



## Research article

Nitrogen content of the exuviae of *Coptotermes gestroi* (Wasmann) (Blattodea: Rhinotermitidae)Reina L. Tong<sup>\*</sup>, Daniel Aguilera-Olivares, Thomas Chouvenc, Nan-Yao Su

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## ABSTRACT

Subterranean termites are hemimetabolous social insects where most of the individuals in a colony molt on a regular basis until they die. Nitrogen is a limiting growth factor in wood-feeding insects, such as termites. Because the exuviae of molting termites are consumed by nestmates, it is possible that exuviae represent a potential source of nitrogen that could be recycled and be part of the overall nitrogen conservation strategy of the colony. Although it was documented that cockroach exuviae can contain relatively high levels of nitrogen, the nitrogen content of subterranean termite exuviae has not been examined. This study determines the nitrogen content of *Coptotermes gestroi* (Wasmann) exuviae collected from four-year-old laboratory colonies using a carbon/nitrogen analyzer. *Coptotermes gestroi* exuviae contained  $11.24 \pm 0.64\%$  N (Mean  $\pm$  SD). The exuviae had a higher proportion of nitrogen than whole bodies of termites ( $\sim 10.46\%$ ), wood ( $\sim 0.12\%$ ), and organic soil ( $\sim 2.49\%$ ). These results support the importance of exuviae consumption by nestmates during the ecdysis process as an aspect of nitrogen conservation strategies in *Coptotermes* colonies.

## 1. Introduction

Nitrogen is considered a limiting factor for herbivores, as such organisms, when supplemented with nitrogen, can exhibit enhanced growth, reproduction, and survival (Mattson 1980). Termites are decomposers of woody tissues, which are limited in nitrogen ( $\sim 0.03$ – $0.15\%$  N) (Cowling and Merrill 1966), while termite bodies contain  $\sim 8$ – $13\%$  N (Higashi et al., 1992). The sharp difference of nitrogen content between wood and termites highlights how nitrogen acquisition and conservation is a critical process for wood-feeding termites (Nalepa 1994; Machida et al., 2001; Mullins and Su 2018; Chouvenc 2020). Mauldin and Smythe (1973) noted that some of the possible mechanisms of nitrogen acquisition and conservation available to termites include obtaining dietary nitrogen, associations with nitrogen-fixing symbionts, and digesting symbionts or their waste products. In addition, recycling termite waste products (e.g., feeding on eggs, egg cases, exuviae, conspecific bodies, feces, carton, etc.) could also be part of the nitrogen conservation mechanisms available to termites (La Fage and Nutting 1978; Nalepa 1994).

Termites are hemimetabolous social insects in the order Blattodea (Inward et al., 2007). Contrary to social Hymenoptera, where the worker force within a colony is composed of adult individuals that went through

complete metamorphosis, the worker force of termite colonies is composed of juvenile individuals produced through arrested development (Nalepa 2011). As a result, termite workers must undergo periodic molting while retaining juvenile traits for the rest of their lives, which implies a possible cost associated with this physiological constraint (Nalepa 1994, 2015). The reason feeding on exuviae is a possible nitrogen conservation strategy is that the cuticle is composed mainly of protein and chitin (Kramer et al., 1991). In the cockroach *Periplaneta americana* (L.), exuviae are  $\sim 9.45\%$  N by weight, and it was proposed that post-molt feeding on exuviae is a means to recycle nitrogen, as over 58% of the nitrogen from exuviae was recycled through post-molt feeding (Mira 2000). Further, the reuse of exuviae as a nitrogen conservation strategy was also observed in psocids (Opit and Thorne 2008). Psocids that fed on their exuviae developed faster than those that did not, especially at lower temperatures (Opit and Thorne 2008).

Subterranean termites may have inherited and possibly optimized this nitrogen conservation strategy from their wood roach ancestors (Nalepa 1994, 2015) – exuviae are not only rapidly consumed by nestmates during the molting process (Raina et al., 2008; King et al., 2013; Kakkar et al., 2016a), the location in which molting occurs is not random. It was recently found that *Coptotermes formosanus* Shiraki workers always return to the central nest (location of the reproductives and brood) to

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molt, leading to the hypothesis that workers centralize the nitrogen recovery process and potentially provision the queen and/or brood with nitrogen recycled from exuviae (Kakkar et al., 2017).

However, while the importance of exuviae consumption in termites for nitrogen conservation has been assumed, the nitrogen content of subterranean termite exuviae has actually not been confirmed. Therefore, in order to understand nitrogen conservation processes in termites within the context of their inherent molting cycle, there is a need to first determine the nitrogen content of exuviae relative to their whole body and their food source. If exuviae are a potential source of recyclable nitrogen, molting at the central nest would be an efficient means to provide for castes with higher nitrogen requirements, such as the reproductives, which invest nitrogen into eggs, or developing larvae (Mullins and Su 2018). Since exuviae are consumed, the relative nitrogen of exuviae may be compared to other possible sources of dietary nitrogen, such as whole bodies of termite workers (through cannibalism), wood, and organic soil.

Using *C. gestroi* as a subterranean termite model, the objective of this study was to compare the nitrogen proportion in termite exuviae, whole termite bodies, organic soil, and wood.

## 2. Materials and methods

### 2.1. Exuviae collection

Due to termites ceasing feeding during what is known as the pre-molt fasting period (Xing et al., 2013), a dietary dye can be used to identify non-feeding workers, as workers that do not uptake dye may be separated and monitored to collect exuviae (Raina et al., 2008; Xing et al., 2013; Kakkar et al., 2016a). Following Tong et al., (2020), one thousand *C. gestroi* workers from a four-year-old laboratory colony ( $n = 4$  colonies) were placed into a glass Petri dish (90 mm in diameter) with a media pad (47 mm in diameter, AP10, Millipore SAS, Molsheim, France) dyed with  $\sim 1$  ml 0.05% Nile Blue A, covered, and kept at  $28 \pm 1$  °C for 48 h. Blue and white workers were then counted and separated. White workers (presumably undergoing pre-molt fast) were placed into a new glass Petri dish (90 mm in diameter) with moistened filter paper (90 mm in diameter) dyed with 0.05% Nile Blue A to identify any potential workers that might resume feeding, as these workers might consume exuviae (Xing et al., 2013; Tong et al., 2020). Petri dishes were checked every hour (10:00 to 22:00) for seven days, and exuviae were removed and placed into a vial (Shell Type 1 Glass, 15 × 45 mm 1 Dram with Plug) and frozen. The collection methods were repeated until at least 100 exuviae per colony of origin were collected.

### 2.2. Nitrogen analysis

All termite and exuviae samples were oven-dried at 60 °C for 24 h before being weighed and analyzed. All wood and soil samples were oven-dried at 60 °C for two weeks. A carbon/nitrogen (CN) analyzer (CN 628 Series, LECO, St. Joseph, MI) was used to quantify the nitrogen content in each sample of exuviae, workers, wood, and organic soil, which are some of the possible dietary sources of nitrogen available to the colony. For all analyses, the furnace temperature of the CN analyzer was 950 °C, oxygen was used as the combustion gas, helium was used as the carrier gas, and EDTA was used as the calibration standard, following

the recommendations of the “Organic Application note” for soil and plant tissue analyses provided by the LECO Corp (<http://www.leco.com/>). To meet the sample minimum weight and minimum detectable nitrogen requirements of the CN analyzer, each “exuviae sample” ( $n = 4$  colonies) consisted of 100 exuviae, each “worker sample” ( $n = 3$  colonies) consisted of 30 *C. gestroi* workers, each “wood sample” ( $n = 7$ ) consisted of *Picea* sp. sawdust weighing about  $70.9 \pm 4.4$  mg (Mean  $\pm$  SD, dry weight), and each “organic soil sample” (Timberline Top Soil, Oldcastle Lawn & Garden, Inc., Atlanta, GA) weighed about  $169.7 \pm 34.6$  mg (Mean  $\pm$  SD,  $n = 8$ , dry weight).

### 2.3. Statistical analysis

The nitrogen percentages (measurement) of exuviae ( $n = 4$ ), workers ( $n = 3$ ), wood ( $n = 7$ ), and organic soil ( $n = 8$ ) were arcsine transformed and compared by source (factor) using a one-way analysis of variance (ANOVA) (JMP 15.0.0, SAS Institute, Cary, NC). Results are presented in Mean  $\pm$  SD. Significant differences among means were assessed using Tukey's honestly significant difference (HSD) post-hoc test ( $\alpha = 0.05$ ).

## 3. Results and discussion

Using dry weight to compare samples, this study showed that the exuviae of *C. gestroi* contained  $\sim 11.24 \pm 0.64\%$  nitrogen (Table 1) and had a significantly higher percentage of nitrogen content than worker bodies, wood, and organic soil ( $F = 1635.1$ ;  $df = 3, 18$ ;  $P < 0.001$ ) (Table 1).

It was estimated that  $\sim 1\%$  of *C. formosanus* workers molt per day (Kakkar et al., 2016b); therefore, if we assume that the molting rate is conserved within the *Coptotermes* genus, a *C. gestroi* colony with one million workers may free up to  $\sim 20$  mg N from exuviae per day to be recycled (Table 1). If *C. gestroi* recycle nitrogen from exuviae with the same efficiency as *P. americana* as observed by Mira (2000) ( $\sim 58$ – $70\%$  N recycled from exuviae),  $\sim 11.6$ – $14$  mg N per day may be reutilized within the colony, for the nitrogen equivalent to produce 254 to 306 workers per day. Some of the chitin may be digested through gut chitinases, as are found in *Reticulitermes* species (Arquette and Rodriguez 2013).

Although our study highlights the potential importance of exuviae recycling as part of a nitrogen conservation strategy, it did not address the potential source of such nitrogen. Beyond the potential acquisition of nitrogen with the help of symbiotic diazotrophic bacteria (Noda et al., 1999), subterranean termites can uptake micronutrients from the soil (Janzow and Judd 2015), and laboratory colonies reared with supplements of organic soil tend to be healthier (Mullins and Su 2018), suggesting that subterranean termites such as *Coptotermes* may trophically obtain nutrients from organic soil. Although the percentage of nitrogen in organic soil is lower than in exuviae, the amount of organic soil in the foraging environment of subterranean termites may still provide a reliable source of nitrogen when compared to wood. Wood also varies in nitrogen content because of the degree of decay by microorganisms (Hungate 1941; La Fage and Nutting 1978), but Mullins and Su (2018) argued that wood could not be the sole source of nitrogen to colonies. Further, organic soil and wood are available to foragers, who must then transfer them back to the other nestmates. Therefore, transferring

**Table 1.** Nitrogen content (Mean  $\pm$  SD) comparison among wood, organic soil, and *C. gestroi* workers and exuviae. Each worker sample contains 30 workers, and each exuviae sample contains 100 exuviae. Means followed by different capital letters within a column are statistically different (one-way ANOVA followed by Tukey's HSD test,  $\alpha = 0.05$ ).

Sample	N	Mass (mg)	Nitrogen (mg)	Nitrogen (%)
Wood	7	70.9 $\pm$ 4.4	0.083 $\pm$ 0.013	0.12 $\pm$ 0.02 A
Organic Soil	8	169.7 $\pm$ 34.6	4.172 $\pm$ 0.770	2.49 $\pm$ 0.15 B
Workers	3	13.5 $\pm$ 0.8	1.369 $\pm$ 0.113	10.46 $\pm$ 0.37 C
Exuviae	4	1.8 $\pm$ 0.2	0.197 $\pm$ 0.016	11.24 $\pm$ 0.64 D

nitrogen from exuviae may be an efficient way to conserve and reuse nitrogen at the colony level.

Further, La Fage and Nutting (1978) consider cannibalism to be an incidental behavior likely rooted in sanitation that happens to conserve nutrients. Cannibalism may also serve to regulate the number and size of castes within the colony (La Fage and Nutting 1978), and soldiers are starved or cannibalized as a possible emergency food ration before workers (Su and La Fage 1986). However, in an experiment on *C. gestroi*, Chouvenec (2020) concluded that cannibalism was mortality-driven and not an active energy conservation strategy, but a mechanism for nitrogen conservation imposed on termites over evolutionary time (Chouvenec 2020). Our study also confirmed that, when compared to soil and wood, termite bodies and termite exuviae both have relatively high nitrogen content, and confirmed that both cannibalism and exuviae consumption could critically be involved in nitrogen conservation in termites.

Although exuviae had the highest percentage of nitrogen among workers, wood, and organic soil, individual exuvia only contain ~0.002 mg N (Table 1). However, the nitrogen stored in exuviae may be more easily assimilated through digestion than unlocking the poor nitrogen content stored in wood or organic soil (La Fage and Nutting 1978; Higashi et al., 1992), and post-molt feeding on exuviae may represent a reliable, possibly easily digestible source of nitrogen. It is therefore possible that nestmates feeding on the exuviae of a termite molting at the central nest can recover some of this nitrogen. During this process, the reacquired nitrogen can be transferred and recycled to other colony members (Machida et al., 2001), such as reproductive individuals (i.e., active egg-laying queens) and developing larvae in the central nest, and toward the production of alates with more sclerotized exoskeletons and nitrogen reserves in mature colonies.

Because the current study provides only an elemental analysis of the nitrogen content of termite exuviae, termite bodies, wood, and organic soil, the specific forms of the nitrogenous compounds (e.g., ammonium, nitrate, protein, chitin, etc.) are not differentiated. The uptake of nitrogen by termites when provided with these various forms may differ, and therefore, comparisons should be taken with caution. However, the amount of nitrogen in termite exuviae was quantified, and will allow insights into the effects of exuviae consumption on termite colony growth in further studies. Further studies may also include finding the specific forms of nitrogen in the exuviae, as well as the presence and amounts of other elements that are limited in wood.

## Declarations

### Author contribution statement

Reina L. Tong: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Daniel Aguilera-Olivares: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Thomas Chouvenec: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nan-Yao Su: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### Data availability statement

Data included in article/supplementary material/referenced in article.

## Declaration of interests statement

The authors declare no conflict of interest.

## Additional information

No additional information is available for this paper.

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