting with three or six larvae gained similar mass after 60 days (Fig. 3a; $T_{4,15} = 27.0$, $p = 0.211$), but on average they gained 10.6 ± 3.4% more mass than males nesting without larvae ($T_{13,19} = 162.0$, $p = 0.046$). Similarly, average testis diameter was the same for males nesting with three or six larvae (Fig. 3b; $T_{8,30} = 122.5$, $p = 0.237$), but the testes of these males were 24.7 ± 6.5% larger in diameter than those of males nesting without larvae ($T_{18,38} = 366.0$, $p = 0.010$). Comparing day-60 males nesting with six larvae, secondaries gained similar body mass ($T_{19,19} = 392.0$, $p = 0.540$) but had significantly larger testes ($T_{38,38} = 1073.0$, $p < 0.001$) than primaries.

Effect of co-maturing males on growth and testicular development, and behavioral interactions in multi-male colonies

Secondary males in multi-male colonies developed at the same rate as males in monogamous colonies. By day 60, males in monogamous colonies gained more body mass (Fig. 4a, $T_{15,36} = 488.0$, $p = 0.044$), but testis diameter did not differ between groups (Fig. 4b, $T_{30,72} = 1558.5$, $p = 0.924$). For physiological and behavioral comparisons, males within multi-male colonies were separated into two groups; those with the largest (hypertrophic) and those with smaller (hypotrophic) testes. There was no difference in mass gain

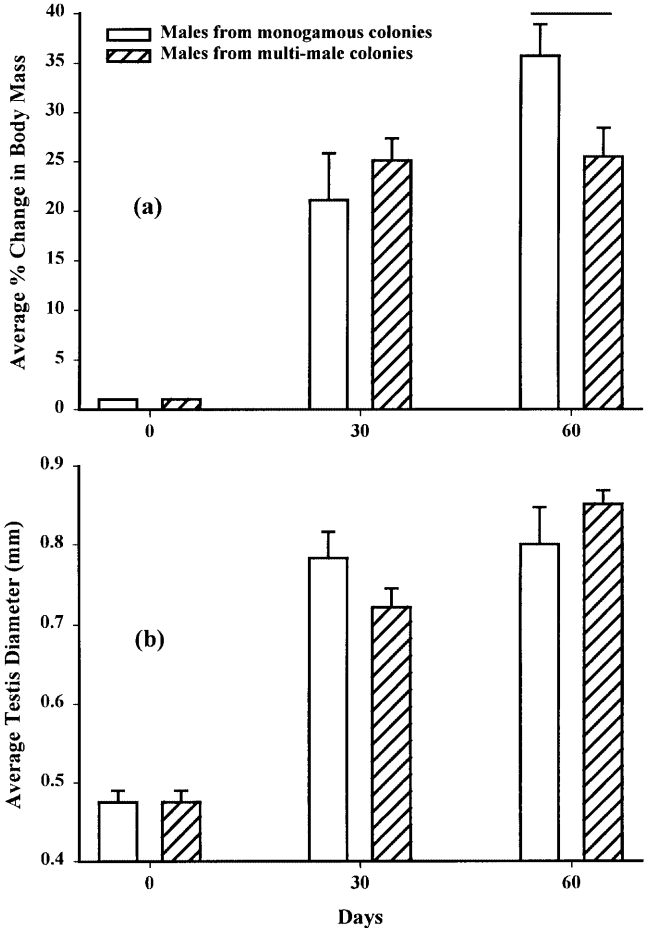

Figure 4. Average (a) change in body mass (% ± SE) and (b) testis diameter (mm ± SE) in maturing secondary males nesting in single- and multi-male colonies. Legend as in Figure 2

(89% < p < 93%) or testis diameter (47% < p 50%) between males sampled from the same multi-male colony.

Co-maturing males did not appear to be treated differently by reproductive females and larvae. During an average ten-minute sampling bout, males with hyper- and hypotrophic testes were allogroomed by a female 0.24 ± 0.06 and 0.40 ± 0.06 times ($T_{13, 18}$ = 164.0, p = 0.081), and by larvae 0.28 ± 0.06 and 0.43 ± 0.11 times ($T_{13, 18}$ = 196.5, p = 0.660), respectively. Trophallactic exchanges were rare, with a total of only five events noted. Males with hyper- and hypotrophic testes engaged in trophallactic exchanges with a female 0.02 ± 0.02 and 0.01 ± 0.01 times ($T_{13, 18}$ = 211.0, p = 0.919), and with larvae 0.01 ± 0.01 and 0.01 ± 0.01 times ($T_{13, 18}$ = 210.5, p = 0.935), respectively. The only significant difference concerned the frequency with which males with hyper- and hypotrophic testes were approached and antennated. The respective frequencies of these interactions for females were 0.70 ± 0.11 and 0.43 ± 0.12 ($T_{13, 18}$ = 257.5, p = 0.050), and for larvae were 1.06 ± 0.15 and 0.68 ± 0.12 ($T_{13, 18}$ = 256.5, p = 0.055). We observe no aggressive behavior between neotenic males or evidence of injury. On rare occasions males were observed allogrooming each other.

Discussion

Relationship between body mass, testis diameter and sperm number

Testis diameter was found to be a reliable indicator of sperm number and reflects the ability to produce and/or store sperm for both primary and secondary male reproductives of *Z. angusticollis*. Similar increases in testis size and sperm number have been observed in *Kalotermes flavicollis*, and the morphological and ultrastructural changes accompanying increasing testis size appear to be associated with spermatogenesis (Grandi, 1992a, b). The increase in the number of accessory gland tubules may also enhance a male's reproductive capability. Although the exact function of accessory gland secretions is not known for termites (Miller, 1969) they may facilitate sperm transfer, enhance female fecundity or induce oogenesis and oviposition (reviewed in Chapman, 1998). The correlation between testis diameter and sperm number was much greater for secondaries than for primaries. This may be due to the very small changes that occurred in testis size and sperm number in primaries (relative to secondaries) during the sample period. Sampling primaries that are more mature may help to clarify this relationship.

There was also a significant correlation between body mass and testis diameter for both primaries and secondaries, and again the relationship was stronger for secondaries than for primaries. Similarly, body mass and sperm number were correlated in secondaries, but the relationship was very weak in primaries. Unlike primary males, newly molted neotenics do not have well-developed fat bodies and wing muscles to metabolize (Grassé, 1949; Nutting, 1969) and they are likely to have lower stores of uric acid (Shellman-Reeve, 1990), an endogenous nitrogen source (Potrikus and Breznak, 1981; Shellman-Reeve, 1990, 1996). Therefore, secondaries may be initially more dependent on exogenous resources than primaries. An increase in body mass may indicate that more nutrients are available for gonadal development and spermatogenesis. This dependence may be further exacerbated by the large initial investment in testis growth and sperm production made by secondaries. In contrast, primaries invest relatively fewer resources, as is apparent in their smaller testes and declining sperm number during the sample period. This reduction is likely the result of sperm loss through ejaculation coupled with low spermatogenic activity.

Effect of reproductive females on growth and testicular development

The presence of a female had no effect on primary male mass gain and testicular development. The rate at which a primary male matures may be more dependent on diet (Cook and Scott, 1933; Waller and LaFage, 1987; Shellman-Reeve, 1990; Nalepa, 1994) than on stimuli from reproductive females. If feeding is inhibited while alates remain in their parent colony, as Heath (1903) suggested, separation from the natal nest may be sufficient to promote the rapid mass

gain observed in newly dealate males nesting with or without a female, which in turn may stimulate gonadal development. In contrast, secondary male mass gain and testis development were significantly promoted by the presence of a reproductive female. Whether the changes in mass and testis diameter were independent processes or whether the increase in mass increased the endogenous resources that a male could allocate towards testicular growth is not known. The increased mass gain may have resulted from a female supplementing the diet of neotenic male, however, the females in our study were newly molted and likely to retain nutritional resources for their own reproductive maturation. The presence of a female may have stimulated a male's rate of food consumption directly or indirectly through an effect on the rate at which larvae provided trophallactic secretions to the reproductives. The female may emit a pheromone or a caste-specific odor, or she may engage in specific behaviors to induce these physiological changes.

Although *Z. angusticollis* secondary males may postpone testicular development and spermatogenesis until a female is present, neotenics nesting without a female still developed testes that were much larger than those of primary males. This size difference between primaries and secondaries was due in part to a greater initial testis diameter in neotenics. However, the testes of neotenics may mature quickly because of the much greater sperm demand that they may face after molting. Unlike *Z. angusticollis* primary reproductives, which normally have a 1 : 1 sex ratio (Jones et al., 1988), the sex ratio of neotenics is skewed toward females (Myles, 1999), largely because immature females are more likely to molt into neotenics than are males (Greenberg and Stuart, 1982). Each replacement female is capable of producing 55–130 eggs in her first clutch (Greenberg et al., 1978); this considerably higher than the upper limit of 30 eggs produced in a primary's first clutch (Heath, 1903; Castle, 1934). The skewed sex ratio coupled with the high initial fecundity of neotenic females makes it likely that neotenic males will experience a higher sperm demand than primary males.

Effect of larvae on growth and testicular development

The presence of as few as three larvae had a significant effect on male development in both primary and secondary reproductives. Compared to males nesting with only a reproductive female, primary males gained less mass with larvae present but developed larger testes, while secondary males nesting with larvae had increased in both mass and testis diameter. Similar results have been found in females of this species (Brent and Traniello, 2001). Third instar and older larvae perform many non-reproductive tasks within the colony and provide reproductives with trophallactic secretions that may be rich in nitrogen (Shellman-Reeve, 1990; Nalepa and Jones, 1991; Rosengaus and Traniello, 1993). Larvae may promote male development by improving the energetic and nutritional environment of reproductives, as suggested for *Macrotermes michaelseni* (Sieber and Leuthold, 1982). Social stimuli from larvae may also induce neuroendocrine responses in the reproductives that promote food consumption in reproductives, reduce the rate of colony task performance, and/or increase the rate of testicular development. For example, primaries may gain less body mass as a result of simply perceiving nutritionally-independent larvae within the nest, rather than in response to physical manipulation by the larvae. Primary reproductives make a considerable investment in producing and rearing their first brood (Nalepa and Jones, 1991). This may necessitate storing sufficient reserves to offset this expenditure. Having larvae present that are old enough to tend brood may induce both female and male primaries to store fewer resources for long-term brood care and to allocate additional resources towards gonadal development.

Effect of co-maturing males on growth and testicular development, and behavioral interactions in multi-male colonies

The presence of another male did not affect secondary male testicular development. Although the extent of testicular development in many insect species depends on whether reproductive competitors are present (Svärd and Wiklund, 1989; Gage, 1991, 1994; Gage and Baker, 1991), *Z. angusticollis* neotenic males in multi-male colonies developed at the same rate as neotenics in monogamous colonies (Fig. 4b). There were also no indications of agonism between secondary males, which in some termite species is used to suppress the development or eliminate supernumerary reproductives (Ratcliffe et al, 1952; Buchli, 1961; Ruppli, 1969; West-Eberhard, 1969, 1981; Fletcher and Ross, 1985; Kinomura and Yamauchi, 1987). Although intrasexual aggression may be used by *Z. angusticollis* primary males to displace potential competitors during colony foundation (Shellman-Reeve, 1999), we found no agonism or evidence of aggression in over 42 h of observations during the first 60 days of secondary male maturation. Also, we found no differences in the types or frequencies of social interactions between males, females and larvae, except that males with larger testes were approached and antennated more often. Secondary males maturing within the same colony are usually close relatives, therefore selective pressure to reduce the loss of resources caused by local mate competition (Hamilton, 1964; Alexander and Sherman, 1977) may hinder the development of mechanisms that would allow neotenics to engage in reproductive competition. The skewed sex ratio common in associations of secondary reproductives (Myles, 1999) may also help to ensure that each male will have an equal opportunity to reproduce, further reducing the likelihood of local mate competition. However, because our study examined only the initial development of a male, reproductive competition may not have been observed if it only occurred in more mature males.

References

Alexander, R.D. and P.W. Sherman, 1977. Local mate competition and parental investment in social insects. *Science 196:* 494–500.

Bordereau, C., 1985. The role of pheromones in termite caste differentiation. In: *Caste Differentiation in Social Insects* (J.A.L. Watson, B.M. Okot-Kotber and Ch. Noirot, Eds.), Pergamon Press, New York. pp. 221–226.

Brent, C.S. and J.F.A. Traniello, 2001. The social influence of larvae on ovarian maturation in primary and secondary reproductives of the dampwood termite *Zootermopsis angusticollis*. *Physiol. Entomol. 266:* 78–85.

Buchli, H.H.R., 1961. Les relations entre la colonie maternelle et les jeunes imagoes ailes de *Reticulitermes lucifugus*. *Vie et Milieu 12:* 628–632.

Castle, G.B., 1934. The dampwood termites of western United Sates, genus *Zootermopsis* (formerly *Termopsis*). In: *Termites and Termite Control* (C.A. Kofoid, Ed.), University of California Press, Berkeley. pp. 273–310

Chapman, R.F. (1998). *The Insects: Structure and Function*. Cambridge University Press, New York. 770 pp.

Child, H.J., 1934. The internal anatomy of termites and the histology of the digestive tract. In: *Termites and Termite Control* (C.A. Kofoid, Ed.), University of California Press, Berkeley. pp. 53–83.

Cook, S.F. and K.G. Scott, 1933. The nutritional requirements of *Zootermopsis (Termopsis) angusticollis*. *J. Cell. Comp. Physiol. 4:* 95–109.

Cowling, E.B. and W. Merrill, 1966. Nitrogen in wood and its role in wood deterioration. *Can. J. Bot. 44:* 1539-1554.

Fletcher, D.J.C and K.G. Ross, 1985. Regulation of reproduction in eusocial Hymenoptera. *Ann. Rev. Ent. 30:* 319-343.

Gage, M.J., 1991. Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. *Anim. Behav. 42:* 1036–1037.

Gage, M.J.G., 1994. Associations between body size, mating pattern, testis size, and sperm length across butterflies. *Proc. R. Soc. Lond. B 258:* 247–254.

Gage, M.J. and R.R. Baker, 1991. Ejaculate size varies with socio-sexual situation in an insect. *Ecol. Ent. 16:* 331–332.

Grandi, G., 1992 a. Preliminary observation on spermatogenesis in immature stages and fertile and sterile castes of *Kalotermes flavicollis* (Fabr.) (Isoptera Kalotermitidae). *Ethol. Ecol. Evol., Special Issue 2:* 99–104.

Grandi, G., 1992 b. Ultrastructural study of testis development and spermatogenesis in *Kalotermes flavicollis* (Fabr.) (Isoptera, Kalotermitidae). *Boll. Zool. 59:* 225–238.

Grandi, G., 1994. Ovary and testis development in different stages and in the sterile caste of *Kalotermes flavicollis* (Fabr.) (Isoptera Kalotermitidae). *Ethol. Ecol. Evol., Special Issue 3:* 17–23.

Grassé, P.-P., 1949. Ordre des Isoptères ou termites. In: *Traité de Zoologie* Vol. IX. (P.-P. Grassé, Ed.), Masson, Paris. pp. 408–544.

Grassé, P.P., 1984. La fondation des nouvelles sociétés. *Termitologia*, Tome II, Masson, Paris. pp. 65–132.

Greenberg, S.L.W. and A.M. Stuart, 1979. The influence of group size on ovarian development in adult and neotenic reproductives of the termite *Zootermopsis angusticollis* (Hagen) (Hodotermitidae). *Int. J. Invert. Reprod. 1:* 99–108.

Greenberg, S.L.W. and A.M. Stuart, 1982. Precocious reproductive development (neoteny) by larvae of a primitive termite *Zootermopsis angusticollis* (Hagen). *Insectes soc. 29:* 535-547.

Greenberg, S.L.W., J.G. Kunkel and A.M. Stuart, 1978. Vitellogenesis in a primitive termite, *Zootermopsis angusticollis*. *Biol. Bull. 155:* 336–346.

Hamilton, W.D., 1964. The genetical evolution of social behavior, I, II. *J. Theor. Bio. 7:* 1–52.

Heath, H., 1903. The habitats of California termites. *Biol. Bull. 4:* 47–63.

Imms, M.A., 1919. On the structure and biology of *Archotermopsis*, together with descriptions of new species of intestinal protozoa, and general observations in the Isoptera. *Phil. Trans. R. Soc. Lond. B 209:* 75–180.

Jones, S.C., J.P. LaFage and R.W. Howard, 1988. Isopteran sex ratios: phylogenetic trends. *Sociobiology 14:* 89–156.

Kinomura, K. and K. Yamauchi, 1987. Fighting and mating behaviors of dimorphic males in the ant *Cardiocondyla wroughtoni*. *J. Ethol. 5:* 75-81.

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

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

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

Light, S.F., 1943. The determination of caste of social insects. *Q. Rev. Biol. 18:* 46–63.

Light, S.F. and F.M. Weesner, 1951. Further studies on the production of supplementary reproductives in *Zootermopsis* (Isoptera). *J. Exp. Zool. 117:* 397–414.

Lüscher, M., 1972. Environmental control of Juvenile Hormone (JH) secretion and caste differentiation in termites. *Gen. Comp. Endo. Suppl. 3:* 509–514.

Lüscher, M., 1974. Kasten und Kasten differenzierung bei neideren Termiten. In: *Sozialpolmorphismus bei Insekten* (G.H. Schmidt, Ed.), Wissenschaftl. Verlagsges., Stuttgart. pp. 694–739.

Lüscher, M., 1976. Evidence for an endocrine control of caste determination in higher termites. In: *Phase and Caste Determination in Insects, Endocrine Aspects* (M. Lüscher, Ed.), Pergamon Press, New York. pp. 91–103.

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

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

Myles, T.G., 1999. Review of secondary reproduction in termites (Insecta: Isoptera). *Sociobiology 33:* 1–91.

Nalepa, C.A. and S.C. Jones, 1991. Evolution of monogamy in termites. *Biol. Rev. 66:* 83–97.

Nalepa, C.A., 1994. Nourishment and the origin of termite eusociality. In: *Nourishment and Evolution in Insect Societies* (J.H. Hunt and C.A. Nalepa, Eds.), Westview Press, Boulder, Colorado. pp. 57–104.

Noirot, Ch., 1985. Pathways of caste development in the lower termites. In: *Caste Differentiation in Social Insects* (J.A.L. Watson, B.M. Okot-Kotber and Ch. Noirot, Eds.), Pergamon Press, New York. pp. 41–58.

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

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

Parker, G.A., 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev. 45:* 525–527.

Potrikus, C.J. and J.A. Breznak, 1981. Gut bacteria recycle uric acid nitrogen in termites – a strategy for nutrient conservation. *Proc. Nat. Acad. Sci. 78:* 4601–4605.

Ratcliffe, F.N., F.J. Gay, and T. Greaves, 1952. *Australian Termites. The Biology, Recognition, and Economic Importance of the Common Species.* C.S.I.R.O., Melbourne.

Rosengaus, R.B. and J.F.A. Traniello, 1991. Biparental care in incipient colonies of the dampwood termite *Zootermopsis angusticollis* Hagen. *J. Insect Behav. 4:* 633–647.

Rosengaus, R.B. and J.F.A. Traniello, 1993. Temporal polyethism in incipient colonies of the primitive termite *Zootermopsis angusticollis*: a single multiage caste. *J. Insect Behav. 6:* 237–252.

Ruppli, E., 1969. Die Elemination überzähliger Ersatzgeschlechtstiere bei der Termite *Kalotermes flavicollis* (Fabr.). *Insectes soc. 16:* 235–248.

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

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

Shellman-Reeve, J.S., 1999. Courting strategies and conflicts in a monogamous, biparental termite. *Proc. R. Soc. London* B *266:* 137–144.

Sieber, R. and R.H. Leuthold, 1982. Repeated copulation and testes enlargement in *Macrotermes michaelseni*. *Physiol. Ent. 7:* 457–465.

Sokal, R.R. and F.J. Rohlf, 1995. *Biometry*. W.H. Freeman and Company, New York. 887 pp.

Svärd, L. and C. Wiklund, 1989. Mass and production rate of ejaculates in relation to monandry-polyandry in butterflies. *Behav. Ecol. Sociobiol. 24:* 395–402.

Thorne, B.L., 1997. Evolution of eusociality in termites. *Ann. Rev. Ecol. Sys. 28:* 27–54.

Thorne, B.L. and M.I. Haverty, 1991. A review of intracolony, intraspecific, and interspecific agonism in termites. *Sociobiology 19:* 115–145.

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

Waller, D.A. and J.P. La Fage, 1987. Nutritional ecology in termites. In: *Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates* (F. Slasky Jr. and J.G. Rodriguez, Eds.), John Wiley & Sons, New York. pp. 497–532.

Weesner, F.M., 1955. The reproductive system of young primary reproductives of *Tenuirostritermes tenuirostris* (Desneux). *Insectes soc. 2:* 323–345.

Weesner, F.M., 1969. The reproductive system. In: *Biology of Termites,* Vol. 1 (K. Krishna and F.M. Weesner, Eds.), Academic Press, New York. pp. 125–160.

West-Eberhard, M.J., 1969. The social biology of polistine wasps. *Misc. Pubs. Mus. Zool., Univ. Michigan 140:* 1–101.

West-Eberhard, M.J., 1981. Intragroup selection and the evolution of insect societies. In: *Natural Selection and Social Behavior* (R.D. Alexander and D.W. Tschinkel, Eds.), Chiron Press, New York. pp. 3–17.