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Population Growth Characteristics of Incipient Colonies of the Eastern Subterranean Termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae)

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Abstract

We investigated growth of inbred monogamous *Reticulitermes flavipes* (Kollar) colonies monthly during their first year, thereby assessing colony growth at a much finer temporal scale than previous studies. Detailed data were obtained on important growth parameters, including numbers of each caste and/or developmental stage in each colony and their biomass. Growth of 180 *R. flavipes* incipient colonies was documented via destructive sampling of 15 unique colonies each month for 12 months. King and queen biomass greatly decreased from the 1 mo to 3 mo census coinciding with egg production and colony foundation, but their biomass remained relatively constant thereafter. Three cohorts of eggs were produced, with the greatest number of eggs (mean = 14.5) being produced during the first month. Larvae were present at 1 mo, and workers were first observed at 2 mo. The survival rate of the initial brood was less than 100%, most likely due to cannibalism. A single soldier was first noted in some colonies at 6 mo. Offspring biomass was equal to that of the reproductive pair at the 2 mo census, double at 3 mo, and quadruple at 11 mo. One-year-old colonies ranged in size from 20 to 40 individuals, with a mean of 28.9 individuals; mean total colony biomass was 39.8 mg. The growth rate of these *R. flavipes* colonies was much slower than previously reported, but our observations are consistent with previous models of colony growth. Our detailed baseline information on *R. flavipes* colony growth is expected to allow for more refined hypotheses and growth model development in future studies of this economically important termite.

Introduction

Subterranean termites are the most economically important structural pests in the United States, and approximately $11 billion is spent annually for prevention and treatment measures and repair of termite damage (Su, 2002). *Reticulitermes flavipes* (Kollar) is the most widespread and economically important native species. Better insights into initial colony growth will improve our understanding of colony establishment and the spread of pest species.

The termite colony's life cycle begins with the dispersal of winged alates to colonize new resources. The alates pair, shed their wings, and establish a new nest. The castes are determined during post-embryonic development, and any larva (first or second instars) can become a worker, soldier, or reproductive (Lainé & Wright, 2003).

Early colony development has been studied in various rhinotermitids. For example, alate-founded colonies have been studied for the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (King & Spink, 1974, 1975; Fei & Henderson, 2003; Morales-Ramos & Rojas, 2003; Sun, 2007); the western subterranean termite, *Reticulitermes hesperus* Banks (Light & Weesner, 1955); and the eastern subterranean termite, *R. flavipes* (Snyder & Popenoe, 1932; Thorne et al., 1997; Grube & Forschler, 2004). In addition, development of new colonies from isolated groups of workers and nymphs has been studied in *R. urbis* Bagneres (Ghesini & Marini, 2009).

R. flavipes is an economically significant pest species that is widely distributed in the eastern United States. Incipient colony growth has been studied by various researchers. Snyder and Popenoe (1932) observed laboratory-reared

colonies and documented the abundance of castes at various times, but they did not conduct a rigorous census. Thorne et al. (1997) used a destructive sampling technique to census 2-yr-old colonies, noting the abundance of castes and colony size range. Grube and Forschler (2004) studied incipient colony growth of monogamous and polygynous colonies during the first 2 yr. They censused a total of 36 monogamous colonies, inbred and outbred, during the first year of colony growth. These colonies were assigned to three groups of 12 colonies, which were censused at 4, 8, and 12 mo, but the researchers provided no colony size averages, only ranges. In spite of this important study, information is needed on colony growth at a much finer temporal scale. More frequent inspection intervals would be useful to better understand *R. flavipes* colony growth during the first year.

The objective of our descriptive study was to investigate growth dynamics of inbred monogamous colonies of *R. flavipes* during the first year through the parameters of numbers, castes and/or developmental stages, and biomass. This detailed baseline information on colony growth will allow for more refined hypotheses and growth model development.

Materials and Methods

Experimental Setup

Thousands of *R. flavipes* swarming alates were collected on May 20, 2011, in Orient, OH (39°46'19.99"N, 83°09'22.30"W). The swarm was from a presumably single large colony and it originated in a barn on a rural farm. The termites were individually sexed by examining the posterior abdominal sternites with the aid of a dissecting microscope (Stemi SV11 Apo, Zeiss, Germany). In the Isoptera, female alates lack styli and have a noticeably elongated seventh abdominal sternite compared to the males (Jones & La Fage, 1980; Zimet & Stuart, 1982; Roisin & Lenz, 1999). A male and female reproductive pair was placed into a plastic "nest" container (3.8 cm ht x 5.4 cm dia, with lid; Pioneer Plastics, Inc., North Dixon, KY) that had been lined with a moist filter paper pad and provisioned with a combination of moist softwood and hardwood mulch. Approximately 800 colonies were maintained in the lidded containers in an environmental chamber in the dark at 26 ºC with 85-90% relative humidity. Water was added to the colonies as needed throughout the study.

Census Methods

Only established colonies headed by a single queen and king were censused. During a 1-yr period, 15 different colonies were randomly selected each month from the initial pool of ~800 colonies, providing a grand total of 180 censused colonies. The census procedure involved careful removal and inspection of small portions of the mulch until aggregated termites were located (often near the nuptial chamber). At that point, any remaining mulch and filter paper was placed on a large tray. All termites and eggs were individually removed by hand from the mulch using a fine-tip paint brush. Immature termites were examined using a dissecting microscope and categorized as larvae, workers, nymphs, or soldiers (see Thorne 1996), then the biomass of each caste and/or developmental stage was obtained using an analytical balance (±0.01 mg, Sartorius AG, Model CPA225D, Goettingen, Germany). The primary reproductives were microscopically examined to determine the queen and king, then each was individually weighed. Each colony was re-established after censusing, but no colony was re-sampled during the study due to the extensive disturbance caused by the indepth inspection process.

Results and Discussion

Throughout the first year of colony development, the number of individuals varied dramatically from month to month (Fig. 1). Egg production was intermittent, with the greatest number of eggs (mean = 14.53; SE = 0.95; n = 15 colonies) observed during the first month (Fig. 1). There were three cohorts of eggs--the first occurred during the first and second month, the second occurred in the sixth and seventh month, and the third spanned months nine through twelve (Fig. 1).

Larvae were observed at 1 mo, and workers were first observed at 2 mo (Fig. 1). The number of larvae in the colony remained between 0 and 3 throughout the first year, with the exception of the initial cohort which consisted of 7.6 larvae at 2 mo (Fig. 1). One-year-old colonies ranged in size from 20 to 40 individuals (all stages), with an average of 28.9 individuals. Workers constituted the majority of colony members from the second month onward, and 1-yr-old colonies had an average of 18 workers (range 13-27). No nymphs were observed during the first year of colony development, which is consistent with Grube and Forschler's (2004) observation that nymphs were first found in 24-mo-old colonies.

There was an initial peak in total colony members that decreased dramatically from an average of 22.3 termites and eggs at 2 mo to 13.9 at 5 mo (Fig. 1). This sharp decline indicates that not every egg hatched and not all offspring survived. Snyder and Popenoe (1932) observed cannibalism of eggs, larvae, and workers in incipient colonies, with healthy young being eaten despite adequate food, and this may have been a contributing factor. It is uncertain whether trophic eggs contributed to the decline in numbers observed in our study since there are no published reports of trophic eggs in *R. flavipes*, and Yamamoto and Matsuura (2011) indicated that trophic eggs did not occur in a closely-related species, *R. speratus* (Kolbe). However, Buchli (1950) indicated that *R.*

lucifugus Rossi sometimes produced unfertilized eggs, which were cannibalized.

At the 6 mo census, a single soldier was observed in each of two colonies with total populations of 12 and 13 individuals. A soldier was present in 47% of the colonies at the 12 mo census and comprised 1.6% of the colony (Fig. 1). Similarly, soldiers were present during the first year in Grube and Forschler's (2004) study, but they occurred earlier (4 mo) and comprised 3% of the colony at 1 yr. In Snyder and Popenoe's (1932) study, soldiers were first observed in 7-mo-old colonies. The appearance of soldiers later in the first year for *R. flavipes* is consistent with a closely related rhinotermid, *R. hesperus*. Light and Weesner (1955) observed that groups of *R. hesperus* colonies produced soldiers either in the initial cohort or in the second cohort.

The biomass of both the king and queen greatly decreased from the 1 mo to 3 mo census (Fig. 2), which corresponds with the first cohort of eggs and care of larvae (Fig. 1). This is the first report of this phenomenon in *R. flavipes*. The dramatic decrease in biomass of both primary reproductives is likely due to their use of fat stores to foster the developing young. The reproductives' biomass continued to decrease until 4 mo when relatively large numbers of workers (mean = 12.67; SE = 0.67; n = 15 colonies) were present in the colonies (Fig. 1). The reproductives' biomass remained relatively constant thereafter through the 1 yr census (Fig. 2), which is consistent with Grube and Forschler (2004). Eventually, as the colony grows, brood care tasks and nest construction will be taken over by workers, then egg production may become continuous. Grube and Forschler (2004) noted that *R. flavipes* queens experience significant weight gain during the second year of colony development.

The total biomass of each colony gradually increased over time, and total biomass of 1-yr-old colonies averaged 39.8 mg (Fig. 2). Offspring biomass was equal to that of the reproductive pair at the 2 mo census, double at 3 mo, and quadruple at 11 mo (Fig. 3).

The growth rate of *R. flavipes* incipient colonies observed in our study was much slower than observed by Grube and Forschler (2004). Regional diversity among termite colonies is expected to be an important factor influencing colony growth rates (reviewed by Lenz 2009). We expect that the genetic diversity of our inbred colonies, all headed by reproductives from a single location in Ohio, would be somewhat limited. For example, at the 12 mo census, there was some variation among our 15 sampled colonies, which ranged from 13-27 workers, but nothing as extreme as observed by Grube and Forschler (2004) whose smallest *R. flavipes* colony had 15 workers and largest colony had 259 workers (colony averages not provided). Grube and Forschler's study involved alates collected from numerous sites in Georgia, USA (University of GA, Athens, GA [33°56'52.82"N, 83°22'38.36"W]; Whitehall Forest, Athens, GA [33°53'05.42"N, 83°21'27.57"W]; Westbrook Farm, Kennesaw, GA [33°59'24.92"N, 84°41'56.50"W]),

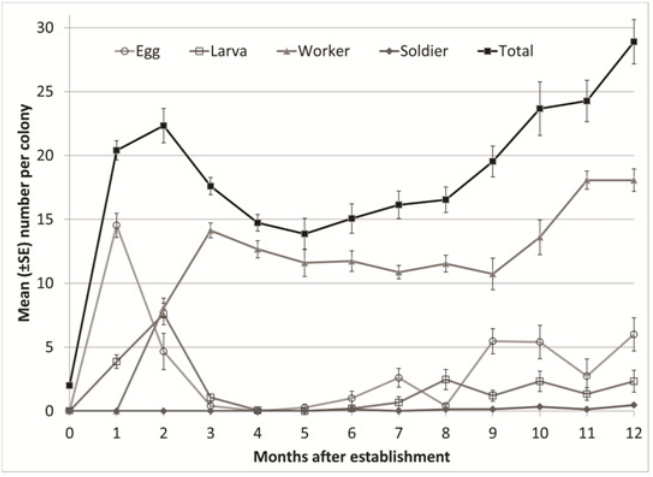

Fig. 1. Numbers of each caste and/or developmental stage (mean±SE) per colony at monthly intervals during the first year of incipient colony growth in *Reticulitermes flavipes.* Total colony size includes the founding pair of primary reproductives. A grand total of 180 colonies was examined, with 15 unique colonies dismantled each month for 12 months.

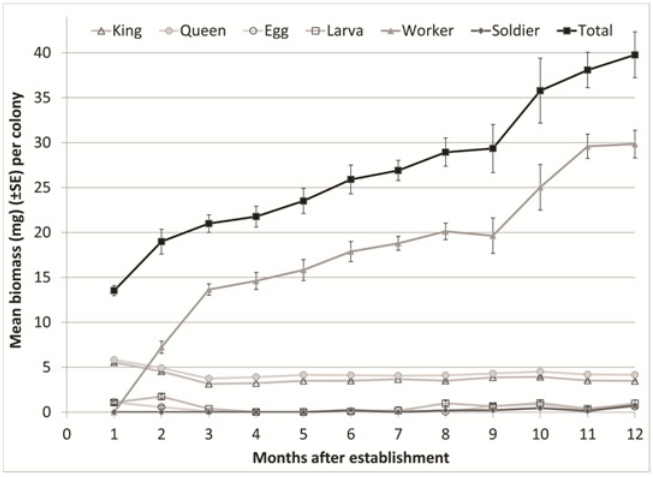

Fig. 2. Mean biomass of each caste and/or developmental stage in *Reticulitermes flavipes* incipient colonies (n=15 colonies per monthly observation) during the first year of colony growth.

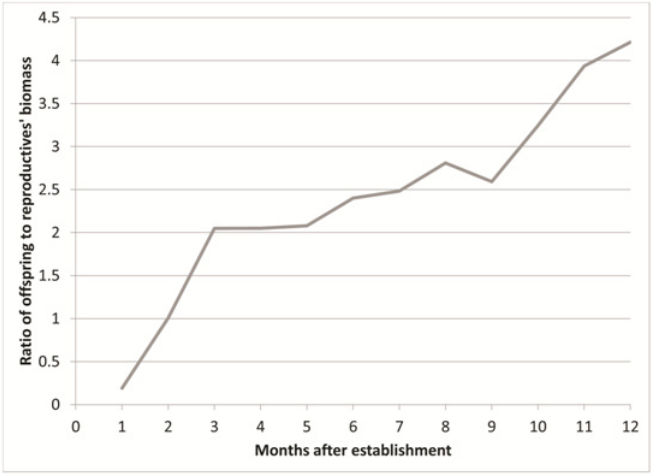

Fig. 3. Ratio of offspring to reproductives' biomass at monthly intervals in *Reticulitermes flavipes* incipient colonies (n=15 colonies per monthly observation) during the first year of colony growth.

and data were combined for inbred and outbred colonies because no differences in colony growth parameters were evident (B.T. Forschler, Professor of Entomology, University of Georgia, USA, personal communication, July 25, 2013).

Fei and Henderson (2003) specifically studied the effect of mate relatedness in a total of 338 sibling and non-sibling incipient colonies of *C. formosanus*, and they found that outbred colonies had significantly increased fecundity compared to inbred colonies. Hence, inbreeding may be an important factor in the slower growth rate of our *R. flavipes* colonies compared to some other studies.

Rearing conditions (food type, temperature, relative humidity, container size, etc.) also are expected to influence colony growth rates (reviewed by Lenz 2009). For example, Grube and Forschler (2004) used sawdust as a food source for newly founded colonies compared to our use of a mixture of soft and hardwood mulch, which is more coarse and may be more difficult for termites to consume. They used a slightly lower temperature (24 ºC) than we did, but lower temperatures would be expected to result in slower growth rates; both studies involved comparable relative humidity (85%). Furthermore, our colonies were maintained in their original small container (80.7 cm^3) whereas Grube and Forschler's colonies were moved from 87.9 cm^3 containers to much larger containers (1312.5 cm^3) between 6 and 18 mo when they had consumed more than 50% of their sawdust allotment. These larger containers were provisioned with a much larger food supply, including solid pine wood and sawdust. These conditions may have contributed to increased colony growth rates.

Our study is the first to provide extensive monthly numerical data to track growth trends in *R. flavipes*. However, general colony trends are paralleled in related studies of *R. flavipes* (Snyder & Popenoe, 1932, Grube & Forschler, 2004). Our study also reveals similar growth trends as observed in rhinotermitids such as *R. hesperus* (Light & Weesner, 1955) and *C. formosanus* (King & Spink, 1974, 1975; Fei & Henderson, 2003; Morales-Ramos & Rojas, 2003; Sun, 2007).

In conclusion, the growth rate of our *R. flavipes* colonies was much slower than observed by Grube and Forschler (2004), but our results are consistent with previous models of colony growth. Several distinct colony growth trends are revealed in our study. Biomass of the king and queen greatly decreased from the 1 mo to 3 mo census, coinciding with egg production and colony foundation, but the reproductives' biomass remained relatively constant thereafter. Egg production was intermittent throughout the first year of *R. flavipes* colony development, with three distinct cohorts of eggs as well as periods without eggs, implying that the king and queen partition their initial investment between reproduction and initial brood care. The survival rate of the initial brood was less than 100%, most likely due to cannibalism. The total biomass of each colony gradually increased over time and 1-yr-old colonies consisted primarily of the worker caste, with an occasional soldier. Offspring biomass was equal to that of the reproductive pair at the 2 mo census, double at 3 mo, and quadruple at 11 mo. One-year-old colonies ranged in size from 20 to 40 individuals, with an average of 28.9 individuals; total colony biomass was 39.8 mg.

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