Female-biased sex allocation and lack of inbreeding avoidance in *Cubitermes* termites

Veronica M. Sinotte | Benjamin H. Conlon | Elena Seibel | Jan W. Schwitalla | Z. Wilhelm de Beer | Michael Poulsen | Nick Bos

Abstract
Sexually reproducing organisms face a strong selective pressure to find a mate and ensure reproduction. An important criterion during mate-selection is to avoid closely related individuals and subsequent potential fitness costs of resulting inbred offspring. Inbreeding avoidance can be active through kin recognition during mate choice, or passive through differential male and female-biased sex ratios, which effectively prevents sib-mating. In addition, sex allocation, or the resources allotted to male and female offspring, can impact mating and reproductive success. Here, we investigate mate choice, sex ratios, and sex allocation in dispersing reproductives (alates) from colonies of the termite *Cubitermes tenuiceps*. Termites have a short time to select a mate for life, which should intensify any fitness consequences of inbreeding. However, alates did not actively avoid inbreeding through mate choice via kin recognition based on genetic or environmental cues. Furthermore, the majority of colonies exhibited a female-biased sex ratio, and none exhibited a male-bias, indicating that differential bias does not reduce inbreeding. Sex allocation was generally female-biased, as females also were heavier, but the potential fitness effect of this costly strategy remains unclear. The bacterium *Wolbachia*, known in other insects to parasitically distort sex allocation toward females, was present within all alates. While *Wolbachia* is commonly associated with termites, parasitism has yet to be demonstrated, warranting further study of the nature of the symbiosis. Both the apparent lack of inbreeding avoidance and potential maladaptive sex allocation implies possible negative effects on mating and fitness.

Keywords
alates, kin recognition, mate choice, reproductive investment, sex ratio bias, *Wolbachia*
1 | INTRODUCTION

For sexually reproducing organisms, selecting a mate and securing reproduction are critical steps in maximizing individual fitness. Mating with a relative can have high costs, including the expression of deleterious recessive mutations through increased homozygosity (Andersson & Hughes, 1996; Charlesworth & Willis, 2009; Pusey & Wolf, 1996). Inbreeding is avoided in many animals through kin recognition (Blouin & Blouin, 1988; Pusey & Wolf, 1996), sex-biased dispersal (Noirot, 1989; Pusey & Wolf, 1996), and differential sex ratios, where individuals in a population specialize on producing predominantly males or females, consequently reducing the likelihood of mating with a sibling (Vargo & Husseneder, 2010). Sex allocation, defined as the energy invested in male and female offspring, can influence the mating, resources procurement, and reproductive success of these individuals or their kin, ultimately impacting inclusive fitness (Fisher, 1930: West, 2009). For monogamous organisms, in particular, inbreeding or maladaptive sex allocation may carry substantial fitness costs.

The lifetime monogamy of termites means that their single mate choice has lasting consequences with regards to inbreeding. Unlike social Hymenoptera, termites are diplo-diploid, and different selection pressures can affect whether inbreeding is avoided, preferred, or tolerated. Although inbreeding has been predicted to often be tolerated in many organisms due to increased fitness benefits of passing on related genes and increased parent-offspring relatedness (Duthie & Reid, 2015), egalitarian parental care (such as in termites) is predicted to undermine this and lead to inbreeding avoidance (Kokko & Ots, 2006); for example through kin recognition or differential sex ratios. Furthermore, inbreeding should not be favored in monogamous organisms unless inbreeding avoidance carries a cost (Waser et al., 1986). Reproductive termites alates leave their natal nest in a nuptial flight, during which they must rapidly choose a mate for life amidst substantial environmental risk (Dial & Vaughan, 1987; Nutting, 1969). This environmental risk during the mating flight could represent a high potential cost to inbreeding avoidance. This in turn could select for behavior that minimizes time spent aboveground, thus reducing the amount of mates that can be encountered, leading to higher inbreeding tolerance (Kokko & Ots, 2006). After the mating flight, the alate pair starts a colony that will undergo multiple reproductive episodes (Eggleton, 2010); thus, mate choice during this brief period can influence lifetime reproductive success across decades. Inbreeding between related alates regularly occurs in termite populations and can result in negative fitness effects (Calleri et al., 2006; DeHeer & Vargo, 2006), which makes it likely that inbreeding should preferentially be avoided if possible. Although sufficient dispersal distances can facilitate outbreeding (Husseneder et al., 2006; Vargo & Husseneder, 2010), termites are generally not considered as apt flyers (Nutting, 1969). Therefore, if inbreeding avoidance is important in this termite species, it is likely that kin recognition or differential sex ratios could also play a role. Alates of basal termite clades (lower termites) do not display kin recognition (but see Aguilera-Oliva et al., 2015; DeHeer & Vargo, 2006; Husseneder & Simms, 2008; Kitade et al., 2004; Shellman-Reeve, 2001), yet the capacity for kin recognition in derived termite lineages (higher termites) is poorly understood (Vargo & Husseneder, 2010). Differential bias in sex ratios between colonies has only been observed in lower termites (Husseneder et al., 2006). Thus, in higher termites, kin recognition and/or sex ratios could potentially play a vital role in reducing the risk of inbreeding when choosing a monogamous mate.

Organisms are predicted to equally allocate resources to male and female offspring (Fisher, 1930), and biased allocation can have positive or negative effects on offspring’s mating and reproductive success. Sex allocation measurements, as opposed to sex ratio, not only incorporate the number of offspring, but also an energetic estimate of each sex. Generally, bias is maladaptive because it increases the reproductive value of the rarer sex or sex with lesser allocation (Fisher, 1930). However, adaptive bias may occur if a specific sex reaps higher fitness benefits under good environmental conditions, and thus receives greater allocation (Trivers & Hare, 1976). Bias can also improve mating success or resource procurement in cases of cooperation or competition with kin, such that allocation maximizes inclusive fitness (Hamilton, 1967; Clark, 1978; Taylor, 1981). In termites, adaptive sex allocation bias can further arise through within-colony mother-son or father-daughter mating, which generates differential fitness between sexes (Hellemans et al., 2019; Kobayashi et al., 2013; Roisin & Lenz, 2002; Vargo et al., 2012). Conversely, maladaptive bias can be caused by sex allocation distorters like the bacterial symbiont Wolbachia, which commonly occurs in insects (Charlat et al., 2003; Correa & Ballard, 2016). Wolbachia is vertically transmitted by females, and thus the parasite can increase its fitness by inducing female bias through male-killing, feminization, and parthenogenesis (Werren et al., 2008). Multiple termite taxa associate with Wolbachia (Bandi et al., 1997; Bordenstein & Rosengaus, 2005; Hellemans, Kaczmarek, et al., 2019; Lo & Evans, 2007; Roy & Harry, 2007; Salunke et al., 2010) but parasitic manipulation has yet to be observed.

Here, we evaluate inbreeding avoidance and sex allocation to infer the potential effects on termite mating and reproductive success. We utilized reproductive alates from the soil-feeding higher termite Cubitermes tenuiceps, since moderate inbreeding and symbiosis with Wolbachia have been observed within the genus (Roy et al., 2010; Roy & Harry, 2007). Inbreeding avoidance was assessed based on kin recognition during a behavioral assay with a male choosing between a nestmate and non-nestmate female from a local or foreign location; thus, determining if genetic or environmental cues affect mate choice. Differential sex ratio bias among colonies was examined by counting all alates from each colony prior to the nuptial flight. Sex allocation was then determined based on the number and dry weights of each sex, as dry weight is a common proxy used for energetic investment in termites and other social insects (Cremer & Heinze, 2002; Hellemans, Fournier, et al., 2019; Kobayashi et al., 2013; Sundstrom et al., 1996; Trivers & Hare, 1976). Finally, the incidence of Wolbachia infection in alates was examined to investigate whether this co-occurred with sex allocation, indicating potential parasitism.
MATERIALS AND METHODS

2.1 | Colony sampling and termite identification

Twelve nests of *Cubitermes tenuiceps* from two locations around Pretoria, South Africa, (Table S1) were systematically excavated in October 2019 when no rain had fallen for 5 months (South African Weather Service, weathersa.co.za), with the aim to collect all alates. Upon collection, alates were stored in boxes humidified with moist tissue paper, where they were kept for 1–2 hr before being brought to the laboratory at FABI, University of Pretoria. Alates were collected 1–2 days before the first rains, after which *Cubitermes* has its nuptial flight (Nutting, 1969). The day after the first rain, several colonies from both locations were excavated and no alates were found. Therefore, the alates collected should represent the full annual reproductive investment per colony.

2.2 | Species identification

Soldiers from each colony were used for genus-level classification (Uys, 2002) while DNA barcoding was used for species identification. Legs were removed from four males and four females from each colony for a DNA extraction using Chelex (0.2 ml 10% Chelex solution, 99.9°C for 15 min). We then amplified **Cytochrome c oxidase subunit II** (**COXII**) for each sample (Primers: A- *tLeu* 5′- CAGATAAGTGCATTGGATTT- 3′; (Miura et al., 1998); and B- *tLys* 5′- GTTTAAGAGACCAGTACTTG- 3′; (Liu & Beckenbach, 1992). The PCR mix consisted of 12.5 µl Red Taq master mix (Ampliqon), 1.0 µl forward primer, 1.0 µl reverse primer, 0.1 µl bovine serum albumin (BSA), and 8.4 µl H₂O for a total volume of 25 µl. PCR conditions were as follows: 94°C for 5 min, 35 cycles of 94°C for 10 s, 50°C for 20 s, and 72°C for 45 s. The program ended with a final elongation of 72°C for 7 min. PCR success was determined using agarose gel electrophoresis. PCR products from 2 specimens (1 female and 1 male) from each colony were then purified using MSB Spin PCRapace (STRATEC Molecular, Germany), and underwent Sanger sequencing at Eurofins (Ebersberg, Germany). The resulting sequences (1 for the forward and 1 for the reverse primer) for each specimen were then aligned using a pairwise Geneious alignment with default settings in Geneious 2019 (Biomatters Ltd., New Zealand). The highest-quality consensus sequence from each colony was checked against the NCBI nucleotide database using BLASTn.

2.3 | Inbreeding avoidance: kin recognition during mate choice and sex ratios

In *Cubitermes*, similar to many termites, initial mate choice during pairing is performed by males. Female *Cubitermes* raise their abdomen in calling behavior, presumably releasing sex-pairing pheromones to attract a male (Bordereau & Pasteels, 2010; Williams, 1959). Subsequently, males choose and follow the female in a tandem run until they establish a nest. Males occasionally exhibit choosiness by changing female partner during the tandem run (Williams, 1959). Therefore, we examine male mate choice between females during pairing and tandem running. Mate choice assays were conducted by observers who did not know the origin of the female. The observers monitored male choice and a coordinator that recorded the colony of origin of the termites. Two females, a nestmate and a non-nestmate from either the same or foreign collection location, were placed away from each other, in an arena (90 mm Ø Petri dish) with soil from the local environment. The observer followed one of the females, assigned as the focal female, for the remainder of the assay. The females were given 1 min to acclimatize, during which they would often raise their abdomens. Sometimes females would walk around for some time before standing still. In the rare occasion that females met each other, they appeared to ignore each other and continue walking until raising their abdomen. After acclimatizing, a male was added (Figure 1a). After 3 min, his choice of female (focal, nonfocal, or no choice) was communicated to the coordinator. The coordinator marked whether the chosen female was nestmate or non-nestmate. During the observation period, few males changed their choice of female, and only the final choice was recorded.
Termites were only used for a single assay, resulting in 78 assays of mate choice (n = 35 non-nestmate from foreign location, n = 43 non-nestmate from same location), in which eleven colonies were used (Table S2). The data were analyzed using a binomial generalized linear mixed model (GLMM) with "mate choice" as the dependent variable, "female origin" the predictor, and "male colony of origin" a random effect. "No choice" occurred 14 times and was not considered in the data analysis. To test whether mate choice was random, we implemented an intercept offset using the logit of 0.5.

Heterogeneity between colonies was tested using a G-test. Sex ratios were determined by quantifying the number of male and female alates found in each colony (n_colony = 11; Table S2). Alates were sexed using morphological characteristics (Krishna et al., 2013), and sex ratio was calculated by dividing the number of males by the total number of alates. For each colony, we analyzed whether sex ratio deviated from 0.5, representative of a balanced sex ratio, using a test of equal proportions (prop.test function, R).

3 | RESULTS

3.1 | Termite identification

All colonies except one were identified to Cubitermes tenuiceps, with 97%–98% nucleotide sequence similarity to the BLASTn hit MN685946.1 in GenBank. The remaining colony also matched best Cubitermes tenuiceps, but with a lower match of 92%, so we removed it from further analyses.

3.2 | Inbreeding avoidance: no apparent kin recognition during mate choice but female-biased sex ratios

Our observations matched previously published literature in that only males appear to be involved in mate choice. As soon as a male taps a female on the abdomen with his antennae, or attaches to the female abdomen with his mandibles, the female starts running and finding a place to dig a nest. The female never appears to turn around to contact the male, and the male continuously remains in contact with the female’s abdomen during this process. Overall, mate choice appeared random (Figure 1, GLMM, intercept, z = 1.60, p = .11). When males had a choice between a nestmate female and a non-nestmate female from the same location, males chose a nestmate female during 45.5% of the trials. When the non-nestmate female was from a different location, males joined a nestmate female in 62.9% of the trials. This difference in choice depending on location was, however, not significant (Figure 1; GLMM: location, G = 12.618, df = 10, p = .25, Figure S1). The sex ratios of colonies were largely female-biased. The average sex ratio was 0.33 ± 0.11 (mean ± SD) and significantly female-biased in 8 out of 11 colonies (Figure 2a, Table S4). No colonies exhibited male-bias.

3.3 | Female-biased sex allocation and ubiquitous presence of Wolbachia

Sex allocation was also found to be largely female-biased. Dry weights differed significantly between sexes (Figure 2b; LMM, Sex, χ² = 213.73, p < .001, Table S5), with females (4.92 ± 0.35 mg, mean ± SD) being heavier than males (4.23 ± 0.21 mg). Thus, sex
significantly affected the total energetic investment (LMM, Sex, $\chi^2 = 30.80, p < .001$), represented by the estimated cumulative dry weight of each sex per colony (Figure S2). The ratio of female-to-male dry weight remained female-biased with the power conversion factor (Table S6), further corroborating our findings of skewed sex allocation.

Wolbachia was successfully amplified for both regions for alates from all colonies (Table S2). All sequenced PCR products were closest to Wolbachia from the termite Kalotermes flavicollis (98.4%–98.8% sequence similarity; GenBank: AJ292345), with the exception of Wolbachia from colony B, which matched closest to Wolbachia from a bush cricket (Bit score: 1,229; sequence identity: 96.9; GenBank: DQ536100) but had highest sequence identity to the K. flavicollis Wolbachia (Bit Score: 1,227; sequence identity: 97.0%). Wolbachia from both of the closest BLAST hits are within the Wolbachia group F (Lo & Evans, 2007; Panaram & Marshall, 2007), which is commonly associated with termites (Lo & Evans, 2007).

4 | DISCUSSION

We sought to investigate characteristics of inbreeding avoidance and sex allocation, which may influence the mating and reproductive success of the higher termite Cubitermes tenuiceps. No apparent male mate choice was found, and sex ratios were female-biased. This suggests that C. tenuiceps neither avoids inbreeding through kin recognition nor through differential sex ratios. As females were heavier than males, sex allocation was also female-biased. Wolbachia was consistently present in alates, warranting further research into potential parasite distortion of termite sex allocation.

Although the cost of inbreeding has not been quantified in C. tenuiceps, there is evidence from other termites that costs can be high (DeHeer & Vargo, 2006), for example in regards to compromised immune defenses (Calleri et al., 2006). If inbreeding has a tangible fitness cost in this monogamous species, reproductive alates must either rarely encounter related individuals, for example, due to sufficient dispersal during mating flights (Vargo & Husseneder, 2010), or trade-offs / evolutionary constraints must exist that prevent the evolution of inbreeding avoidance. Mating flights are extremely dangerous (Nutting, 1969), which could lead to males choosing the first female they encounter regardless of relatedness (Waser et al., 1986). Another potential trade-off may be related to pathogen pressure. In the termites Zootermopsis angusticollis and Coptotermes formosanus, mating with an unrelated individual increases mortality of the mated pair, likely due to exposure to foreign fungi and bacteria (Fei & Henderson, 2003; Rosengaus & Traniello, 1993). Increased risk of infection could outweigh any inbreeding costs. Our results show a trend, although nonsignificant, that males prefer nestmate females when the non-nestmate female is from a different location. If different locations harbor different pathogens, inbreeding could be adaptive to avoid novel pathogens.

Differentially biased sex ratios, where colonies specialize in producing either males or females to prevent sib-mating, can effectively reduce the effect of inbreeding (Noirot, 1989) and have been observed in Coptotermes formosanus (Husseneder et al., 2006). However, we found female-biased sex ratios for all but three colonies, which did not demonstrate male-bias, indicating that sex ratio biases would not counter inbreeding. The deviation from equal investment in the sexes suggests other traits potentially drive female bias and impact fitness.

The drivers and fitness consequences of the female-biased sex allocation remain unclear, although certain adaptive cases are unlikely due to termite biology. Local mate competition, resource competition, or resource enhancement can promote sex allocation bias to improve mating success or resource procurement of offspring or kin (Hamilton, 1967; Clark, 1978; Taylor, 1981). Mate competition between related males can cause female bias in order to enhance male reproductive success through multiple mating (Hamilton, 1967; Taylor, 1981). However, monogamy, as exhibited by most termites, nullifies any benefit of multiple mating and reduces mate competition, making the optimal sex allocation equally favor males and females (West et al., 2000). Additionally, alates of higher termites rarely remain in their natal nest or interact during nest-founding, reducing the potential for resource enhancement via cooperation among kin (but see Eggleton, 2010; Roisin, 2000), and Cubitermes'
food source (soil) (Uys, 2002), is a ubiquitous resource, likely mini-
mizing resource competition.

Two other traits predicted to affect investment, within-colony mating (Kobayashi et al., 2013; Roisin & Lenz, 2002) and environment-
mental conditions (Trivers & Willard, 1973), could potentially cause the sex allocation bias to benefit the termites. Within-colony mating with son or daughter reproductive replacements can benefit-
ally bias sex allocation to favor the parent with greater genetic contribu-
tion (Kobayashi et al., 2013; Roisin, 2000; Roisin & Lenz, 2002). However, it is unlikely that within-colony mating promotes the fe-
male bias in C. tenuiceps. Reproductive replacements normally occur in a smaller fraction of termite colonies than observed in this study (Hellemans, Fournier, et al., 2019; Kobayashi et al., 2013; Vargo et al., 2012), and replacements are thought to be uncommon in Cubitermes (Myles, 1999; Noirot, 1956). Alternatively, sex allocation may be biased if fitness of the sexes varies with environmental qual-
ity, such that the sex that reaps higher fitness benefits from a better environment should receive greater allocation in good conditions (Kümmerli & Keller, 2011; Trivers & Willard, 1973). Thus, termites could theoretically invest more in females if they had greater fitness under favorable environmental conditions, reinforcing the produc-
tion of this energetically costly sex. However, conditional sex allo-
cation related to environmental quality remains to be demonstrated in termites. Overall, the lack of evidence for within-colony mating or advantageous environmental conditions prevents any firm conclu-
sions of adaptive sex allocation.

The female-biased sex allocation may be maladaptive and poten-
tially relates to the ubiquitous prevalence of Wolbachia. The inflated number of females during the nuptial flight may reduce the mating success and increase the reproductive value of the rarer male sex (Fisher, 1930). Thus, biased allocation toward females potentially reduces fitness, and the frequency-dependent nature of selection should cause colonies to invest more in males, unless a parasitic sex distorter is driving the female bias (Fisher, 1930; West, 2009). Wolbachia is well-known female-biased sex allocation distorter in ar-
thropods (Werren et al., 2008). This bacterial symbiont is vertically transmitted through females, and the symbiont can maximize trans-
mission through reproductive parasitism (Miller & Schneider, 2012; Werren et al., 2008) in the form of feminization of males, male-
killing, and/or parthenogenesis induction (Charlat et al., 2003; Correa & Ballard, 2016). All alates surveyed were infected with Wolbachia, similar to the high incidence in alates of Cubitermes sp. affinis subarquatus (Roy et al., 2015), and other termites across de-
rived (higher termites) and basal (lower termites) clades also host Wolbachia (Bandi et al., 1997; Bordenstein & Rosengaus, 2005; Hellemans, Kaczmarek, et al., 2019; Lo & Evans, 2007; Roy & Harry, 2007; Salunke et al., 2010). However, the exact nature of the symbiosis in termites remains largely unknown (but see Hellemans, Kaczmarek, et al., 2019), and a parasitic association cannot be as-
sumed without causal proof. For example, variable sex allocation, which is driven by parthenogenesis and within-colony inbreeding, and Wolbachia co-occur in some other termites without being caus-
ally linked (Hellemans, Fournier, et al., 2019; Matsuura et al., 2004; Yashiro & Lo, 2019). If Wolbachia caused the observed female-biased sex allocation, this may generate fitness costs due to reduced mating success during the nuptial flight and diminished reproductive suc-
due to male-killing of offspring (Charlat et al., 2003). Thus, the symbiotic nature of Wolbachia in Cubitermes is deserving of further research, particularly if it acts as a reproductive parasite and conse-
quentially reduces host fitness.

ACKNOWLEDGMENTS
We thank the University of Pretoria, Forestry and Agricultural Biotechnology Institute and the Agricultural Research Council for access to field sites. We thank Sylvia Mathiasen for her help in the laboratory, Jacobus J. Boomsma for helpful discussion, and the Social and Symbiotic Evolution Group for comments on the manus-
script. We would also like to thank two anonymous reviewers for the helpful comments.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
Veronica M. Sinotte: Investigation (equal); methodology (equal); resources (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal). Benjamin H. Conlon: Conceptualization (equal); data curation (equal); formal analy-

sis (equal); investigation (equal); methodology (equal); writing-
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DATA AVAILABILITY STATEMENT
Sequences generated in this study were deposited to GenBank (Table S3). Data on mate choice and sex ratios have been uploaded to datadryad: (https://datadryad.org/stash/share/35OzcGoQ1Yo vGwOmqtD2gbXVMl4YzilocKQc1_srY0Yf).

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REFERENCES
Aguilera-Olivares, D., Flores-Prado, L., Veliz, D., & Niemeyer, H. M. (2015). Mechanisms of inbreeding avoidance in the one-piece dry-
wood termite Neotermes chilensis. Insectes Sociaux, 62(2), 237–245. https://doi.org/10.1007/s00040-015-0399-1
Andersson, D. I., & Hughes, D. (1996). Muller’s ratchet decreases fitness of a DNA-based microbe. *Proceedings of the National Academy of Sciences*, 93, 906–907. https://doi.org/10.1073/pnas.93.2.906

Bandi, C., Sironi, M., Nalepa, C., Corona, S., & Sacchi, L. (1997). Phylogenetically distant intercellular symbionts in termites. *Parasitologia*, 39, 71–75.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Blouin, S. F., & Blouin, M. (1988). Inbreeding avoidance behaviors. *Trends in Ecology & Evolution*, 3(9), 230–233. https://doi.org/10.1016/0169-5347(88)90164-4

Boomsma, J. J. (1989). Sex-investment ratios in ants: Has female bias been systematically overestimated? *The American Naturalist*, 133(4), 517–532.

Boomsma, J. J., Keller, L., & Nielsen, M. G. (1995). A comparative analysis of sex ratio investment parameters in ants. *Functional Ecology*, 9(5), 743–753.

Bordenstein, S., & Rosengaus, R. B. (2005). Discovery of a novel Wolbachia supergroup in isoptera. *Current Microbiology*, 51(6), 393–398. https://doi.org/10.1007/s00284-005-0084-0

Bordereau, C., & Pasteels, J. M. (2010). Pheromones and chemical ecology of dispersal and foraging in termites. In D. E. Bignell, Y. Roisin, L. Sire, & N. Lo (Eds.), *Behavioral biology of termites: A Modern synthesis* (pp. 279–320). Springer.

Calleri, D. V., McGrail Reid, E., Rosengaus, R. B., Vargo, E. L., & Traniello, J. A. F. (2006). Inbreeding and disease resistance in a social insect: Effects of heterozygosity on immune competence in the termite Zootermopsis angusticollis. *Proceedings of the Royal Society B*, 273, 2633–2640.

Charlat, S., Hurst, G. D. D., & Mercot, H. (2003). Evolutionary consequences of Wolbachia infections. *Trends in Genetics*, 19(4), 217–223. https://doi.org/10.1016/S0168-9525(03)00024-6

Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics*, 10(11), 783–796. https://doi.org/10.1038/nrg2664

Clark, A. B. (1978). Sex ratio and local resource competition in a prosimian primate *Science*, 201, 163–165. https://doi.org/10.1126/science.201.4351.163

Correa, C. C., & Ballard, J. W. O. (2016). Wolbachia associations with insects: Winning or losing against a master manipulator *Frontiers in Ecology and Evolution*, 3, 1–18.

Cremer, S., & Heinze, J. (2002). Adaptive production of fighter males: Queens of the ant Cardiocondyla adjust the sex ratio under local mate competition. *Proceedings. Biological Sciences*, 269, 417–422.

DeHeer, C. J., & Vargo, E. L. (2006). An indirect test of inbreeding depression in the termites Reticulitermes flavipes and Reticulitermes virgicus. *Behavioral Ecology and Sociobiology*, 59, 753–761. https://doi.org/10.1007/s00265-005-0105-9

Dial, K. P., & Vaughan, T. A. (1987). Opportunistic predation on alate termites in Kenya. *Biotropica*, 19, 185–189. https://doi.org/10.2307/2388744

Duthie, A. B., & Reid, J. M. (2015). What happens after inbreeding avoidance? Inbreeding by Rejected relatives and the inclusive fitness benefit of inbreeding avoidance. *PLoS One*, 10, e0125140. https://doi.org/10.1371/journal.pone.0125140

Eggleton, P. (2010). An introduction to termites: Biology, taxonomy and functional morphology. In D. E. Bignell, Y. Roisin, & N. Lo (Eds.), *Biology of termites: A modern synthesis* (pp. 1–26). Springer.

Fei, H. X., & Henderson, G. (2003). Comparative study of incipient colony development in the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera, Rhiolithmitidae). *Insectes Sociaux*, 50, 226–233. https://doi.org/10.1007/s00040-003-0666-4

Fisher, R. A. (1930). The *genetical theory of natural selection*. Oxford University Press.
Nutting, W. L. (1969). Flight and colony foundation. In K. Kumar & F. M. Weesner (Eds.), *Biology of termites* (pp. 233–282). Elsevier.

O'Neill, S. L., Giordano, R., Colbert, A. M., Karr, T. L., & Robertson, H. M. (1992). 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proceedings of the National Academy of Sciences, 89*, 2699–2702. https://doi.org/10.1073/pnas.89.7.2699

Panaram, K., & Marshall, J. L. (2007). F supergroup Wolbachia in bush cickeets: What do patterns of sequence variation reveal about this supergroup and horizontal transfer between nematodes and arthropods? *Genetica, 130*, 53–60. https://doi.org/10.1007/s10709-006-0020-7

Pusey, A., & Wolf, M. (1996). Inbreeding avoidance in animals. *Trends in Ecology & Evolution, 11*, 201–206. https://doi.org/10.1016/0169-5347(96)10028-8

R Core Team (2018). *R: A language and environment for statistical computing*. Austria.

Roisin, Y. (2000). Diversity and evolution of caste patterns. In T. Abe, D. Roisin, & L. Zootermopsis. *Termites: Evolution, sociality, symbioses, ecology* (pp. 95–119). Springer.

Roisin, Y., & Lenz, M. (2002). Origin of male-biased sex allocation in orphaned colonies of the termite, Coptotermes lacteus. *Behavioral Ecology and Sociobiology, 51*, 472–479.

Rosengaus, R. B., & Traniello, J. F. (1993). Disease risk as a cost of outbreeding in the termite Zootermopsis angusticollis. *Proceedings of the National Academy of Sciences, 90*, 6641–6645. https://doi.org/10.1073/pnas.90.14.6641

Roy, V., Dupont, L., & Harry, M. (2010). Parentage analysis in Gabonese colonies of soil-feeding termites belonging to the Cubitermes sp. *affinis subarquatus* complex of species (Termitidae: Termitinae). *Insect Science, 17*, 84–90.

Roy, V., Girondot, M., & Harry, M. (2015). The distribution of Wolbachia in Cubitermes (Termitidae: Termitinae) castes and colonies: A modelling approach. *PLoS One, 10*, e0116070.

Roy, V., & Harry, M. (2007). Diversity of Wolbachia isolated from the Cubitermes sp. *affinis subarquatus* complex of species (Termitidae), revealed by multigene phylogenies. *FEMS Microbiology Letters, 274*, 102–111.

Salunkhe, B. K., Salunkhe, R. C., Dhotre, D. P., Khandagale, A. B., Waluliker, S. A., Kirwale, G. S., Ghathe, H. V., Patole, M. S., & Shouche, Y. S. (2010). Diversity of Wolbachia in Odontotermes spp. (Termitidae) and Coptotermes heimi (Rhinotermitidae) using the multigene approach: Phylogenetic diversity of Wolbachia in Indian termites. *FEMS Microbiology Letters, 307*, 55–64.

Shellman-Reeve, J. S. (2001). Genetic relatedness and partner preference in a monogamous, wood-dwelling termite. *Animal Behaviour, 61*, 869–876.

Sundström, L., Chapuisat, M., & Keller, L. (1996). Conditional manipulation of sex ratios by ant workers: A test of kin selection theory. *Science, 274*, 993–995. https://doi.org/10.1126/science.274.5289.993

Taylor, P. D. (1981). Intra-sex and inter-sex sibling interactions as sex ratio determinants. *Nature, 291*, 64–66. https://doi.org/10.1038/291064a0

Trivers, R. L., & Hare, H. (1976). Haploidploidy and the evolution of the social insect. *Science, 191*, 249–263. https://doi.org/10.1126/science.108197

Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179(4068), 90–92.

Uys, V. M. (2002). *A guide to the termite genera of Southern Africa*. Plant Protection Research Institute. Agricultural Research Council.

Vargo, E. L., & Hynesseneder, C. (2010). Genetic structure of termite colonies and populations. In D. E. Bignell, Y. Roisin, & N. Lo (Eds.), *Biology of termites: A modern synthesis* (pp. 321–347). Springer.

Vargo, E. L., Labadie, P. E., & Matsuura, K. (2012). Asexual queen succession in the subterranean termite *Reticulitermes virginicus*. *Proceedings of the Royal Society B, 279*, 813–819.

Waser, P. M., Austad, S. N., & Keane, B. (1986). When should animals tolerate inbreeding? *The American Naturalist, 128*, 529–537. https://doi.org/10.1086/284585

Werren, J. H., Baldo, L., & Clark, M. E. (2008). Wolbachia: Master manipulators of invertebrate biology. *Nature Reviews Microbiology, 6*, 741–751. https://doi.org/10.1038/nrmicro1969

West, S. A. (2009). *Sex allocation*. Princeton University Press.

West, S. A., Smith, T. G., & Read, A. F. (2000). Sex allocation and population structure in aipocomplexum (protozoa) parasites. *Proceedings of the Royal Society of London, Series B: Biological Sciences, 267*, 257–263. https://doi.org/10.1098/rspb.2000.0995

Williams, R. M. C. (1959). Flight and colony foundation in two Cubitermes species (Isoptera: Termitidae). *Insectes Sociaux, 6*, 203–218. https://doi.org/10.1007/BF02225952

Yashiro, T., & Lo, N. (2019). Comparative screening of endosymbiotic bacteria associated with the asexual and sexual lineages of the termite *Glyptotermes nakajimai*. *Communicative & Integrative Biology, 12*, 55–58.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Sinotte VM, Conlon BH, Seibel E, et al. Female-biased sex allocation and lack of inbreeding avoidance in Cubitermes termites. *Ecol Evol* 2021;11:5598–5605. https://doi.org/10.1002/ece3.7462