Sample Size Requirement in the Study of Subterranean Termite (Isoptera: Rhinotermitidae) Sex Ratio[1]

Jian Hu[2] and Brian T. Forschler[3]

Department of Entomology, University of Georgia, Athens, Georgia 30602, USA

J. Entomol. Sci. 46(1): 23-29 (January 2011)

Abstract The choice of an appropriate sample size is a neglected topic in termite sex ratio research because the literature contains a variety of sample sizes with most < 50 individuals. We compared the effect of sample size (from 25 - 800) on matching the ratio obtained from several *Reticulitermes flavipes* (Kollar) populations of 1600 individuals. This study showed that a sample size of < 50 is too small to estimate the actual sex ratio of a population. A sample size of at least 100 termites is required to provide ±10% error in a census of *Reticulitermes* sex ratios. The results should improve confidence in estimates of termite colony sex ratios, and we propose a standard sample size for such studies.

Key Words termite, sex ratio, sample size, *Reticulitermes flavipes*

The study of sex ratio evolution is an active area of evolutionary biology (Bourke and Franks 1995). Sex ratio information has far-reaching evolutionary consequences for both parents and offspring providing insights into resource partitioning and reproductive potential within populations (Dean and Gold 2004, Shuker et al. 2009). Investigations of sex ratios are essential to the study of termite mating systems and caste development. Progress has been made in understanding sex allocation in termites, such as *Amitermes* (Noirot 1955), *Coptotermes* (Roisin and Lenz 2002), *Macrotermes* (Darlington 1986), *Nasutitermes* (Thorne 1983) and *Reticulitermes* (Hayashi et al. 2007). However, a variety of sample sizes are reported, and most studies used sample sizes of 25 - 50 individuals (Howard and Haverty 1980, Jones et al. 1988) and provide variable results (Table 1).

According to mark-release-recapture estimates of *Reticulitermes* and *Coptotermes* foraging populations, the numbers range from 127 - 6.8 × 10^6 termites per colony and workers account for 95 - 98% of that number (Grace 1990, Su et al. 1993, Forschler and Townsend 1996). The question arises, based on the aforementioned variability reported in the literature, of sample size influence on sex ratio data from such large populations. The choice of sample size is affected by several factors, including population size, the desired level of precision, the level of confidence for risk, and the degree of variability in the attributes being measured (Miaoulis and Michener 1976).

In this study, we attempted, using worker caste members from *Reticulitermes flavipes* (Kollar), to identify the sample size appropriate to meet several levels of precision

[1]Received 04 April 2010; accepted for publication 27 June 2010.
[2]Current address: Guangdong Entomological Institute, Guangzhou 510260, China.
[3]Corresponding author (e-mail: bfor@uga.edu).

Table 1. Examples of sample size from the termite sex ratio literature.

Species	Class	Sex ratio (♂/♀)	Sample size	Reference
	Worker	0.22 - 3.40	20 - 25	Husseneder et al. 2008
C. formosanus	Soldier	3.00	209	Henderson and Rao 1993
	Alate	0.71 - 1.73	57 - 111	Husseneder and Simms 2008
C. gestroi	Neotenic	all male to all female	1 - 28	Costa-Leonardo et al. 2004
C. lacteus	Larvae	0.61 - 5.67	9 - 103	Roisin and Lenz 2002
	Worker	0.52 - 1.17	100 - 106	
C. domesticus	Soldier	1.00	52	Muller and Korb 2008
R. flavipes		1.00	Unknown	Jones et al. 1988
	Worker	1.03	25	Dean and Gold 2004
		1.33	32 - 100	Zimet and Stuart 1982
		0.75	1 - 9	Zimet and Stuart 1982
	Soldier	0.78	25	Dean and Gold 2004
		1.00	200	Matsuura 2006
	Nymph	0.88	25	Dean and Gold 2004
		2.67	1 - 9	Zimet and Stuart 1982
	Alate	0.89	24 - 52	Dean and Gold 2004
R. speratus	Worker	1.00 to all male	100 - 605	Hayashi et al. 2007
R. speratus	Nymph	all male to all female	100 - 605	Hayashi et al. 2007

for termite sex ratio studies. Our study assumed a worker population of >300,000 and applied 2 strategies to determine appropriate sample size: (1) A sex ratio census of 4 field-collected populations, and (2) application of a statistical model to calculate sample size.

Materials and Methods

Four distinct populations of *R. flavipes* were collected for this study: 3 from Athens in Clarke Co. (Populations A, B, & C) and one from Sapelo Island in McIntosh Co., (population D) Georgia, USA. Termites were fixed in 100% ethanol. One thousand six hundred workers from each population were sexed as determined by the arrangement of sternal plates described by Zimet and Stuart (1982). The sternal plate character

was verified as a correct indication of sex in workers by dissecting 10 - 20 workers of each sex to confirm the presence of ovaries or testes. The seventh sternite of males is similar in size to the other sternites and is not convex, whereas females are characterized as having an enlarged, elongated and distally-convex seventh sternite. The sex ratio for each population of 1600 workers was then compared with the ratios obtained from randomly assigning individuals from the larger population into sample sizes of 25 (64 replications), 50 (32 replications), 100 (16 replications), 200 (8 replications), 400 (4 replications) and 800 (2 replications) within ±5%, ±10% or ±20% error.

Results and Discussion

The sex ratios of workers from the 4 *R. flavipes* populations were 1.35 for population A, 0.89 for population B, 0.39 for population C and 0.99 for population D. Three of the populations were considered to represent a neutral sex ratio based on our classification of 1.5 - 0.67 as neutral. Population C, however, was considered female biased at 0.39. According to the results, 24.9 ± 8% of the 25 sample size replicates were within ±5% error, and the sample size of 100 provided 34.3 ± 6% of the replicates consistent with the sex ratio of the sample of 1600 (Fig. 1A). Whereas the sample size of 25 had 44.7 ± 7% of the replicates within ±10% error, 84% of the 100 sample size replicates were consistent with the sample size of 1600 (Fig. 1B). And, within ±20% error, 76.3 ± 11% of replicates of sample size of 25 and 100% replicates of sample size of 100 were consistent with the sample size of 1600 (Fig. 1C).

The sex ratio estimate for the sample size of 25 varied as much as 9 fold from the population estimate (Fig. 2A), and a sample size of 50 varied as much as 3 times. Within either ±5%, ±10%, or ±20% error, a sample size of 25 or 50 is too small to accurately estimate a colony's sex ratio. A sample size of 100, however, is sufficient to estimate a population's sex ratio within ±20% error with 100% confidence (Fig. 2B), but we usually allow sampling error within ±5% or ±10%. Though the sample size of 400 is more accurate within ±10% error (Fig. 2C), it is often impractical to obtain such numbers from a field collection.

Using the model of Cochran (1963) for proportions,

$$N_o = \frac{Z^2pq}{e^2},$$

where n_0 is the sample size, the value for Z is the upper $\alpha/2$ point of the normal distribution, Z = 1.96, p is the estimated proportion of an attribute that is present in the population, q is 1-p (assume its maximum value when $P = 0.5$), and e is the desired level of precision, we determined that a sample size of 96 or 97 is sufficient to estimate the actual sex ratio with ±10% precision whereas meeting the criteria. According to our results, a sample size of 100 yields a ± 16% error. This is likely due to, first, we assumed a 100% confidence level using a census, whereas the statistical model provided a 95% confidence level, and second, the mean value of 1600 specimens we compared has an error of ±3% for being the true value in a large population. Although the remaining ±2.5% error of the sample size of 100 could not be explained, using a census was consistent with the statistical model calculation because they vary less than 10%. Therefore, we suggest a sample size of 100 be adopted in sampling for termite sex ratios.

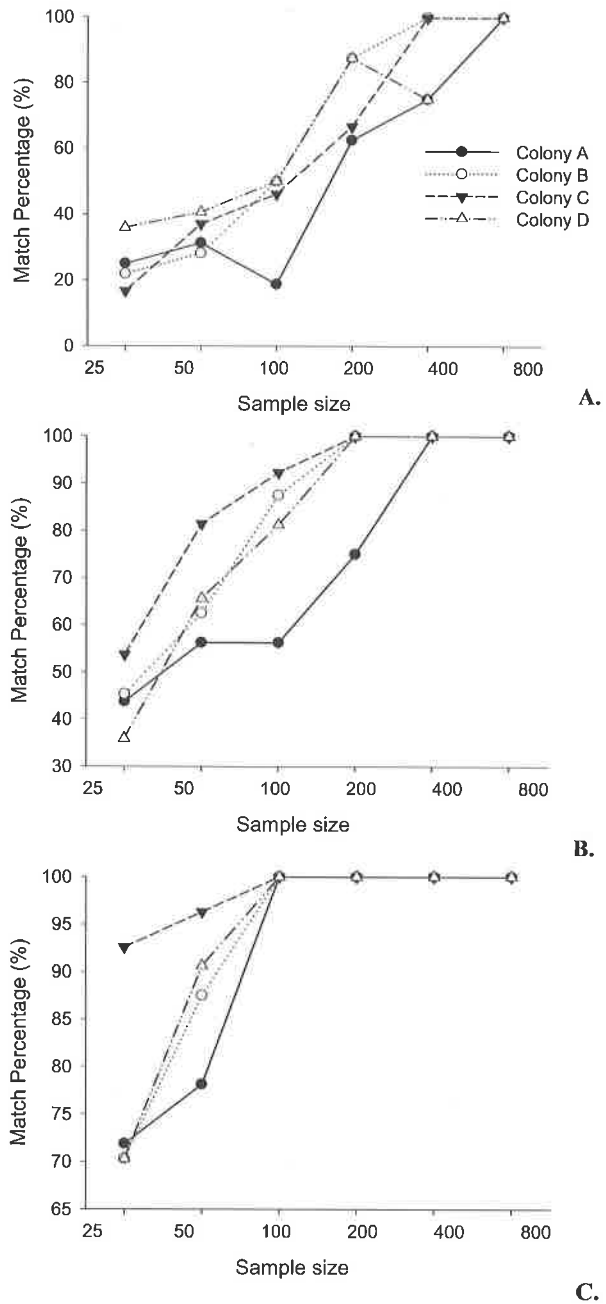

Fig. 1. Match of percentage of female termites compared with 1600 observations within +5% (A), +10% (B) and +20% (C) error between different sample sizes (Four colonies: A, B, C, and D).

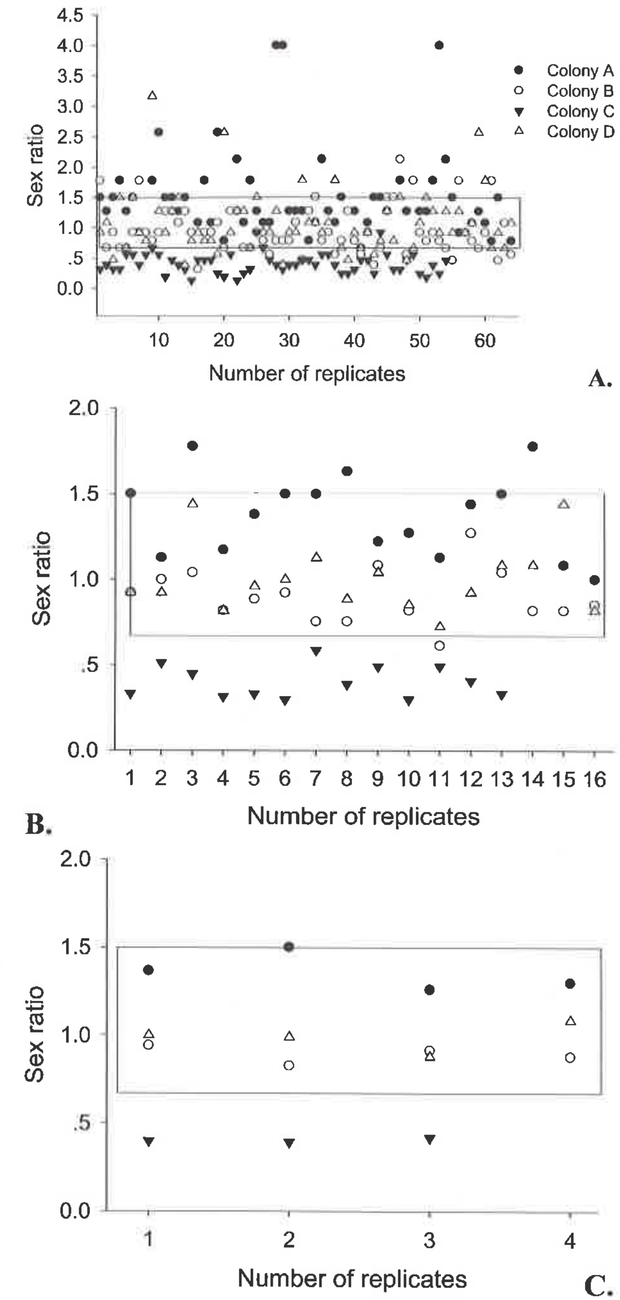

Fig. 2. Sex ratio's distribution for a sample size of 25(A), 100(B) and 400(C) (Four colonies: A, B, C, and D). Rectangular blocks show the data within the distribution of neutral sex ratio: 0.67 - 1.5.

Although sex ratio is easily understood as an expression of the numbers of males and females in a population, the error caused by the ratio transformation of the data can double the number expressed as a percentage. That is because in a ratio, both numerator and denominator have proportional errors (Boomsma and Grafen 1991). The sex ratio can, therefore, double the error compared with the percentage. If a ratio is chosen as the method to express the number of sexes, one must augment the sample size beyond our recommendation of 100.

In the strictest condition, only 50:50 is neutral and any increase in either sex is biased. We suggest the range that determines, within an acceptable confidence level, if a colony is neutral or biased. For example, with a sample size of 100 we are willing to accept ±10% error, a sex ratio between 40:60 or 60:40 is a reasonable error in reporting a sample as representative of a neutral (50:50) distribution. Thus, a sex ratio of 0.67 - 1.5 is neutral and <0.67 and >1.5 are biased for a sample size of 100. However, neutral and biased are man-made definitions and should be clearly described and understood by researchers examining proportions of sexes in termite societies.

Acknowledgments

This work was funded by State appropriations from the Georgia Department of Agriculture and a Start-up grant from the Guangdong Entomological Institute, China.

References Cited

Boomsma, J. and A. Grafen. 1991. Colony-level sex ratio selection in the eusocial Hymenoptera. J. Evol. Biol. 4: 383-407.

Bourke, A. F. G. and N. R. Franks. 1995. Social evolution in ants. Princeton Univ. Press, Princeton, NJ.

Cochran, W. G. 1963. Sampling techniques. John Wiley & Sons. Inc, NY.

Costa-Leonardo, A. M., A. Arab and F. E. Casarin. 2004. Neotenic formation in laboratory colonies of the termite *Coptotermes gestroi* after orphaning. J. Insect Sci. 4: 1-6.

Darlington, J. 1986. Seasonality in mature nests of the termitem *Macrotermes michaelseni* in Kenya. Insectes Soc. 33: 168-189.

Dean, S. R. and R. E. Gold. 2004. Sex ratios and development of the reproductive system in castes of *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 97: 147-152.

Forschler, B. T. and M. L. Townsend. 1996. Mark-release-recapture estimates of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) colony foraging populations from Georgia, USA. Environ. Entomol. 25: 952-962.

Grace, J. K. 1990. Mark-recapture studies with *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology 16: 297-303.

Hayashi, Y., N. Lo, H. Miyata and O. Kitade. 2007. Sex-linked genetic influence on caste determination in a termite. Science 318: 985-987.

Henderson, G. and K. S. Rao. 1993. Sexual dimorphism in soldiers of Formosan Subterranean Termite (Isoptera: Rhinotermitidae). Sociobiology 21: 341-345.

Howard, R. W. and M. I. Haverty. 1980. Reproductives in mature colonies of *Reticulitermes flavipes*: abundance, sex-ratio, and association with soldiers. Environ. Entomol. 9: 458-460.

Husseneder, C. and D. M. Simms. 2008. Size and heterozygosity influence partner selection in the Formosan subterranean termite. Behav. Ecol. 19: 764-773.

Husseneder, C., J. E. Powell, J. K. Grace, E. L. Vargo and K. Matsuura. 2008. Worker size in the Formosan subterranean termite in relation to colony breeding structure as inferred from molecular markers. Environ. Entomol. 37: 400-408.

Jones, S. C., J. P. La Fage and R. W. Howard. 1988. Isopteran sex ratios: phylogenetic trends. Sociobiology 14: 89-156.

Matsuura, K. 2006. A novel hypothesis for the origin of the sexual division of labor in termites: which sex should be soldiers? Evol. Ecol. 20: 565-574.

Miaoulis, G. and R. D. Michener. 1976. An introduction to sampling. Kendall/Hunt Publ. Co., Dubuque, IA.

Muller, H. and J. Korb. 2008. Male or female soldiers? An evaluation of several factors which may influence soldier sex ratio in lower termites. Insectes Soc. 55: 213-219.

Noirot, C. 1955. Recherches sur le polymorphisme des termites supérieurs (Termitidae). Ann. Sci. Nat. Zool. (11e sér.) 17: 399-595.

Roisin, Y. and M. Lenz. 2002. Origin of male-biased sex allocation in orphaned colonies of the termite, *Coptotermes lacteus*. Behav. Ecol. Sociobiol. 51: 472-479.

Shuker, D. M., A. M. Moynihan and L. Ross. 2009. Sexual conflict, sex allocation and the genetic system. Biol. Lett. 5: 682-685.

Su, N. Y., P. M. Ban and R. H. Scheffrahn. 1993. Foraging populations and territories of the eastern subterranean termite (Isoptera, Rhinotermitidae) in southeastern Florida. Environ. Entomol. 22: 1113-1117.

Thorne, B. 1983. Alate production and sex ratio in colonies of the neotropical termite *Nasutitermes corniger* (Isoptera; Termitidae). Oecologia 58: 103-109.

Zimet, M. and A. M. Stuart. 1982. Sexual dimorphism in the immature stages of the termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology 7: 1-7.