ABSTRACT

Title of Dissertation: *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE) COLONIES: REPRODUCTIVE LIFESPANS, CASTE RATIOS, NESTING AND FORAGING DYNAMICS, AND GENETIC ARCHITECTURE

Catherine Everitt Long, Doctor of Philosophy, 2005

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The eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) is a major decomposer of wood and a significant pest of lumber and paper. Despite its economic and ecological importance, key aspects of its colony dynamics are poorly understood. In 1993, laboratory colonies were initiated with alate pairs. In 2003, the colonies contained an average of 12,600 individuals. Ninety-seven percent of kings and 72% of primary queens survived; 29% of colonies contained replacement female reproductives. In addition to providing unprecedented demographic information regarding colony growth rate and longevity, lifespan of founding reproductives, and the response of colonies to the loss of primary kings and queens, these complete colonies demonstrated foraging and nesting activities of socially intact families.

The laboratory colonies foraged in multi-resource networks. Travel between the resource nodes was observed, and after 30 weeks all spatial networks were censused. None of the castes was distributed equally among the three resources. Reproductives, which were found in a satellite node in 71% of colonies, and brood did not share the same node a significant portion of the time, suggesting that the nesting strategy was polydomous rather than monodomous. Mark-recapture data indicate that workers were significantly more likely to be found in the resource where they had been located previously, indicating i) they feed non-randomly among the multiple resources and ii) they feed extensively at one location rather than shuttling regularly between satellite- and central nodes (as in a central-place foraging model). Such site fidelity violates an assumption of the Lincoln Index, leading to significant misestimation of actual colony population totals established by census. Worker genotypes, as determined by microsatellite markers, indicate that despite the absence of obvious physical isolation, genetic differentiation had occurred among the workers in one of the queenless colonies. Inbreeding coefficients generated by the queenless colony genotypes did not differ significantly from the predicted *F*-statistics for colonies of similar breeding structure, confirming that sampled workers can accurately estimate the breeding regime of field populations.

RETICULITERMES FLAVIPES (ISOPTERA: RHINOTERMITIDAE) COLONIES: REPRODUCTIVE LIFESPANS, CASTE RATIOS, NESTING AND FORAGING DYNAMICS, AND GENETIC ARCHITECTURE

By

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1 INTRODUCTION

Order Isoptera is composed of over 2,600 universally eusocial termite species (Kambhampati & Eggleton 2000). Subterranean termites from the genus *Reticulitermes*, including the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae), range throughout much of the United States. As decomposers of dead cellulose tissue from trees and woody plants, including lumber and paper products, *Reticulitermes* are responsible for a large portion of the billions of dollars spent annually on preventative and remedial pest treatments in the USA.

Reticulitermes colonies are founded by pairs of dispersing alates. If either parent is lost, the colony's reproductive viability can be salvaged; offspring can quickly differentiate into replacement or supplemental reproductives, collectively termed neotenics. To take advantage of their patchy resources and to avoid competition and predation, *Reticulitermes* colonies are mobile and feed on several resources simultaneously. The ebb and flow of workers among feeding sites is poorly understood. Colonies were long thought to occupy single (monodomous) nests which formed the hub of their foraging excursions (central-place foraging); however, a colony may be distributed throughout a series of interconnected nest sites (polydomous) where workers feed preferentially without regular travel to the other nest nodes (dispersed central-place foraging) (see Wilson 1971, Crozier & Pamilo 1996).

If workers exhibit localized feeding site preferences within a network (Evans, et al. 1998, 1999), the frequency of contact between subsets of workers may be reduced. If neotenics develop within these subsets and begin reproducing (Reilly 1987; Vargo 2003;

DeHeer & Vargo 2004), a network of interconnected bud nests with distinct genetic frequencies might develop. If true, these scenarios would fundamentally alter our understanding of *R. flavipes* biology.

Laboratory colonies initiated in 1993 by pairing *R. flavipes* alates and culturing their complete families afforded the opportunity to observe and manipulate whole, large nests. Chapter 2 presents demographic information regarding colony growth rate and longevity, lifespan of founding reproductives, the response of colonies to the loss of founding kings and queens, and the contribution of intrinsic factors in determining caste ratios and individual body sizes. These fundamental elements of colony ontogeny and demography delineate previously unknown patterns of growth, investment, reproduction, and survivorship that impact both intra- and extra-colony dynamics. Chapter 3 examines the foraging patterns and caste distribution of *R. flavipes* colonies across a network of three spatially separated feeding sites. The more that is understood about how colonies occupy their space and distribute individuals among feeding sites within that space, the better able we will be to predict and influence the movement and growth of these ecologically and economically important social insects. Chapter 4 examines genetic profiles and *F*-statistics of four queenless *R. flavipes* colonies. These results offer a rare opportunity to compare inbreeding coefficients of a population of known breeding structure and age with outcomes predicted by computer modeling.

2 LONG-TERM ASSESSMENT OF REPRODUCTIVE LIFESPAN, CASTE RATIOS, AND COLONY SIZE IN *RETICULITERMES FLAVIPES*

2.1 INTRODUCTION

This chapter presents demographic information regarding *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) colony growth rate and longevity, lifespan of founding reproductives, the response of colonies to the loss of founding kings and queens, and the contribution of intrinsic factors in determining caste ratios and individual body sizes as observed in 11-year old, complete, laboratory-reared colonies. These fundamental elements of colony ontogeny and demography delineate previously unknown patterns of growth, investment, reproduction, and survivorship that impact both intra- and extra-colony dynamics.

2.1.1 General eusocial research

Eusocial colony studies, which have typically featured Hymenoptera, have examined the impact of resource availability and quality on colony growth (Tschinkel 1988), caste ratios (McGlynn & Owen 2002) and the production of sexual offspring (McGlynn, et al. 2002), as well as the impact of various reproductive dynamics on colony growth and survivorship (Arcila, et al. 2002). Isopteran colony ontogeny research has focused primarily on families of termites which occupy centralized mounds (Costa-Leonardo, et al. 1996), trees (Luykx 1986, 1993) or epigeal nests (Thorne 1984). These structures house the reproductives throughout the life of the colony, allowing for repeated, relatively non-destructive observation and manipulation.

2.1.2 Subterranean termite research

Relatively little work has been done with subterranean termites, such as Rhinotermitidae (Lenz & Barrett 1982; Lenz & Runko 1993), which forage and nest in diffuse resources and underground galleries. Colonies may abandon their nesting sites in response to disturbance or environmental change, and a non-destructive method of repeatedly surveying and assessing entire field colonies is currently unavailable. Thus, analysis of subterranean termite colony structure depends on destructive sampling (Howard & Haverty 1980; Howard, et al. 1982) or indirect methods such as mark-release-recapture field sampling (Grace, et al. 1995; Grace 1996), inferences based on genetic analysis of collected insects (Reilly 1987; Jenkins, et al. 1998, 1999; Vargo 2000; Bulmer, et al. 2001) and the direct observation of laboratory-reared colonies (Haverty & Howard 1981; Thorne, et al. 1997).

Exhaustive population data were gathered from *R. flavipes* laboratory colonies twice during this research. The colonies were initiated with sibling alate pairs in March 1993. Full colony censuses were conducted in 2001, when the colonies were 8 years old, and again in 2003, when they were 10 years old. According to the literature, these are the oldest, complete, *R. flavipes* colonies studied to date. The termites lived in confined spaces free of predation and competition, ate *ad libitum*, and were shielded from seasonal extremes of temperature and humidity. These factors are likely to have impacted individual life spans, robustness, and rates of colony growth. Although the magnitude of values provided by these colonies may differ from field values, the patterns and trends they illustrate augment our knowledge regarding otherwise unattainable elements of

R. flavipes colony ontogeny, demography and development, including colony growth and the lifespan of primary reproductives, the number and sex ratio of neotenics, and a potential genetic basis for caste proportions.

2.2 METHODS

2.2.1 Culturing techniques and population census

In 1993, 82 incipient colonies were established using pairs of sibling alates from three separate dispersal flights in Prince George's County, Maryland, USA. These alates were identified as *R. flavipes* with a taxonomic key (Weesner 1965). In order to delineate the colonies produced by alates from the three distinct field collections, the laboratory colonies were classified as deriving from either Lineage 1, 2, or 3, with each lineage defined as the product of alates from a single field colony. The geographic distance between the original alate sources was sufficient (several miles) to assume that the resulting laboratory lineages are not closely related. Thus, differences observed between the identically-raised lineages are likely to be genetic in origin.

The establishment and initial rearing of the colonies is described in Thorne, et al. (1997). In 1994, the termite diet of decayed pine and hardwood sawdust was replaced with a combination of weathered pine survey stakes and decayed paper birch *Betula papyrifera* (Marshall) (Betulaceae). Wood was replenished when inspection indicated depletion. Distilled water was provided regularly. In 1998, the colonies were transferred to 185 x 187 mm (5.1 liter) clear plastic containers (Pioneer Plastics, Inc., North Dixon, Kentucky). The laboratory was maintained at a constant temperature of 23° C.

Since their founding in 1993, nearly two-thirds of the original 82 colonies have died, including all of the pairings involving Lineage 2. Some of this mortality was attributed to resource depletion or desiccation, but often no explanation was evident. Within the first two years, 20 colonies died and 7 lost at least one of their primary reproductives, leaving the 55 colonies containing functional primary reproductives

censused in 1995 (Thorne, et al. 1997). Twenty-five additional colonies were lost over the next 6 years. Subsets of colonies were randomly selected for census in 1997 and 1999. Data from the 1999 tallies are included here, but too few of the colonies examined in 1997 survived in order to provide robust comparative analysis. In 2001, 8 years after initiation, 29 colonies remained, including 22 Lineage 1 colonies and 7 Lineage 3 colonies. Three colonies expired between 2001 and 2003, leaving 22 Lineage 1 and 4 Lineage 3 colonies for the last complete census in 2003. Finally, primary and secondary reproductives were counted and weighed in 2004.

Census totals from five dates are included here, although the first, 1995 count was not conducted by C.E. Long. To accommodate for the irregular time intervals at which these demographic measurements were made, the growth rates are presented as changes per year and the elapsed times named Interval 1, Interval 2, etc. Interval 1 refers to the changes noted between the 1995 and 1999 censuses, over which time the colonies aged from 2 to 6 years old. Interval 2 spans the time between 1999 and 2001, when the colonies aged from 6 to 8 years old. Interval 3 runs between 2001 and 2003, reflecting growth from age 8 to 10 years. Finally, Interval 4 reflects 2003-2004, when the colonies matured from age 10 to 11 years old.

At each census, the total number of primary and secondary reproductives, workers, soldiers and presoldiers (hereinafter collectively termed soldiers), larvae (instars 1-3) and pre-alate nymphs were tallied (caste terminology follows Thorne 1996 b). Egg number was categorized into one of four ranges: 1-50, 51-100, 101-300, or >301. Mean worker and soldier wet weights were calculated for each colony using three replicates of ten individuals. Wet weights were collected for each primary reproductive and, if

applicable, for the total number of male and female neotenics within each colony. Colonies with functional primary queens were designated “queenright” (Wilson 1971; Hölldobler & Wilson 1977), and by extension those with primary kings were termed “kingright.”

2.2.2 Statistical analyses

Comparative analyses were performed (SAS 2002) on data from both queenright and queenless colonies, between colonies founded by alates from Lineage 1 and Lineage 3, and among data from the five censuses. For paired analyses, the Student’s t-test determined whether significant differences existed ($a=0.05$). When variances were equal, data were pooled; the Satterthwaite adjustment was used to accommodate for unequal variances (SAS 2002). Correlations were calculated with Pearson Correlation Coefficients. For analyses over time, such as colony growth rate and neotenic production, analyses of variance with repeated measures were undertaken.

2.3 RESULTS

2.3.1 Summary statistics of 8 and 10 year censuses

In 2001, the 8-year old nests ranged from 3,620 to 11,641 individuals, averaging 7,193 (SE $\pm$ 357). Workers composed 82% of this total. Soldiers constituted a mean of 2.6 $\pm$ 0.03 % of the nest, which is consistent with the expected average of 2% for *R. flavipes* (Banks & Snyder 1920; Howard & Haverty 1980; Grace 1996) All colonies contained larvae, with totals ranging from 37 to 2,672. Mean queen weight was 9.3 $\pm$ 0.4 mg, almost double the mean king weight, 4.6 $\pm$ 0.01 mg.

In 2003, average colony size increased by 63% to 12,600 $\pm$ 4,761 individuals, ranging from 4,292 to 25,437. With an average of 10,333 $\pm$ 3,937 individuals, workers again comprised 82% of this total. At 2.4 $\pm$ 1.7%, the proportion of soldiers within the colony was essentially unchanged between 2001 and 2003. Larvae totals ranged from 74 to 6,108. Mean primary reproductive weights increased to 15.5 $\pm$ 0.5 mg for queens and to 5.1 $\pm$ 0.06 mg for kings.

2.3.2 Rates of change over 10 years

Although the demographic profiles of the two unrelated lineages differed significantly in many respects during the 10 year observation period, minimal variance existed among their rates of change. Thus, data from Lineage 1 and Lineage 3 were pooled for the growth rate analyses. Colony populations increased dramatically during Interval 1 when the colonies aged from 2 to 6 years old, rising by over 1,000%. Adjusted for growth per year (277%), the change in this Interval was significantly greater

(P=0.0112, t=9.35, df=2) than in the two subsequent periods, when growth slowed to 22% and 33%, respectively (Figure 1).

In both lineages, individual workers, queens, and kings experienced two phases of growth separated by an interval of either greatly reduced or negative growth. Mean worker weights increased by an average of 9.5% per year in Interval 1, only to decrease by 4% in Interval 2. This significantly different rate of change (P=0.0005, t=46.96, df=2) reversed in Interval 3, when growth accelerated to 14.5% per year (Figure 2).

Queens grew at an uneven pace over the 10-year time period, with annual growth measured at the following rates: 15.0%, 1.2%, 36.4%, and 6.0%. The rapid increase during Interval 3, which occurred when the queens aged from 8 to 10 years of age, was notably higher (P=0.0184, t=7.26, df=2) than either of the two slowest rates, but not significantly different from Interval 1, which recorded growth experienced between 2 and 6 years of age (Figure 3.). King body weights exhibited a similar dip in growth rate during Interval 2 (1.25%), but otherwise showed a steady rise in the rate of increase. The fastest gains were observed during Interval 4 (16%). This two rates differed significantly from each other (P=0.0248, t=1.85, df=2), but neither differed significantly from the other two Intervals (Figure 4).

2.3.3 Differences between parental lineages

Data from the two unrelated alate lineages were analyzed for differences in total population size, caste proportions and individual body weights. Table 1 provides a condensed comparison of this demographic information. Across both founding lineages, colonies contained a wide array of soldier proportions, ranging from 0.55 to 6.9%.

Figure 1. Mean change in *R. flavipes* population growth rates in three time intervals. Error bars are standard errors of the mean. Significant differences (P<0.05) denoted with an asterisk (*).

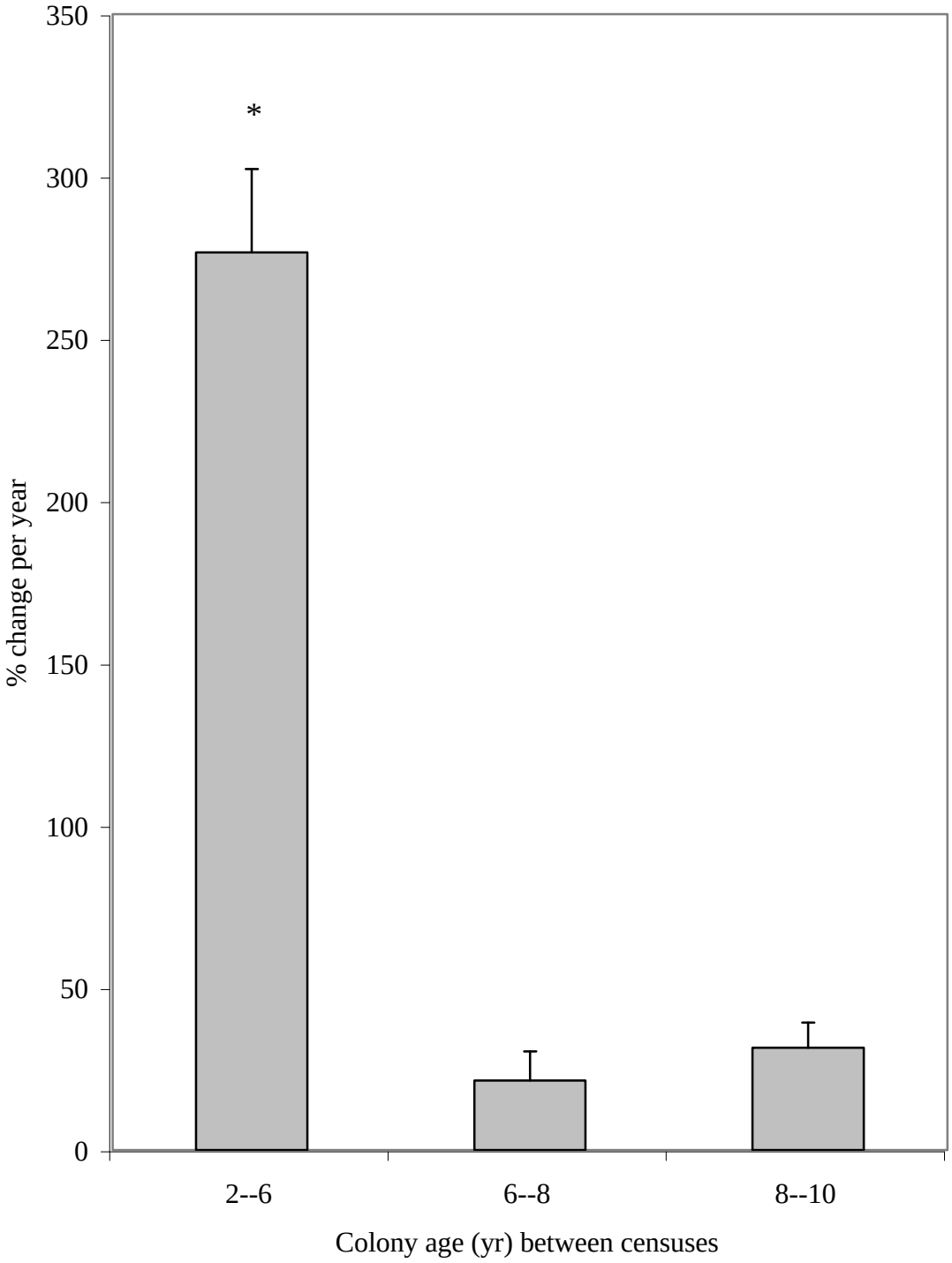


Figure 2. Mean change in *R. flavipes* worker growth rates in three time intervals. Error bars are standard errors of the mean. Significant differences (P<0.05) denoted with an asterisk (*).

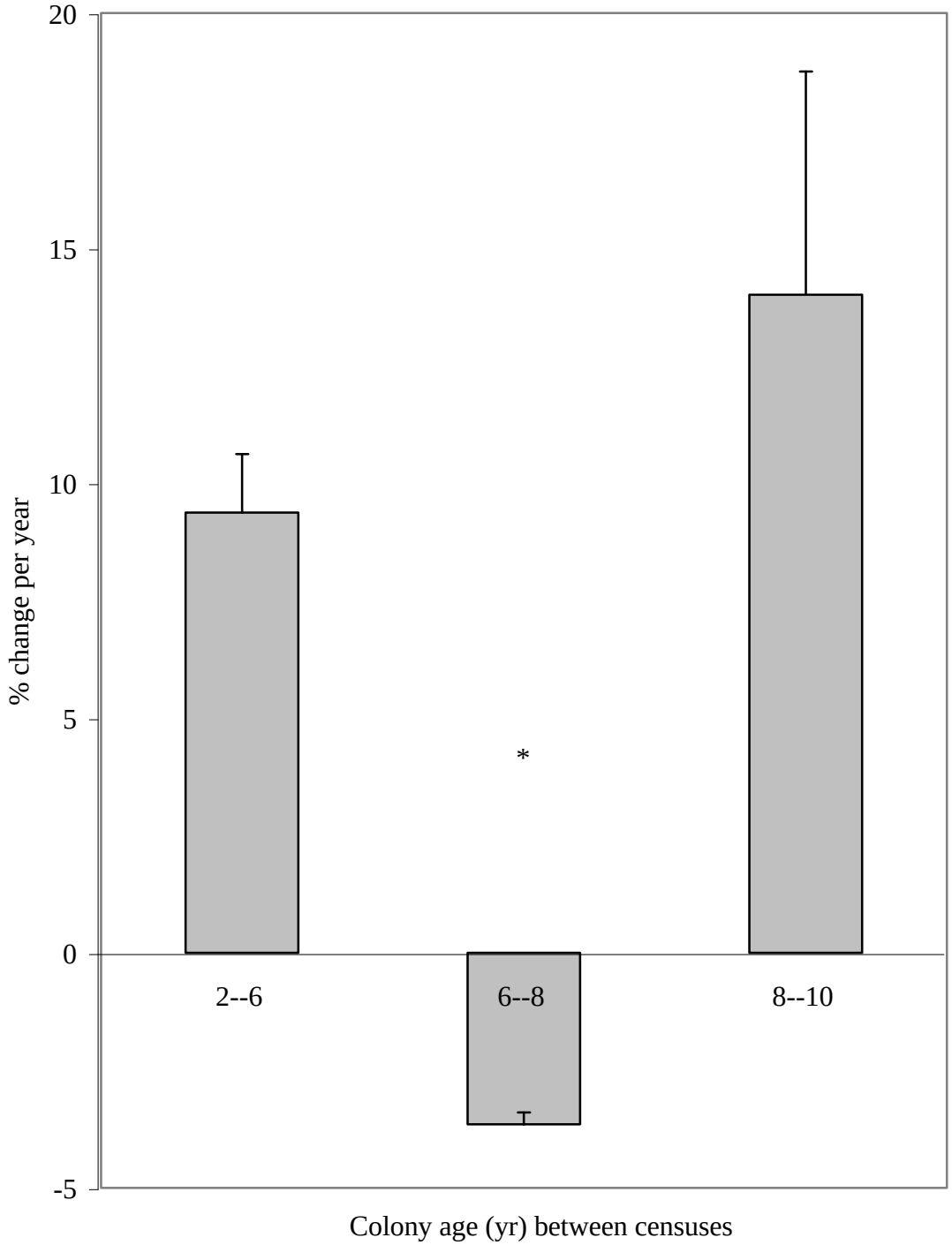


Figure 3. Mean change in *R. flavipes* queen growth rates in four time intervals. Error bars are standard errors of the mean.

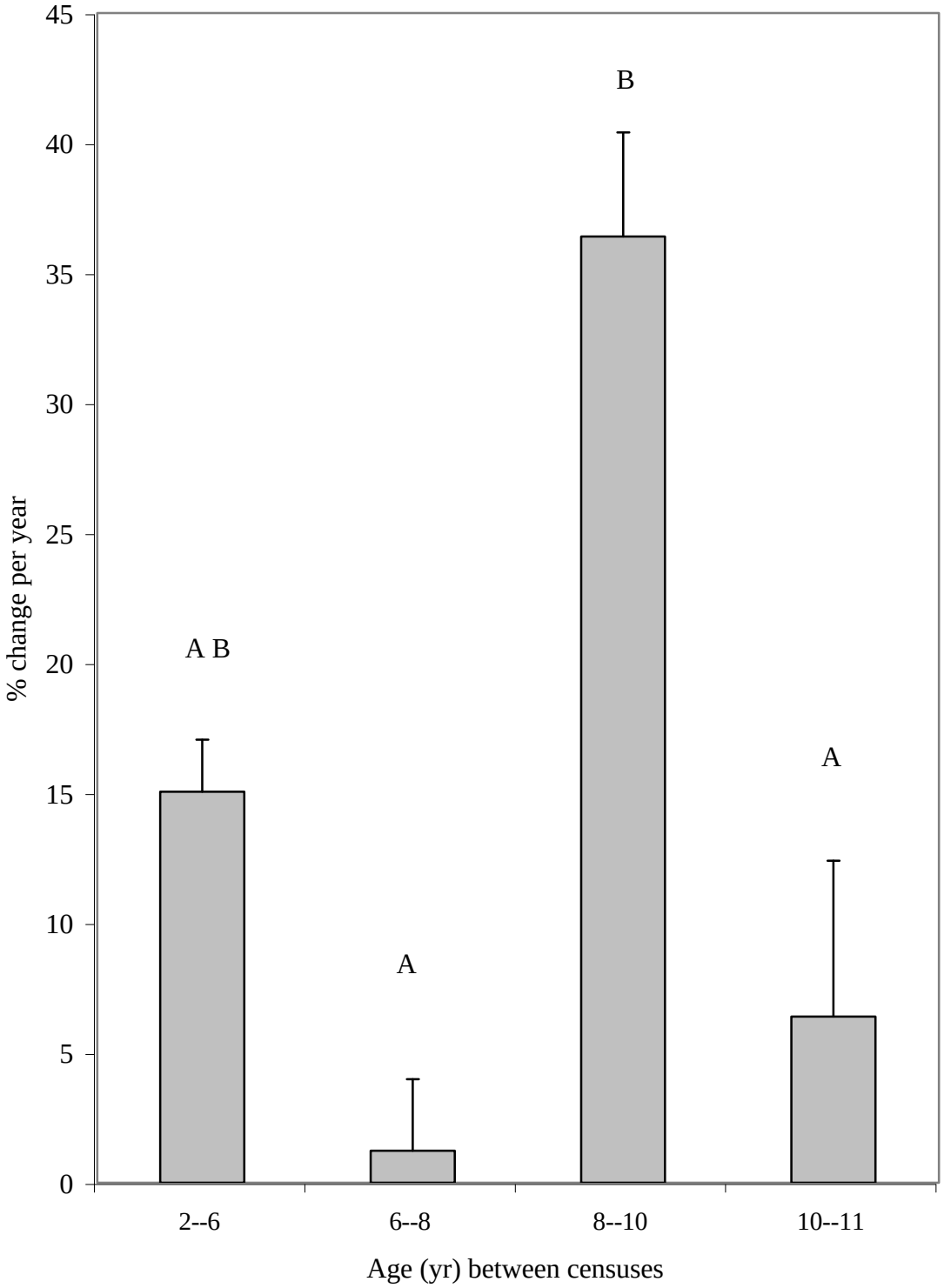


Figure 4. Mean change in *R. flavipes* king growth rates in four time intervals. Error bars are standard errors of the mean. Significant differences (P<0.05) are indicted by different letters (LSM).

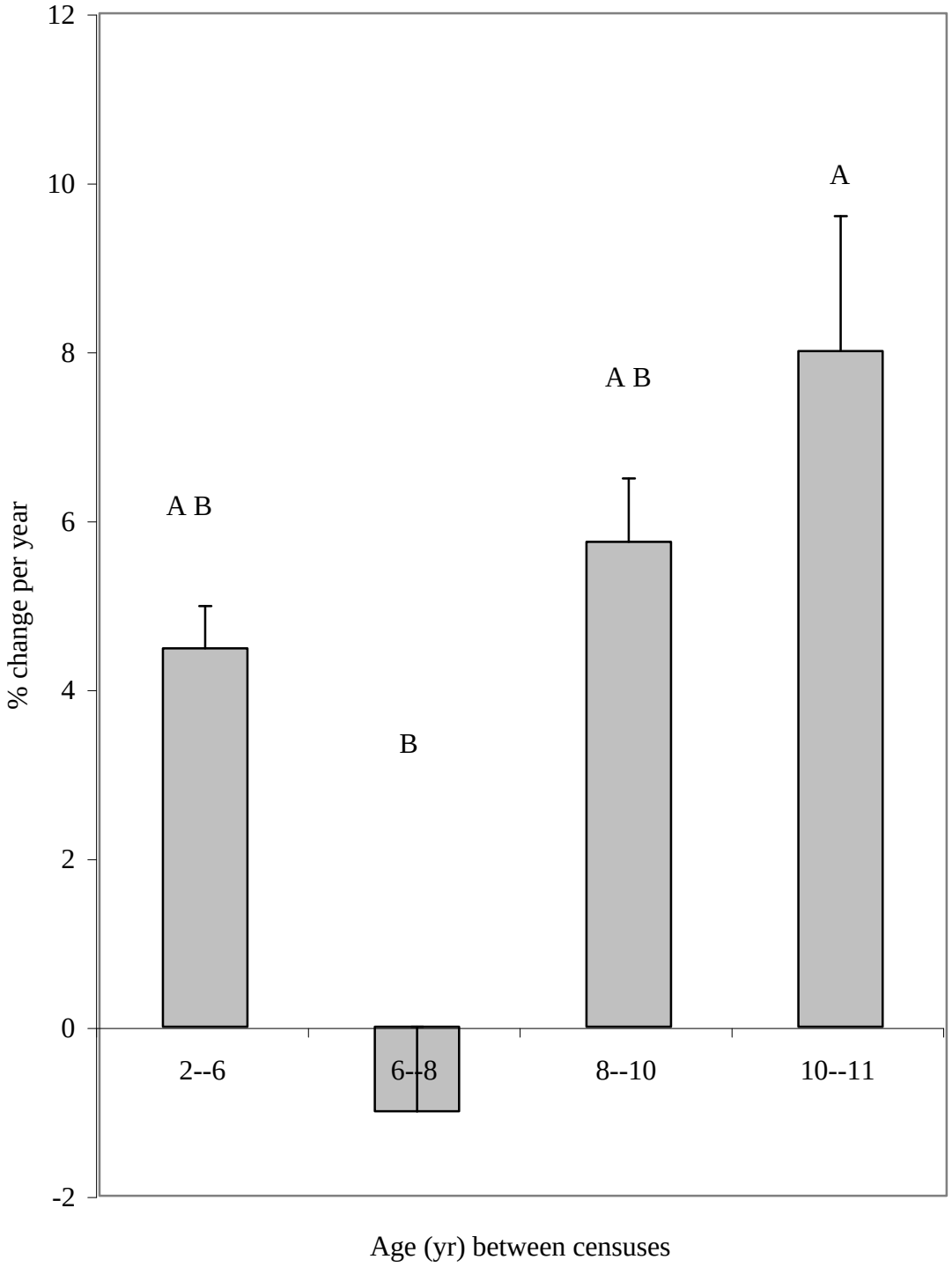


Table 1: A comparison of demographic data (mean ± SE) from two unrelated lineages of *R. flavipes* gathered on five dates. Significant differences (P<0.05) are noted with an asterisk (*).

	Age (yr)	Year	Lineage 1	Lineage 3	t-test results
Colony size	2	1995[a]	348 ± 35	500 ± 49	P=0.0320*
	6	1999[b]	4,555 + 478	5,518 + 559	P=0.2130
	8	2001[c]	7,324 ± 470	6,891 ± 487	P=0.5870
	10	2003[d]	13,060 ± 1,102	10,187 ± 718	P=0.2780
Worker wt	2	1995[e]	2.1 ± 0.04	1.9 ± 0.06	P=0.0060*
(mg)	6	1999	2.9 ± 0.1	2.6 ± 0.1	P=0.0910
	8	2001	2.7 ± 0.1	2.4 ± 0.1	P=0.0141*
	10	2003	3.2 + 0.1	3.3 + 0.1	P=0.6870
Queen wt	2	1995	6.4 + 0.2	5.0 + 0.2	P<0.0001*
(mg)	6	1999	8.6 ± 0.4	9.3 ± 0.5	P=0.3000
	8	2001[f]	9.3 ± 0.4	9.0 ± 0.4	P=0.6220
	10	2003[g]	15.3 ± 0.2	16.3 ± 0.2	P=0.7780
	11	2004[h]	16.3 ± 1.4	15.4 ± 1.4	P=0.6645
King wt	2	1995	4.1 ± 0.2	3.6 ± 0.4	P=0.0160*
(mg)	6	1999	5.0 + 0.1	4.1 + 0.2	P=0.0002*
	8	2001	4.8 ± 0.1	4.1 ± 0.1	P<0.0001*
	10	2003	5.2 + 0.01	4.7 + 0.02	P=0.0500*
	11	2004[i]	5.8 ± 0.3	5.0 ± 0.1	P=0.0972

a Unless noted, Lineage 1 N=42 and Lineage 3 N=13 for 1995 data.
b Unless noted, lineage 1 N=9, Lineage 3 N=10 for 1999 data.
c Unless noted, Lineage 1 N=20, Lineage 3 N=9 for 2001 data.
d Unless noted, Lineage 1 N=20, Lineage 3 N=4 for 2003 data.
e Lineage 1 N=32, Lineage 3 N=12.
f Due to queen deaths, Lineage 1 N=17, Lineage 3 N=9.
g Due to queen deaths, Lineage 1 N=16, Lineage 3 N=4.
h Limited colonies were sampled in 2004; Lineage 1 N=5, Lineage 3 N=3.
i Limited colonies were sampled in 2004; Lineage 1 N=9, Lineage 3 N=3.

Lineage 1 colonies repeatedly exhibited a significantly higher proportion of soldiers than Lineage 3 nests, containing more than twice as many soldiers in all time periods (Figure 5).

The colonies derived from Lineage 3 contained significantly more members than those founded by Lineage 1 alates during the initial 1995 census, but in subsequent years total colony population sizes from the two parental lines were equivalent (Figure 6). Across all castes, individuals from Lineage 1 were generally larger than their Lineage 3 counterparts at each census. In the first 8 years, Lineage 1 workers weighed more at all three census dates, significantly so in 1995 and 2001 (Figure 7). Workers from the queenless colonies were excluded from the 2001 analysis, when colony reproductive status appeared to influence worker size. Insufficient data exist to compare soldier weights in the first three censuses; however, in 2001 and 2003, Lineage 1 soldiers were significantly larger than those in Lineage 3 ($P=0.0360$, $t=0.63$, $df=28$ and $P=0.0211$, $t=2.48$, $df=22.2$, respectively). In all time periods, Lineage 1 kings were heavier than Lineage 3 kings, significantly so at all but the 2004 census (Figure 8). With the exception of 2003, Lineage 1 queens were larger than their Lineage 3 counterparts, significantly so in 1995 (Figure 9).

2.3.4 Queenright vs. Queenless colonies

The 1995 population census focused on incipient colonies; seven of the 62 surviving colonies had lost their primary reproductives and were excluded from the count. Colonies which had lost primary reproductives were included in surveys beginning in 2001 in order to gain insight into the effects of shifting reproductive

Figure 5. Mean proportion of *R. flavipes* colony constituted by soldier caste. Error bars are standard errors of the mean. Significant differences (P<0.05) denoted with an asterisk (*).

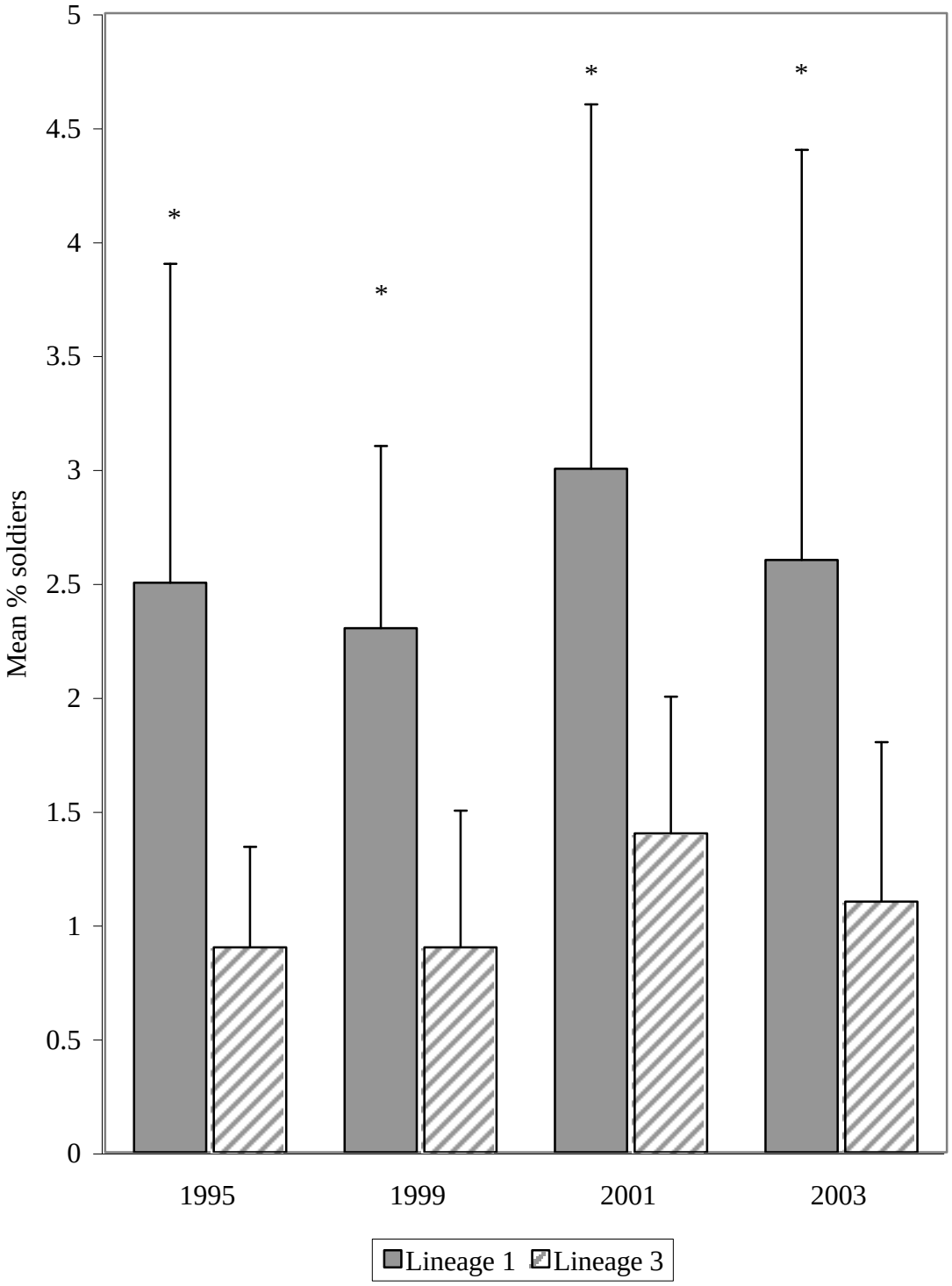


Figure 6. mean total colony growth for two *R. flavipes* lineages over time. Error bars are standard errors of the mean. Significant differences (P<0.05) denoted with an asterisk (*).

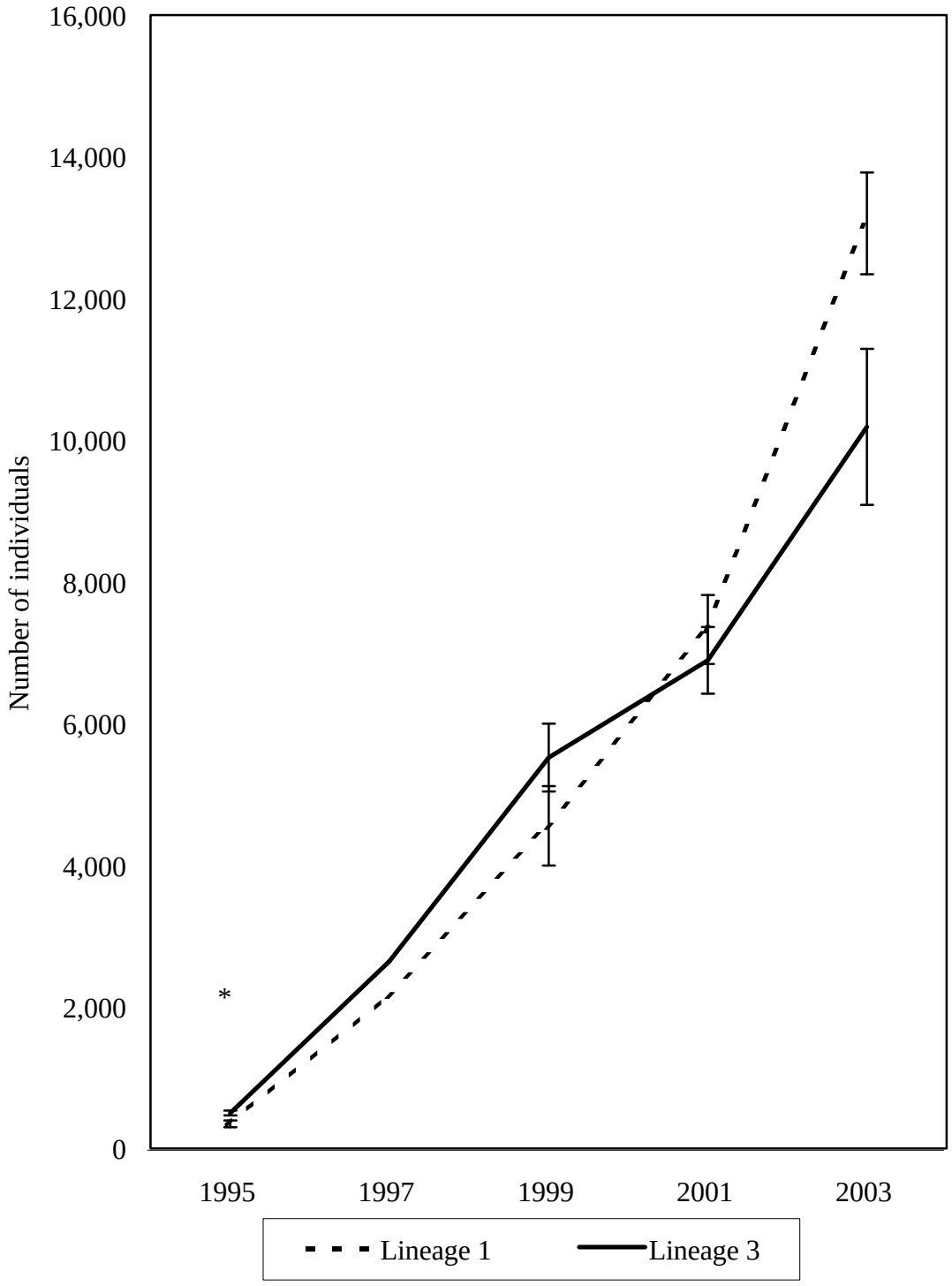


Figure 7. Mean *R. flavipes* worker weights over time. Error bars are standard errors of the mean. Significant differences (P<0.05) denoted with an asterisk (*).

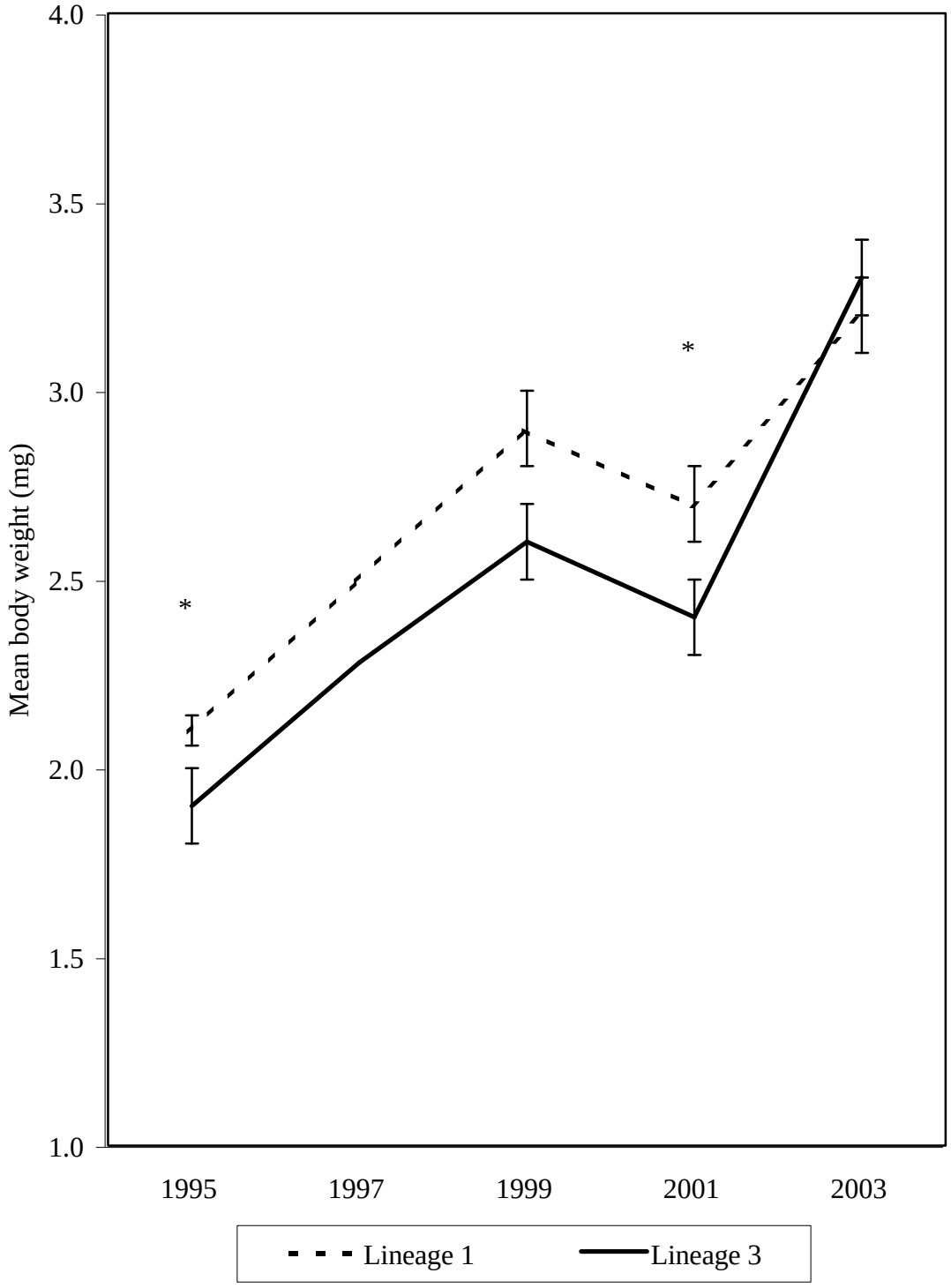


Figure 8. Mean *R. flavipes* king weights over time. Error bars are standard errors of the mean. Significant differences (P<0.05) denoted with an asterisk (*).

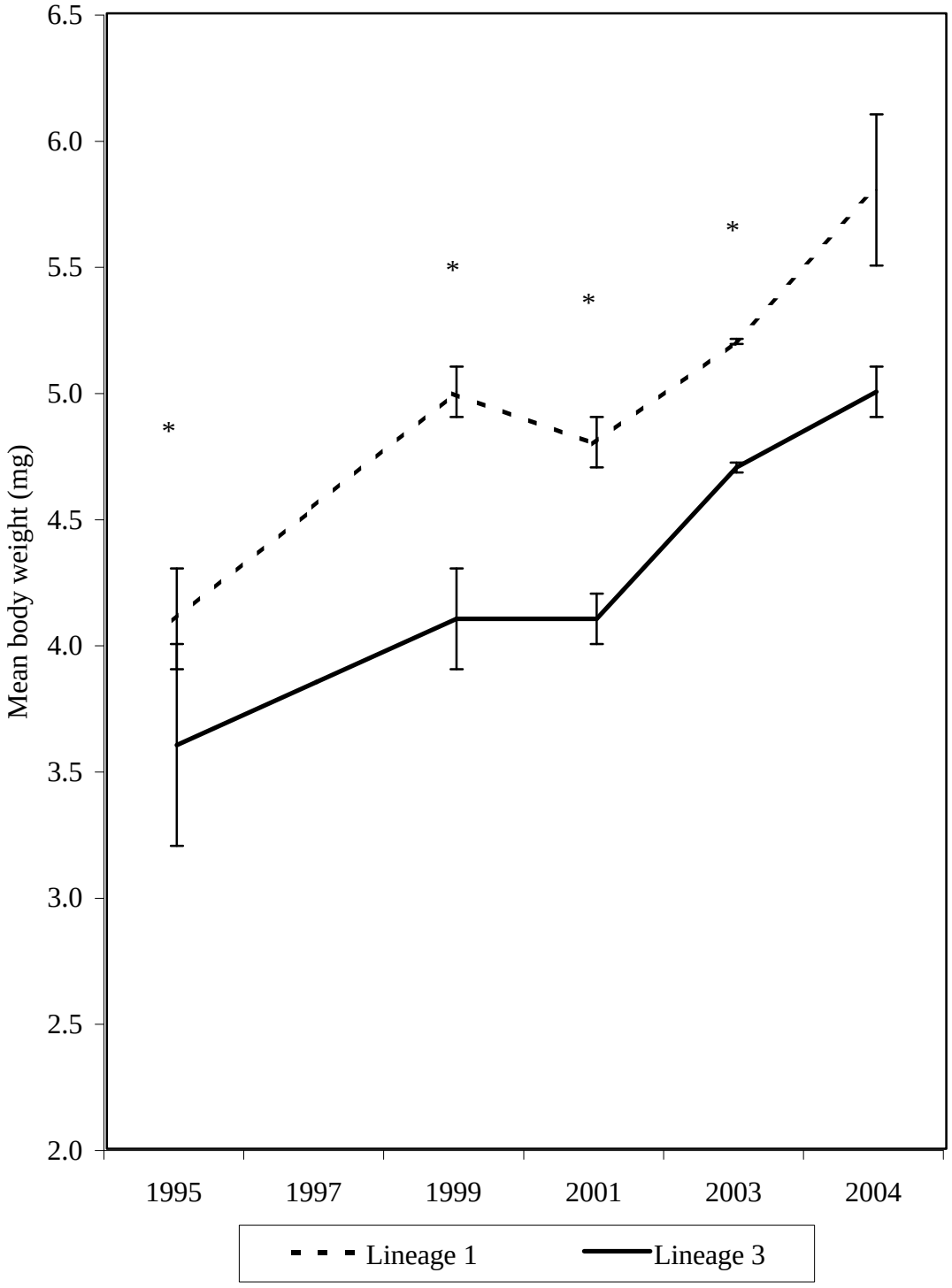
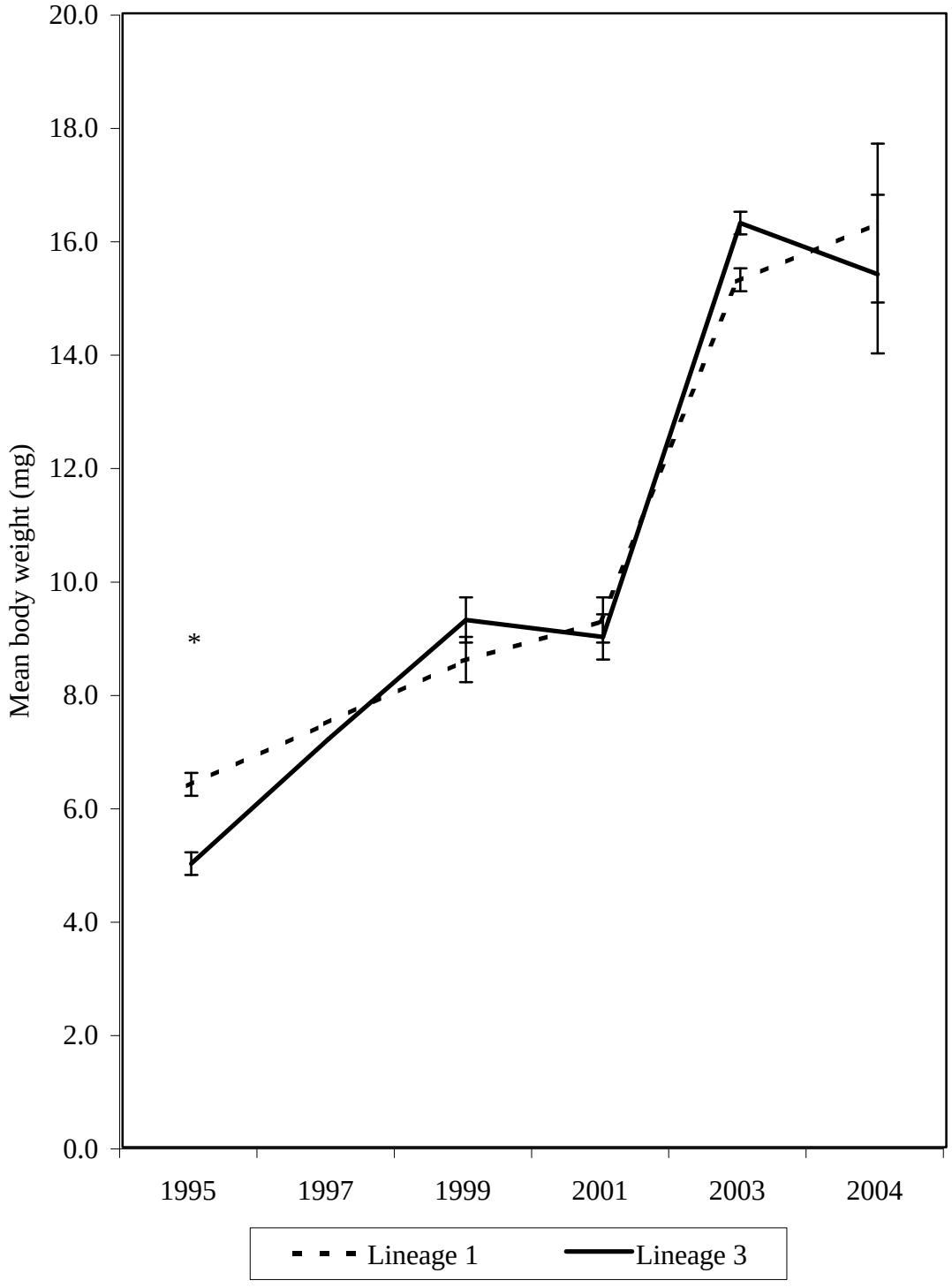


Figure 9. Mean *R. flavipes* queen weights over time. Error bars are standard errors of the mean. Significant differences (P<0.05) denoted with an asterisk (*).



dynamics on colony demographics. In 2001, at 8 years of age, 25 of 29 (86%) colonies retained their primary queens and 28 of 29 (97%) their primary kings. Over the next three years, two additional queens were lost, dropping the queenright proportion to 22 of 28 (78%). Chi-squared analysis indicated that queen survivorship was similar for both lineages (x^2=0.160). Table 2 summarizes the demographic data for the queenright and queenless colonies.

On each census date, queenless colonies were equivalent to their queenright counterparts in total size, worker and soldier number, soldier weight, and egg rank. Although queenright colonies contained significantly more larvae (P=0.0007, t=-4.16, df=17) and smaller workers (P=0.0422, t=2.15, df=24) in 2001, these differences subsided in 2003. Beginning in 1999, the queenless (and one kingless) colonies contained vastly more nymphs than the queenright colonies.

2.3.4.1 Female neotenics

Among the queenless families, the number and size of female neotenics generated by the colonies were analyzed relative to the length of time the colony had been without its primary queen, to the size of the colony, and to the number of neotenic females within the nest. Neotenic totals were available beginning in 1999; individual weights were collected in 2003 and 2004. There was no way to determine whether the same neotenic individuals were present from year to year.

The length of time a colony was queenless was estimated by summing the years the queen was known to be deceased. For example, although the Colony 36 queen died between the 1999 and 2001 censuses, 2001 was considered its first “queenless year.”

Table 2: A comparison of demographic data (mean + SE) from queenright and queenless *R. flavipes* colonies in 2001 and 2003 when the colonies were 8 and 10 years old, respectively. Significant differences (P<0.05) are noted with an asterisk (*).

	Year	Queenright		Queenless		t-test results
Colony total	2001 [a]	7,548	+ 535	6,373	+ 923	P=0.3390
	2003 [b]	12,944	± 1,084	11,511	± 2,103	P=0.5318
Workers	2001	6,560	± 460	4,999	± 705	P=0.1560
	2003	10,758	± 902	8,104	± 742	P=0.2238
Larvae	2001	729	+ 139	63	+ 30	P=0.0007*
	2003	2,190	± 328	2,332	± 790	P=0.8460
Soldiers	2001	163	± 18	229	± 51	P=0.1890
	2003	320	± 58	194	± 57	P=0.2582
Nymphs	2001	0.72	± 0.28	550 [c]	± 86	P=0.0001*
	2003	5.6	± 1.5	586	± 250	P=0.0003*
Eggs	2001	175	± 64	63	± 30	P=0.1300
	2003	710	± 182	616	± 201	P=0.7895
Worker wt (mg)	2001	2.7	± 0.1	3.0	± 0.2	P=0.0422*
	2003	3.2	± 0.09	3.2	± 0.18	P=0.9016
Soldier wt (mg)	2001	3.1	± 0.1	3.0	± 0.1	P=0.9400
	2003	3.4	± 0.2	3.4	± 0.26	P=0.8789

a Unless noted, 2001 Queenright N=17, Queenless N=4.

b 2003 Queenright N=19, Queenless N=6.

c One recently orphaned colony was excluded from this computation, thus N=3.

Thus, Colony 36 was considered queenless for four years (2001-2004), although this may have been an underestimation of up to two years. The length of time a colony was queenless was independent of the number of female neotenics produced (r=0.15, P=0.6285) (Figure 10) and had no significant effect on neotenic weights (P=0.6860, F=0.19, df=1).

Neotenic production was not significantly impacted by the size of the colony (P=0.1658, F=20.94, df=6). Although the queenless colonies increased in total size between 1999 and 2003, the average number of female neotenics per colony decreased significantly over this period (P=0.0491, F=4.07, df=3). In 1999, queenless nests contained an average of 11.0 female neotenics (range: 3-15). By 2004, totals ranged from 1-12 individuals with a mean of 5.7 (Table 3, Figure 11). Rather than being evenly distributed across these ranges, neotenics were produced in clusters of either a few, large individuals or numerous, small neotenic sisters (Figure 12). When between 1 and 3 sisters differentiated within a colony, their mean weight (15.8 $\pm$ 2.0 mg) was significantly higher (P=0.0500, F=7.13, df=1) than the average weight (8.1 $\pm$ 2.1 mg) of sisters within groups of between 6 and 14 individuals. In fact, in both 2003 and 2004, the mean weight of the larger neotenics was not significantly different from the female primary reproductives in the queenright colonies (P=0.1388, t=-1.53, df=24).

Regardless of group size, the differentiation of female neotenics resulted in significantly greater female reproductive biomass per colony than in queenright colonies of equivalent age (P=0.0414, t=2.18, df=20). Primary queens comprised an average of 17.4 $\pm$ 1.4 mg of biomass per colony. Weights of individual members of the small assemblies were statistically indistinguishable from primary queens, but the sisters

Figure 10. Relationship between the minumum time a *R. flavipes* was queenless and the number of female neotenics produced. The number of female neotenics was statistically independent of the time the colony had been queenless (r=0.15, P=0.6285).

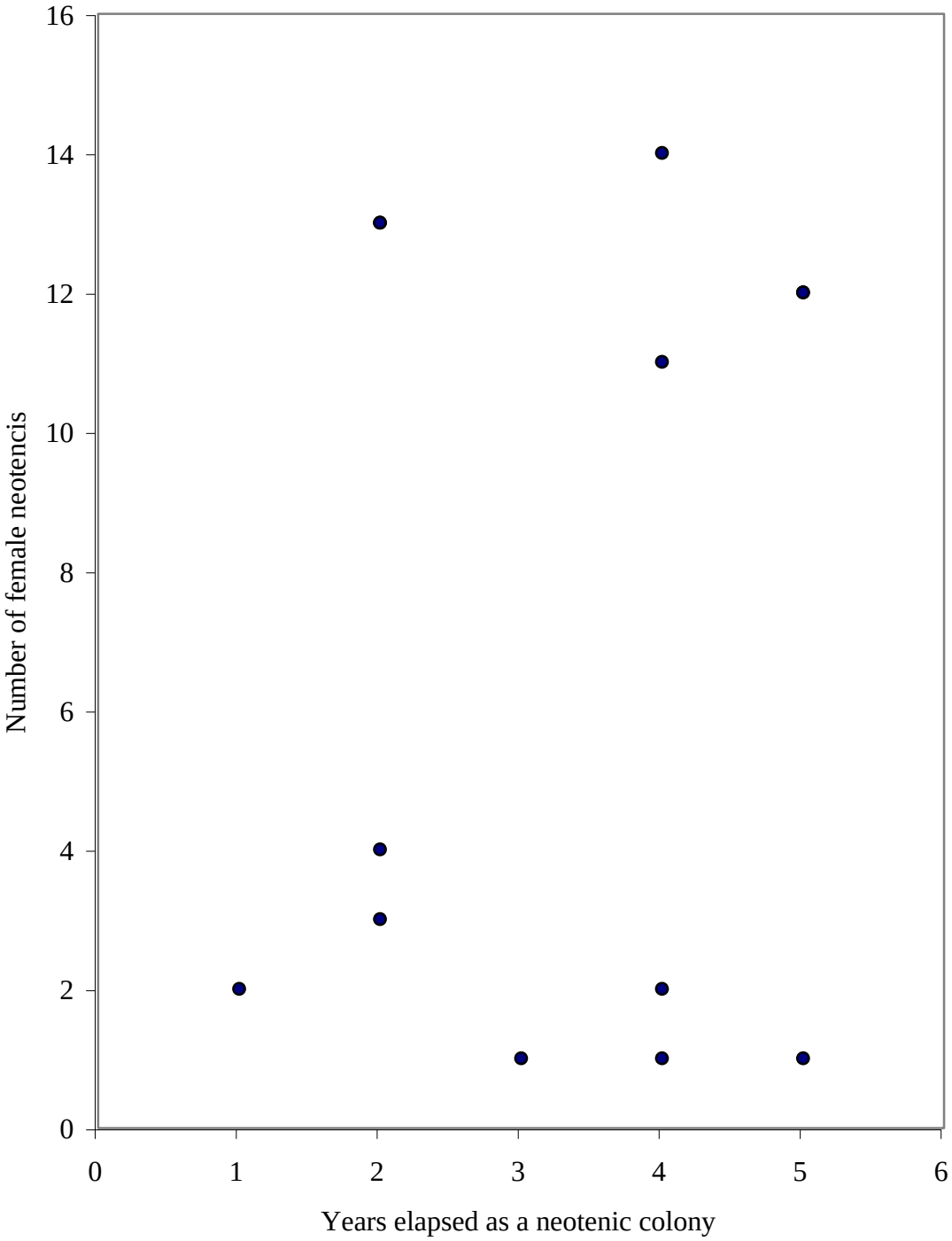


Table 3: The number of female neotenics present in queenless *R. flavipes* colonies at 6, 8, 10 and 11 years of age. The mean numbers of neotenics changed significantly over time (P=0.0491, F=4.07, df=3). Significant differences (P<0.05) are indicted by different letters (LSM).

Year	Number of colonies	Range of neotenic females	Mean ± SE
1999	3	3 - 15	11.0 ± 4.0 a
2001	4	1 - 13	7.8 + 3.1 ab
2003	5	1 - 14	6.2 ± 2.6 b
2004	6	1 - 12	5.7 ± 5.2 b

Figure 11. Numbers of female neotenics in queenless *R. flavipes* colonies. Error bars are standard errors of the mean. Significant differences (P<0.05) are indicted by different letters (LSM).

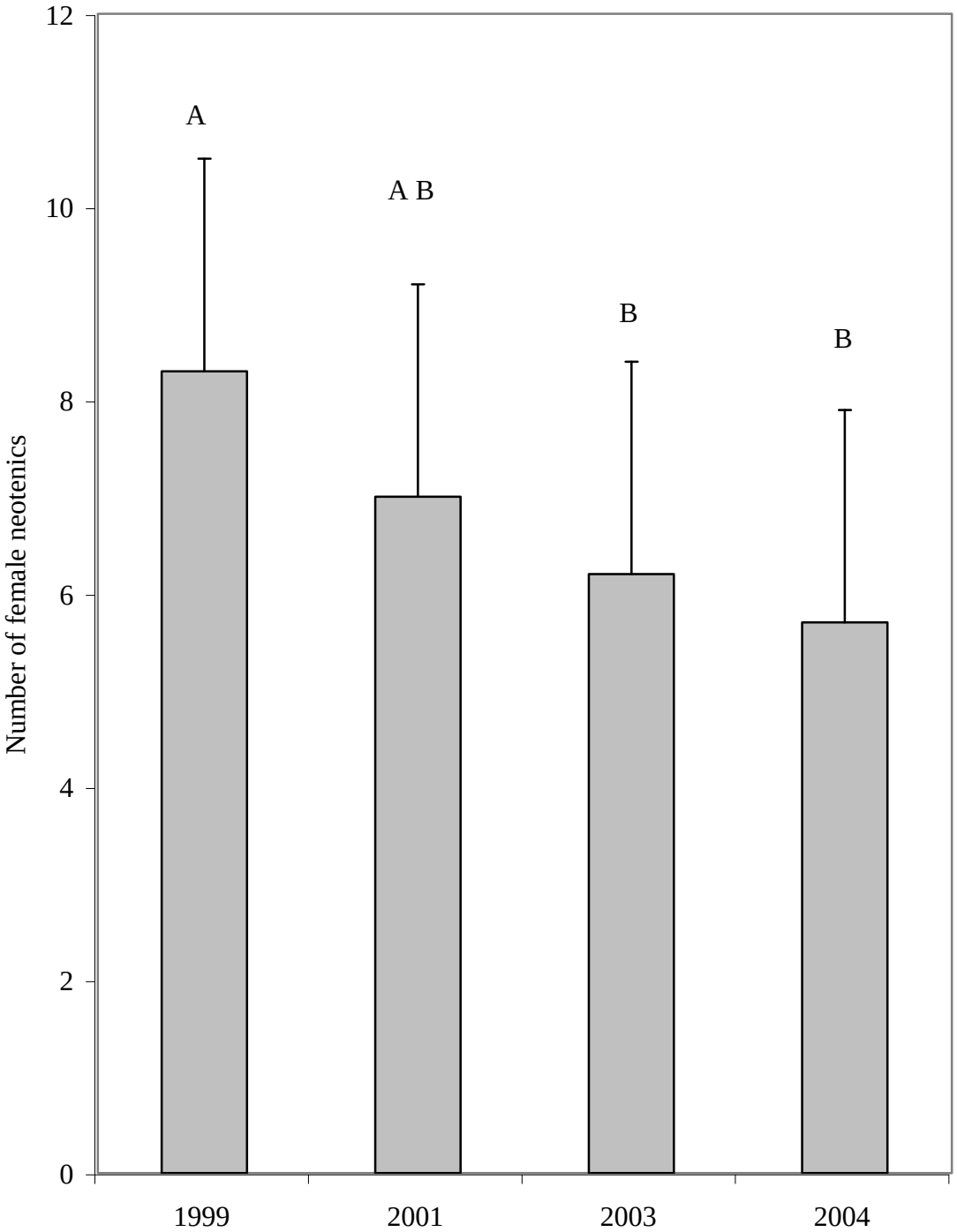
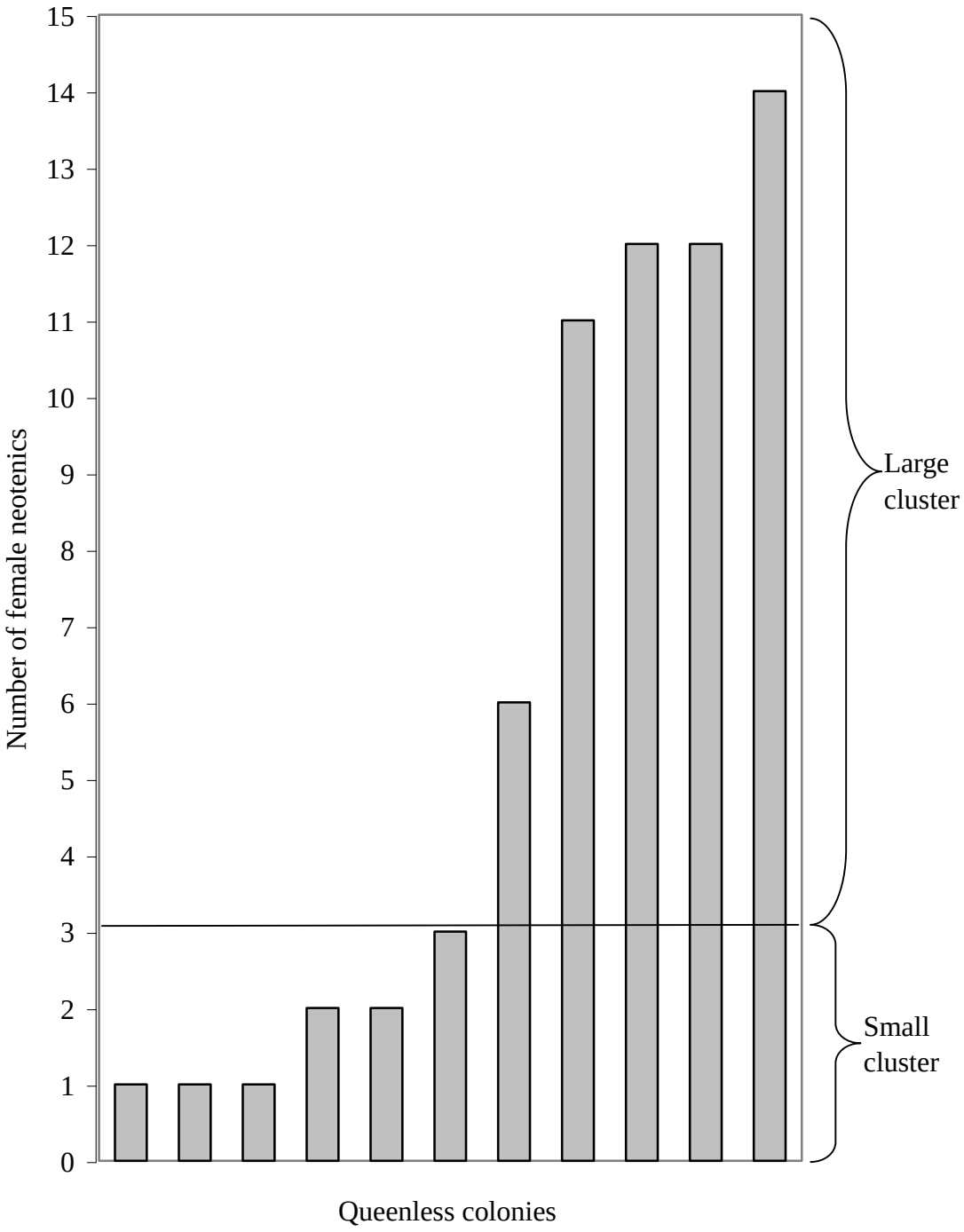


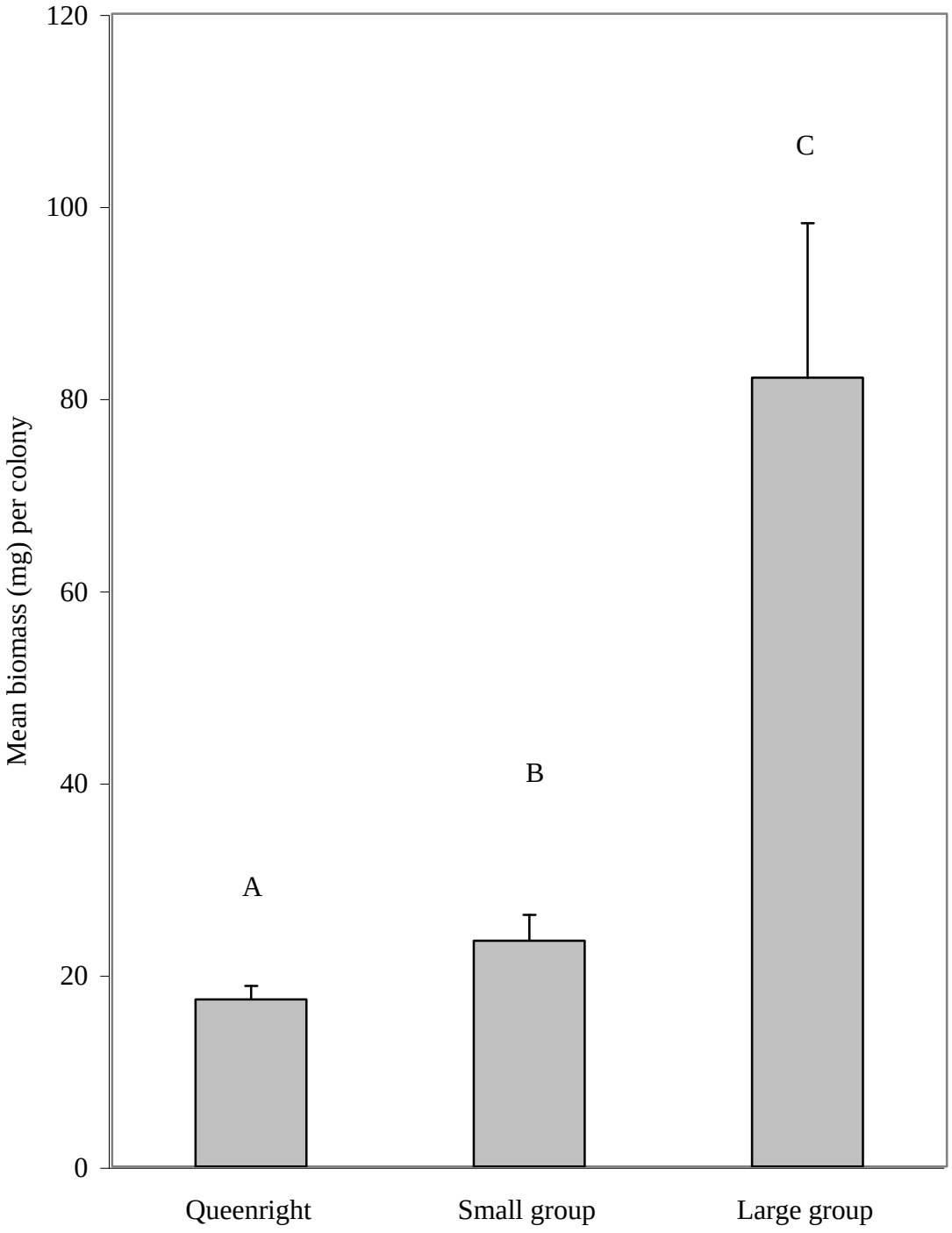
Figure 12. Rank order of the total number of *R. flavipes* female neotenics found during the 2001 and 2003 censuses. A neotenic cluster with >3 individuals was considered large, one with <3 was considered small.



combined for a mean of 23.5 $\pm$ 2.7 mg of reproductive biomass per colony. Large clusters contained a mean aggregate biomass of 82.1 $\pm$ 16.1 mg (Figure 13).

In the single colony in which the primary queen outlived the king, 75 female and 52 male neotenic offspring developed. Five of these females were visibly physogastric. In order to test the egg-laying capability of the queen and her coexisting neotenic daughters, the queen and a random subset of the female neotenics were held individually for 72 hours. In this time period, each of the females, including several non-physogastric individuals, produced eggs. The viability of these eggs and the maternal origin of the nearly 1,000 eggs found in this colony is unknown. Pooled together, the 75 neotenic sisters (6.8 $\pm$ 1.1) weighed significantly less than females from a typical large cluster of secondary reproductives ($P=0.0132$, $t=-2.53$, $df=82$); however, when the five physogastric individuals (8.6 $\pm$ 0.3) were analyzed separately, their weights were statistically comparable to those in the populous cluster ($P=0.4326$, $t=0.83$, $df=8$).

Figure 13. Mean female reproductive biomass in 10-year old queenright and queenless *R. flavipes* colonies. Error bars are standard errors of the mean. Significant differences ($P<0.05$) are indicted by different letters (LSM).



2.4 DISCUSSION

2.4.1 Lifespan of primary reproductives

These laboratory colonies provided a rare, and possibly unique, opportunity to monitor complete *R. flavipes* colonies for an extended period of time. In 2001, when the colonies were 8 years old, 88% of the founding queens and 97% of the founding kings were alive. At age 11, king survivorship was unchanged and 72% of queens endured. These laboratory cultures add novel data to Keller's (1998) compilation of ant and termite queen lifespans, which illustrated broad variability in maximum queen lifespans, ranging from a 5.9 year old *Zootermopsis angusticollis* (Hagen) (Isoptera: Termopsidae) queen to *Macrotermes michaelseni* (Sjöstedt) (Isoptera: Macrotermitidae) queens that lived for 19 years.

2.4.2 Colony and individual growth rates

Colony size and individual body weights are likely the product of genetic predisposition, environmental factors, and colony age (Su & Sheffrahn 1988 a, 1988 b, Grace, et al. 1995). Colony sizes in this study's two unrelated *R. flavipes* lineages were equivalent, but individual body weights for all castes were consistently different over time. Given the identical age of these colonies and their common rearing conditions, differences in body size were likely a heritable trait.

There is evidence from *Coptotermes formosanus* (Shiraki) field colonies that as colonies age, total population size and worker weight become inversely related (Shimizu 1962, Grace, et al. 1995). Over an eleven year period, wild *C. formosanus* body weights increased by approximately 15%, but colony sizes fell by 66% (Grace, et al. 1995). The

R. flavipes in the current study did not exhibit this trend. Individual and population growth rates were directly correlated over 10 years. Between 1999 and 2001, when the colonies aged from 6 to 8 years, growth rates in every category either slowed dramatically or, in the case of workers, dropped. Subsequently, rates of growth were equivalent to those recorded before the downturn, suggesting that the reduction was unrelated to colony age. This trend is not linked to any known modification in conditions or handling procedures. It is possible that the quality of the laboratory-wide food resources, which were collected from the field annually, was suboptimal at some point during this period.

2.4.3 Number, size, and sex ratio of neotenics

Neotenic individuals were identified in 25% of the 11 year old *R. flavipes* colonies in this study. All of these colonies retained one of their primary parents: six contained a surviving king and one a surviving queen. Three of the queenless colonies lost their primary females prior to 1999, providing data from colonies whose egg production had been assumed by secondary reproductive females for at least 6 years. All neotenics in the six queenless colonies were female. The one kingless nest contained a female-skewed group of neotenics containing both genders.

In the family Rhinotermitidae, it is thought that multiple female neotenics differentiate simultaneously after queen loss; however, "multiple" is rarely quantified. In the field, *R. flavipes* neotenics have been observed in quantities ranging from a couple, to a few dozen, to several hundred (Banks & Snyder 1920; Snyder 1920, 1954; Esenther 1969; Howard & Haverty 1980). With the exception of Howard & Haverty's (1980)

judgment that each of their field colonies contained at least 50,000 workers, none of the other sources estimated the population size of the sampled colonies. Thus, it is difficult to gauge a colony's expected neotenic output relative to its size and age.

In the current, 11-year study, no more than 15 female neotenics were ever found in a queenless colony. While these colonies increased in total size over time (a mean of over 11,000 in 2003), the average number of female neotenics declined significantly between 1999 and 2004. These seemingly low numbers of female neotenics may have been associated with small colony size (relative to field colonies) or spatial constraints of the laboratory nesting areas. However, the one kingless colony in this study contained 75 female neotenics, suggesting that larger assemblies of neotenics were possible in these conditions.

The *R. flavipes* colonies in this study responded to the loss of a primary queen with two distinct strategies. Regardless of population size or the time spent as a queenless colony, female primaries were replaced by groups of either a few, large females or numerous, small females. Colonies pursued each strategy with equal likelihood. Similar dual strategies have been observed in *Coptotermes lacteus* (Froggatt) (Isoptera: Rhinotermitidae) (Lenz & Runko 1993) and *Porotermes adamsoni* (Froggatt) (Isoptera: Termopsidae) (Lenz 1985). In several *Nasutitermes* species, there is a negative correlation between the number of primary queens in a polygynous association and the egg-laying capacity (as estimated by physogastry) of each queen (Thorne 1984; Roisin & Pasteels 1985, 1986 a, 1986 b).

As predicted by numerous authors, (Miller 1969; Nutting 1970; Myles 1999), the replacement responses of my queenless *R. flavipes* colonies produced significantly more

female reproductive biomass than queenright colonies of equal age. However, the larger clusters generated almost 250% more reproductive biomass than the small groups. Although large field populations (estimated to be at least in the hundreds of thousands) have been attributed to the relatively substantial reproductive output of neotenics (Snyder 1920, 1934, 1954; Pickens 1932; Myles & Nutting 1988; Grace 1996), the queenless colonies in this study grew on pace with their queenright counterparts.

Gender ratios vary within neotenic cohorts that develop in response to the loss of male, female, or both primary reproductives. *C. lacteus* nests that lost both primary reproductives produced cohorts of heavily male-biased neotenics (Lenz & Runko 1993), while comparably orphaned *R. flavipes* colonies produced female-skewed neotenic subpopulations (Howard & Haverty 1980). Grassé & Noirot (1960) determined that the loss of *Kalotermes flavicollis* (Fabricius) (Isoptera: Kalotermitidae) primary queens resulted in development of both male and female neotenics but loss of the king led only to the differentiation of males. In contrast, colonies of *Armitermes euamignathus* (Silvestri) (Isoptera: Termitidae) (Costa-Leonardo, et al. 1996) that lost their queens but retained their kings produced only female neotenics.

In addition to development of secondary reproductives, it has been observed that recently orphaned *C. lacteus* nests produce numerous nymphs of both genders (Lenz & Runko 1993). It has been calculated that orphaned colonies are at greater risk of failure than complete nests; thus, nymph production is undertaken to maximize the survival of a colony's genes via alate dispersal (Lenz & Runko 1993). In the current study, the queenless and (one) kingless colonies contained vastly more nymphs than the queenright colonies. Nymphal individuals may have been produced in anticipation of an alate

dispersal flight (Lenz & Runko 1993) or subterranean colony budding (Snyder 1920), neither of which was possible in the laboratory.

2.4.4 Potential genetic basis for caste proportions

Among polymorphic ant species, caste proportion may be influenced by both environmental variables, including food availability (McGlynn & Owen 2002), conspecific competition (Passera, et al. 1996), and predation (Herbers 1980), as well as internal variables such as colony age (Gibson 1989) and the number of queens in the colony (Kenne, et al. 2000). There has been little examination of the role intrinsic, genotypic factors may play in the determination of social insect caste ratios.

The termite soldier caste is physically distinct and exclusively defensive; soldiers are unable to collect food and are wholly dependent on their nestmates for sustenance (Haverty 1977). The percentage of soldiers in a colony is thought to be consistent within species (Howard & Haverty 1980; Noirot & Darlington 2000), although interspecific competition, predation pressure and seasonality account for some fluctuation (Haverty & Howard 1981; Howard & Haverty 1981). By working in the laboratory with colonies derived from two groups of unrelated alates, the external challenges that might impact caste proportion were greatly reduced, increasing the likelihood that differences in caste ratios were due to heritable traits.

Soldier ratios within *R. flavipes* field colonies range from 0.67 to 10.8% (Banks & Snyder 1920; Grace 1996). Across both lineages, the study colonies contained a mean of 2.6 $\pm$1.6% soldiers, consistent with the published average of 2% for *R. flavipes* (Howard & Haverty 1980). However, Lineage 1 colonies repeatedly exhibited a significantly

higher proportion of soldiers than Lineage 3 colonies, containing more than twice as many soldiers at each census date. These proportions were unaffected by the reproductive status of the colonies; soldiers appeared in equivalent numbers in the presence of either primary or secondary reproductives, a finding consistent with Luykx's (1986) work with colonies of *Incisitermes schwarzi* (Banks) (Isoptera: Kalotermitidae). These findings suggest that genotypic variation underlies a portion of the deviation in soldier ratios observed in conspecific field colonies.

2.4.5 Conclusion

This analysis of 11 year old, whole, laboratory-reared *R. flavipes* colonies provides demographic information regarding colony growth rate and longevity, lifespan of founding reproductives, the response of colonies to the loss of founding kings and queens, and the contribution of intrinsic factors in determining caste ratios and individual body sizes. These fundamental elements of colony ontogeny and demography delineate patterns of growth, investment, reproduction and survivorship that impact both intra- and extra-colony dynamics in these eusocial societies.

Similar ontogenetic field studies have been used to predict territorial expansion rates of both feral (Oldroyd, et al. 1997) and Africanized honey bees (Otis 1982); forecasts of *R. flavipes* reoccupation of disturbed landscapes would be valuable. Insight gained from these *R. flavipes* colonies may aid in the development of nest growth models similar to those available for species of ants many of which, like *R. flavipes*, depend on heterogeneous and unpredictable food resources (Chew 1987; McGlynn, et al. 2002) and

which may have the flexibility to be either mono- or polygynous (Tschinkel 1988; Arcila, et al. 2002).

3 THE IMPACT OF MULTIPLE, SPATIALLY SEPARATED FOOD RESOURCES ON REPRODUCTION, CASTE DISTRIBUTION, AND FORAGING PATTERNS OF *RETICULITERMES FLAVIPES*

3.1 INTRODUCTION

This chapter examines the foraging patterns and caste distribution of *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) colonies across a network of spatially separated feeding sites in the laboratory. Multi-resource foraging arenas provided an unprecedented opportunity to observe the behavior of entire, mature *R. flavipes* colonies as they investigated, occupied, and moved among three resources. Did colonies utilize the resources simultaneously? Did groups of foragers become isolated? Did they exhibit resource fidelity? How were the castes, including reproductives, distributed among the feeding sites? The influence of the spatial environment on termite colonies is poorly understood despite the fact that subterranean termite colonies can occupy territories spanning hundreds of square meters. The more we know about how colonies occupy their space, and disperse among resources within that space, the better able we will be to predict and influence the movement and growth of these ecologically and economically important social insects.

3.1.1 General nesting and foraging strategies

Isopteran nesting and foraging strategies are inextricably linked. In the most primitive families, an entire colony lives and feeds within a single food resource. These "one-piece nesters" do not forage away from the nest; a colony's life-long potential for

territorial expansion and thus population growth is determined by the size and quality of the wood initially chosen by newly mated alates. Intermediate nesters live and feed in a network of resources linked by subterranean galleries. Separate nesters construct earthen mounds or arboreal nests distinct from their food resources (reviewed in Noirot & Darlington 2000). Both derived groups may occupy a single location (monodomy) or the population may be distributed across a series of interconnected nest sites (polydomy or polycalism) (Oster & Wilson 1978; Crozier & Pamilo 1996). A range of social nesting strategies have been defined as polydomous. Traniello & Levings (1986), Pfeiffer & Linsenmair (1998), and Holway & Case (2000) focused on colony foraging patterns and thus classified nests with multiple entrances or satellite feeding areas as polydomous. Alternatively, Stuart (1985), Banschbach & Herbers (1999), and Walin, et al. (2001) defined a polydomous colony as one with numerous, physically discrete caches of eggs and larvae.

3.1.2 *R. flavipes* foraging

R. flavipes are intermediate nesters; it is unclear whether the colonies can also be polycalic. As consumers of dead wood, they depend on fallen timber which is a spatially unpredictable, exhaustible food resource (Myles 1999). Colonies are essentially nomadic, living in subterranean galleries excavated near or within their food resources and moving in response to disturbance, resource exhaustion, competition, predation, or unfavorable environmental conditions. Foragers hunt for food in a systematic, branching pattern that is thought to minimize search redundancy (Robson, et al. 1995; Reinhard, et al. 1997). Estimates of foraging territory dimensions vary widely. In addition to

biological sources of variation such as colony size, food quality and availability, competition, and predation, the available sampling methods are imprecise (Thorne, et al. 1996; Evans, et al. 1998). Mark-recapture studies suggest that *R. flavipes* territories range from 0.6 - >2,000 m^2 (Su, et al. 1993; Forshler & Ryder 1996) and that colonymates can be found in resources up to 79 m apart (Grace, et al. 1989). Using genetic markers to distinguish distinct colonies, DeHeer & Vargo (2004) identified workers from a single colony feeding on at least 16 different resources over the course of 3 years.

Reticulitermes colony mobility, non-random search patterns, and multi-site feeding are well supported experimentally, but many other aspects of their foraging behavior remain unclear. The ebb and flow of workers among colony resources is poorly understood. Although not formally described as such in the literature, subterranean termite foraging is thought to follow the tenets of central-place foraging. In this scenario, foragers leave the nest, gather food, and retrace their steps back to the central nest (Orians & Pearson 1979). In one of the earliest mark-recapture experiments conducted with termite field colonies, Su, et al. (1984) concluded that after depositing their nutritional payload at the main nest, *Coptotermes formosanus* (Shiraki) (Isoptera: Rhinotermitidae) workers redeployed randomly among the colony's established feeding sites; thus, a colony's entire population cycled through all of its feeding sites at random.

Because this random remixing conclusion is a principal assumption in both population estimation models and pesticide delivery strategies, it has been closely scrutinized. Using three Australian species, Evans, et al. (1998, 1999) showed that marked foragers consistently failed to remix uniformly with their unmarked nestmates.

Similar patterns are evident in previous *Reticulitermes* work (Su, et al. 1993; Forschler & Townsend 1996). Thorne, et al. (1996) and Evans, et al. (1998) call this site fidelity; Delaplane & LaFage (1987, 1989) and Delaplane (1990) term it feeding tenacity.

The Lincoln index is a mark-recapture sampling model developed to study migratory birds (Lincoln 1930) which has been used to evaluate everything from squirrel populations (Harris & Stearns 1991) to computer software defects (Briand, et al. 2000). Five assumptions underlie the model, including the random remixing of marked individuals within the whole population. The index has been used to assess the size of foraging territories and populations in several subterranean termite genera (see Thorne, et al. 1996), including *Reticulitermes* (Esenther 1980; Grace, et al. 1989; Grace 1990; Su, et al. 1993; Su 1994). My research colonies provided an unprecedented opportunity to observe *R. flavipes* foraging patterns for evidence of polydomy and central-place foraging, to test for random remixing, and to compare population estimates generated by mark-recapture sampling with precise census data.

3.1.3 Caste distribution

Intra-nest demography is well understood in many higher termites. Species-specific mounds and arboreal nests often conform to narrow architectural specifications, which may include royals cells, nursery chambers, and fungal gardens (reviewed in Noirot & Darlington 2000). These nest substructures house specific castes and age classes. However, caste distribution within the ephemeral galleries of subterranean colonies is virtually unknown. Subterranean termite researchers often imply that colony activity is centered around a main, resource-rich hub containing reproductives and brood.

This assumption appears to be based on the familiar image of a nest rather than data. Field observations suggest that pre-alate nymphs and brood are each often clustered together (pers. obsv.), but with the exception of Lenz, et al. (2001), [a brief communiqué without data], there are no published accounts regarding the spatial distribution of castes within subterranean feeding networks.

The pattern of contact between reproductives and their offspring profoundly impacts development of the brood and cohesiveness of the family unit. Primary reproductives suppress sexual maturation of their offspring with an inhibitory pheromone (Lüscher 1961). In the absence of this influence, immature individuals can differentiate into replacement or supplemental reproductives (collectively termed neotenics) and establish bud or daughter nests (Snyder 1920; Pickens 1932; Esenther 1969; Howard & Haverty 1980; Thorne 1996 a). Nutting (1969) concluded that neotenics could develop and reproduce only in bud nests which were completely isolated from the parent nest, but evidence suggests that *Reticulitermes* workers may maintain contact between the parental nests and satellite resources which contain actively reproducing neotenics (Reilly 1987; Bulmer, et al. 2001). Although laboratory experiments indicate that neotenics can form within weeks of complete physical isolation from their parents and the bulk of their nestmates (Buchli 1958; Wantanabe & Noda 1991; Pawson & Gold 1996), our understanding of neotenic development within a whole, spatially widespread colony is scant.

To examine *R. flavipes* exploitation of multiple, physically separated feeding sites, whole laboratory colonies were introduced to multi-resource foraging networks. Visual observation, periodic censuses, and mark-recapture sampling provided data which

allowed determination of how workers investigated and occupied those resources, how castes were distributed among feeding sites, whether subgroups of termites became isolated in the resources, and if not, whether workers and reproductives traveled among the resources at random. The resulting patterns of travel and brood distribution may alter the informal categorization of *R. flavipes* as a central-place forager and suggest an alternate strategy that utilizes a series of nest nodes in which workers feed and rear brood.

3.2 METHODS

3.2.1 The termites

Thirteen complete laboratory-reared *R. flavipes* colonies derived from two distinct parental lineages were manipulated in this experiment. Nine of the colonies (five from Lineage 1 and four from Lineage 3) contained their primary reproductives and four (all Lineage 1) contained a primary king and at least one neotenic female (see Chapter 2 and Long, et al. (2003) for lineage descriptions). The manipulation began in November 2001, when the colonies were 8 years old, and terminated in November 2004. Colonies averaged 7,108 $\pm$ 479 individuals at the beginning of the experiment. For 3 years prior to this experiment, the colonies were housed in 185 x 187 mm (5.1 liter) clear, plastic, lidded containers (Pioneer Plastics, Inc., North Dixon, KY) and fed a diet of weathered pine (Pinaceae) survey stakes and decayed paper birch *Betula papyrifera* (Marshall) (Betulaceae).

3.2.2 Creation of multi-resource colony networks

Multi-resource networks were created by providing each colony with three feeding sites linked by two foraging pathways. To construct a central nest, the base of a 5.1 liter canister was pierced with a heating element, creating two holes on opposite sides of the container. To build a satellite resource, a single hole was punctured in the base of a 181 x 76.2 mm (1.2 liter) clear, plastic, lidded container (Pioneer Plastics, Inc., North Dixon, KY). Short lengths of clear Tygon® tubing (12.7 mm interior diameter) (Norton Performance Plastic Corp., Akron, OH) were inserted into the holes and sealed in place with hot glue. The central canister was linked to each of two satellites with sections of

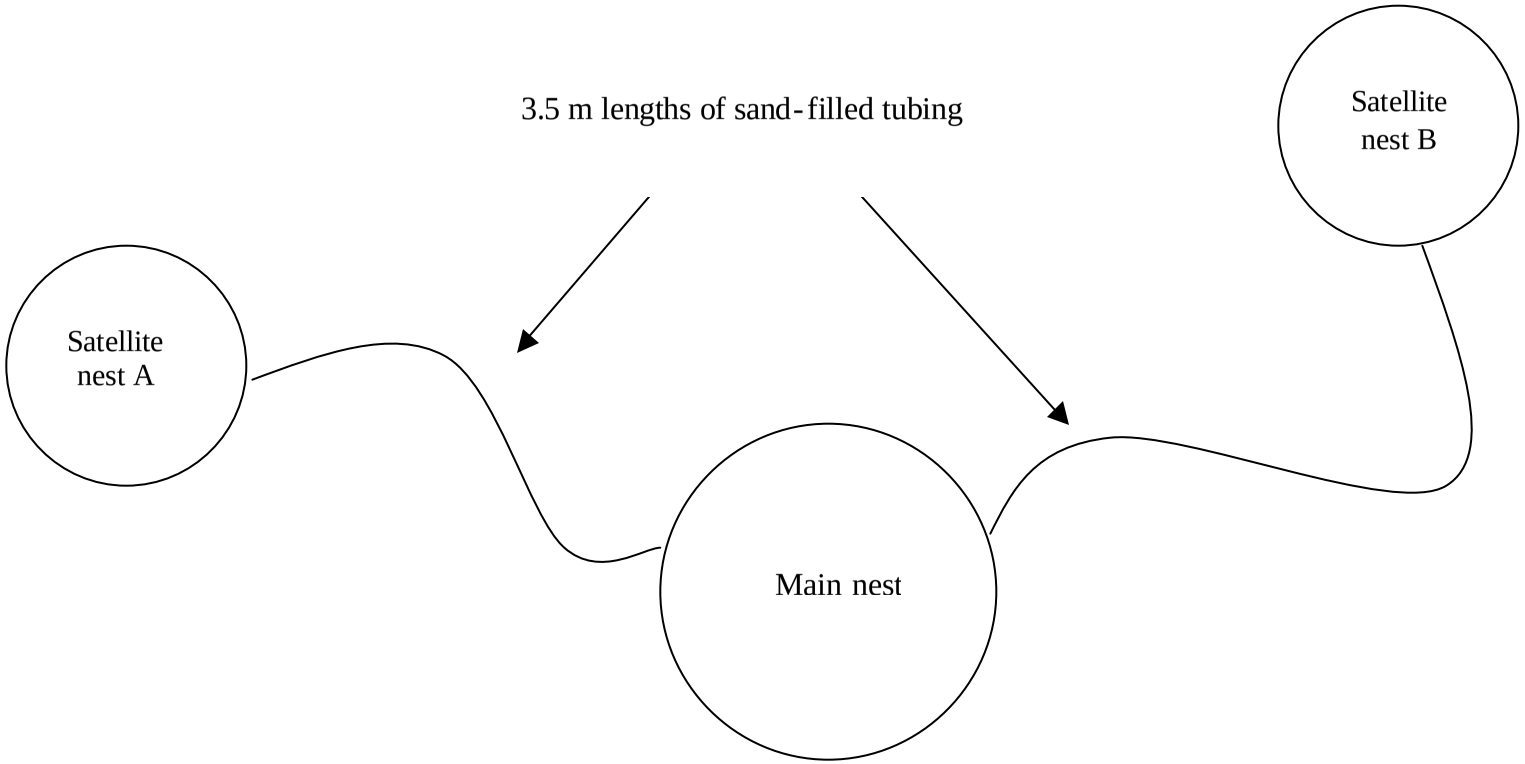
tubing fastened together with quick disconnects (Sigma-Aldrich, St. Louis, MO). The linear distance between the middle chamber and a satellite was approximately 3.5 m. Satellite resource canisters were not directly linked to each other; the distance between them, via the central resource, was about 7 m. Using conventional means to extrapolate foraging territory size from points of observed feeding activity (Grace, et al. 1989; Forschler & Ryder 1996), the experimental networks comprise foraging territories of just over 6 m^2, which are comparable to those observed in the field (Forschler & Ryder 1996; Haverty, et al. 2000; Vargo 2003).

To make the tunneling activity required of foragers realistic, the tubing was loosely filled with moistened, autoclaved play sand (Global Stone Corp., Oakville, Ont.). The pathways were provisioned at 10 cm intervals with birch slivers to encourage the termites' natural investigative behavior and to simulate "snack" cellulose sources, such as roots or debris, found in field soils. All three feeding locations were provisioned with equal volumes (approximately 1 liter) of moistened birch. The birch was prepared by cutting ~ 4 cm diameter logs into 1 cm-thick discs. Discs were stacked, providing the termites with a resource that could be easily dismantled for inspection and census. Figure 14 illustrates this multi-resource colony network.

3.2.3 Travel among the resources

To populate a multi-resource network, an entire termite colony was transferred from its original 5.1 liter nest canister into the central hub of the three-part network.

Figure 14. Schematic of multi-resource colony network. The main nest, a 5.1 liter plastic canister, was linked with two 1.2 liter satellite nests using 3.5 m lengths of sand-filled Tygon® tubing. See text for description of food type and locations.



Workers excavated tunnels through the sand, with the tubing itself forming the floor of the trail; activity was easily observed by placing a mirror under the tubing. Although individual termites were impossible to track throughout the network, the volume and direction of forager traffic provided a surrogate measurement of worker exchange among the three resources. The number of workers and their direction of travel was recorded for 2 minutes at each of the four portals (two in the main nest and one at each satellite). Observations were made weekly for 30 weeks.

The impact of colony parental lineage and reproductive status (either primary or secondary reproductives) on colony travel patterns was analyzed using four metrics: i) the time needed for workers to discover the tunnels and ii) to reach the terminal satellites, iii) the traffic volume within the tunnels, and iv) the travel differential, defined as the number of workers traveling outbound (away from the central nest) subtracted from those moving inbound (toward the central nest). Preliminary analysis indicated that the parental lineage variable could appropriately be nested within the reproductive status variable; thus this combined effect is hereinafter referred to as "colony category." Minimal variation existed between travel means on the two pathways in each network, so data were pooled within colonies for all analyses.

The tunnel and satellite discovery data were analyzed with mixed model ANOVA after undergoing transformation to meet the assumptions of normality. After assumptions testing of the volumetric and differential data sets with ANOVA, more sensitive ANCOVA were performed with time as the covariate. It was hypothesized that no linear relationships existed between the traffic observation times (H_O: b=0), and that when

adjusted to the mean time (week 13.9), means were equal among the three colony categories (H_O: $\mu_1 = \mu_2 = \mu_3$; H_A: $\mu_1 \neq \mu_2 \neq \mu_3$). For both data sets, the initial, full-model ANCOVA was a one-way treatment structure with linear and quadratic covariates in addition to the main, fixed effects. None of the covariate interactions indicated significant differences and were discarded. Thus, the final ANCOVA models were reduced to tests of the covariate (time) and the fixed effect class variable (category).

3.2.4 Population distribution

With three potential feeding locations, two questions emerged regarding distribution of the castes throughout the multi-resource networks: i) were satellite and central chambers equally likely to be inhabited by termites, and ii) independent of resource type, were members of each caste dispersed evenly among the three sites? In August 2003, 20 months after initiation of the foraging networks, the colonies were fully censused. Totals of each caste (primary or secondary reproductives, workers, soldiers, nymphs, larvae, and eggs) were tallied in each of the three resources and the two connecting tubes. Prior to disassembly of a network, the resource portals were blocked to prevent the mass movement of individuals in response to the disturbance. Afterwards, the networks were reassembled and the termites repatriated to their resources.

First, mixed-model ANOVA were performed for each caste to determine whether individuals were more likely to be found in one of the three resources: (H_O: $\mu_{Sat\ A} = \mu_{Sat\ B} = \mu_{Cent\ Res}$; H_A: $\mu_{Sat\ A} \neq \mu_{Sat\ B} \neq \mu_{Cent\ Res}$). Second, t-tests were performed to examine whether, independent of resource type, population distribution patterns were random: (H_O: $\mu_{Rank\ 1} = \mu_{Rank\ 2} = \mu_{Rank\ 3}$; H_A: $\mu_{Rank\ 1} > \mu_{Rank\ 2} > \mu_{Rank\ 3}$).

(Note: the connecting tubes were considered a fourth and fifth potential location for the reproductives. Non-reproductives within the tubes were included in the census totals, but were not considered in the distribution analyses).

To prepare data for t-test analyses of each caste, the per-resource subtotals in each colony were ranked in order of descending abundance. The three groups were not expected to contain equivalent shares of the total caste population. The first rank was expected to be greater than 1/3, the middle to be 1/3, and the last rank to be less the than 1/3. Since expected deviation in the first and third ranks was unknown, formulation of an appropriate null hypothesis was impossible. Therefore, the Monte-Carlo technique was used to generate the expected values needed to devise null hypotheses for each caste (Doucet, et al. 2001).

The Monte-Carlo technique creates a pool of expected values appropriate for the creation of a null hypothesis by generating a pseudo-population. In similar techniques, such as bootstrapping, data are resampled repeatedly in order to create this new pool of expected values. For this Monte-Carlo pseudo-population, SAS created sets of random trinomials comprising 1/3 probabilities (e.g. 0.3443, 0.3333 and 0.3224). Values were then ranked by descending abundance, thus matching the troika of actual, observed values for each colony. For each caste, this procedure was repeated 13,000 times (1,000 for each of the 13 colonies), creating more than 250,000 values in the pseudo-population. Finally, the generated samples were averaged across each rank to create the expected percentage of individuals in each of the three groups. The observed rank values were analyzed in simple t-tests with a Bonferroni adjustment to accommodate for the inherent correlation between the ranks.

To determine whether the majority of each caste were located together, for example to evaluate whether the majorities of brood and workers were found together, the co-location status (together or apart) of the bulk of the following three caste pairs were determined: reproductives and brood, reproductives and workers, and workers and brood. Chi-squared tests for each pairing were performed to establish whether the relative locations differed significantly from a random distribution.

3.2.5 Fast marking technique

In preparation for the mark-recapture components of this research, a novel procedure was developed for marking *R. flavipes* (Long 2004). This innovation was inspired by Evans' (2000) work with *Coptotermes*. In the traditional, slow-dying procedure, insects are fed dry filter paper stained with a histological dye dissolved in solvent. The termites must feed on this paper for 3-10 days to acquire sufficient dye (Grace & Abdally 1989; Su, et al. 1991; Oi & Su 1994). This period of segregation from nestmates, the suboptimal diet, and the exposure to solvent is thought to reduce the termites' vigor (see Evans 2000).

To speed the termites uptake of the dye, *R. flavipes* workers were exposed to ambient laboratory air for approximately 4 hours, or until they had lost 10% of their body weight. The thirsty termites were placed on filter paper discs (Fisherbrand Qualitative P8; Fisher Scientific, Pittsburgh, PA) saturated with either 0.8 g/L Nile Blue (Aldrich Chemical Co., Milwaukee, WI) or 2.8 g/L Neutral Red (Acros Organics, Morris Plaines, NJ) dissolved in deionized water. After 60 hours, the workers acquired stains equivalent

to those imparted by traditional marking techniques. As reported in Long (2004), this treatment did not significantly impact survivorship (P=0.9801, F=0.14, df=5).

3.2.5.1 Mark-recapture sampling

Data for both the fidelity analysis and the Lincoln index evaluation were generated by two mark-recapture sampling events. Workers were collected from both satellite resources, dyed either red (Satellite A) or blue (Satellite B), counted, and returned to their collection points. Termites were regathered from each satellite as well as from the central nest. In August 2003, dyed workers were recollected from all of the colonies after 7 days. In September 2004, the experiment was rerun with lengthier intervals before recollection. Six colonies were resampled after 15 days and seven colonies reexamined after 30 days. Marker impermanence made longer recollection intervals infeasible.

3.2.5.2 Resource fidelity

The relative proportion of marked termites recovered from each of the three resources provided insight regarding redistribution patterns of workers. The percentages of red and blue workers present in the recollection samples were calculated for each location. Marked workers were classified as being recovered from either the original collection point, the intermediate point, or the most distant point. Where necessary, data were transformed in order to meet the assumptions of normality. Colony-level variation was minimal, allowing pooled analysis of all colonies. Mixed model ANOVA determined whether equal proportions of dyed workers were collected from each

location. If the same proportion of dyed workers was recovered in each of the three resources, (H_O: $\mu_1 = \mu_2 = \mu_3$), it would suggest that termites redistributed evenly throughout the network. Alternatively, if marked termites were recaptured disproportionately (H_A: $\mu_1 ? \mu_2 ? \mu_3$), then workers failed to redistribute randomly.

3.2.5.3 Test of population estimation

The Lincoln index is based on the following logic: the number of recaptured marked individuals (r) is to the total number in the second sample (n) as the number of originally marked individuals (m) is to the whole population (N); thus N = mn/r. For this analysis, the dye marker simply denotes a subset of previously captured individuals rather than establishes the site of origin, so red- and blue-stained individuals were simply categorized as "marked." The index depends on resampling from the same sites; thus recapture totals from the central nest, which was not sampled initially, were excluded from this analysis.

To evaluate accuracy of the Lincoln index, population estimates generated by the index protocol were compared to the precise 2003 full colony census. Raw census totals were contrasted with the 7-day recapture interval experiment, which was performed in August 2003. For the 14- and 30-day interval experiments, conducted in September 2004, likely population growth was extrapolated based on growth rates observed over the previous 4 years. Lineage 1 colonies increased in size by approximately 39%; Lineage 3 colonies by approximately 23%. Chi-squared tests determined whether significant differences existed between the census totals (observed values) and the Lincoln index estimates (expected values).

3.3 RESULTS

3.3.1 Travel patterns

After introduction to the central chambers of the multi-resource networks, workers located the foraging pathways leading towards the satellites within a week, and in some cases within hours of exposure to the novel environments. Mean discovery time was 6.3 $\pm$ 4.5 days. Broad variation existed among the colonies, but discovery time was not significantly impacted by colony category (the nested effect of lineage and reproductive status) (P=0.6625, F=0.43, df=2). Workers advanced through the tubes at a consistent pace, taking 20.7 ($\pm$ 7.1) days to traverse 3.5 m to the satellite resources. This exploration time was statistically equivalent for all colonies (P=0.1649, F=2.17, df=2).

Over the 30-week course of the experiment, colony category had no significant impact on either the volume (P=0.2000, F=1.68, df=2) or the direction (P=0.9773, F=0.02, df=2) of termite traffic on the foraging pathways. The relationship between the traffic differential (outbound minus inbound workers relative to the central node) and time was not significantly different from zero (P=0.6503, F=0.21, df=1), signifying that no significant linear relationship existed between the two. This suggests that once the resources were occupied, there was a lack of net change in the distribution of termites among the three resources over time (Figure 15). Significant differences were noted in the relationship between traffic volume and time (P=0.0051, F=8.52, df=1), indicating a significant linear relationship. Traffic volume increased by an average of 0.79 individuals each week over the 30 week experiment (Figure 16).

Figure 15. Linear regression of the net loss of *R. flavipes* workers from the central nest over 30 weeks. The mean number of workers leaving the central hub decreased by 0.009 per week. This change was not significantly different from zero (P=0.6503, F=0.21, df=1), indicating no net change in the number of termites in the central node. All three colony categories responded consistently over time (P=0.9773, F=0.02, df=2).

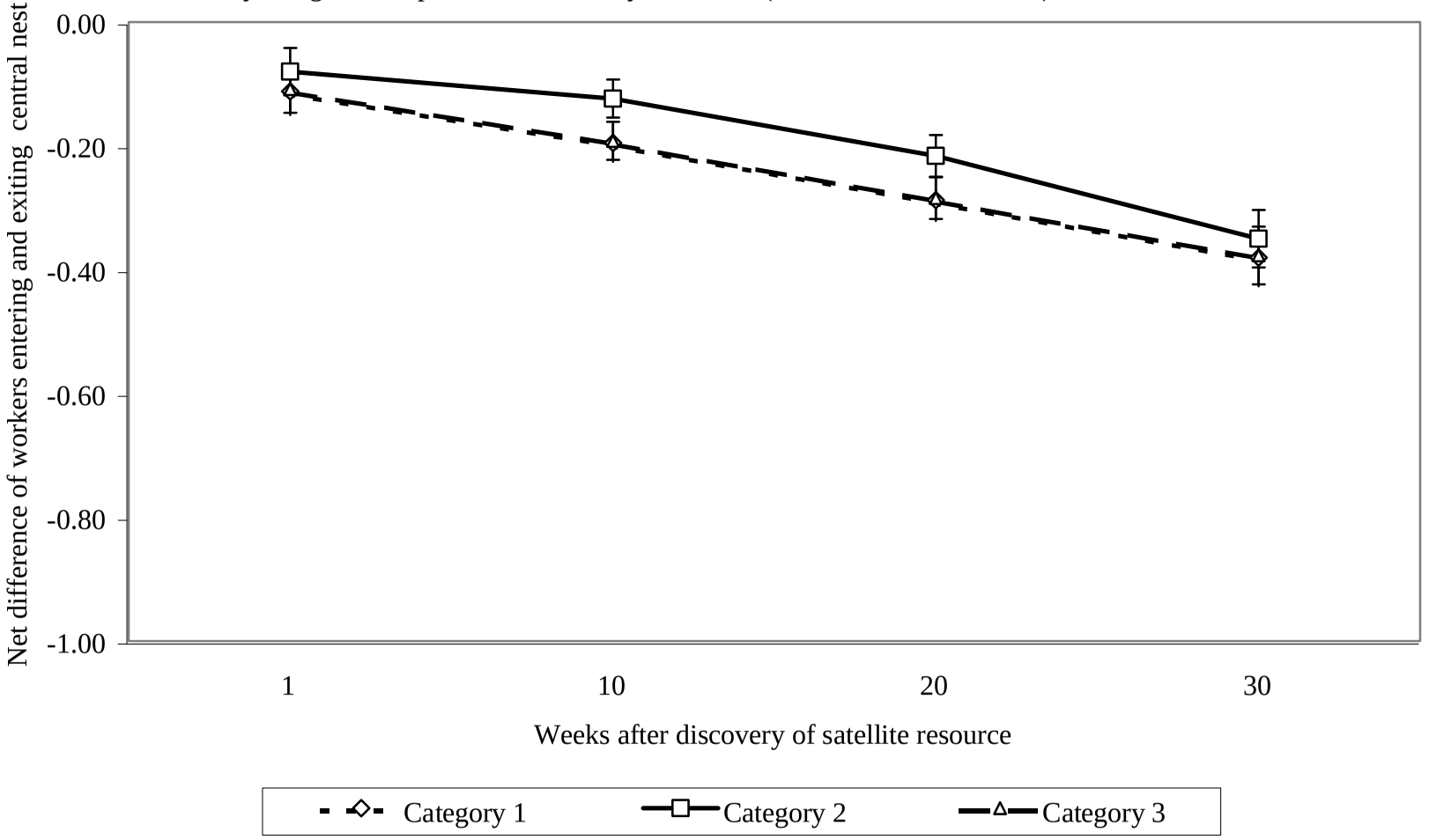
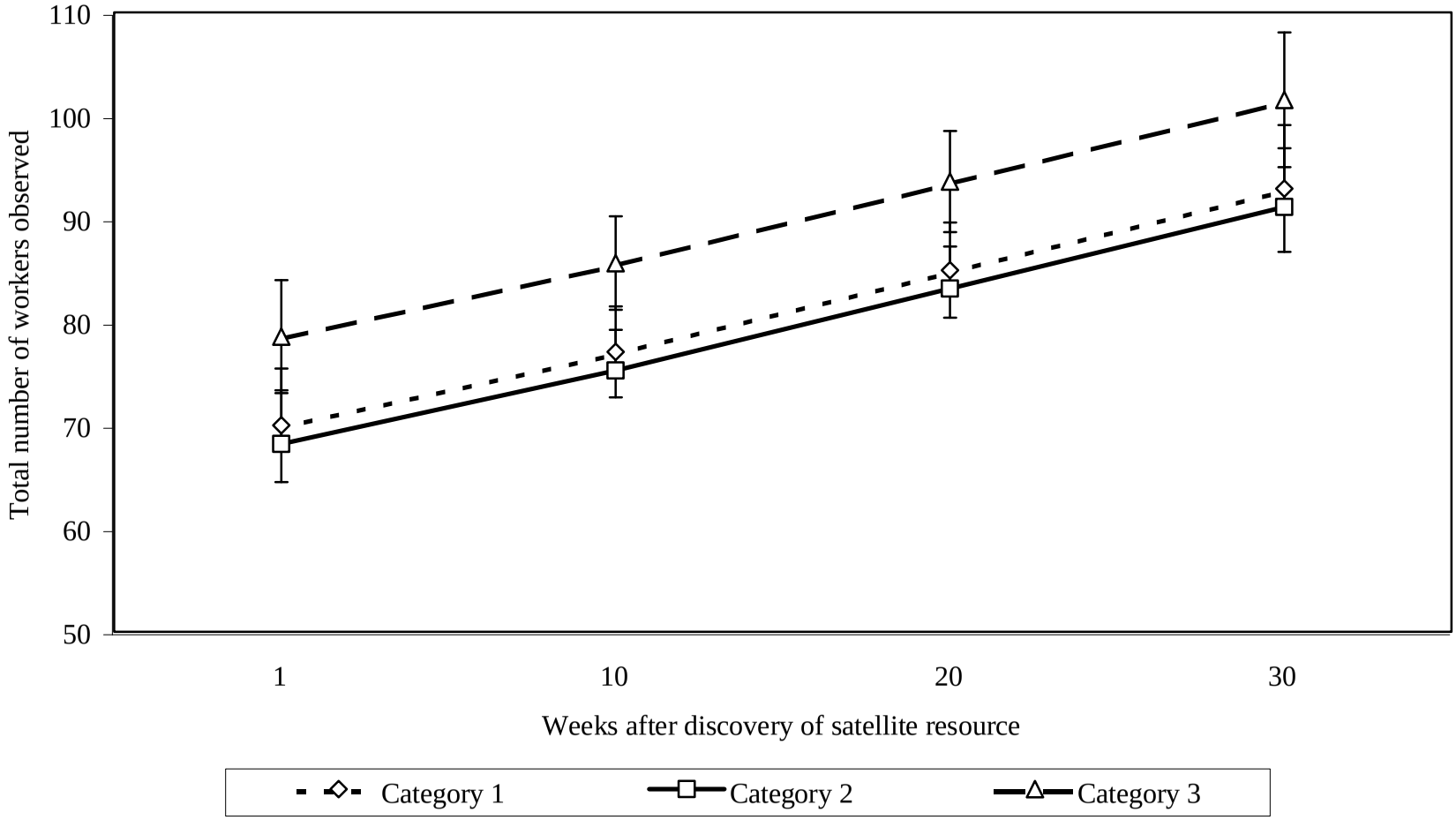


Figure 16. Linear regression of traffic volume over 30-week time period. *R. flavipes* traffic increased significantly over time (P=0.0051, F=8.52, df=52.6), with 0.79 more workers traveling per week. The three colony categories responded consistently over time (P=0.2000, F=1.68, df=2).



3.3.2 Population distribution

3.3.2.1 Reproductives

Location type (central or satellite node) did not significantly impact the residence of the reproductive caste (P=0.7553, F=0.47, df=4). Four of 14 king-queen pairs or neotenic clusters were found in the central resource, 7 were established in a satellite resource, and 3 were located in a connecting tube. Reproductives and brood were never found co-mingled in the same gallery. In 6 of 14 colonies, reproductives were in the same resource container as their brood, but housed in separately constructed wood or frass chambers. In the remaining 8 colonies, reproductives were located in a separate resource entirely. The relative location of reproductives and the majority of both their brood and the workers was not significantly different from a random distribution ($?^2$=0.14, df=1, p=1.0 and ($?^2$=1.3, df=1, p=1.0, respectively). In 13 of 14 colonies, the king and the female reproductive(s) were found together. In the one remaining colony, the king and two female neotenics were located in the central chamber, while two other neotenic females were positioned in a satellite. In this case, 10% of the colony's larvae were found in the satellite with the male-less neotenic sisters; the remainder of the larvae and all of the eggs were found in the central resource with the king.

3.3.2.2 Non-reproductives

Distribution analyses for the five non-reproductive castes comprised two distinct components. First, the potential impact of resource type on population dispersal was examined. Then, independent of location, the caste distribution patterns were scrutinized. Table 4 summarizes statistical results for both analyses. With the exception of the soldier

Table 4. Statistical results regarding distribution of castes and age groups among three resources in a multi-resource network. Significance (P<0.05) is denoted with an asterisk (*).

	Was the location of the majority of the population random? [a]	P-values	Mean % of pop in each resource in descending rank order	SE	Bonferroni-adjusted P-values [b]	t values
Reproductives	Y	P=0.7553 F=0.47 df=4	--	--	--	--
Workers	Y	P=0.5157 F=0.67 df=2	49.6 30.5 2.0	2.0 1.9 1.9	P=0.000010* p=0.458885 p=0.000033*	t=7.7, df=2 t=-1.5, df=2 t=-6.9, df=2
Soldiers	N	P=0.0178* F=4.48 df=2	58.3 28.0 137	3.8 2.2 2.2	P=0.000589* P=0.101469 P=0.000058*	t=5.1, df=2 t=-2.4, df=2 t=-6.5, df=2
Larvae	Y	P=0.3672 F=1.03 df=2	81.2 14.5 4.4	4.1 3.2 1.7	P=0.000000* P=0.000174* P=0.000000*	t=11.5, df=2 t=-5.8, df=2 t=-16.4, df=2
Eggs	Y	P=0.7074 F=0.35 df=2	96.1 3.9 0	2.6 2.6 --	P=0.000000* P=0.000000* --	t=23.4, df=2 t=-11.5, df=2 --
Pre-alate nymphs	Y	P=0.5305 F=0.67 df=2	70.0 24.4 5.7	8.0 6.7 3.7	P=0.005479* P=0.623321 P=0.000127*	t=3.9, df=2 t=-1.3, df=2 t=-6.0, df=2

a This hypothesis was tested with a mixed-model ANOVA, with resource sites as the fixed effects.

b Independent of resource type, t-examined whether population distribution was random across the network.

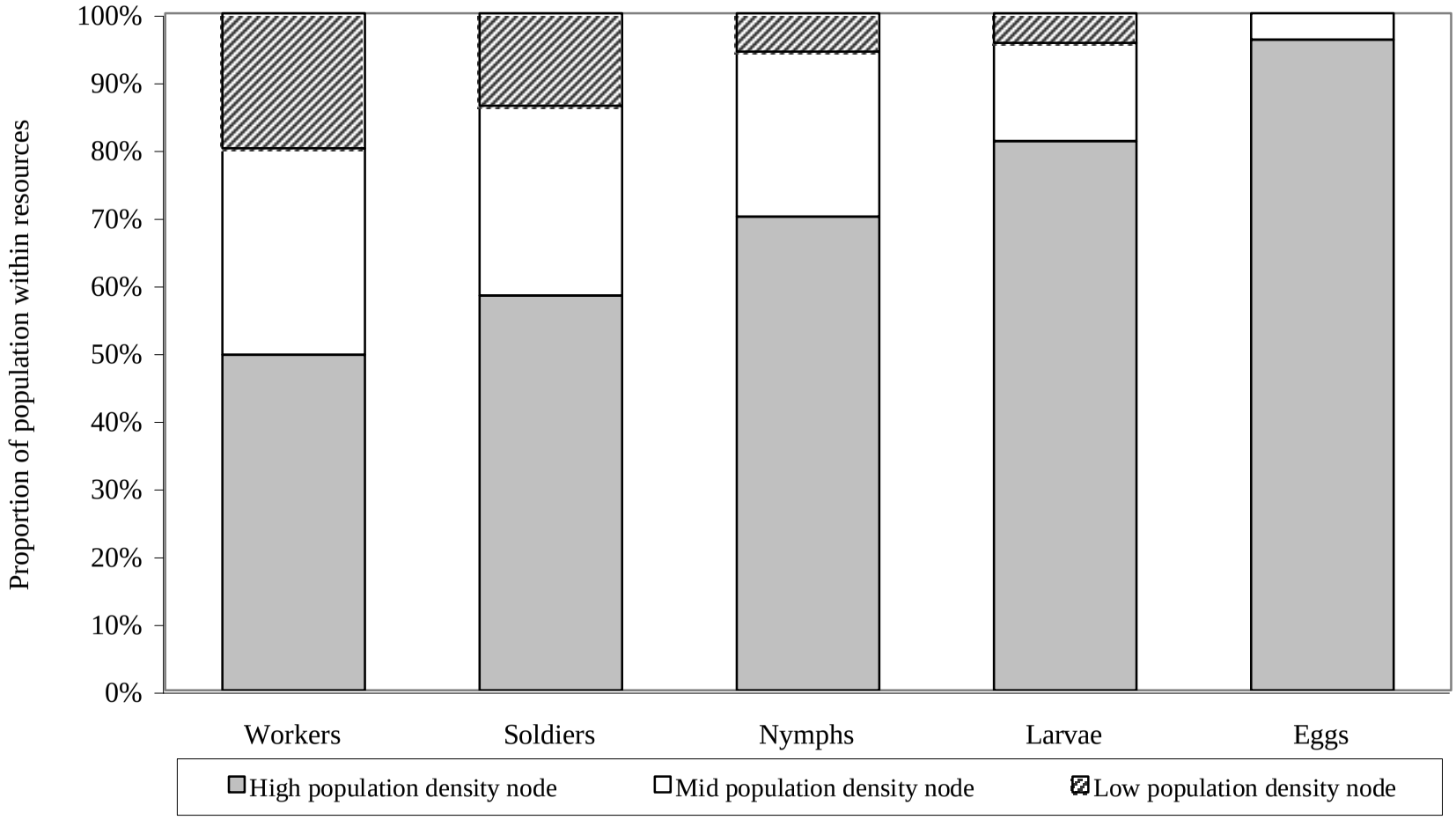
caste, *R. flavipes* did not exhibit a resource node preference. Soldiers were significantly more likely to be found in the central chamber than in either of the satellite resources (P=0.0178, F=4.48, df=2).

None of the non-reproductive castes were distributed randomly across the network. The density of each caste within each of the three locations was ranked in descending order: high- , mid- , and low-density. If the distributions were random, these densities would vary but not be significantly different from 0.33. Although workers, soldiers, and pre-alate nymphs were present in the mid-density locations at the expected, approximately one-third ratio, these castes were significantly over- and under-represented in the high- and low-density locations, respectively. Larvae and egg distributions were markedly disproportionate, with 81% and 96% of the respective totals located together in a single locale (Figure 17). In 93% of colonies (13 of 14), the larvae and the eggs were co-mingled in not only the same resource but a common nursery chamber within the larger arena. In 72% of cases (11 of 14 colonies), the majority of the brood were located in the resource that also contained the majority of the workers; this was significantly different from a random distribution ($?^2$=5.0, df=1, P<0.05).

3.3.3 Resource fidelity

To evaluate diffusion of termites among the resources, marked workers were sampled from the three locations at three time intervals. A week after marked termites were returned to their satellite of origin, they were significantly more likely to be recovered from that same resource than from either of the other two available feeding

Figure 17. Distribution of *R. flavipes* castes within a multi-resource network, independent of node type. Each column represents the total caste within a colony; shaded sections illustrate the relative distribution of that caste across the three potential locations.



sites (P=0.0007, F=8.25, df=2). This difference persisted after 14- and 30 days (P=0.0051, F=6.94, df=2 and P=0.0410, F=3.52, df=2, respectively) (Figure 18). For all three recollection schedules, workers were equally likely to be recovered, regardless of dye type (7-day P=0.6511; F=0.21; df=1; 14-day P=0.3283, F=1.06, df=1; 30-day P=0.1116, F=2.67, df=1).

3.3.4 Population estimation

The Lincoln index was an inaccurate estimator of the colony population totals. Chi-squared values for data collected at 7, 14, and 30 days after the initial collection indicated a significant difference between the estimated and actual population sizes (7-day: $?^2$=47747.2, df=10, p=0.001; 14-day: $?^2$=9298.4, df=5, p=0.001; 30-day: $?^2$=34271.0, df=6, p=0.001). Using the mark-recapture data gathered 7 days after the release of the marked termites, the index estimates accounted for between only 9 and 50% of the known population totals. In a single case, the index overestimate was 248% of the direct census total (Figure 19). The index generated eight population overestimates and five underestimates with data collected 14 and 30 days after the marking period (Figure 20). Using the 14-day interval data, one colony's estimate varied from the true total by 5%, but the remaining indices accounted for between 45% and 173% of the census counts. With the exception of one prediction that was short 101 individuals (a 1% deviation), the 30-day data misestimates were between 24% and 476% of the known totals.

Figure 18. Illustration of resource fidelity. After 7, 14, and 30 days, *R. flavipes* workers were significantly more likely (P=0.0007, P=0.0051, P=0.0410) to be recovered from their original feeding site than at either of two more distant sites. Significant differences (P<0.05) are indicted by different letters (LSM).

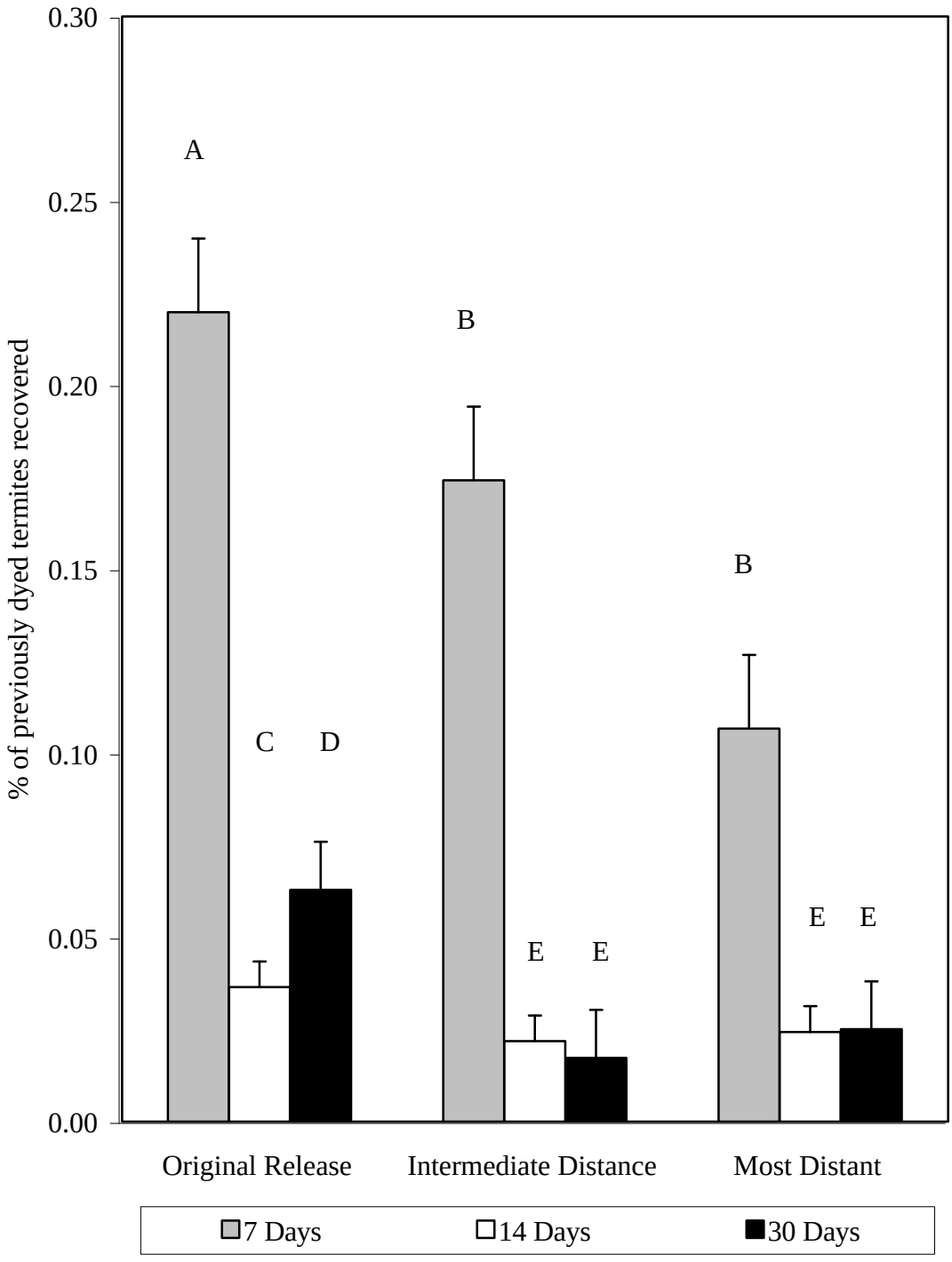


Figure 19. A comparison of *R. flavipes* population estimates generated by the Lincoln index with actual census totals using data from a 7-day recapture interval. The totals are significantly different (P=0.001 , $?^2$=47747.2, df=10)

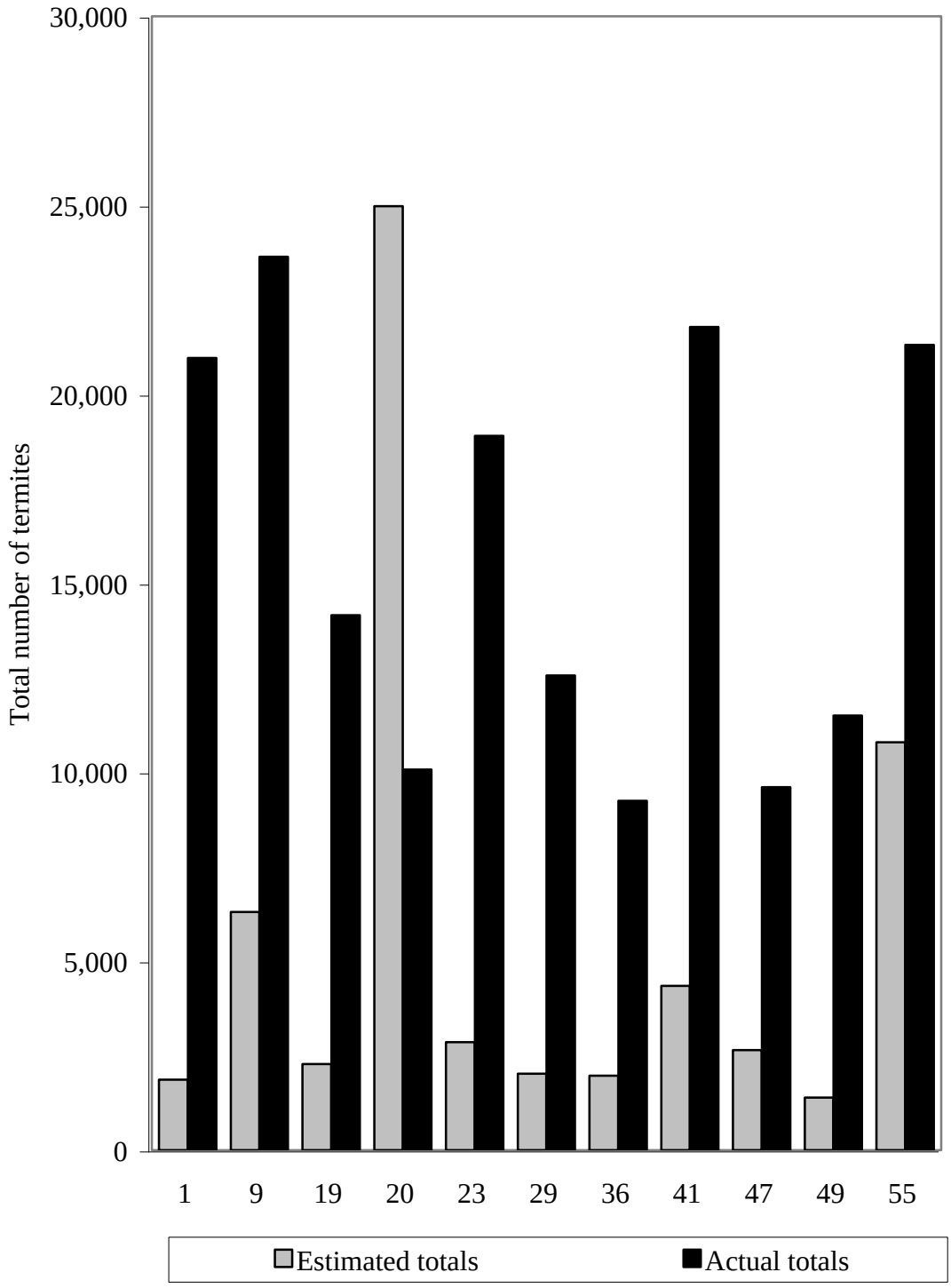
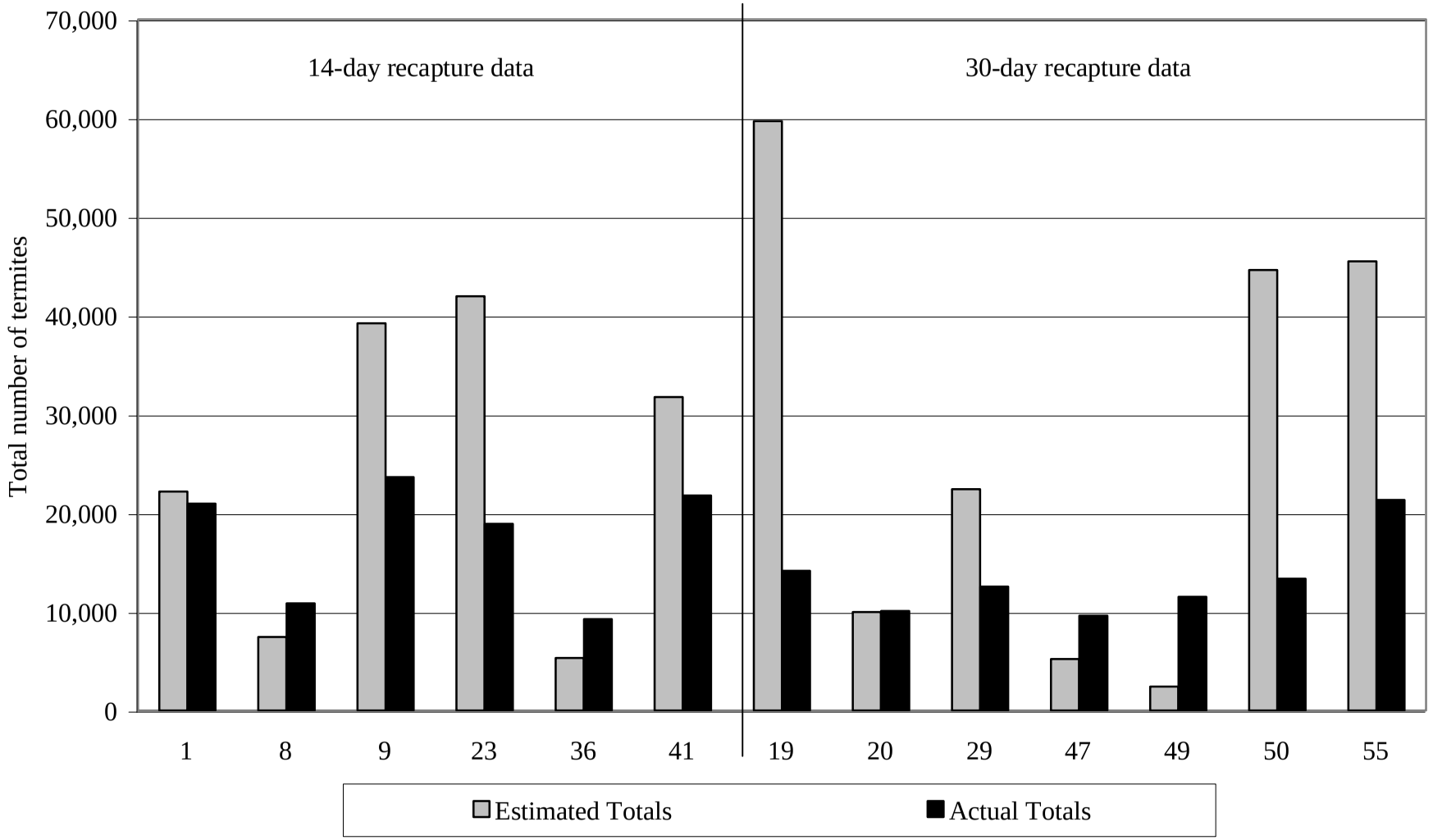


Figure 20. A comparison of *R. flavipes* population estimates generated by the Lincoln index with actual census totals. The left half of the chart is based on data from a 14-day recapture interval, the right half from a 30-day interval. The totals are significantly different (14-day: P=0.001, ?2=9298.4, df=5; 30-day: P=0.001, ?2=34271.0, df=6).



3.4 DISCUSSION

Results of this unique multi-resource manipulation confirm the growing evidence that *R. flavipes* do not forage randomly among available feeding sites. This study presents an unparalleled comparison between direct, precise census data and population estimates generated by the Lincoln index, provides novel data regarding the relative position of reproductives and brood within the nesting galleries, and suggests ways in which *R. flavipes* diverge from the classic central-place foraging model.

3.4.1 Practical implications of resource fidelity

The standard tool for subterranean termite population assessment and many modern termite control strategies depend on the assumption that workers deploy to the colony's satellite resources at random after visiting the large, central nest. Mark-recapture sampling of complete *R. flavipes* laboratory colonies indicate that even after 30 days, workers were significantly more likely to be found where they had been previously collected than at different feeding sites. This result has a profound impact on both population modeling and the theory and practice of pest management.

3.4.1.1 Population estimates

The non-random distribution of marked individuals within a population violates one of the five assumptions underlying the Lincoln index (Lincoln 1930; Southwood 1971). If workers mingle evenly across a feeding network, marked workers released in one site would exit at random and be replaced by unmarked nestmates prior to the second sampling. If, as the present results indicate, termites are significantly more likely to be

recovered from their original resource, marked individuals will be over-represented in the recapture, thus leading to an underestimation of the population.

For example, census data indicated that Colony 36 contained a total of 9,245 individuals (N). In the first sample, 204 workers were collected and dyed (m). A week later, 145 workers were recollected (n). The Lincoln index (N = mn/r) predicts that 3.2 marked termites should be collected in the recapture sample (r); however, 15 termites were actually recollected. According to the index, this colony should have contained 1,972 termites, which is 7,273 individuals short of the accurate total. Ten of the 11 estimates evaluated 7 days after the release of the marked individuals produced totals that greatly underestimated the true population.

Failure of workers to reintegrate with the colony as a whole may explain the model's striking underestimation of the population when a relatively short recapture interval was used; however, data from 8 of the 13 lengthier intervals greatly overestimated the colony totals. The previous example illustrated how a relatively large number of marked individuals in the recapture sample will reduce the estimated total. Conversely, proportionately fewer dyed termites in the second collection will exaggerate a predicted total. Despite the finding that workers were still significantly more likely to be in their original location after a month, marker impermanence may have contributed to a paucity of marked workers in the recapture samples and to the subsequent inflation of population estimates.

In five of the colonies for which the Lincoln index overestimated the total population [data was available for only one interval for the remaining three colonies], marked workers constituted a smaller percentage of recaptured foragers in the 14- or 30-

day interval samples than they had in the 7-day samples. Dye trials conducted prior to this experiment indicated that some termites gradually lose their stain after 3 weeks, particularly those treated with Nile Blue (Long 2004). Thorne, et al. (1996) noted significant marker loss among foragers who were, contrary to many dye persistence studies (Esenther 1980; Lai, et al. 1983; Su, et al. 1983, 1988, 1991; Grace & Abdally 1989; Salih & Logan 1990), permitted limited mobility and fed a wood diet.

Marker impermanence would not have affected the outcome of the site fidelity study, which depended on the relative recovery of marked workers from the three feeding sites and was independent of previous recapture rates. Fading should have impacted the entire colony and thus calculations based on relative proportions should have been unaffected. Thorne, et al. (1996) concluded that foraging activity and a wood diet may hasten metabolism of vital stains. If the act of traveling to the far end of the network increased the likelihood that the study termites would lose their marks, the conclusion of feeding site fidelity would be false. However, equivalent proportions of marked termites were consistently recovered from both the intermediate- and most-distant feeding sites over time (Figure 18). Although this finding does not preclude the possibility that simply leaving the original resource sapped the dye from a significant number of foragers, if the distances traveled were tightly linked to the amount of dye lost, significant differences should have been evident between the recovery rates at the intermediate and most-distant resources.

3.4.1.2 Colony-wide termiticide delivery

Several slow-acting, non-repellent termiticides are on the market, including (but not limited to) imidacloprid, fipronil, and noviflumuron. In the laboratory, efficacy of these products, applied at their legally labeled rates, varied widely. Fipronil (a phenylpyrazole) resulted in mortality rates of nearly 70% after 24 hours and almost 100% after 72 hours (Ibrahim, et al. 2003). Depending on soil type, imidacloprid (a nicotinoid) accounted for the death of between 8.8% and 95.8% workers after 21 days (Ramakrishnan, et al. 2000). Termites exposed to noviflumuron (a growth inhibitor) for 14 days experienced mortality rates of 86% after 26 days (Karr, et al. 2004).

In order to fulfill their colony control potential, these products depend on two elements of random remixing: i) exposed workers are constantly replaced at a treated site by naïve nestmates; ii) toxicant is transferred from initially exposed foragers to their remote nestmates via grooming, trophallaxis, and cannibalism (Bayer 2004; BASF 2005; DowAgro Science 2005; McNally, et al. 2005). The aforementioned feeding tenacity results demonstrate that although foragers may travel from one end of the multi-resource network to another within a 7-day period, they are significantly more likely to be found within their original resource for at least a month. Given that fipronil mortality was nearly 100% after only 72 hours (Ibrahim, et al. 2003) and that workers can experience impaired mobility within minutes of exposure to imidacloprid (Thorne & Breisch 2001), many of the potential termiticide carriers might be dead or incapacitated before they have the chance to leave the site of exposure and contact distant nestmates. When these treatments fail to eliminate an infestation, feeding site fidelity and the subsequent failure

of termiticide-exposed foragers to fully circulate throughout the colony may be contributing factors.

3.4.2 Inter-network travel

Feeding site tenacity among subsets of workers in this study did not trigger the physical isolation of bud or daughter nests. Once the satellite nodes were populated, the net travel differential, or the number of foragers entering and exiting the central nest, was not significantly different from zero, indicating an even exchange of workers between the sites and uninterrupted communication between the resources. Forager traffic volume grew steadily over the 30-week experiment, increasing from 37.5 to 48.1 individuals per minute. Data to gauge fluctuations within a 24-hour time span were not gathered, but these rates suggest between 54,000 and over 69,000 termite passages through the tunnels per day. The largest colony in the study contained 13,054 workers.

The steady worker traffic in the tunnels coupled with strong evidence of feeding site fidelity suggest that inter-node travel may be a specialized task. Strict age- and size-specific polyethism is widespread among social insects, appearing in nearly all the social Hymenoptera studied (Wilson 1971; Robinson 1992). Isopteran polyethism is less common, occurring most often in the highly derived Termitidae (Pasteels 1965; Badertscher, et al. 1983; Miura & Matsumoto 1995, 1998; Hinze, et al. 2002). Among *Macrotermes* spp., hunting for resources and food acquisition are performed by major and minor workers, respectively, with little or no overlap (Badertscher, et al. 1983; Lys & Leuthold 1991). *Hodotermes mossambicus* (Hagen) (Isoptera: Hodotermitidae) subdivides resource acquisition tasks so narrowly that dried grasses are sliced from their

stalks and carried back to the nest by separate groups of workers (Leuthold, et al. 1976). Polyethism among lower termites appears to be much less distinct. The task repertoires of younger, and thus smaller, *Reticulitermes fukienensis* (Light) (Isoptera: Rhinotermitidae) workers were equal to their older (= larger) nestmates, but their tunnel construction and repair, grooming, and paper consumption rates were slower (Crosland & Traniello 1997; Crosland, et al. 1997, 1998). Younger *Reticulitermes lucifugus santonensis* (Feytaud) (Isoptera: Rhinotermitidae) workers appeared less independent than older ones, but there were no strict divisions among tasks or locations within the nest (Garnier-Sillam 1983). Work with *R. flavipes* field colonies in North Carolina suggests that foraging columns may be subdivided into scouts, who investigate new resources, and harvesters, who follow previously established pathways (Dal Molin 1978).

3.4.2.1 Bucket brigades

In contrast to multistage task partitioning in which specialized "gatherers" deposit food in a predetermined cache and "carriers" transport it to the nest (see Leuthold, et al. 1976), Anderson, et al. (2002) proposed that insect societies may also employ "bucket brigades" to convey materials to their nests. In a bucket brigade, laden workers transfer their loads directly to unladen nestmates they meet along the trail. At least three of these opportunistic transfers must occur between the food source and the nest (Bartholdi, et al. 1999). This highly dynamic model is free of the potential queuing delays inherent in systems with fixed caching locations (Ratnieks & Anderson 1999 a, 1999 b) and can be continuously adjusted to optimize efficiency. Bucket brigades offer an advantage when foragers have difficulty passing each other on the trail, as in a narrow tunnel or on a

slender branch, and allow for workers to "specialize" on various stretches of trail (Bartholdi & Eisenstein 1996; Anderson, et al. 2002).

In a laboratory foraging arena similar to mine, *Macrotermes subhyalinus* (Rambur) (Isoptera: Macrotermitidae) workers who met on the trail briefly interacted and antennated. Workers would then usually pass each other, but occasionally the pair would exchange stomodeal materials and then reverse course so that each termite returned in the direction from which it had come (see Anderson, et al. 2002). Similar behavior was observed among *R. flavipes* in the foraging arenas. In some sections of the tubing, an "upper deck" pathway was constructed above the trail laid down on the floor of a tube. Each level carried one-way traffic so workers traveled unimpeded by oncoming individuals. However, where inbound and outbound traffic shared a common path, workers briefly interacted with almost every individual they encountered. Occasionally, workers would reverse course after these interactions. The frequency and location of these exchanges were not quantified, and it was unclear whether trophallaxis was a component.

If workers and their food resources could be tracked separately throughout a colony network, researchers might be able to determine whether *R. flavipes* employ the bucket brigade strategy to transport materials among their nests sites. Using the dual marking technique developed by Suárez & Thorne (1999), workers could be marked with vital stains or topical acrylic paint and their alimentary materials tagged with a radioactive tracer. Tracking a colony's food resources across a multi-resource network independent of the workers who performed the initial feeding would complement my research and further expand our understanding of colony feeding dynamics.

3.4.3 Distribution of brood and reproductives

Results from this research dispel the belief that *R. flavipes* maintain a central nesting chamber containing brood and reproductives. The glimpse of colony life offered by direct censuses of the nodes suggest that the reproductives were mobile throughout the experiment. Twenty months after establishment of the multi-resource systems, 71% of royal pairs were found outside the original, central chamber, and they were no more likely to be found with their brood than would occur randomly. When networks were dismantled 14 months later, all but one pair were found in a different location. These observations did not capture the frequency of reproductive relocation, but suggest that despite the absence of obvious distress (such as predation, resource depletion, or environmental changes) they were itinerant. One pair, flanked by soldiers, was observed moving along the pathway between two resources.

3.4.4 Polydomy and foraging strategy

By demonstrating feeding site fidelity at multiple, interconnected resources, these *R. flavipes* colonies clearly exhibit the forager-based polydomous nesting strategy described by Traniello & Levings (1986), Pfeiffer & Linsenmair (1998), and Holway & Case (2000). Although an average of 81% of larvae and 96% of eggs were clustered together in a single node, small numbers of immatures were present in the remaining resources; thus, these colonies also met the brood-based criteria for polydomy (Stuart 1985; Banschbach & Herbers 1999; Walin, et al. 2001). This combination of factors suggest a decentralized, dynamic network rather than a classic central-place foraging

model. Such dispersed central-place foraging seems particularly well-suited to *Reticulitermes*, given the genus' exploitation of fallen timber, which is a spatially patchy, unpredictable, and exhaustible food resource. Among ants, foraging from multiple nest loci reduces a colony's total foraging costs (Hölldobler & Lumsden 1980) either by reducing overlap in the pathways of individual foragers or by allowing groups of foragers to concentrate on high-quality resource patches (Pfeiffer & Linsenmair 1998).

3.4.5 Conclusion

This research answers several long-standing questions regarding *R. flavipes* foraging and nesting strategies. It was known that these nomadic, subterranean colonies fed simultaneously on multiple resources, but information regarding travel between the resources and the distribution of the population among them was scarce. Insights from this laboratory study of complete colonies exploiting multiple resources filled in some of these details. Although subsets of workers feed preferentially on single resources, communication between resources is uninterrupted. There is evidence that this exchange may be accomplished via a bucket brigade populated by a subset of "couriers." Counter to the popular image of a centralized nest containing reproductives and young, these *R. flavipes* colonies established a polydomous network, seeding all their feeding sites with at least a few larvae and housing the royal pair and the bulk of the colony brood at separate locations across the network. These factors suggest that *R. flavipes* are at least facultatively dispersed central-place foragers rather than obligate central-place foragers. Discovery of an alternative strategy modifies our understanding of the population

dynamics and spatial distribution of these colonies, and has implication for the theory and practice of subterranean termite pest management.

4 INBREEDING COEFFICIENTS AND BREEDING STRUCTURE WITHIN LABORATORY-REARED *RETICULITERMES FLAVIPES* COLONIES: INFERENCES BASED ON DIRECT CENSUS AND GENETIC POLYMORPHISM

4.1 INTRODUCTION

This chapter examines the genetic profiles and *F*-statistics for four entire *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) colonies that had lost their founding female reproductive, the queen. Microsatellite markers provided worker genotypes which were used to classify each colony as either a simple or an extended family, to examine each family for evidence of genetic differentiation, and to generate *F*-statistics and a relatedness coefficient for the total, four-colony population. These results offered a rare opportunity to compare inbreeding coefficients colonies of known breeding structure and age with outcomes predicted by a simulation model. If inbreeding coefficients generated by the model are congruent with those found in the study colonies, researchers will have increased confidence in predictions of field colony breeding structures based on genotypes of sampled workers.

4.1.1 Nesting and breeding schemes

Chapter 3 of this dissertation shows that the *Reticulitermes* nesting and foraging repertoire includes establishment of interconnected, polydomous networks in which feeding and brood rearing occur in multiple locations. Polydomy and colony mobility are

effective strategies for a species that feeds on dead wood, which is a poor quality, patchy resource. Feeding concurrently at multiple sites may allow colonies to optimize available resources and minimize exposure to poor habitats; however, groups of foragers may have reduced contact with their nestmates or become completely isolated.

Primary reproductives suppress sexual maturation of their offspring with an inhibitory pheromone (Lüscher 1961). In the absence of this influence, immature individuals may differentiate into neotenics (replacement or supplemental reproductives) and possibly establish bud nests (Pickens 1932; Esenther 1969; Howard & Haverty 1980; Thorne 1996a). This reproductive flexibility maximizes colony fitness by increasing the likelihood that alates will be produced despite eventual loss of primary (founding) reproductives. Production of offspring by neotenic siblings does not alter fitness to a parent colony's primary reproductives: the primaries' average degree of relatedness is equivalent (r=0.5) to both their direct offspring and to any "grandoffspring" produced by their inbreeding sons and daughters (Thorne, et al. 1999). Nutting (1969) concluded that neotenics could develop and reproduce only in bud nests which were completely isolated from their parents' nest, but recently calculated fixation indices suggest that *Reticulitermes* workers may maintain contact between their parental nests and satellite resources where neotenics have differentiated and begun to reproduce (Reilly 1987; Vargo 2003; DeHeer & Vargo 2004).

Wright (1921) developed the fixation index (*F*-statistics) to quantify the effect of inbreeding on population substructure. By subdividing a population into groups more or less remote in ancestry, the level of inbreeding in one subset can be examined with reference to various base populations (Falconer & Mackay 1996; Hartl & Clark 1997).

Thus, effects of inbreeding on gene frequencies can be examined relative to a region or to the population as a whole. Allozyme (Clément 1981, 1984; Reilly 1987) and mitochondrial DNA (Jenkins, et al. 1998, 1999; Bulmer, et al. 2001) analyses have generated *F*-statistics and relatedness coefficients (*b*) which confirm that, in addition to *Reticulitermes* living in simple families headed by outbred kings and queens, inbreeding occurs between siblings and parent-offspring pairs in both single nests and in multi-bud nest complexes. Vargo (2003) and DeHeer & Vargo (2004) deduced the same range of breeding schemes using microsatellite analyses for *R. flavipes* and *Reticulitermes virginicus* (Banks) (Isoptera: Rhinotermitidae).

4.1.2 Microsatellites analysis

Microsatellites are tandem repeats of simple DNA sequences which occur throughout eukaryotic genomes. These highly variable, codominant markers can be a sensitive tool for establishing pedigree relationships among individuals (reviewed in Vargo 2003). Vargo (2000) identified nine polymorphic *R. flavipes* microsatellites with between 2 and 12 alleles apiece. Only 30% of the allozyme markers Reilly (1987) resolved were polymorphic, and of these only two different alleles were possible per locus. The high degree of polymorphism within some microsatellites provides the best resolution of *Reticulitermes* intra-colony relationships available at this time (Vargo 2000).

The allelic and genotypic data generated by the microsatellite sequences facilitated a three-pronged genetic analysis of the queenless colonies. First, individual colonies were classified as either simple or extended families. Second, each colony was

examined for evidence of genetic differentiation, defined as allele frequencies that differed within subsets of a colony. Finally, *F*-statistics and the relatedness coefficient for the total four-colony "population" were generated to examine relative degrees of inbreeding at various levels of population substructure (Hartl & Clark 1997).

4.2 METHODS

4.2.1 The termites

In 1993, incipient *R. flavipes* colonies were established in the laboratory using pairs of sibling alates from dispersal flights in Prince George's County, Maryland (Thorne, et al. 1997). Thirteen of these colonies were included in my research. Between November 2001 and October 2004, the colonies were allowed to forage in multi-resource feeding networks. Nine of these colonies housed primary reproductives and four contained a primary king and at least one neotenic female. Three of the queenless colonies had lost their queens at least 4 years before the present sampling, and the fourth had been queenless at least 2 years. In August 2003, 20 months after the multi-resource networks were established, the colonies were completely censused and samples were collected for genetic analysis. Thirty workers and 30 immatures were selected randomly from each of a colony's three resources. All samples were placed in 95% ethyl alcohol and refrigerated (42° F).

4.2.2 Microsatellite genotyping

DNA was extracted from 20 workers and 20 larvae per resource from each of the four queenless colonies and 20 workers from one of the nine queenright nests. Because the king and queen were the only two individuals to ever reproduce in the queenright family, intra-colony genetic differentiation was impossible; however, this colony's heterozygosity ratios allowed comparison with the queenless colonies. The preparation and analysis of the DNA followed Vargo (2003). Whole termites were crushed and their genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen, Valencia, CA).

Microsatellite genotypes were revealed in a two step process. First, the extracted DNA was amplified with a polymerase chain reaction (PCR) (PTC-100 thermal cycler; MJ Research, Watertown, MA) using eight pairs of primers and a fluorescently labeled primer (Vargo 2000). Second, the PCR products were separated on polyacrylamide sequencing gels run on an automated Li-Cor 4300 DNA Analyzer (Li-Cor Biosciences, Lincoln, NE). A total of 320 individuals were genotyped at each of eight microsatellite loci: Rs 16, Rs 33, Rs 62, Rf 1-3, Rf 6-1, Rf 5-10, Rf 15-2, and Rf 24-2. Additionally, workers from the queenright colony were genotyped at locus Rs 15.

4.2.3 Data analysis

The alleles and genotypes revealed by the sequencing gels were used to assess basic genetic information about both individuals and their families, to determine whether genotypic differentiation was evident within each colony, and to estimate hierarchical *F*-statistics for the population of four colonies. Colonies were classified as either simple or extended families. In simple families, worker genotypes at all loci are consistent with those of the direct offspring of monogamous pairs of reproductives and observed genotype frequencies are not significantly different from frequencies expected for offspring from a single mating pair. Extended family colonies exhibit worker genotypes inconsistent with simple family parentage, such as more than four genotypic variants or more than two classes of homozygotes. Various polygamous combinations of neotenics and primary reproductives may produce such outcomes. A colony whose number of genotypes is consistent with the simple family model but whose observed and expected genotypic frequencies differ significantly would also be classified as an extended family.

To test for significant deviation from expected intra-colony genotype frequencies (H_O: the genotypic distribution is uniform within each colony), G-tests were performed for each locus and then summed for an overall estimate of significance ($P<0.05$). A G-test is an unbiased log-likelihood or "G-based" exact test which generates a P-value (Raymond & Rousset 1995; Goudet, et al. 1996). The G-tests were executed with the program, GenePop on the Web, using option 3/suboption 4 (Raymond & Rousset 2004). This option combination performed tests of genotypic differentiation between all pairs of samples at each locus (GenePop 2004).

F-statistics and the relatedness coefficient for the queenless *R. flavipes* colonies were generated using analyses of molecular variance (Weir & Cockerham 1984) as performed by the web-based Genetic Data Analysis software program (Lewis & Zaykin 2001). Resultant *F*-values were used to calculate the relatedness coefficient: $b = 2 F_{CT} / (1 + F_{IT})$ (Pamilo 1984). These results were compred to those predicted by simulations (Thorne, et al. 1999) which calculated expected *F*-statistics and relatedness coefficients based on models of various *R. flavipes* breeding schemes. Values with non-overlapping 95% confidence intervals (generated by bootstrapping over loci with 1,000 replicates) were considered significantly different.

I followed the notational conventions of Thorne, et al. (1999) and Bulmer, et al. (2001), in which genetic variation is partitioned on three levels: the individual (I), the colony (C), and the total population (T). Thus, F_{IT} is equivalent to the standard inbreeding coefficient, F_{IS}. F_{CT} represents genetic differentiation among colonies and is similar to F_{ST}. The within colony inbreeding coefficient, F_{IC}, which has no equivalent in

non-social populations, is sensitive to the effective number of reproductives and their mating patterns.

4.3 RESULTS

4.3.1 Basic information

Scorable PCR products were generated using all nine microsatellite primers. Locus Rf 6-1 was fixed in all five colonies and is thus excluded from further discussion. The genotypic profiles of larvae were identical to those of workers and will not be discussed separately. The eight polymorphic loci contained an average of 3 alleles (range=2-5). Table 5 presents the genotypes found in each colony. Table 6 compares expected and observed heterozygosity ratios. Although all of these colonies were founded by sibling alates, average heterozygosity was 0.54 (0.31-0.90). This ratio is comparable to values observed in the North Carolina field populations sampled by Vargo (2000) and DeHeer & Vargo (2004).

The worker genotypes in Colonies 1, 9, 36, and 47 were consistent with those from simple families. The genotypic profile of Colony 49 workers was indicative of an extended family. Locus Rf 24-2 contained three alleles but five genotypic classes; four homozygous genotypes were scored at Rs 33. Both of these scenarios are possible only if at least three and four parents, respectively, contribute to the offspring; however, the genotypes cannot indicate exactly how many parents are contributing.

4.3.2 Genotypic differentiation within colonies

The three queenless colonies exhibiting simple family structure showed no evidence of genotypic differentiation. The queenright colony also, predictably, demonstrated a simple family structure and thus no sign of differentiation. The proportion of worker genotypes in Colony 49 differed significantly from the expected

Table 5. Numbers of each genotype found among *R. flavipes* workers sampled from five colonies. Colony 1 was queenright; the others were queenless and contained at least one female neotenic. Twenty-five alleles were identified at nine loci. Missing data (--) indicate either non-scorable PCR product for that locus, or that the locus was not sequenced in that colony.

	Colonies				
Genotypes	1[a]	9	36	47	49
Rs 15[b]		--	--	--	--
317/269	9				
290/269	8				
Rs 16					
305/305	9		60	60	38
305/295	10	30			17
295/295		29			
Rs 33					
259/259	13	16	60	60	4
267/259		32			5
255/255					21
263/255					17
263/263					7
267/267	6	10			1
Rs 62					
315/315	19	60	21	19	55
319/319			9	9	
319/315			29	32	
Rf 1-3	--				
236/221			16		22
224/224			17		
224/221			16		
236/224			8		
224/218				4	
236/218					7
221/218		25			13
221/221					17
218/218		33			
245/245				2	
245/224				8	
245/218				7	
Rf 5-10	--				
153/153		59	60	31	24
153/147				25	33

Table 5 (cont.). Numbers of each genotype found among *R. flavipes* workers sampled from five colonies. Colony 1 was queenright; the others were queenless and contained at least one female neotenic. Twenty-five alleles were identified at nine loci. Missing data (--) indicate either non-scorable PCR product for that locus, or that the locus was not sequenced in that colony.

	Colonies				
Genotypes	1	9	36	47	49
Rf 6-1					
170/170	18	60	60	60	55
Rf 15-2					
235/232		31	30	36	23
235/235	19		29	24	22
232/232		29			10
Rf 24-2					
106/106	4		30	1	10
196/106		31	30		6
169/106	4			15	20
169/169	4			27	6
196/169					16
199/106	7				
196/196		28			
199/169				16	

a Twenty workers were analyzed for this queenright colony. Sixty workers were examined from each of the other four queenless colonies. Failure of individual samples to yield readable data account for discrepancies between these totals and the number of genotypes presented.

b Only Colony 1 was sequenced at locus Rs 15.

Table 6. The number of alleles and observed and expected heterozygosity ratios at seven loci for four queenless *R. flavipes* colonies. Missing data (--) indicate either a non-scorable PCR product for that locus, or that the locus was not sequenced in that colony.

Loci	Colony	No. of alleles	Observed heterozygosity	Expected heterozygosity
Rs 15	1	3	0.47	050
Rs 16	1	2	0.53	0.50
	9	2	0.50	0.50
	36	1	0.00	0.00
	47	1	0.00	0.00
	49	2	0.31	0.50
Rs 33	1	2	0.00	0.50
	9	2	0.55	0.50
	36	1	0.00	0.00
	47	1	0.00	0.00
	49	4	0.40	1.00
Rs 62	1	1	0.00	0.00
	9	1	0.00	0.00
	36	2	0.49	0.50
	47	3	0.53	0.50
	49	1	0.00	0.00
Rf 1-3	1	--	--	--
	9	2	0.43	0.50
	36	3	0.70	0.75
	47	3	0.90	0.75
	49	3	0.71	0.75
Rf 5-10	1	--	--	--
	9	--	--	--
	36	1	0.00	0.00
	47	2	0.45	0.50
	49	2	0.58	0.50
Rf 15-2	1	1	0.00	0.00
	9	2	0.52	0.50
	36	2	0.51	0.50
	47	2	0.60	0.50
	49	2	0.42	0.50
Rf 24-2	1	3	0.63	0.50
	9	2	0.53	0.50
	36	2	0.50	0.50
	47	3	0.53	0.50
	49	3	0.72	0.57

pattern ($P<0.0001$, $G<0.001$, $df=12$). G-test results for all four colonies are presented in Table 7. There is evidence that Colony 49's population differentiation had a spatial component. At two different loci, alleles or genotypes were not observed in all three of the available resources: at Rs 33, alleles 259 and 267 were missing in two resources, and the genotype 196/106 on locus Rf 24-2 was absent from one of the sites.

4.3.3 *F*-statistics

In this population of four queenless *R. flavipes* colonies, $F_{IT} = 0.52$, $F_{CT} = 0.59$, $F_{IC} = -0.17$, and $b = 0.78$. Table 8 reports these values as well as the predicted *F*-statistics and relatedness coefficients for simple families, for inbred colonies with multiple secondary reproductives, and for interconnected bud nests. My results are identical to values predicted for an inbred colony with two female neotenics and a single male (Thorne, et al. 1999).

Table 7. G-like test P-values for seven polymorphic loci in four queenless colonies. These values were pooled to test for genotypic differentiation within each colony. Significant differentiation on this level is denoted with an asterisk (*). Missing data (--) indicate fixed alleles.

Colony	Loci	G-like test p-value	(SE)
9	Rs 16	0.3050	(0.0047)
	Rs 33	0.8608	(0.0021)
	Rs 62	--	--
	Rf 1-3	0.1485	(0.0054)
	Rf 5-10	--	
	Rf 15-2	0.7620	(0.0039)
	Rf 24-2	0.6534	(0.0032)
	Total: P=0.8508, $?^2$=4.1, df=8		
36	Rs 16	--	--
	Rs 33	--	--
	Rs 62	0.3904	(0.0057)
	Rf 1-3	0.1869	(0.0061)
	Rf 5-10	--	--
	Rf 15-2	0.1974	(0.0037)
	Rf 24-2	0.1872	(0.0042)
	Total: P=0.1589, $?^2$=11.8, df=8		
47	Rs 16	--	--
	Rs 33	--	--
	Rs 62	0.6868	(0.0039)
	Rf 1-3	0.0381	(0.0015)
	Rf 5-10	0.8910	(0.0016)
	Rf 15-2	0.2468	(0.0042)
	Rf 24-2	0.9748	(0.0011)
	Total: P=0.4090, $?^2$=10.4, df=10		
49	Rs 16	0.3612	(0.0046)
	Rs 33	<00001	(0.0000)
	Rs 62	--	--
	Rf 1-3	0.1483	(0.0055)
	Rf 5-10	0.6317	(0.0043)
	Rf 15-2	0.1360	(0.0047)
	Rf 24-2	0.0101	(0.0011)
	Total: P<0.0001*, $?^2$<0.001, df=12		

Table 8. *F*-statistics and relatedness coefficients (*b*) for actual and simulated *R. flavipes* colonies. The parenthetical values are 95% confidence intervals.

Breeding scheme	F_{IT}	F_{CT}	F_{IC}	*b*
My queenless colonies:	0.52	0.59	-0.17	0.78
N_f = 7.5[a], King = 1	(0.37-0.65)	(0.48-0.69)	(-0.27- -0.08)	
Simulation results [b]:				
Simple families	0.00	0.25	-0.34	0.50
Inbreeding among neotenics:				
$N_f = N_m = 1$, X = 1	0.33	0.42	-0.14	0.62
$N_f = N_m = 1$, X = 3	0.57	0.65	-0.22	0.82
$N_f = N_m = 10$, X = 3	0.37	0.37	-0.02	0.56
$N_f = 2$, $N_m = 1$, X = 3	0.52	0.59	-0.17	0.78
$N_f = 200$, $N_m = 100$, X = 3	0.34	0.34	0.00	0.51
Interconnected nest buds:				
$N_f = N_m = 1$, X = 3, p = 0.5	0.66	0.56	0.22	0.68
$N_f = N_m = 1$, X = 3, p = 0.9	0.66	0.64	0.04	0.77
$N_f = N_m = 100$, X = 3, p =0.9	0.43	0.41	0.03	0.58

a This is the mean number of female neotenics in the four queenless colonies.

b Simulations values from Thorne, et al. (1999)
X = number of generations,
N_f and N_m = the number of female and male neotenics, respectively
p = proportion of workers collected from each bud nest

4.4 DISCUSSION

The results of the microsatellite analysis provided information regarding the family structure of the queenless colonies, revealed evidence of genetic differentiation within one of the colonies, and produced *F*-statistics for colonies whose number and gender of reproductives were confirmed by census.

4.4.1 Family structure and genetic differentiation

Based on their genotypic profiles, three of the queenless colonies were classified as simple families despite census evidence that they had contained female replacement reproductives (1, 4, and 11 individuals) for at least 4 years. Colony 36, which contained a single female neotenic, had been queenless the least amount of time (a minimum of 4 years), but the nest could have contained the offspring of this replacement reproductive and of the dead queen (Evans 2004). The *R. flavipes* colonies in this study exhibited expected levels of heterozygosity (mean=0.54); however, if levels of polymorphisms per locus had been higher the neotenic sisters would have been more likely to possess distinct genotypes and thus the profiles of their offspring would have resembled those of an extended family.

G-test results indicated that Colony 49 (which had been queenless for at least 6 years) was undergoing genetic differentiation. The genotypic classes represented among the workers indicated that at least four parents were contributing alleles, but it was impossible to determine how many of the 14 female neotenics were actively reproducing. The non-uniform distribution of alleles across the multi-resource network suggested that differentiation may have had a spatial component, either in offspring production or

preferred distribution (i.e. associations of closest kin). The resource whose workers harbored the two unique alleles also contained the king, the 14 neotenic sisters, and the entire brood clutch. Travel and mark-recapture data indicate that worker exchange occurred among all three sites throughout the Colony's 3-year tenure in the multi-resource network. Co-habitation of all reproductives does not imply nest budding in this particular case, but genetic isolation of a subset of workers despite constant contact with less genetically differentiated individuals lends credence to the hypothesis that physical or functional budding can occur without complete isolation from nestmates (Thorne, et al. 1999).

4.4.2 Hierarchical population structure

Wright's *F*-statistics provide a direct measure of population structure: the proportionate reduction in heterozygosity of individuals relative to both their colony (F_{IC}) and the total population (F_{IT}) and the proportionate reduction in heterozygosity of colonies relative to the total population (F_{CT}) (Pamilo 1984; Hartl & Clark 1997). In other words, F_{IT} is an estimate of total inbreeding within a population and can be partitioned into intra-colony effect (F_{IC}) and the genetic contrast between colonies (F_{CT}) (Bulmer, et al. 2001).

Thorne, et al. (1999) predicted that a simple family generated by monogamous, outbred, primary reproductives from an infinitely large, panmictic population should have an F_{IT} of 0.00, with virtually no chance that alleles at a given locus are identical by common descent. Moderate contrast should be evident between colonies (F_{CT}=0.25). Because this coefficient reflects shared alleles in the same subpopulation rather than in

the panmictic, global population considered when calculating the F_{IT}, even outbred colonies will share some autozygous alleles (Hartl & Clark 1997). Within a colony, alleles at the same locus should be negatively associated relative to what would occur in the population as a whole, thus F_{IC}= -0.34. The expected relatedness coefficient (*b*) in a population of diploid, simple families is 0.50.

F-statistics in the study colonies were identical to expected results for an extended family with two female and one male replacement reproductives that had been undergoing inbreeding for three generations (Thorne, et al. 1999). Census data revealed that the queenless colonies diverged from the model at three points. First, the laboratory colonies contained primary kings and an average of 7.5 (range=1-14) female neotenics. Second, the model forecast results of sibling-sibling pairings; however, the parent-offspring matings in the queenless colonies should have had an equivalent effect on the inbreeding coefficients (Luykx 1985). Finally, because individual neotenics were not traced over time, the number of elapsed inbred generations is unknown. However, 5 years of census data indicate that the number and weights of the females in each colony were consistent over time, suggesting a single generation of long-lived neotenics.

4.4.2.1 F_{IT}

An F_{IT} value of 0.52 and a relatedness coefficient of 0.78 indicates marked inbreeding in this *R. flavipes* "population." The founding of these colonies by probable siblings (individuals collected from the same flight) undoubtedly accounted for a portion of this loss of heterozygosity, but regional variation may have also contributed. *R. flavipes* F_{IT} estimates range from 0.08 in Massachusetts (Bulmer, et al. 2001),

to 0.21 in North Carolina (Vargo 2003), to 0.68 in Tennessee (Reilly 1987). Ecological conditions may foster varying levels of colony insularity which would in turn impact outbreeding opportunities. Colony age may also affect F_{IT} estimates; older colonies have more opportunity to experience inbreeding. These laboratory colonies were founded in April 1993, but precise data regarding field colonies are unknowable.

4.4.2.2 F_{IC}

Replacement of one or both primary reproductives with sibling neotenics increases the likelihood that alleles will become fixed and increase the ratio of homozygous loci in a colony. F_{IC} will approach zero as the effective number of neotenics rises, and become positive if subsets of individuals within the colony become homozygous for different alleles at the same locus, as they might if differentiation occurred at interconnected bud nests (Thorne, et al. 1999; Vargo 2003). Positive F_{IC} values could also be generated if distinct colonies were inadvertently collected together (Bulmer, et al. 2001) or if previously unrelated colonies fused together (Clément 1986; Leponce, et al. 1996; DeHeer & Vargo 2004). The queenless colonies' F_{IC} of -0.17 indicates an intermediate loss of heterozygosity, but not existence of differentiated bud nests.

4.4.2.3 F_{CT}

As colonies within a population become more inbred and different alleles are lost via drift, the genetic contrast between them will increase as each colony becomes homozygous for different alleles. The Thorne, et al. (1999) simulation demonstrated that

F_{CT} values rise with either increasing numbers of fecund replacement reproductives or multiple, inbred generations. An F_{CT} of 0.59 in this laboratory population indicates relatively high contrast between colonies.

4.4.3 Conclusion

Molecular analyses are valuable, revealing genetic relationships between individuals and within populations that are indistinguishable using morphological or behavioral characteristics. For example, worker movement within the Colony 49 foraging network suggested that individuals were part of a uniform family, but genotypic data revealed that genetic differentiation existed among spatially fragmented clusters within the group. This development, in the absence of physically isolated worker subsets, bolsters the hypothesis that genetically distinct "bud nests" might arise within an interconnected polydomous network.

Inference of breeding structure in the four laboratory-reared *R. flavipes* colonies has little intrinsic worth; each colony's reproductive status was directly deduced via census and thus the overall hierarchical structure revealed by the *F*-statistics was unsurprising. The value of this exercise lay in testing the model. Although one of the model scenarios perfectly matches the laboratory colonies' inbreeding coefficients, the actual breeding situations may depart from the model at two points: numbers of females present and/or breeding and number of generations. Based on the genetic analyses, two female neotenics were forecast by the simulation. The laboratory colonies contained an average of 7.5 female neotenics; however, genotypic data cannot determine the number of females actually contributing alleles (the effective number of females). In the

simulation, colonies underwent inbreeding for three generations. The number of elapsed generations in the laboratory was unclear.

Despite these nuances, the model correctly forecast a group of inbred colonies containing more females than males. The expected *F*-statistics for inbreeding among neotenics are sufficiently distinct from other broad categories of breeding schemes (i.e. bud nests) that minor differences in effective reproductive population size or number of inbred generations should not impact the model's accuracy. This result confirms that analysis of genotypes from sampled workers can correctly predict breeding structures of wild *R. flavipes* populations. Such resolution of relatedness and genetic architecture within eusocial insect colonies is invaluable.

5 CONCLUSION

Despite their ecological and economic impact, many aspects of subterranean termite biology are unclear because of challenges associated with the study of cryptic, social insects. Laboratory colonies initiated in 1993 by pairing *R. flavipes* alates afforded the rare opportunity to observe facets of colony ontogeny in whole, large families, to investigate the effect of remote food resources on foraging patterns, reproduction, and caste distribution, and to compare expected and observed inbreeding coefficients from colonies of known breeding structure.

Eleven years of demographic data revealed that primary reproductives can live up to a decade, the longest on record. Replacement female reproductives can be generated after queen death in clusters, and this differentiation coincides with the development of pre-alate nymphs. Heritable traits appear to underlie body sizes of individuals across all castes, as well as the proportion of a colony represented by soldiers

Results from manipulations using multi-resource foraging network suggest that long-standing assumptions regarding *R. flavipes* foraging and nesting dynamics require revision. Worker travel data, full-colony censuses, and mark-recapture sampling indicate that socially-intact laboratory colonies established a polydomous nest network and exploited resources using a dispersed central-place strategy rather conforming to an obligately monodomous, classic central-place foraging system. Rather than containing a single, reproductive nucleus, the reproductives and the brood cluster were not located in the same node a significant portion of the time. Workers were significantly more likely to be repeatedly collected from the same node, indicating that they did not forage

regularly among the other nodes nor did they shuttle habitually between their preferred satellite and the central resource. This fidelity, coupled with long-term marker impermanence, violated assumptions of the Lincoln Index, which consequently produced significantly inaccurate estimates of colony population totals.

Census results revealed the breeding structure of the colonies (i.e. simple family, neotenics) and microsatellite analysis generated genotypes from queenless workers. Laboratory-population *F*-statistics calculated with these genotypes provided valuable comparison to expected inbreeding coefficients. The observed and expected values were not significantly different, indicating that *F*-statistics can be an accurate predictor of *R. flavipes* family structure and thus an important tool in the evaluation of field colonies.

These results suggest that the subterranean termite *R. flavipes* exercises novel nesting and foraging dynamics. These data offer unique insight into long-term colony ontogeny and demography, as well as the relationship between breeding structure and inbreeding coefficients. Hopefully, this refined understanding of *R. flavipes* will inform and inspire future research of this ecologically vital and economically important social insect.

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