

Census of Monogyne and Polygyne Laboratory Colonies Illuminates Dynamics of Population Growth in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

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Ann. Entomol. Soc. Am. 97(3): 466–475 (2004)

ABSTRACT One hundred and three laboratory colonies of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), ranging from 4 mo to 9 yr of age, were destructively sampled, and the numbers of eggs and other life stages were counted. Polygyny was maintained in 9.7% ($n = 31$) of the groups censused at four months, whereas monogyny was the only condition found thereafter. Polyandry was recorded in 17% of colonies sampled over a 2-yr period. Colonies set up with multiple males/females achieved similar rates of progeny (numbers, caste proportions, and biomass) compared with colonies initiated by a single royal pair. The soldier caste was present at the 4-mo census, whereas nymphs and neotenic first occurred in 2-yr-old groups. Primary queens reached a mean body mass of 7.06 mg (SD 2.4) after 2 yr and did not gain considerable weight between 2 and 4 yr. Two 6-yr-old queens weighed 17 and 18 mg, respectively, and produced the highest number of eggs per day. A simple computer model of colony growth, using both egg-laying rates obtained from our census and hypothetical egg-laying rates, revealed that the expected number of larvae matched the numbers censused in colonies up to 2 yr of age, although worker numbers were consistently overestimated. The data indicate that a monogyne colony of *R. flavipes* could not attain the population size occasionally described in field studies. Extrapolating laboratory data and potential reproductive mechanisms that can account for field data are discussed.

KEY WORDS subterranean termites, population model, reproductive strategies, polyandry

THE OCCURRENCE OF PRIMARY polygyny in termites has been reported for 38 species, all belonging to the higher termites (reviewed in Thorne 1985, Roisin 1993). Extensive studies on polygyny have been carried out on several *Nasutitermes* species (Termitidae: Nasutitermitinae) (Thorne 1982, 1984; Roisin and Pasteels 1985, 1986) and *Macrotermes michaelseni* (Sjoestedt) (Termitidae: Macrotermitinae) (Darlington 1985). These species create a royal cell in a distinct aboveground termitarium. This conspicuous structure, permanently housing the walled-in primary reproductives, enables scientists to open the royal cell and thereby determine the number and gender of the reproductives within that colony.

Polygyny in the presence of primary males has not been demonstrated for imaginal queens in lower termites. The majority of laboratory studies on colony development and caste composition of the lower termites (Kalotermitidae, Rhinotermitidae, and Termopsidae) have been conducted with a single royal pair (Buchli 1950; Weesner 1956; Ruppli 1969; King and Spink 1974; Howard and Haverty 1981; Jones et al. 1981; Rosengaus and Traniello 1991; Thorne et al. 1997, 1999). Therefore, it has been assumed that each colony starts independently with a single, alate-derived primary reproductive pair who creates a nuptial cham-

ber where, within a few weeks, eggs and then larvae are present (Weesner 1956, Nutting 1969).

Maternal relatedness of *Reticulitermes* species collected over time from a single field inspection port (feeding site) in Georgia suggested a multimaternal colony composition (Jenkins et al. 1999). Additional studies using allozymes and double-stranded conformation polymorphism techniques with *R. flavipes* in Massachusetts found a variety of breeding strategies, from monogynous to polydomous, within populations collected in a limited area (Bulmer et al. 2001). Furthermore, it has been demonstrated in France and the United States that termite populations of *Reticulitermes* species can be "open" or "closed," depending on species, season, and locality (Clément 1986, Forschler and Jenkins 2000). Allozyme allelic frequencies found in an open colony implies that multiple reproductives coexist in a single colony (Clément 1981). Two recent studies on cooperative colony foundation in *Reticulitermes speratus* Feytaud revealed that in polygynous groups containing a primary male, only one primary female reproductive survived and that no pairings set up with multiple males (plus females) established viable colonies (Matsuura and Nishida 2001, Matsuura et al. 2002). We, however, have observed in the field, on two separate occasions, *Reticulitermes* nuptial

chambers containing multiple dealates with nearly identical proportions of males and females (B.T.F., unpublished data).

It is valid to assume that termite colonies housing more than one egg-laying primary female should achieve higher colony growth rates than those containing a single reproductive pair. Polygynous or polyandrous colonies may be established through several mechanisms, including pleometrotic colony foundation (primary polygyny or polyandry), by recruiting or producing neotenic (secondary polygyny or polyandry), or by coalescence or cooperation between separate colonies (polydomy). Each of these mechanisms could be used to explain the hundreds of thousands to millions of termites per colony documented in field studies of *Reticulitermes* spp. (Howard et al. 1982; Grace et al. 1989, 1995; Grace 1990; Su et al. 1993; Forschler and Townsend 1996; Su and La Fage 1999). However, the possibility of monogyne colonies attaining populations in the millions cannot be ruled out without information on the egg production of primary reproductives.

The purpose of this study was to examine the growth rates of *Reticulitermes flavipes* (Kollar) laboratory colonies initiated either by a single royal pair or by multiple males/females. This was accomplished using measures of egg production, number of progeny and worker biomass over time. We estimated daily egg-laying rates of primary female reproductives in relation to their age and weight, and we used our census data to develop a best-case scenario for colony growth.

Methods

Termites. *R. flavipes* alates were collected in March 1993, April 1996, January 1997, and in March/April of 1998, 1999, 2000, and 2001. They originated from either termite-infested buildings on the University of Georgia campus or from fallen pine logs gathered in Whitehall Forest, Clarke County, or Westbrook Farm, Spalding County, Georgia. Logs were transported to the laboratory where all emerging alates were collected. Alates collected from swarms at infested buildings were acquired on site. All alates were maintained according to swarm location by placement in petri dishes containing moist filter paper (Whatman no. 1) in an environmental chamber at 24°C, 85% RH until sexed and paired to initiate incipient colonies. Voucher specimens from all termite colonies were preserved in 70% ethanol and are maintained in our laboratory in Athens, GA.

Pairing and Culturing Procedure. The sex of all alate termites was determined by examination of the sexual dimorphism evident in the caudal sternites (Weesner 1965, Grassé 1982, Zimet and Stuart 1982) because our observations as well as previous experiments (Buchli 1950) have shown that formation of tandems during the initial phases of pairing can occur between termites of the same sex.

Single male/female pairs and multiple male/female combinations were censused according to availability

at the time of census (most of the colonies we used in this study were set up months or years before we conducted the census). Those numbers include single male/female combinations: colony age of 4, 8, or 12 mo, $n = 12$ colonies; 24 mo, $n = 8$; 36 mo, $n = 5$; 48 mo, $n = 2$. Multiple male/female units: subscripts show the number of males (m) or females (f) initially placed in each set up: 4 mo, $n = 12$; A1 (m_1f_2), A2 (m_1f_3); A3–6 (m_2f_1); A7–9 (m_2f_2); A10 (m_3f_1); A11–12 (m_3f_3); 8 mo, $n = 12$, B1–4 (m_2f_1); B5–7 (m_2f_2); B8 (m_2f_3); B9–12 (m_3f_3); 12 mo, $n = 12$; C1–2 (m_1f_2); C3–4 (m_2f_1); C5–6 (m_2f_2); C7–9 (m_2f_3); C10 (m_2f_4); C11 (m_3f_3); C12 (m_4f_1); 24 mo, $n = 8$; D1 (m_1f_2); D2 (m_2f_1); D3–4 (m_2f_2); D5–6 (m_3f_3); D7 (m_4f_3); D8 (m_4f_4); and 48 mo, $n = 2$; E1–2 (m_2f_1).

Demographic data from our *R. flavipes* laboratory colonies between the ages of 4–24 mo were gathered from groups intentionally set up to collect specimens for genetic baseline studies. The older colonies were randomly selected from groups we set up when alates were available and maintained in the laboratory for a variety of studies. Therefore, the number of colonies with multiple males/females varied in and between each age class, and each age class contains different numbers of male/female combinations due to availability of termites from the various swarms.

All colonies were established and maintained in the same manner. Termite dealate combinations were placed in lidded plastic jars ($\varnothing 5.5$ cm, 87.9 cm³) filled with coarse, weathered, moist pine sawdust. Water was added to the sawdust as needed to maintain sufficient moisture [15–20% (wt:wt)] throughout the experiment. All containers were incubated at 24°C, 85% RH in permanent darkness and examined monthly by looking through the sides of the lidded jar. Between 6 and 18 mo, when consumption of >50% of the original allotment of sawdust was noted, colonies were positioned, inside their original jar, into bigger containers (17.5 cm in length by 12.5 cm in width by 6 cm in height; 1312.5 cm³) filled half-way with stacks (2–4 pieces) of solid pinewood (2 by 2 by 12 cm) and more of the aforementioned sawdust. As that substrate was consumed the container was filled to 75% capacity with more pinewood and sawdust (between 26 and 34 mo). At 3 yr, termite colony containers were placed into the next container size (26 by 20 by 9.5 cm; 4940 cm³) with additional substrate and maintained as described previously for the next 1 to 2 yr. Two colonies were placed in lidded "aquariums" (50 by 30 by 40 cm; 0.06 m³) at 5 yr of age and censused 1 yr later.

Determination of Caste Composition and Colony Growth. At the age of 4, 8, 12, 24, 36, and 48 mo as well as 5, 6, and 9 yr, colony containers were opened, the contents sorted, and the number of eggs, instars 1 and 2, workers, presoldiers, soldiers, nymphs, and reproductives (primary and/or neotenic) recorded. Larval 2 (L2) and worker 3 (W3) stages were discriminated according to the classification of Buchli (1958) by a noticeable brownish color to the base of the mandibles and clypeus. Neotenic was determined by the four convergent, pigmented bands observable in a dorsal

view of the head capsule and differentiated according to Buchli (1958).

Differences in the number of progeny (eggs, larvae, workers, soldiers, and nymphs) from single pair and multiple male/female units were analyzed using Kolmogorov-Smirnov two-sample test for each age class. The Mann-Whitney *U* test was performed for comparison of means of soldier:worker ratios between single pair and multiple males/females colonies.

Live (wet) weight of all worker, soldier, nymph, and reproductive castes was taken separately and immediately after collection. All workers used for measurement of live weights were developmental stage 4 or older (according to Buchli 1958). We used Kruskal-Wallis analysis of variance (ANOVA) to test the assumption that worker biomass (BM) from colonies started with two, three, or four females, was equivalent to BM in colonies founded by single females. We also tested the null hypothesis, that colonies containing multiple males (≥ 2) and a single female, achieved equal numbers of progeny compared with colonies housing a single male by using Kruskal-Wallis ANOVA. We again used Kruskal-Wallis ANOVA to test whether queen weight was related to queen age.

Only colonies with kings and queens that were alive at the time of the census were included in the statistical analyses used in this study. Histological or molecular tests were not performed to examine the reproductive status of males and females. Therefore, it is unknown whether more than one specimen of each gender was reproductively involved in colonies set up with multiple males/females. The number of 4-yr and older colonies was restricted to a maximum of four in each age class because they were chosen from laboratory cultures set up and maintained for various studies and the destructive sampling used in this protocol precluded their use in future research. Due to the low number of colonies we did not include the 5- to 9-yr-old colonies in our statistical analysis of the BM or numbers of offspring data sets.

Best-Case Scenario of Colony Growth. We used a simple simulation model to explore the limits of termite colony growth with the data we generated from our census of laboratory colonies. The model can be described by the following formula: $P = ER \times (n - 33)$, where *P* is worker population, *ER* is egg-laying rate, and *n* is an integer defined as the number of days at which one desires a population estimate (*n* has an upper limit set by the worker life span). Two scenarios were simulated using the above-mentioned formula.

In the first scenario, data included the egg-laying rates obtained in our census (ranging from 0.38 up to 107.95 eggs per day, depending on colony age) by using developmental times for the various stadia and castes, as reported in the literature (Weesner 1956, Buchli 1958). The simulations assumed a death rate equal to a worker life span and a closed population, i.e., no immigration or emigration. We chose a 4-yr upper limit for worker life spans as a point of discussion for the model results (Nutting 1969).

The second scenario used hypothetical egg-laying rates (a multiple of the highest egg laying rate

Table 1. Number of workers found in published studies on laboratory colony growth in the family *Rhinotermitidae*

Species	Colony age/days	No. of workers (range)	Reference
<i>R. speratus</i>	100	8-14	Matsuura et al. (2002)
<i>R. flavipes</i>	120	7-34	Grube and Forschler
<i>R. lucifugus</i> Ressi	138	12-17	Buchli (1950)
<i>R. hesperus</i> Banks	89-151	8-13	Light and Weesner (1955)
<i>R. hesperus</i>	180	2-7	Weesner (1956)
<i>R. flavipes</i>	240	14-80	Grube & Forschler
<i>R. flavipes</i> ; <i>R. virginicus</i>	365	18-26	Howard et al. (1981)
<i>R. flavipes</i>	365	23-51	Beard (1974)
<i>R. flavipes</i>	365	15-259	Grube and Forschler
<i>R. flavipes</i>	730	30-783	Thorne et al. (1997)
<i>R. flavipes</i>	720	128-2,137	Grube and Forschler
<i>C. formosanus</i>	135	11-31	King and Spink (1974)

The census results presented in this study are added in bold type.

achieved in our laboratory colonies) to obtain theoretical numbers of worker termites per monogyne colony. We assumed a worker life span of either 2- or 4 yr. Results reflect the expected number of eggs and workers in a monogyne colony at 4 yr of age.

We used χ^2 analysis to test the fit of the model's predictions to our census data. The simulation model was a program developed by Dr. Glen Ware (University of Georgia, Athens) and was performed using the SAS Statistical Analysis Software (SAS Institute 2002). A copy of the program is available upon request from the authors.

Results

Colony Growth. The mean number of worker termites counted in our census was higher, per age class, than previously reported in the literature for *Reticulitermes* spp. (Table 1), indicating that our technique provided conditions appropriate for discussion of optimal laboratory colony growth. Variation in the number of offspring was high within and between colonies and age classes for all groups (Table 2). The mean number of eggs, larvae, and workers recorded a nine-fold increase between 12 and 24 mo, coincident with moving the colonies to larger containers (Table 2). An additional increase in numbers was recorded in the 6-yr-old groups, again after movement into larger arenas. Compared with the mean of $1,501 \pm 969$ progeny (number of eggs and all other stages) for the 48-mo-old groups (Table 2), the two 6-yr-old colonies contained 10,537 (7,582 workers) and 12,233 (8,084 workers) progeny, respectively.

Caste Composition. In all age classes, workers represented the dominant life stage. In the 4- through 12-mo-old colonies eggs and larval stages represented $\approx 40\%$ of the population, whereas worker proportions ranged from a low of 45% in the 8-mo census to 84.5% in the 4-yr census.

Soldiers occurred in all but one 4-mo old colony. Twenty-two of the remaining 4-mo-old colonies ($n = 24$) contained a single soldier, whereas one housed two soldiers. During the first year, the average pro-

Table 2. Range of offspring censused from *R. flavipes* laboratory colonies destructively sampled by caste for each age class for both monogyne (A) and polygyne (B) setups as well as the combined data

Colony age (mo)	Caste	Range of colony offspring from the census		Combined data
		A Monogyne setup (1♂/1♀)	B Polygyne setup (x♂/1♀)	Mean of all data A + B
4 mo <i>n</i> = 12 (A) <i>n</i> = 12 (B)	Eggs	0-16	2-21	7.75 ± 6.1
	Larvae 1-2	0-13	2-13	4.2 ± 3.5
	Workers	7-24	14-34	19.3 ± 6.2
	Soldiers	0-1	0-2	0.9 ± 0.3
	Nymphs Neotenics			
8 mo <i>n</i> = 12 (A) <i>n</i> = 12 (B)	Eggs	3-61	3-47	21.8 ± 15.1
	Larvae 1-2	0-25	0-39	10.9 ± 10.8
	Workers	14-47	15-80	35.7 ± 15.5
	Soldiers	1-3	1-4	2.2 ± 1.0
	Nymphs Neotenics			
12 mo <i>n</i> = 12 (A) <i>n</i> = 12 (B)	Eggs	1-109	0-101	42.2 ± 38.3
	Larvae 1-2	8-92	3-75	33.8 ± 25.9
	Workers	21-259	15-227	94.2 ± 65.3
	Soldiers	0-9	1-10	3.8 ± 2.6
	Nymphs Neotenics			
24 mo <i>n</i> = 8 (A) <i>n</i> = 8 (B)	Eggs	0-432	78-686	326.5 ± 185.8
	Larvae 1-2	46-510	2-635	214.6 ± 195.0
	Workers	128-2,137	290-1,589	823.8 ± 556.8
	Soldiers	5-57	8-57	26.7 ± 17.5
	Nymphs Neotenics	0-1	1-15 0-1	3.3 ± 5.7
36 mo <i>n</i> = 5 (A)	Eggs	0-389	No data available	149.0 ± 183.3
	Larvae 1-2	68-657		282.6 ± 232.5
	Workers	66-3,014		1,348.8 ± 1,204.6
	Soldiers	3-60		28.2 ± 23.9
	Nymphs Neotenics	0-9 0-2		3.4 ± 3.8 0.6 ± 0.9
48 mo <i>n</i> = 2 (A) <i>n</i> = 2 (B)	Eggs	159-212	0	92.7 ± 109.3
	Larvae 1-2	226-227	8-18	119.7 ± 123.3
	Workers	1,401-1,974	129-1,578	1,270.5 ± 797.8
	Soldiers	15-24	11-22	18.0 ± 6.1
	Nymphs Neotenics	0-3		1.5 ± 2.1

portion of soldiers was $3.0\% \pm 1.8$ (relative to all castes, including eggs), colonies 2 yr and older maintained between 1.0 and 2.5% soldiers. Comparison of the soldier:worker ratio between single pair and multiple male/female colonies in each age class (Fig. 1) showed no significant difference ($P > 0.05$; Mann-Whitney *U* test). The high ratio in 48-mo-old multiple male/female colonies set up with two males/one female was caused by a low number of workers (129) in one of the units; therefore, it was considered an anomaly (Fig. 1).

Nymphs were first found in 24-mo old colonies and thereafter in each age class sampled. Nymphs never exceeded 1% of the total population except in one 9-yr-old colony (15%) where neotenics had replaced the primary pair.

The Reproductive Caste. After 4 mo, three (25%) of the multiple female groups (A1, A7, and A11) retained two queens (Table 3). These pairs of primary female reproductives seemed, at this stage, healthy and well nourished with no signs of mutilation (missing legs or

antennae segments). By the 8-mo census, monogyne was the state of all colonies started with more than one female.

Supplemental neoteny (neotenics in the presence with the original queen) occurred in 25% ($n = 12$) of the groups we examined between the ages of 3 and 5 yr. Two 3- and one 5-yr-old colony contained brachypterous female neotenic reproductives, but their abdomens showed no signs of physogastry (i.e., about the size of older nymphs). A female replacement apterous neotenic (neotenic present without the original queen) was found in one 2-yr and one 5-yr-old colony. The 9-yr old colony contained a primary male, no primary female, and nine female neotenics that were classified as either apterous ($n = 4$), pseudapterous ($n = 3$) or brachypterous ($n = 2$). None of these nine neotenics seemed to be particularly physogastric, but the apterous females weighed twice as much (between 10 and 12 mg) as the pseudapterous (between 5 and 6 mg) and brachypterous (between 4 and 5 mg).

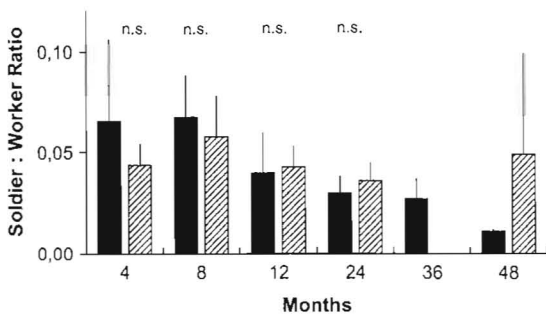


Fig. 1. Mean soldier:worker ratio (\pm SD) of colonies set up with a single royal pair and with multiple males/females. Ratio 1 female:1 male (black bars) versus ratio x males: x females (striped bars): $P > 0.05$ in each age class (Mann-Whitney U test). No statistical analysis was performed for 48-mo-old colonies ($n = 2$). No colonies were available with multiple males/females at 36 mo of age.

Primary polyandry was more readily accepted by *R. flavipes* than polygyny. A total of 41 colonies were censused that started with multiple males and 17% displayed polyandry when censused between 2 and 4 yr of age, including six colonies containing two males (A12; B3, 4; C4, 8; D7) and one (D5) with three males (Table 3).

Worker Biomass as an Indicator of Reproductive Productivity. The fact that only a single female was found in groups older than 4 mo does not necessarily indicate that all surplus females died without being reproductively active. Therefore, we compared the reproductive productivity of colonies set up with a single royal couple to those with multiple females. Total worker biomass per colony served as the measure for comparison, because the worker stage accounted for the major proportion of termites in each unit. Colonies set up with at least one male and one, two, three, or four primary females achieved similar worker biomass compared with the control groups (1 female/1 male) at 4, 8, and 24 mo (Fig. 2). A statistically significant difference occurred in the 12-mo-old units started with two females ($P = 0.0209$, $df = 3$, $n = 23$, $H = 9.7367$; Kruskal-Wallis ANOVA) but was within the range of the single pair data and therefore considered biologically insignificant. Colonies that retained two females after 4 mo (A1, A7, and A11) achieved a worker biomass of 0.036 ± 0.01 mg ($n = 3$), which was similar to the 4-mo-old monogyne colonies (0.031 ± 0.02 mg, $n = 9$, $P > 0.10$; Kolmogorov-Smirnov two-sample test). Monogyne units retaining multiple males produced offspring within the range of colonies of the same age with a single male ($P > 0.05$; Kruskal-Wallis ANOVA).

The presence of multiple reproductives during the early stages of colony development did not provide significant differences in the mean number of progeny compared with single male/female colonies. We therefore combined these data for analysis and discussion of queen weight, fecundity, and estimates of colony growth rates.

Table 3. Survivorship of the primary reproductive caste from colonies initiated by multiple males/multiple females by colony and age class

Colony age (mo)	Initial no. of primary reproductives		Colony code	<i>n</i>	Surviving no. of primary reproductives		
	♀	♂			♀	♂	
4	1	2	A 3-6	4	1	1	
	1	3	A 10	1	1	1	
	2	1	A 1	1	2	1	
	2	2	A 7	1	2	1	
	2	2	A 8, 9	2	1	1	
	3	1	A 2	1	1	1	
	3	3	A 11	1	2	1	
	3	3	A 12	1	1	2	
	8	1	2	B 1, 2	2	1	1
		1	2	B 3, 4	2	1	2
		2	2	B 5-7	3	1	1
		3	2	B 8	1	1	1
3		3	B 9-12	4	1	1	
12	1	2	C 3	1	1	1	
	1	2	C 4	1	1	2	
	1	4	C 12	1	1	1	
	2	1	C 1, 2	2	1	1	
	2	2	C 5, 6	2	1	1	
	3	2	C 7, 9	2	1	1	
	3	2	C 8	1	1	2	
	3	3	C 11	1	1	1	
	4	2	C 10	1	1	1	
	24	2	1	D 1	1	1	1
1		2	D 2	1	1	1	
2		2	D 3, 4	2	1	1	
3		3	D 5	1	1	3	
3		3	D 6	1	1	1	
3		4	D 7	1	0 ^a	2	
4		4	D 8	1	1	1	
48		1	2	E 1, 2	2	1	1

^a Primary female reproductive was replaced by an apterous neotenic reproductive.

Live Weight and Egg-Laying Capacity. Body weight of female primary reproductives did not significantly change during the first year but after movement into larger rearing containers, between 12 and 24 mo of age, showed a significant weight gain (Fig. 3A). For the next two sample periods, 36 and 48 mo, queen weights remained equivalent (ranging between 4 and 11 mg). Again, 1 yr after moving colonies into larger arenas queen weight increased, this time at the 6-yr census. The two 6-yr-old queens weighed 17 and 18 mg, respectively, and although remarkably physogastric, were also mobile. The apterous neotenic female, in a 2-yr-old colony, weighed more (12 mg) than the primary queens in the same age class whose weights ranged from 4 to 11 mg.

Male primary reproductives gained little weight. At 4 mo, primary males weighed between 2 and 3 mg (the typical weight of both males and females at swarming), and after 6 yr their weight ranged between 3 and 4 mg. Worker weights at 4 mo ranged from 1.2 to 1.8 mg; at 8 mo from 1.2 to 2.4 mg; at 12 mo from 1.2 to 2.6 mg; at 24 mo from 1.4 to 2.7 mg; at 36 mo from 1.5 to 2.3 mg; and at 48 mo from 1.7 to 2.6 mg per termite.

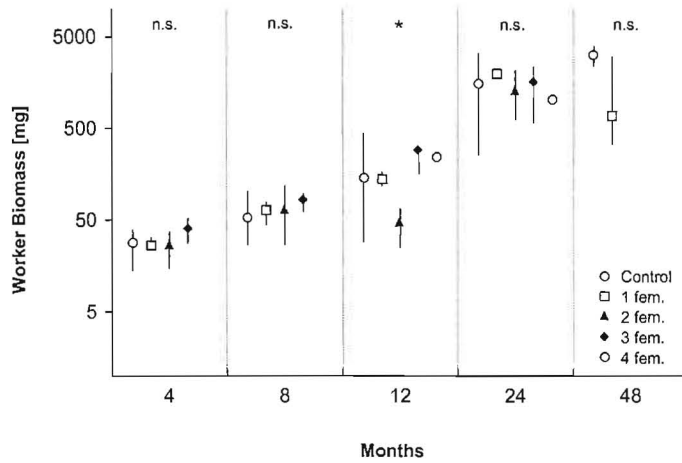


Fig. 2. Worker biomass (mean \pm SD, log-transformed ordinate scale) of colonies set up with one, two, three, or four females in each age class ($P > 0.05$ n.s.; $*P \leq 0.05$; Kruskal-Wallis ANOVA). All control groups contained one male/one female. The remaining categories (1-4 females) were initiated with more than one male and the appropriate number of females. The number of colonies censused in each age class and set up are mentioned in the text. No statistical analysis was performed for 48-mo-old colonies ($n = 2$). No colonies were available with multiple males/females at 36 mo of age.

We estimated the daily number of eggs laid by a single queen at a given age by dividing the number of eggs found in each colony census by 20, assuming a 20-d incubation period at 24°C. This assumption is based on published data concerning egg and larval developmental times for *Reticulitermes santonensis* Feytaud (Buchli 1958) and *Reticulitermes hesperus* Banks (Weesner 1956). The estimated number of eggs per day produced by primary reproductives remained low during the first 12 mo (averaging 0.4, 1.0, and 2.1 eggs per day at 4, 8, and 12 mo, respectively) but increased eight-fold at 2 yr of age (Fig. 3B). The highest number of eggs per day was provided by one 6-yr-old primary reproductive at 145.7 eggs per day.

Best-Case Scenario for Optimal Colony Growth. The SAS model simulating colony growth was first run with the mean egg-laying rates obtained from our census (Table 4). The number of larvae provided by the computer simulation for colonies between the ages of 4 mo to 2 yr matched the mean number of

larvae censused ($P > 0.05$, $df = 23$ for 4-mo to 12-mo-old groups; χ^2 test). For older colonies, the model either underestimated (36-mo-old groups) or overestimated (48-mo-old groups), the theoretically expected number of larvae ($P < 0.01$, $df = 4$ and 3, respectively; χ^2 test). Only two colonies were censused in the 6-yr age class, but the number of larvae in each group (1,154 and 1,564 larvae) was close to the expected (1,470).

The predicted number of workers in each age class (assuming a 6-mo or 2-yr worker life span) exceeded the census data by 38.9% (SD 33.9) and 73.8% (SD 14.5), respectively. Only with a worker life span of 3 mo did the mathematical model produce worker numbers statistically similar to what we found in our census between the 4- and 24-mo age classes ($P > 0.05$, $df = 23$ for 4-mo to 12-mo-old groups; χ^2 test).

A hypothetical high egg-laying rate of 300 eggs per day (more than twice the maximum number of eggs found in our census) deposited from the very first day

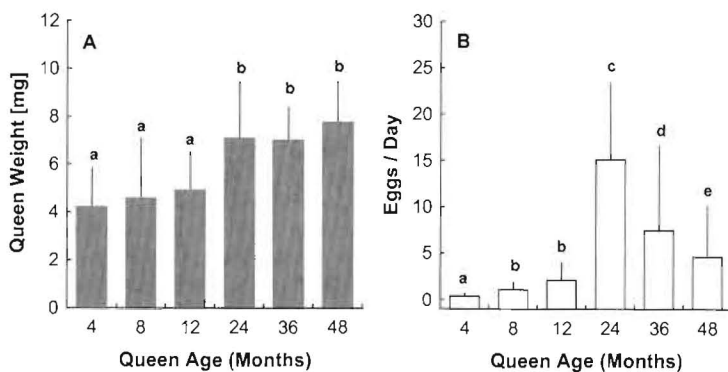


Fig. 3. Changes in (A) queen weight (mean \pm SD) and (B) eggs per day by census date. Different lowercase letters indicate significant differences ($P \leq 0.05$; Kruskal-Wallis ANOVA).

Table 4. Results from the simulation model of termite colony growth assuming a monogyne colony by using the mean egg laying rates obtained from laboratory colonies in this study and hypothetical egg laying rates

Input		Egg laying rates obtained from census					Hypothetical egg-laying rate		
		Worker life span: 6 mo		Worker life span: 2 yr			Input	Output	
Egg-laying rate (eggs/d)	Colony age (yr)	No. of larvae (instars 1-2)	No. of workers (instars ≥ 3)	No. of eggs ^a	No. of larvae (instars 1-2)	No. of workers (instars ≥ 3)	Egg-laying rate (per day)	No. of eggs ^a	No. of workers (instars ≥ 3)
0.38	0.33	5	33	45	5	33	145.7**	212,722	101,553
1.09	0.66	15	118	176	15	141	300	423,000	209,400
2.11	1	30	255	440	30	372	1,000	1,440,000	697,000
14.41	2	202	2,133	5,699	202	5,238			
7.45	3	104	1,103	8,419	104	7,740			
9.27	4	130	1,372	11,802	130	5,806			
10.60	5	149	1,568	15,671	149	6,913			
107.95	6	1,470	15,532	50,829	1,470	35,947			

A worker life span of either 6 mo or 2 yr was assumed.

^a Number of eggs is cumulative.

** Highest egg-laying rate recorded from the *R. flavipes* colony census.

and with a worker life span of 6 mo resulted in a population of 44,400 workers and, with a 4-yr worker life span, of 422,000 workers (Table 4). A daily egg-laying rate of 1,000 eggs from the first day of colony foundation theoretically allows the primary female to produce 688,000 workers with a 2-yr worker life span or 1.4 million workers with a 4-yr worker life span.

Discussion

A better understanding of the reproductive biology of subterranean termites has implications for such diverse topics as the evolution of eusociality in insects to implementation of population management strategies (Thorne et al. 1997; Forschler and Jenkins 2000). Due to the cryptic nesting and feeding habits of *R. flavipes*, rearing and maintaining colonies in the laboratory can serve as the basis for a general description of the principles of reproductive organization and population growth in this subterranean termite species.

The data obtained in this study implies that a monogyne colony cannot reach the population size occasionally found in field studies. This is the first study in *Reticulitermes* to assess the number of eggs, larvae, and workers toward development of a population growth model. By assuming a worker life span of 6 mo and 2 yr, our model overestimated the number of workers compared with what we found in our laboratory colonies; only with a worker life span of 3 mo did the model predict worker numbers that were within the range of what we censused in the 1-2-yr-old colonies. The low number of workers found in our study (in contrast to the best-case scenario model data) may either be explained by the fact that the average worker life span in laboratory colonies tends to be short or that termites can adjust their population size to resource availability. The two greatest increases in colony growth (i.e., egg-laying rate, progeny numbers, biomass, and queen weights) were recorded during the year 2 and year 6 census, in age classes censused 1 yr after movement to larger arenas.

This trend indicates that *R. flavipes* can detect the size of available resources and adjust their egg-laying rate to accommodate exploitation of new resources. Colonies that were censused in the 2- to 5-yr age classes were moved from 1,312.5-cm³ arenas to 4,940-cm³ arenas and did not show a corresponding increase in any of the colony growth parameters we measured. The first move, after 12 mo, was to an arena 55× the initial/nuptial chamber. The second move (between 2 and 4 yr) was to an arena 3.8× the size, whereas the last move (at 5 yr) was 12× the size of the previous arena. Perhaps a resource four times larger is not sufficient to elicit a population response, but a difference of at least 10× can trigger a population increase. If the arenas we used were limiting the growth potential of the colonies and older workers (perhaps involved more in exploration and exploitation of new food reserves) were sacrificed to maintain younger workers (perhaps involved more in broad care tasks) we would find fewer workers than predicted by the model. This hypothesis fits well with the live weights we found for workers during the census that were lower than the average field collected *R. flavipes* (census average = 2.4 mg per termite; field average = 3.0 mg per termite). An additional indication comes from the number of larvae predicted in the model and censused in colonies up to 2 yr of age; a steady increase in larva per colony by age class suggest that the young colonies were supplied with sufficient space and food. After 2 yr, production of larvae leveled off until the 5-yr-old colonies were moved into larger containers providing additional resources. This potential to respond to resource size should be examined in further studies to verify the veracity of drawing inference from laboratory studies for field population growth. Our study cannot prove a positive relationship between food volume and colony growth rates but as Lenz (1994) pointed out, adjustment of feeding rates to characteristics of food supply could be important for colony growth and maintenance of caste proportions.

Further evidence of the ability of *R. flavipes* to respond to resource size can be found in examining queen body weight and egg laying rates that increased at the year 1 and year 6 census. Extrapolating body weight and egg-laying capacity of the 6-yr-old primary female recorded in our census (18 mg/145.7 eggs per day) a body mass of 125 mg is needed to produce 1000 eggs per day, a mass never reported in *R. flavipes* primary reproductives. Additionally, our mathematical model revealed that a primary female would have to laying 685 eggs per day to attain 1 million workers given a 4-yr worker life span and 1,370 eggs per day with a 2-yr worker life span, rates that seem unattainable from our data.

Yet, questions remain in reconciling our laboratory data with field estimates of subterranean termite populations. We assumed in our best-case colony growth model that egg laying was continuous. In June 2003, we found a physogastric *R. flavipes* primary queen in a field-collected log that weighed 77 mg. Ten days after placing this queen in a petri dish with several hundred workers, she lost 50 mg (down to 27 mg) and laid 884 eggs. We would have predicted using the rate recorded from our laboratory data that this queen should have laid 6,160 eggs (eight eggs per day per milligram of body weight) in 10 d. We can assume the conditions that enabled this reproductive to reach such proportions were not maintained due to disturbance and other unknown factors because she lost weight so quickly and provided fewer eggs than expected. It can be argued that this large queen was involved in a seasonal push to replace those colony members that followed the imaginal line toward adulthood and left in the springtime swarm. Regardless, this single capture highlights the dynamic nature of subterranean termite reproductive capabilities and hints at periodic episodes (determined by either resource availability or season) of egg laying that would likely reduce the growth rates for field colonies compared with our model.

Our laboratory data point toward reproductive strategies where more than a single female is responsible for the production of offspring in field colonies containing >50,000 workers. Field data from two disparate approaches, molecular techniques and mark-recapture population estimates, indicate that polygyny is an option for *Reticulitermes*; the former directly and the latter by inference with our data set. Alloenzymes, mtDNA markers, and multilocus fingerprinting imply social structures that include polydomy or polygyny (Clément 1986, Jenkins et al. 1999, Bulmer et al. 2001).

In *R. flavipes* field colonies, pleometrotic founding may occur, but this study suggests it is not maintained because after the 4-mo census, all arenas established with several females contained only one. The prevalence of haplometrosis is in accordance with the previous literature on Rhinotermitidae incipient colony foundation and growth (Buchli 1950, Light and Weesner 1955, Weesner 1956, Beard 1974, King and Spink 1974, Howard et al. 1982, Thorne et al. 1997). Matsuura et al. (2002) conducted a study designed to

examine the question of pleometrosis, by using *R. speratus*, and found that only in the absence of males did two females successfully initiate a colony.

The fate of the surplus female primary reproductives cannot be discerned from our census data, although at 4 mo none of the multiple queens we found showed any signs of aggressive interaction. It might be possible that the first workers neglect all but one reproductive, resulting in malnourished and un-groomed females that eventually die. Perhaps the first worker generation kills the females (Ruppli 1969) or severe mutilation of all competing females caused by the successful primary reproductive results in cannibalism by the first worker generation (Emerson 1933). Despite the uncertainty surrounding the fate of "extra" female reproductives, laboratory studies repeatedly indicate that monogynous colony foundation is the essence of *Reticulitermes* social structure.

Acceptance of polyandry by the colonies we surveyed is in contrast to work conducted with *R. speratus* by Matsuura and Nishida (2001) who found no survivorship in multiple male incipient colonies. However, polyandry is consistent with our occasional recovery of multiple primary males from field collections (B.T.F., unpublished data). Although there was no evidence from our data that the presence of more than one primary male resulted in an increased rate of colony growth, it remains to be determined whether both males contributed toward the population gene pool. The incidence of polyandry in field colonies should be considered when multilocus fingerprinting techniques are used to examine reproductive strategies that heretofore have assumed allele frequencies based only on female inputs (Clément 1986, Bulmer et al. 2001).

The occurrence of neotenic reproductives could explain the disparity between laboratory colony studies that highlight monogyny and field data that indicates input from multiple females. In the lower termite families Kalotermitidae and Rhinotermitidae, the occurrence of neotenic reproductives has been described both under field conditions (Lenz and Barrett 1982, Luykx 1993, reviewed in Myles 1999) and in laboratory colonies (Buchli 1956, Howard and Haverly 1980, Watanabe and Noda 1991; Pawson and Gold 1996). A review of secondary reproduction in termites with comments on its role in termite ecology and social evolution has been published recently by Myles (1999). It is interesting that, in contrast to field collections, laboratory groups tend to produce more apterous (third form) than brachypterous (second form) neotenic reproductives; this may be another indicator of the ability to "estimate" food resources: the production of nymphal-derived brachypterous neotene forms is more energy-consuming than smaller, worker-derived apterous forms. In one 2-yr-old colony, we found 32 apterous neotenes (all females) and 143 large nymphs but no primary reproductives (thus not integrated into the analyzed data set). In another 2-yr-old colony, we recorded a physogastric apterous neotenic female, two primary males, and the highest number of eggs ($n = 686$) and

larvae ($n = 635$) in that age class. Although there is little doubt that the absence of a primary reproductive results in the production of neotenics, it is evident from our data that formation of neotenics is an independent process that occurs in the presence or absence of primary reproductives as reported by Buchli (1956). Neotenic polygyny could explain the disparity between the population estimates generated by our best-case scenario of monogynous colony growth and published population estimates from field studies (Grace et al. 1989, Su et al. 1993, Forschler and Townsend 1996). The presence of numerous neotenics could boost the reproductive capacity of a colony to reach 0.5–1 million workers. It is conceivable that in the field, given the mobility of the primary reproductives and the location of separate feeding sites, these neotenics could become functional and contribute to the population.

In conclusion, this article reports data from the census of >100 laboratory colonies of *R. flavipes* over all life stages, including eggs, and several age classes. The information gained from this study provides insights into the reproductive dynamics of *Reticulitermes* kept in laboratory culture that includes the lack of primary polygyny, prevalence of polyandry, potential for population adjustments in response to resource availability, formation of neotenics in the presence of primary females, and the need for more knowledge to reconcile the occurrence of large field populations. Future research should address several key components of colony growth models to better predict colony growth in field populations. These would include worker life spans, the prevalence of periodic or continuous egg production, and the role of resource size and seasonality in influencing maintenance of colony size.

Acknowledgments

Lisa J. Stabler, Jonathan Patterson, Lee Johnson, and Christopher Chappell helped count termites and their assistance was invaluable. Special thanks go to Glen Ware and Caroline Clancy for help with the SAS model. We also thank Susan Jones for valuable comments on previous drafts of this manuscript.

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Received 8 July 2003; accepted 15 December 2003.