

# Sex Ratios in Field Populations of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) on Sapelo Island, Georgia, USA

by

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## ABSTRACT

Social insects provide some of the most striking examples of sexual division. Thirty termite inspection ports were sampled four times in 11 months (September 2008 – July 2009). A total of 90 collections of a not-yet described *Reticulitermes* spp. were examined to estimate sex ratios. The range of sex ratios (male to female) observed, by caste, were: 0.49-3.50 for workers, 0.75-5.25 for soldiers and 0.02-26.00 for nymphs. These results provide new minimum and maximum values of sex ratios for each caste in *Reticulitermes* and reveal that there is a great deal of variation in field population sex ratios. The variation could be attributed to sample size, termite population movement between sample dates, and species differences. The data are discussed relative to interpretation of termite sex ratio data.

Key words: Isoptera, *Reticulitermes*, sex ratio, sample size, fluctuations of sex ratios

## INTRODUCTION

The study of sex ratio evolution is an active area of evolutionary biology (Bourke & Franks 1995). Fisher (1929) suggested that frequency-dependent selection maintains equal sex ratios when parental expenditure on offspring is equal, resulting in a 1:1 sex ratio. However, biased sex ratios have been reported in the Isoptera reflective of a remarkably complex and diversified set of developmental pathways (Crosland *et al.* 1994, Lenz & Runko 1993, Roisin 2000). Recent advances in sexual selection in termites have created growing interest in using the Isoptera as models for research of non-Fisherian sex ratios (Hayashi *et al.* 2007, Korb *et al.* 2009, Lo *et al.* 2009).

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Previous studies of sex ratios from termite genera such as *Nasutitermes* (Thorne 1983), *Coptotermes* (Roisin & Lenz 2002) and *Reticulitermes* (Dean & Gold 2004, Zimet & Stuart 1982) have given important baseline data. Much of the data in the literature is based on sample sizes of 25 to 50 termites. However, a recent study using census data from laboratory cultures of *R. flavipes* suggest that a minimum sample size of 100 individuals is necessary to represent a population's true sex ratio although these numbers are not often attained when sampling field populations (Hu & Forschler 2011). It is therefore important to recognize when interpreting results that the confidence values for a sex ratio estimate obtained from a sample of 25 termites provides 80% accuracy with an accepted error of 20% (Hu & Forschler 2011).

While the application of a G-test for heterogeneity has been used to identify departures from a 1:1 sex ratio (Matsuura 2006, Muller & Korb 2008), that method combines data and can mask variation associated with individual castes, sample location or sampling interval. For example, a G-test of two collections, one male-skewed accompanied by an equal number of female-skewed individuals are recorded as 1.00 (male to female) or equal despite the fact that the two collections were heavily biased towards one sex. Thus, examining ratios by caste, or some other categorization, within and between collection dates or sites might provide insights into developmental pathways over a G-test when describing sex ratios from termite populations.

Recent analyses of mitochondrial DNA (sequence of the *cox II* gene) has revealed a novel species of *Reticulitermes* from Sapelo Island, Georgia, USA (Sillam-Dusses & Forschler 2010). Further investigation based on soldier and alate morphology and flight times also separates *R. spp* from the described species of *Reticulitermes* (Forschler unpublished data). This paper reports sex ratios of that novel species from 30 collection sites on Sapelo Island, Georgia, USA. Sex ratios were determined for collections that provided a sample size of at least 100 workers and we defined sex ratios as neutral (0.67-1.50), female-skewed (<0.67), and male-skewed (>1.50) based on individuals counted within a collection site and date. The results illustrate the tendency of sexual bias, by caste, within a single collection and variation of caste sex ratios over time from the same collection site which are discussed relative to interpreting termite sex ratio data.

## MATERIALS AND METHODS

### Termite Collections

The field site was on Sapelo Island in MacIntosh County, Georgia, USA. Thirty termite inspection ports (IP) located on the south end of the island were sampled every 3 months from September 2008 to July 2009. An IP consisted of a PVC pipe receptacle that was 16-cm long by 10-cm diameter. Termite sandwiches were placed in each IP and were composed of 9 pieces of southern yellow pine (*Pinus* spp) (2 cm × 2 cm × 12 cm) separated by wooden dowels (0.2 cm × 0.5 cm) and held together with a plastic cable tie (24-cm length). Termite IP's were capped using a 10-cm-diameter plastic knock-out plug (Forschler 1996). We collected 90 samples that contained more than 100 termites (including workers, soldiers and nymphs) that were stored in 100% ethanol for use in this study as well as being placed in our voucher collection.

### Sex and Species Identification

Sex was determined by the arrangement of sternal plates as described by Zimet and Stuart (1982). The sternal plate character was verified as a correct indication of sex by dissecting 10-20 individuals of each sex to confirm the presence of ovaries or testes (Roisin & Lenz 2002). Species identification was made using soldier characters in published keys (Scheffrahn & Su 1994), worker live weight (unpublished data indicated that *R. hageni* - the species designation according to published keys – mean worker live weight, an average of 5 groups of 10 workers, is 1.8 mg per termite while *R. spp.* average 1 mg), and DNA sequence from the *cox II* gene (Sillam-Dusses & Forschler 2010). We assumed all termites used in this study were representative of the *R. spp.* designation because worker live weight and DNA were consistent over the 11 months of this study, .

### Sample Size

Obtaining a sample size for this study of 100 for each caste per collection site/date was not possible so we randomly selected 100 workers from each IP and those collections that provided more than 25 soldiers and/or nymphs. Sex ratios of the aforementioned castes were compared using Statistcix 9.0 (Analytical Software 2008). We define a sex ratio (male to female) within 0.67-1.50 as neutral, <0.67 as female-skewed and >1.50 male-biased.

## RESULTS AND DISCUSSION

The sex ratios for our worker samples ranged from 0.49-3.50 (N=90) (Table 1). Of the worker samples we examined 3.3% were female-skewed, 64.4% neutral and 32.2% male-skewed. Sex ratios for soldiers ranged from 0.75-5.25 (N=19). The soldier collections were classified as neutral 47.4% of the time, 52.6% were male-skewed and none found to be female-skewed. Sex ratios for nymphs ranged from 0.02-26.00 (N=16) with 62.5% female-skewed, 25.0% neutral and 12.5% male-skewed (Fig. 1). The mean sex ratio for the combined data (sex ratio by collection site/date and caste) indicated that worker and soldier collections were not significantly different even though, by our definition, the soldiers provided a male biased mean (Kruskal-Wallis multiple range test at  $\alpha=0.05$ ) (Table 1). The mean value of the combined data for the nymphal caste was significantly different yet the mean indicated a male bias despite

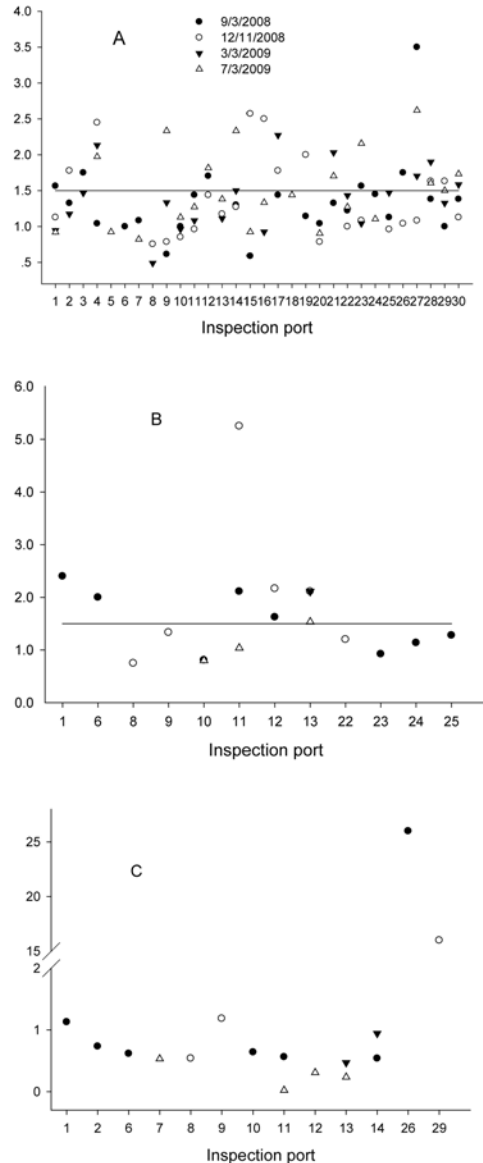


Fig. 1 Distribution of sex ratios of *Reticulitermes* spp. during one year by collection site. A: workers (sample size=100), B: soldiers ( $25 \leq$  sample size  $\leq 100$ ) and C: nymphs ( $25 \leq$  sample size  $\leq 100$ ).

Table 1. Sex ratios of 3 castes of *Reticulitermes* spp.

Class	n	Mean±S.E.	Range	Female-skewed	Neutral	Male-skewed
Worker	90	1.41±0.05a	0.49-3.50	3.3%	64.4%	32.2%
Soldier	19	1.70±0.25a	0.75-5.25	0	47.4%	52.6%
Nymph	16	3.15±1.80b	0.02-26.00	62.5%	25.0%	12.5%

Means followed by different letters were significantly different (Kruskal-Wallis multiple range test at the 0.05 level of probability). n: number of collections studied.

the high proportion of individual samples that were female biased (Table 1). The proportion of sex ratios by collection site/date indicated that soldier and worker sex ratios tended to deviate from neutral in the direction of a male bias while nymphs tended to be female skewed (Table 1).

A variety of sex ratios have been reported in *Reticulitermes*. Zimet and Stuart (1982) found that sex ratios from field populations were 1.33 for workers, 0.76 for soldiers, and 2.70 for nymphs in *R. flavipes*. Jones *et al.* (1988) and Matsuura (2006) recorded that sex ratios of workers and soldiers of *R. flavipes* were essentially 1:1. Dean and Gold (2004) reported sex ratios of 0.97 for workers, 0.79 for soldiers and 0.88 for nymphs of *R. flavipes* – all neutral according to our classification of sex ratios. Our data range (0.49-3.50 for workers, 0.75-5.25 for soldiers and 0.02-26.00 for nymphs) covered all previous sex ratio data and provided new population sex ratio limits for each caste in *R. spp.* The data from this field survey revealed that there is a great deal of variation in sex ratio estimates in subterranean termites field samples both within and between collection sites/dates.

Eight IP collection sites (IP #1, 6, 8, 9, 10, 11, 12 and 13) provided all three castes over the course of our four sampling dates. Sex ratios were calculated by IP (collection site and date) assuming the same population visited that IP over the 11 months that occupied our sampling interval and tested using a G-test for heterogeneity to identify significant departures from a 1:1 sex ratio. The results ( $P=0.004$  for IP #1;  $P=0.564$  for IP #6;  $P=0.001$  for IP #8;  $P=0.102$  for IP #9;  $P=0.070$  for IP #10;  $P=0.641$  for IP #11;  $P=0.001$  for IP #12;  $P=0.870$  for IP #13) of G-tests showed 3 out of 8 IPs provided an equivalent or neutral sex ratio. However, only 1 out of the 8 IPs provided a neutral sex ratio in every sample for all castes - IP #10 (Fig 1). Comparison of bias results from the G-test and individual sample dates for a particular

IP indicated that the latter provided more variation. Collections from the same IP over time provided worker sex ratios that varied from one classification to another on nearly 80% of the collection dates. An example would be collections from IP #9 that were female-skewed in September 2008, neutral in December 2008 and March 2009, and male-skewed in July 2009 (Fig. 1). The collections from IP #26 on October 2008 and IP #29 on December 2008 provided a male bias in the nymphal and workers castes on those same dates (Fig. 1). However, both IP's had neutral worker sex ratios on all other sample dates – December 2008 and all dates for #26 & #29, respectively (Fig. 1). Collection site #11 provided four worker collections that were deemed neutral, two soldier collections identified as male-skewed and one neutral while the two nymphal collections were female skewed (Fig. 1). The collections from IP #11 represent a population that fits the standard perception of sex ratios in *Reticulitermes* in that we had an overall neutral sex ratio when all castes are considered (Dean & Gold 2004, Hayashi *et al.* 2007, Jones *et al.* 1988, Zimet & Stuart 1982). Yet, examining the data by caste we recorded a sex-linked bifurcation at the soldier/nymphal developmental lines with the soldier caste male biased and the nymphal female biased (Fig. 1). Approximately 1/3 (29%, N=28) of our worker samples, from an IP, provided a consistent neutral sex ratio over 2 or more sample dates which follows the expected outcome assuming the same population visited that site while not one IP provided a consistently biased ratio (Fig. 1).

The variety of sex ratios obtained from these collection sites is interesting from the vantage point of understanding subterranean termite development and interpreting data on sex ratios from field collections. The variation could be the result of sample size as predicted by Hu & Forschler (2011) – particularly with the sample size used for the minor castes, soldiers and nymphs. It could be that the distribution of sexes between feeding sites used by a particular population is unequal at the time of collection or that the populations visiting these IP's changed frequently (Forschler & Jenkins 2000, Haverty *et al.* 2010). Molecular analysis is now underway to ascertain the relatedness of the samples collected over time. These data raise more questions than they answer yet it is important that caution should be exercised when interpreting sex ratio data from field collections. These data also highlight the need for further study of mechanisms involved in determining termite population sex ratios.

Henderson and Rao (1993), working with *Coptotermes*, and Jones *et al.* (1988) and Matsuura (2006) working with *R. flavipes* suggested that the overall sex ratio of a subterranean termite colony is neutral. Recent work has shown that worker sex ratios from 8 laboratory cultures (colonies) of *R. flavipes* headed by adult reproductive pairs (king and queen) were all male skewed (Hu and Forschler, unpublished) revealing that colony-level sex ratios are not necessarily 1:1. One intriguing question remains to be answered - do reproductives lay eggs that are predetermined to be one sex or another resulting in skewed sex ratios (Lenz & Runko 1993)? Hayashi *et al.* (2007) used experimental pairings of neotenic phenotypes to conclude that sex ratios of nymphs and workers followed a single X-linked locus genetic model. Their model predicts that workers sex ratios from fNmN, fEmN and fEmE paired neotenic crossings (f: female, m: male, N: nymphoid and E: ergatoid) would be 1.00, and worker offspring in fNmE would be exclusively male. However, our data of estimates from individual collection sites show that worker sex ratios can vary between 0.49-3.50, and only 22.4% of our worker collections (N=90) fit Hayashi's model. Perhaps this inconsistency is related to the status of primary reproductives as suggested by Hayashi *et al.* (2007) – we did not determine the mating system for the various collections, or a difference between *Reticulitermes* species – Hayashi *et al.* used *R. speratus*, or issues with sample size and true population sex ratios (Hu & Forschler 2011).

The sex ratios from our temporal collections at single collection points showed there were fluctuations over time (Fig. 1). The mechanism(s) involved in determining the sex ratio of a termite population is poorly understood although estimates that use less than 100 individuals should not be considered definitive when interpreting results from field collections. Sex ratios of subterranean termite field populations deserves more hypothesis-driven examination but the value of field observations is that they can illuminate issues that challenge concepts and provide an impetus for future experimentation.

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