Production of Nymphs and Apterous Neotenic Reproductives in Subterranean Termites, *Reticulitermes flavipes* and *R. malletei* (Blattodea: Rhinotermitidae), in Delaware, U.S.A.

by

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ABSTRACT

Colonies of *Reticulitermes malletei* Clément have been found to dominate a study site in Lewes, DE, USA over other species of subterranean termites. This study was initiated to determine factors that would allow *R. malletei* to do so, specifically the rate of development of nymphs and apterous neotenic (ergatoid) reproductives as a result of orphaning. Results showed that for worker termites collected during the swarm of 2004, there was no statistical difference in nymph or ergatoid reproductive production rates between the two species: 52.1% for *R. flavipes* nymphs Vs 55.0% for *R. malletei* nymphs; 0.0% for *R. flavipes* ergatoid reproductives Vs 5.0% for *R. malletei* ergatoid reproductives. In contrast, for worker termites collected post-swarm in 2005, there were statistically significant differences between the species: 93.3% for *R. flavipes* nymphs Vs 80% for *R. malletei* nymphs; 6.6% for *R. flavipes* ergatoid reproductives Vs 93.3% for *R. malletei* ergatoid reproductives. Few individuals of either caste developed in the experimental containers; many times only one replacement reproductive was observed. Neither species showed an advantage in the minimum time required to produce nymphs: 41.6 ± 11.0 days to 53.8 ± 23.2 days for *R. flavipes* and 46.5 ± 22.1 days to 57.4 ± 35.8 days for *R. malletei*.

KEY WORDS: orphaning, ergatoid, dominance, replacement reproductives

INTRODUCTION

Since 1995, populations of three species of subterranean termites have been studied in a 6-hectare pine-scrub and beach in Lewes, Delaware, U.S.A.

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at Cape Henlopen State Park: *Reticulitermes flavipes* (Kollar), *R. virginicus* (Banks) and the recently described species (Austin *et al.* 2007), *R. malletei* Clément. From 1997 through 2001, alates and soldiers were collected from 26 colonies. DNA sequences from 18 of these colonies were identified as *R. malletei;* sequences from seven as *R. flavipes;* and the sequence from one as *R. virginicus* (King *et al.* 2007). Surveys have consistently shown that *R. malletei* colonies outnumber colonies of the other two species in this site (King, unpublished data). The study reported in this paper was initiated to determine factors that would allow *R. malletei* to dominate the study site, specifically the rate of development of nymphs and apterous neotenic (ergatoid) reproductives of *R. malletei* compared to that of *R. flavipes* as a result of orphaning. Termite nymphs are individuals with wing pads that are developing along the imaginal pathway (Thorne 1996). They may be destined to become alates or brachypterous neotenic reproductives. The presence of nymphs in a colony indicates that the colony is in the process of reproducing; either through primary or secondary reproduction. Apterous neotenic reproductives lack wing buds and remain in the colony.

MATERIALS AND METHODS

In 2002, 2004, and 2005, termites and their food source, Southern Yellow Pine block "sandwiches," were removed from monitoring stations that had been established in a 6-hectare site in Lewes, Delaware, U.S.A. at Cape Henlopen State Park according to King *et al.* (2007). Dates of removal coincided either with the swarm for each species or after the swarm; four to five colonies of *R. flavipes* were used and four to seven of *R. malletei* (Table 1). Upon removal from the field, the termites were transferred to the laboratory at the University of Delaware, Newark, DE in one-gallon plastic containers (17.5 cm diameter x 19.0 cm high; Consolidated Plastics Company, Inc., Twinsburg, OH) and maintained there with their original food source.

Within one week of each collection, the laboratory study was initiated. Two pieces of Southern Yellow Pine (approximately 4.0 cm X 4.0 cm X 1.8 cm) were moistened with tap water and placed one on top of the other in the bottom of each 16-oz plastic container (10.5 cm diameter X 7.5 cm high; Fulton Paper and Party Supplies, Wilmington, DE). At least 15 containers were set up for each study (Table 1). Sand obtained from the study site in

Lewes, DE was moistened with tap water until the particles stuck together with no run-off. Approximately 335 g of moistened sand was placed around the wood in each container leaving the top of the wood exposed. Ninety-eight mid-sized workers and two soldiers were added to each container. In only one study duration (*R. flavipes* post-swarm '05) did the number of field-collected termite workers limit the number of containers that could be set up; in all other study durations, field-collected termite workers were abundant.

All containers were capped and holes were punched in the lids with a dissecting needle to prevent mold forming in the containers. The containers were placed in a cupboard with no light and maintained at room temperature. Containers were misted with tap water as needed.

Observations were made monthly for 10 to 32 weeks (Table 1). Numbers of workers, soldiers, nymphs, ergatoid reproductives, and larvae were recorded for each container at each observation date; however, there was no

Table 1. Date termites removed from field, numbers of field colonies from which termites were obtained, numbers of containers in each study and study duration.

		R. flavipes	*R. malletes*
Swarm '02	Date removed from field		5/24/02
	# colonies		6
	# containers		40
	study duration		5/29/02-8/8/02
Swarm '04	Date removed from field	5/10/04	5/13/04
	# colonies	5	4
	# containers	23	20
	study duration	5/10/04-11/20/04	5/13/04-10/18/04
Post-swarm '02	Date removed from field	6/3/02	
	# colonies	4	
	# containers	40	
	study duration	6/7/02-11/11/02	
Post-swarm '04	Date removed from field		7/1/04
	# colonies		7
	# containers		28
	study duration		7/7/04-2/16/05
Post-swarm '05	Date removed from field	7/15/05	7/15/05
	# colonies	4	7
	# containers	15	40
	study duration	7/18/05-1/25/06	7/18/05-1/25/06

way to determine if any one individual was new or had been present in the last count.

The number and percent of containers positive for nymphs, ergatoid reproductives, and larvae for each study duration was calculated, as was the total number and percent of containers positive for either of these castes. Between-species Z tests for pair-wise comparison of proportions were conducted for nymph, ergatoid reproductive and total positive containers.

The maximum number of nymphs and ergatoid reproductives to develop in each container was identified. The range of the maximum numbers for each study duration was determined; the mean and standard deviation for each range were calculated.

The number of days to initial development of nymphs and ergatoid reproductives was determined for each container in which such development took place; the mean and standard deviation were calculated for each study duration. Between-species t-tests were conducted for swarm '04 and post-swarm '05 nymphs.

RESULTS

As shown in Table 2, more than half of the containers were positive for production of nymphs, ergatoid reproductives or larvae. For those termites removed from the field during the swarm of 2004, there was no statistical difference (Z test, $P=0.05$) in nymphs or ergatoid reproductive production between the two species (52.1% *R. flavipes* nymphs Vs 55.0% *R. malletei* nymphs; 0.0% *R. flavipes* ergatoid reproductives Vs 5.0% *R. malletei)*. However, for those termites removed from the field after the swarm of 2005, significantly more *R. flavipes* containers were positive for nymph production (93.3%) than were containers of *R. malletei* (80.0%) (Z test, $P<0.05$). The converse was found for ergatoid reproductives in 2005: significantly more containers of *R. malletei* (93.3%) were positive for ergatoid reproductives than were containers of *R. flavipes* (6.6%) (Z test, $P<0.05$).

Table 3 presents the ranges of the maximum numbers of individual nymphs and ergatoid reproductives that developed per container for each study duration, their means and standard deviations. For example, in those swarm '04 containers in which *R. flavipes* nymphs were produced, in at least one container only one nymph was found; however, in at least one other container

Table 2. Number and percent of containers in which nymphs, ergatoid reproductives and larvae developed. Statistical comparisons were made between species for nymphs, ergatoid reproductives and the total number of containers.

	R. flavipes				*R. malletei*			
	Nymphs	Ergatoid Repro-ductives	Larvae	Total[1]	Nymphs	Ergatoid Repro-ductives	Larvae	Total[1]
Swarm '02					1 2.5%	22 55.0%	3 7.5%	25 62.5%
Swarm '04	12 52.1%[a]	0 0.0%[b]	0 0.0%	12 52.1%[c]	11 55.0%[d]	1 5.0%[b]	2 10.0%	11 55.0%[e]
Post-swarm '02	27 67.5%	24 60.0%	7 17.5%	34 85.0%				
Post-swarm '04					9 32.1%	13 46.4%	2 7.1%	16 57.1%
Post-swarm '05	14 93.3%[d]	1 6.0%[f]	1 6.6%	14 93.3%[a]	24 80.0%[e]	28 93.3%[a]	4 13.3%	29 97.0%[g]

[1] Numbers of containers with nymphs, ergatoid reproductives or larvae.
Values between columns followed by the same letter (a, b, c and h): no significant difference at P>0.05 level (Z test).
Values between columns followed by different letters (d-e and f-g): significant difference at P<0.05 level (Z test).

Table 3. Range of greatest number of individuals to form in containers, mean ± SD.

	R. flavipes		*R. malletei*	
	Nymphs	Ergatoid Reproductives	Nymphs	Ergatoid Reproductives
Swarm '02				range 1-2 1.05 ± 0.21
Swarm '04	range 1-11 2.92 ± 2.81		range 1-8 3.91 ± 2.63	
Post-swarm '02	range 1-5 2.5 ± 1.45	range 1-3 1.48 ± 0.68		
Post-swarm '04			range 1-6 1.56 ± 1.67	range 1-5 2.31 ± 1.32
Post-swarm '05	range 5-24 15.36 ± 7.52		range 1-15 4.78 ± 3.57	range 1-5 2.25 ± 0.97

11 nymphs were found. The mean number of nymphs found was 2.92 ± 2.81. In this study duration, as well as most of the others, the development of nymphs and ergotic reproductives varied greatly. In addition, the dates at

which the greatest numbers of individual developed in each container varied. For example, the greatest numbers of *R. malletei* nymphs in the containers of swarm '04 and the dates on which that value was obtained were: 2 (6/2/04), 6 (6/2/04), 8 (6/2/04), 2 (6/14/04), 4 (6/22/04), 2 (6/28/04), 6 (7/7/04), 8 (7/7/04), 2 (7/26/04), 1 (9/8/04), 2 (9/8/04). Because of the high variability, no conclusions were drawn on this data.

The mean number of days to initial development of individuals is given in Table 4. No significant difference in production of nymphs was found between the two species (t-test, P=0.05). For the swarm of '04, containers of *R. flavipes* were positive for nymphs in 53.8 ± 23.2 days, while containers of *R. malletei* were positive for nymphs in 57.4 ± 35.8 days. For the post-swarm of '05, containers of *R. flavipes* were positive for nymphs in 41.6 ± 11.0 days, while those of *R. malletei* were positive for nymphs in 46.5 ± 22.1 days.

Table 4. Mean number of days to initial development of individuals ± SD. Statistical comparisons were made between species for nymphs.

	R. flavipes		*R. malletei*	
	Nymphs	Ergatoid Reproductives	Nymphs	Ergatoid Reproductives
Swarm '02				47.5 ± 13.9
Swarm '04	53.8[a] ± 23.2		57.4[a] ± 35.8	
Post-swarm '02	21.4 ± 10.8	51.4 ± 7.4		
Post-swarm '04			63.7 ± 31.0	55.2 ± 27.8
Post-swarm '05	41.6[a] ± 11.0		46.5[a] ± 15.8	58.7 ± 22.1

Values between columns followed by the same letter: no significant difference at P=0.05 level (t-test)

DISCUSSION

There are several factors that could explain the dominance of one species over another in a field site: timing of arrival, resource utilization, defense and reproduction. Termite reproduction takes two forms: primary reproduction through alate swarms and secondary (neotenic) reproduction. Myles (1999) reported that neotenic reproductives are found in 61.7% of lower termite genera and that orphaning is the main factor that provokes replacement reproduction. He notes that such reproduction allows the offspring to inherit

established resources and forgo predation from above ground dispersal. Thus, if two species arrive in a new site at the same time, the species that has an advantage in production of replacement reproductives may come to dominate the site, all else being equal. Each laboratory container in our study can be viewed as a potential new colony that has just been separated from the main colony in the field. Thorne *et al.* (1999) in reviewing reproductive dynamics and colony structure of *Reticulitermes* concludes that multiple replacement reproductives give a boost to colony growth and appear to be crucial to colony expansion. Howard & Haverty (1980) examined multiple colonies of *R. flavipes* and found that neotenics made up 1.28% of the colonies with two females for every male. Thorne *et al* (1997), Long *et al.* (2003, 2007) and Grube & Forschler (2004) have conducted long term *R. flavipes* alate-initiated colony development. Long *et al.* (2003) found that when a colony lost a founding parent, more pre-alate nymphs developed than in colonies with both parents. Long *et al.* (2007) reported that queenless colonies produced significantly more female reproductive biomass than those colonies that retained their queen. Grube & Forschler (2004) censused colonies ranging in age from four months to nine years and stated that a single king and queen of *R. flavipes* could not produce colonies with the population size occasionally described in field studies. They concluded that neotenic polygyny could explain this disparity and found that laboratory groups tended to produce more ergatoid reproductives than brachypterous neotenic reproductives. Our data for *R. malletei* supports this observation in most cases; however, our data for *R. flavipes* does not.

Pawson & Gold (1996) and Pichon *et al.* (2007) examined the ability of termite colonies to develop neotenic reproductives upon orphaning. Pichon *et al.* (2007) found that 53% of surviving laboratory colonies of *R. grassei* and *R. santonensis* developed secondary reproductives and concluded that small numbers of *Reticulitermes* could establish new colonies within a few years. For *R. flavipes*, Pawson & Gold (1996) found that reproductives formed within three months after separation from the founding colony, while in our study, *R. flavipes* developed reproductives in as little as three weeks and before 54 days. However, in our study we seeded the containers with two soldiers for every 98 workers, thus the development time probably was the same for the two studies. In regards to the numbers of reproductive individuals developing

in each laboratory container, our data agrees with Pawson & Gold (1996) - in general few individuals develop at any one time.

Howard & Haverty (1981) determined that mature colonies of *R. flavipes* have one complete reproductive cycle each year and that proportions of nymphs peak in early fall and early summer. While it would be tempting to compare our data from workers taken during the swarm with those taken post-swarm, this can be done only for *R. malletei* nymphs in 2004. In the absence of similar data for *R. flavipes*, this analysis was not reported.

While it is obvious that both *R. flavipes* and *R. malletei* are capable of producing nymphs and ergatoid reproductives upon orphaning, it is not clear if this ability contributes to the dominance of *R. malletei* in the Lewes, DE study site. Neither species showed an advantage in the time required to produce replacement reproductives. While *R. malletei* does show an advantage in the production of ergatoid reproductives (post-swarm '05), *R. flavipes* clearly shows an advantage in the production of nymphs. In addition, the number of individual ergatoid reproductives of *R. malletei* are few compared to the number of individual nymphs produced by *R. flavipes*. Grube & Forschler (2004) point out that less energy is required for the production of apterous individuals, perhaps in the long run this would allow *R. malletei* to dominate. Further studies comparing these two species are needed, including a comparison of the long-term reproductive potential of these two forms of neotenic reproductives.

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