Variation in soldier investment is linked to the evolution of termite soldier defense strategies

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Abstract

The evolutionary success of social insects is attributable to their division of labor, often facilitated by specialized castes. One such caste, the soldier, specializes in colony defense. In termites, soldier defense strategies vary across taxa, ranging from modes relying on nest architecture (strong-point strategy) to those employing active engagement with intruders (counter-attack strategy). While previous studies have suggested that soldier morphology and behavior are associated with colony defense strategy, the broader ecological and evolutionary factors shaping variation in defense strategies remain unexplored. In this study, we use data from the literature to examine the relationship between defense strategy, soldier proportion, and nesting strategy within a phylogenetic comparative framework. Our results reveal a clear pattern: species employing strong-point strategies exhibit lower soldier proportions, whereas counter-attack strategists typically invest more heavily in soldiers. Phylogeny-based inference of trait evolutionary history indicated that counter-attack strategies evolved independently from strong-point strategies at least 12 times, while no evidence was found for the reverse pattern. We find that factors such as foraging type (non-foraging vs. foraging), defense behavior (e.g., autothysis, phragmosis), and life-history traits can, at least partly, explain interspecific variation in soldier investment. Notably, foraging strategy was significantly associated with the evolution of defense strategy, with non-foraging termites largely restricted to strong-point strategies. Overall, our findings underscore the multifactorial nature of termite defense strategy evolution.

Introduction

The division of labor underpinning the success of social insects is enhanced by the presence of morphologically specialized castes, enhancing optimization of colony-level activities such as foraging, nest maintenance, and defense (Wilson 1971; Wilson and Hölldobler 2005; Duarte et al. 2011; Torres et al. 2012; Gordon 2016). The colony composition of different castes is thus crucial for efficient colony function (Oster and Wilson 1978; Ulrich et al. 2018; Aguilera-Olivares et al. 2023), and can impact diverse phenomena including colony migration, social immunity, and defense (Oster and Wilson 1978; Hasegawa 1997; Shibao 1998; Kamiya and Poulin 2013; Lagrue et al. 2018; Sakai et al. 2024). Theoretical and empirical studies suggest that caste proportions may be regulated over evolutionary timescales through natural selection, while short-term adjustments rely more on individual behavioral flexibility instead of altering the caste composition (Hölldobler and Wilson 1990; Schmid-Hempel 1992; Baudier et al. 2019). Thus, we can expect that caste proportions are regulated in a species-specific manner, reflecting the ecological and evolutionary drivers of each species.

Some social insect societies possess ‘soldier’ castes, which specialize in colony defense (Anderson, 1984; Roux & Korb, 2004; Tian & Zhou, 2014). Despite their defensive benefits, soldiers can impose significant costs on colony growth, as soldiers are typically larger than ordinary workers and thus require more energetic investment to produce (Oster and Wilson 1978; Prestwich 1984; Chouvenc et al. 2015). In addition, soldiers make limited contributions to general tasks, such as nest maintenance, increasing the load on workers for these tasks (Shibao et al. 2004). As workers and soldiers make up the majority of the colony, we can expect selection to work to optimize the proportion of soldiers by balancing defense efficacy with colony productivity, ensuring efficient resource allocation and colony stability.

The cost of soldiers to colonies of social insects is likely related to their degree of specialization for nest defense, as more specialized castes may be unable to perform alternative tasks such as nest maintenance or brood care (Oster and Wilson 1978). Termites are the perfect example of such specialized behavior, as soldiers cannot even feed independently due to the form of their mouthparts (Cleveland 1925; Badertscher et al. 1983). Termite soldiers thus represent relatively high maintenance costs, likely necessitating strict regulation of soldier production to ensure colony sustainability. By extension, we can expect cross-taxon variation in the degree of soldier investment, reflecting different ecological contexts and life-history traits.

Termite defense strategies can be broadly categorized into two main types: strong-point and counter-attack strategies (Eggleton 2011; Tuma et al. 2020) (Figure 1a). These strategies differ markedly in the demand for soldiers to defend the colony effectively. In the strong-point strategy, even a small number of soldiers can effectively secure the nest by functioning as static barriers that block entrances and tunnels (Anderson & Franks, 2001; Hanus, 2008; Matsuura, 2002; Seid et al., 2008). This strategy thus requires a specific nest architecture that provides suitable strong-points to defend, in addition to the adaptive defensive behavior of the individual soldier. Strong-point strategy soldiers typically possess phragmotic heads, crushing mandibles, snapping mandibles, or autothytic rupture mechanisms that rupture their own bodies and spread sticky, toxic substances (Deligne & De Coninck, 2006; Šobotník et al., 2010a). Conversely, in the counter-attack strategy, soldiers actively engage with invaders independently of nest architecture using specialized mandibles (e.g., slashing or piercing mandibles), or developed secretory glands (e.g., frontal or labial gland) to deploy chemical signals and defensive secretions (Prestwich 1984), and employ recruitment of additional soldiers through chemical or physical signals to provide a rapid collective response to external threats. The counter-attack strategy requires a relatively high proportion of soldiers to maintain defense efficacy, as it mainly relies on coordinated aggression (e.g., Stuart, 1981; Traniello, 1981). As these two strategies require dramatically different numbers of soldiers to be effective, we can expect the defense strategy to strongly influence colony energy allocation.

In addition to variation in defense strategies, termite species differ markedly in life history and ecology. One notable variable characteristic is the foraging strategy. Termite foraging strategies can be broadly classified into one-piece nesters (Noirot 1970), which complete their entire life cycle within a single piece of wood, and foragers, which regularly forage outside and nest in multiple pieces of wood (Abe 1987; Mizumoto et al. 2022). One-piece nesters can be expected to favor strong-point defense, as they benefit from the inherent structural defensibility of the nest itself, while foragers likely face greater predation risks outside the nest, and may therefore rely more heavily on active counter-attack behaviors. Thus, nesting traits may shape or constrain the evolution of caste specialization and defense mechanisms in termites.

Several studies have investigated the colony soldier-to-worker ratio across termite species, with notable work by Haverty (1977) highlighting large variations in soldier proportions among different lineages. Although previous research has suggested a potential correlation between soldier proportions and defense strategies (Eggleton, 2011; Matsuura, 2002; Šobotník et al., 2010b; Tuma et al., 2020; Thompson et al., 2000), this hypothesis remains untested within a phylogenetic comparative framework across multiple lineages. In this study, we examine the evolutionary relationships between termite defense strategies, soldier proportion, and nesting strategies. We address two central questions: (i) does soldier proportion vary with defense strategy, and (ii) is there an association between nesting strategies and defense strategies? To answer these questions, we synthesize data from existing literature and map these traits onto a termite phylogeny. We then quantify the number and direction of evolutionary transitions in defense strategy and test whether nesting strategy and soldier investment have co-evolved with forms of defense. This approach allows us to evaluate the joint influence of foraging strategy and phylogenetic history on the level of investment in soldier castes across termite lineages. Our study provides novel insights into the adaptive significance of caste differentiation and the evolutionary ecology of social defense in termites.

Materials and methods

Data collection

We collected information about i) foraging strategy, ii) the proportion of the soldiers, and iii) defense strategies of termite soldiers from the literature based on searches conducted using Google Scholar. Data were summarized at the genus level because the majority of published information on defense strategy or soldier morphology is available at this level (e.g., Prestwich, 1979; Tian & Zhou, 2014).

We obtained data on nesting strategy from a literature survey by Mizumoto et al. (2022). As one-piece nesters differ significantly from other nesting strategies in that they do not forage outside the nest, previous studies (e.g., Mizumoto & Bourguignon, 2020; Mizumoto et al., 2022) classified termite nesting strategies broadly into two categories: non-foraging termites (one-piece nester) and foraging termites (multiple-site nester and central-site nester). We followed these authors in using a discrete two-state trait of non-foraging (one-piece nesters) or foraging (other termites).

The proportion of soldiers in various termite species was summarized in Haverty (1977), and we thus focused on adding to this dataset with the information published after 1977. We conducted our literature survey by using Google Scholar from January to November 2024 using the search string function including: “Genus-name” AND “Proportion of soldier”, “Genus-name” AND “Caste composition”, and “Genus-name” AND “Soldier ratio”. We repeated searches for each of 265 different genera for which foraging strategy data were described in Mizumoto et al. (2022) (see Supplementary Table S1 for the list of genus names). We checked the recorded data on the proportion of soldiers in the colony or the number of individuals of all castes, including soldiers. The only exception to this was for Sattar et al. (2013), who did not report soldier numbers, but did report the proportion of workers and total colony size in colonies of *Microtermes obesi* and *Odontotermes lokanandi*. For these groups, we thus treated the proportion of non-worker individuals in a colony as an estimate of the soldier proportion, as workers and soldiers constitute the majority of colony members. If the number of soldiers was stated in the paper instead of proportion, the proportion of soldiers in the colony was calculated by dividing the number of soldiers by the total number of individuals in the colony. When data from multiple colonies were available in the paper, we calculated the mean soldier proportions of all colonies as a representative value. Some termite species may have more than one soldier morph (i.e., major soldier and minor soldier), and limited papers provided the proportion for each soldier type separately. In this case, we treated the sum of the proportion of all types of soldiers as the soldier proportion for that colony. If data were available for multiple species in the same genus, we used the mean value of the soldier proportions for all species in the analysis as a representative value for that genus (species-level soldier proportion for these cases can be found in Supplementary Table S2). Finally, we found that the sampling method varied among studies, which could bias estimates of soldier proportions. For example, in some papers, the data on soldier proportions were based on observations of foraging groups rather than the whole colony. To account for this sampling bias, we classified the sampling methods used in the paper into those using entire colonies and those using only colony subsets. We then divided the data into a dataset containing all data (broad dataset) and one containing only the entire colony data (robust dataset). The same statistical analyses were performed on each dataset to see if sampling methods affected our conclusions. Our final datasets comprised data on the proportion of soldiers in 66 termite genera from 72 papers (broad dataset; Supplementary Table S2 for the list of literature), and 29 genera from studies where entire colonies were sampled (robust dataset; see Supplementary Table S3 for the list of genera).

To classify soldier defensive strategies, we performed a separate literature survey (Supplementary Table S2 for the list of literature). We identified 60 studies using the search strings function on Google Scholar, including “Genus-name” AND “colony defense”. For “Genus-name”, we surveyed defense strategies for each of the 66 genera for which we could find data on the proportion of soldiers. Interestingly, there was no overlap between the studies collated for this purpose and those obtained for the soldier proportion survey. Genera were classified into strong-point or counter-attack classes based on the morphological and/or behavioral information described (see below). If there was a clear description of the defensive behavior available for the soldiers of the genus in the literature, we used that description preferentially to determine the defensive strategy (30 genera). If we could not find clear descriptions of the defensive strategies of the soldier castes, we checked publications reporting the type species of the genus and thus descriptions of the morphology of soldier castes (30 genera), and based our classification on soldier morphology (see below). In addition, we looked for photographs of soldiers of species belonging to the genus when it was difficult to classify soldiers based solely on descriptions in the articles (six genera). These photographs were obtained from the Termite Database (Constantino 2020; five genera) and Post Harvest Technology Center (https://phtcenter.com/; *Psammotermes*) and used to check their head morphology (see Supplementary Table S2 for the websites from which the photos were collected).

To determine the defensive strategy of each genus, we followed previous studies (Prestwich 1984; Scholtz et al. 2008; Eggleton 2011; Tuma et al. 2020) and classified genera as employing either a strong-point or counter-attack strategy. Genera were classified as using a strong-point strategy when their soldiers had phragmotic heads (e.g., *Cryptotermes*) or snapping mandibles (e.g., *Cavitermes*), or when the mandibles were described as jagged, large, robust, or paired with a rectangular head shape, except for cases in which the soldier actively attacking with toxic or irritating chemicals against external enemies. For cases of self-sacrificial autothysis, such as in *Glossotermes*, in which soldiers burst their bodies to spread defensive chemicals (Prestwich, 1986; Shorter & Rueppell, 2012; Šobotník et al., 2010a), we followed Tuma et al. (2020) in classifying this single-use behavior as a strong-point strategy. Bursting soldiers cause predators to stick to their bodies, ultimately blocking nest entrances or tunnels and preventing further intrusion by external threats. Genera were classified as using a counter-attack strategy if their soldiers actively attacked external enemies with toxic, irritating, or viscous chemicals. A clear example is the nasute soldiers with elongated, funnel-shaped heads adapted for chemical spraying (“glue squirting” or “daubing brush” in past descriptions). Chemical secretion alone was not considered sufficient for classification as a counter-attack strategy unless it was clearly involved in external aggression. For instance, *Reticulitermes speratus* soldiers secrete caste-specific chemicals (Zalkow et al. 1981), yet they engage primarily in phragmotic defense (Matsuura 2002) and thus were classified under the strong-point strategy. Similarly, some *Cryptotermes* and both symmetrical snapping soldiers (e.g., *Cavitermes*) produce sticky chemicals (Kyjaková et al., 2015; Šobotník et al., 2010b), but their head morphology and defense behavior suggest they function primarily within confined nest spaces (Scholtz et al. 2008; Kuan et al. 2020), supporting a strong-point classification. When a genus included multiple soldier morphs (e.g., *Macrotermes* and *Psammotermes*), we classified the genus based on the morphology of the most common or dominant soldier morph described (see Supplementary Table S2 for details of soldier morph classifications).

Several genera in our dataset involved uncertainty in trait classification, particularly regarding defense strategy and foraging behavior. To assess the impact of such ambiguity on our conclusions, we conducted two supplementary analyses. The first adopted a conservative approach by excluding all genera with uncertain trait classification (see Supplementary Table S4 for this genus-level dataset). We excluded: (i) all Cubitermitinae, a group exhibiting mixed signals—some species show morphological features typical of counter-attack soldiers while also performing phragmotic behavior associated with strong-point defense (Hanus, 2008); (ii) genera lacking detailed behavioral observations of soldiers, where defense strategies were inferred solely from morphology (see Supplementary Table S2 for the list of genera); and (iii) *Prorhinotermes*, categorized as a non-forager in our main analysis but reported to occasionally forage and establish multiple nesting sites, suggesting an intermediate nesting strategy (Rupf & Roisin, 2008). The second analysis focused specifically on the Cubitermitinae. We retained all other genera from the original analysis, and reclassified all members of Cubitermitinae as strong-point strategists based on their consistent nest architecture and morphological traits (Cerdá & Dejean, 2011; Eom et al., 2015; Hellemans et al., 2021). Although detailed behavioral data are lacking for some genera within this group, such as *Noditermes* and *Thoracotermes*, their defense strategies were inferred from shared morphological characteristics and similarities in nesting structure. In these two supplemental analyses, we employed the same methods as in the main analysis (see *Statistical analysis* for details) to analyze the relationship between defense strategies and proportion of soldiers by phylogenetic generalized linear model (PGLM), and the correlation between defense and foraging strategies by Pagel test.

Overall, we obtained both soldier proportion and defense strategy data for 124 termite species across all termite families except Stylotermitidae (Hellemans et al. 2024). For the family Termitidae, soldier proportion data for five of 18 subfamilies (Apicotermitinae, Engelitermitinae, Foraminitermitinae, Forficulitermitinae, and Sphaerotermitinae) could not be included in the dataset as no soldier ratio data were available. Overall, 36 genera used a strong-point strategy, while 30 used a counter-attack strategy (broad dataset; total 66 genera). For our robust dataset, there were 18 strong-point genera and 11 counter-attack genera (robust dataset; total 29 genera). Although the robust dataset did not include some termite families, the datasets showed highly congruent results (see Results). For nesting strategies, 16 genera were classified as non-foraging termites, while 50 genera were foraging termites based on Mizumoto et al. (2022).

Statistical analysis

To investigate the relationship between the defense strategy and the proportion of soldiers while controlling phylogenetic relationships, we used a PGLM. To determine the correlation structure between samples, we used the genus-level time-calibrated Bayesian phylogenetic tree from a previous study, which included the 66 genera used in this study (Mizumoto and Bourguignon 2021). We employed a logistic regression model with the logit function as the link function, with maximum penalized likelihood estimation and 1,000 bootstraps to model the relationship between defense strategy (dependent variable) and soldier proportion (independent variable). This analysis was performed using the phyloglm function of the “phylolm” package (Ho and Ane 2014) in R version 4.3.1 (R Core Team 2023). We also explored the correlation between defense strategy and termite foraging behavior using the Pagel test (ARD model) implemented in the fitPagel function of the package “phytools” version 2.4.4 (Revell 2024). The Pagel test (Pagel 1994) certifies the correlation between two discrete traits while correcting for phylogenetic nonindependence between data points.

We performed the ancestral state reconstruction of the soldier defense strategies, using the ace function provided by the R “ape” package, version 5.8 (Paradis and Schliep 2019). We employed the Equal Rates (ER) model, as we had only two discrete values (counter-attack or strong-point) for this analysis. For ancestral state reconstruction, the ARD model was excluded from model selection because the imbalance in traits resulted in unstable estimates (one of the estimated transition rates was 0 and the Hessian inverse matrix contained missing data). The relative likelihoods of alternative ancestral defense strategies were plotted on each node of the phylogenetic tree as a pie chart using the “ggtree” package (Yu 2020). In addition to the Pagel test for correlated evolution, we used Fisher's exact test to evaluate the relationship between nesting strategies and defense strategies because this test provides a robust method for assessing associations between two categorical variables and complements Pagel's test by directly examining the independence of nesting and defense traits in the absence of phylogenetic information.

Results

Our phylogenetically controlled analysis of soldier proportions at the genus level indicated that soldier proportions varied between defense strategies (PGLM; estimate ± SE = -0.2272± 0.0636, z = -3.57, 95% CI = [-0.3636, -0.1197], $p < 0.001$; Figure 1b), with the average proportion of counter-attack soldiers being approximately three times higher than the average proportion of strong-point soldiers (11.53 ± 9% for counter-attack soldiers, 3.65 ± 2.82% for strong-point soldiers). This trend was consistent when using the robust dataset (PGLM; estimate ± SE = -0.3069 ± 0.1427, z = -2.15, 95% CI = [-0.4565, -0.0870], p = 0.03). Furthermore, the relationship between defense strategy and soldier ratio showed the same trend in the two supplemental analyses; (i) when excluding all ambiguous classification genera (Supplemental material S5) and (ii) when assuming all Cubitermitinae are strong-point strategists (Supplemental material S6). Thus, the correlation between defense strategy and soldier ratio was robust regardless of the dataset used. In the main analysis, variation in soldier proportion among genera within each strategy was high, and of similar magnitude in the two strategies, with standard deviations of 77% and 71% of the mean, respectively. As a result, some strong-point strategy genera had a proportion of soldiers comparable to counter-attack strategists (notably *Glossotermes* (8.4%), *Globitermes* (12.6%), *Paraneotermes* (11.1%), and *Homallotermes* (8.0%)).

Phylogenetically controlled analysis revealed a significant evolutionary association between defense strategy and foraging strategy (Pagel test: likelihood ratio = 11.97, p = 0.02). This pattern was further supported by a strong bias toward the strong-point strategy among non-foraging termites: all non-foraging genera employed the strong-point strategy, with the exception of *Prorhinotermes* and *Termitogeton*. In contrast, foraging termites exhibited a more balanced distribution of defense strategies (Figure 1c), with 28 counter-attack genera and 22 strong-point genera. This contrast was also statistically significant in a non-phylogenetic analysis (Fisher’s exact test: $p < 0.01$, odds ratio = 8.64). While Fisher's exact test was significant in all supplemental analyses ($p < 0.01$ for all analyses), the Pagel test showed non-significant results in two supplemental analyses: (i) when excluding all ambiguous classification genera and (ii) when assuming all Cubitermitinae are strong-point strategists (Supplemental material S7).

Ancestral state reconstruction indicated that the most recent common ancestor of termites most likely employed a strong-point defense strategy, with a probability of 90% (Figure 2a). Counter-attack strategies evolved from strong-point defense at least 12 times independently throughout termite evolution. A comparison with sister genera employing the ancestral strategy suggests that this shift was often, but not always, associated with a marked increase in the proportion of soldiers. On the other hand, there was no evidence of any reversals from the counter-attack strategy to the strong-point strategy.

Discussion

Our analysis reveals a clear relationship between defensive strategy and the investment in soldiers in termite colonies. Specifically, species employing strong-point strategies on average exhibit significantly lower soldier proportions (3.65 ± 2.82%) than those utilizing counter-attack strategies (11.53 ± 9%). This pattern likely reflects the different approaches for effective defense against external threats. Since strong-point strategies rely heavily on nest architecture and structural barriers, far fewer soldiers are required for effective defense (Thompson et al. 2000). In contrast, counter-attack strategies necessitate direct engagement with external threats, making a larger soldier contingent necessary for effective defense.

While our ancestral state reconstruction indicated the ancestral defense strategy was likely the strong-point strategy for termites, *Mastotermes darwiniensis*—the sole extant member of the most basal lineage—was assigned to the counter-attack strategy in our analysis. This assignment is supported by its behavioral and physiological traits: soldiers of *M. darwiniensis* exhibit active defense with piercing mandibles and secrete viscous, toxic substances despite lacking an external fontanelle, which typically delivers chemical defenses in more derived taxa such as Nasutitermitinae (Moore 1968; Prestwich 1979). In addition, this species shows colony-level coordination through chemical alarm signaling and forages over a broad spatial range (Rubenstein and Abbot 2017; Sillam-Dussès et al. 2023), traits otherwise associated exclusively with Neoisoptera. These features suggest that *M. darwiniensis* represents an independent, early evolution of the counter-attack strategy outside Neoisoptera. However, the rarity of such transitions among other basal taxa raises the possibility that this shift was only possible due to the unique combination of derived traits present in *M. darwiniensis*, which may have temporarily relaxed the evolutionary constraints present in other early-branching lineages.

In contrast, the counter-attack strategy evolved repeatedly within Neoisoptera—at least 11 times independently—suggesting that broader evolutionary conditions within this clade favored such transitions. This repeated emergence likely coincided with the evolution of key traits, such as chemical alarm signaling and the elaboration of frontal and labial glands, which underpin effective chemical defenses in termites (Prestwich et al. 1977; Šobotník et al. 2010b; Costa-Leonardo et al. 2023). The coordinated group responses and caste-specific alarm reactions enabled by these traits likely enhanced colony-level defense performance. However, these adaptations may come at an evolutionary cost. Morphological specializations like the elongated heads of Nasutitermes, optimized for precise chemical delivery, are structurally incompatible with phragmotic defense used in strong-point strategies (Matsuura 2002). Furthermore, the behavioral complexity of counter-attack strategies, such as coordinated group attacks and caste-specific responses to alarm pheromones, requires colony-level coordination and higher degrees of behavioral specialization (Šobotník et al., 2010b). This contrasts with strong-point strategies, which may rely more heavily on individual-level traits. This asymmetry in complexity may explain the apparent one-way nature of evolutionary transitions in defense strategy, as counter-attack strategies involve a greater degree of integrated collective behaviors, they may be more prone to behavioral canalization—a process by which repeated, fixed behavioral routines reduce the flexibility to adopt alternative strategies (West-Eberhard 2003; Sih et al. 2004). In contrast, the more individual-based nature of strong-point defense may make transitions toward counter-attack more likely than the reverse.

Together, the combination of morphological, physiological, and behavioral adaptations may mean that once termites evolve a counter-attack strategy, they may enter an evolutionary one-way path that precludes reversion to structurally based defense.

Almost all non-foragers employed the strong-point strategy, suggesting a close link between foraging strategy and defense strategy. Our main analysis revealed a significant evolutionary correlation between foraging strategy and defense strategy, as indicated by both phylogenetically controlled (Pagel test) and uncontrolled (Fisher's exact test) approaches. However, this pattern was not consistently supported in our supplemental analyses: when we either (i) assumed all Cubitermitinae to employ strong-point strategies or (ii) excluded all genera with ambiguous classifications, the trend persisted but did not reach statistical significance (Pagel test, $p = 0.06$ and 0.08). These outcomes suggest that the inference of evolutionary trait correlations may be sensitive to uncertainties in trait classification. In contrast, the correlation between defense strategy and proportion of soldiers, tested using PGLM, remained statistically significant across both the main and all supplementary analyses, supporting the robustness and generality of this pattern. Notably, these patterns persisted even when analyses excluded sources of trait uncertainty individually (e.g., Cubitermitinae, *Prorhinotermes*, or genera lacking detailed behavioral descriptions). Therefore, we conclude that the association between defense strategy and proportion of soldiers is consistently robust, whereas the evolutionary correlation between defense and foraging strategies becomes non-significant in some supplementary analyses. It is also important to note that ecological traits such as nesting behavior likely exist along a continuum rather than in discrete categories (Mizumoto and Bourguignon 2020). For example, *Prorhinotermes*, categorized as a one-piece nester in our main analysis, has been reported to exhibit facultative foraging and utilize multiple nesting sites (Rupf and Roisin 2008), raising the possibility of intermediate foraging strategies. Similar ambiguity may apply to defense strategies, particularly for genera classified solely based on morphological traits. Overall, our supplemental analyses underscore the robustness of the association between defense strategy and soldier investment and highlight how classification uncertainties and trait continua can influence the detection of evolutionary correlations. Continued efforts to refine trait definitions and collect detailed behavioral data, especially for ambiguously classified or understudied taxa, will be important for improving the resolution of future comparative analyses.

While defense strategy is linked to soldier proportions, considerable variation exists even within each strategy (Figure 1d). This variation may be attributed to a combination of variation in sampling biases and variation in life-history traits. For instance, seasonal fluctuations in colony composition, particularly in non-foraging species, may affect soldier proportion, as many non-soldier individuals leave as alates during swarming (Korb and Hartfelder 2008; Rubenstein and Abbot 2017). Life-history traits such as autothysis, nest architecture, and soldier polymorphism may also influence investment in soldiers. Autothysis, a form of self-sacrificial defense, may increase soldier needs due to inevitable loss of soldier individuals (e.g., *Glossotermes*, *Serritermes*, *Globitermes*), yet its presence in soldierless or low-soldier taxa (e.g., *Apicotermitinae*, *Orthognathotermes*) indicates the influence of this defense on soldier investment is still unclear (Bordereau et al. 1997; Šobotník et al. 2010b). Similarly, nest structure can shape defensive demands: species with complex subterranean nests (e.g., *Apicotermes*, *Cylindrotermes*) may require fewer soldiers due to the protective properties of their nest architecture (Eggleton 2011; Tuma et al. 2020; Heyde et al. 2021), and variation in nest complexity may also explain within-genus differences in some genera (e.g., *Nasutitermes* and *Coptotermes*); (Arab et al. 2017; Lee et al. 2017). In some counter-attack genera, soldiers may take on auxiliary roles, such as foraging or social immunity, reducing their cost to the colony (Traniello 1981; Almeida et al. 2016), and polymorphic soldiers may offer defensive flexibility against several types of external threats and allow more efficient resource allocation (Hanus 2008; Feinerman and Traniello 2016). Additionally, soldier body size is largely variable across termite diversity, modifying costs of soldier investment, where, e.g., Natusitermitinae often have smaller soldiers, while phragmotic soldiers are usually the largest caste within the nests (Mizumoto and Bourguignon 2021). Together, these factors likely interact to shape the wide range of soldier proportions observed within same type of defense strategy.

In summary, the evolutionary diversification of termite defense strategies involves repeated, unidirectional shifts towards counter-attack strategies, accompanied by increased soldier investment and the evolution of more complex behavioral adaptations. This suggests that the evolution of specialized defensive castes may often follow constrained trajectories shaped by both functional trade-offs and ecological demands. Such evolutionary one-way transitions, likely facilitated by innovations such as chemical alarm signaling, highlight the interplay between behavioral specialization and morphological commitment in the morphological castes associated with the division of labor. These results emphasize the importance of integrating behavioral, ecological, and phylogenetic perspectives to understand how division of labor evolves and diversifies across social taxa. Future work incorporating more detailed behavioral data, ecological context, and expanded taxonomic sampling will be essential to test whether the canalized patterns observed here are also found in other social insect taxa.

Declarations

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Conflicts of interest

The authors declare no competing interests.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Data files and R scripts for this study are available in GitHub, at https://github.com/Akiya-Satoh/Termite_sol_prop

Code availability

Not applicable

Authors' contributions

Akiya Satoh (Conceptualization [Equal], Data Curation [Lead], Formal Analysis [Lead], Investigation [Lead], Methodology [Lead], Visualization [Lead], Writing – Original Draft [Lead])

Isaac Planas-Sitjà (Conceptualization [Support], Formal Analysis [Support], Visualization [Support], Writing – Review & Editing [Equal], Funding Acquisition [Equal], Supervision [Equal])

Adam L. Cronin (Conceptualization [Support], Formal Analysis [Support], Methodology [Support], Visualization [Support], Writing – Review & Editing [Equal], Supervision [Equal])

Nobuaki Mizumoto (Conceptualization [Equal], Formal Analysis [Support], Methodology [Support], Visualization [Support], Writing – Review & Editing [Equal], Funding Acquisition [Equal], Supervision [Equal])

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Figures

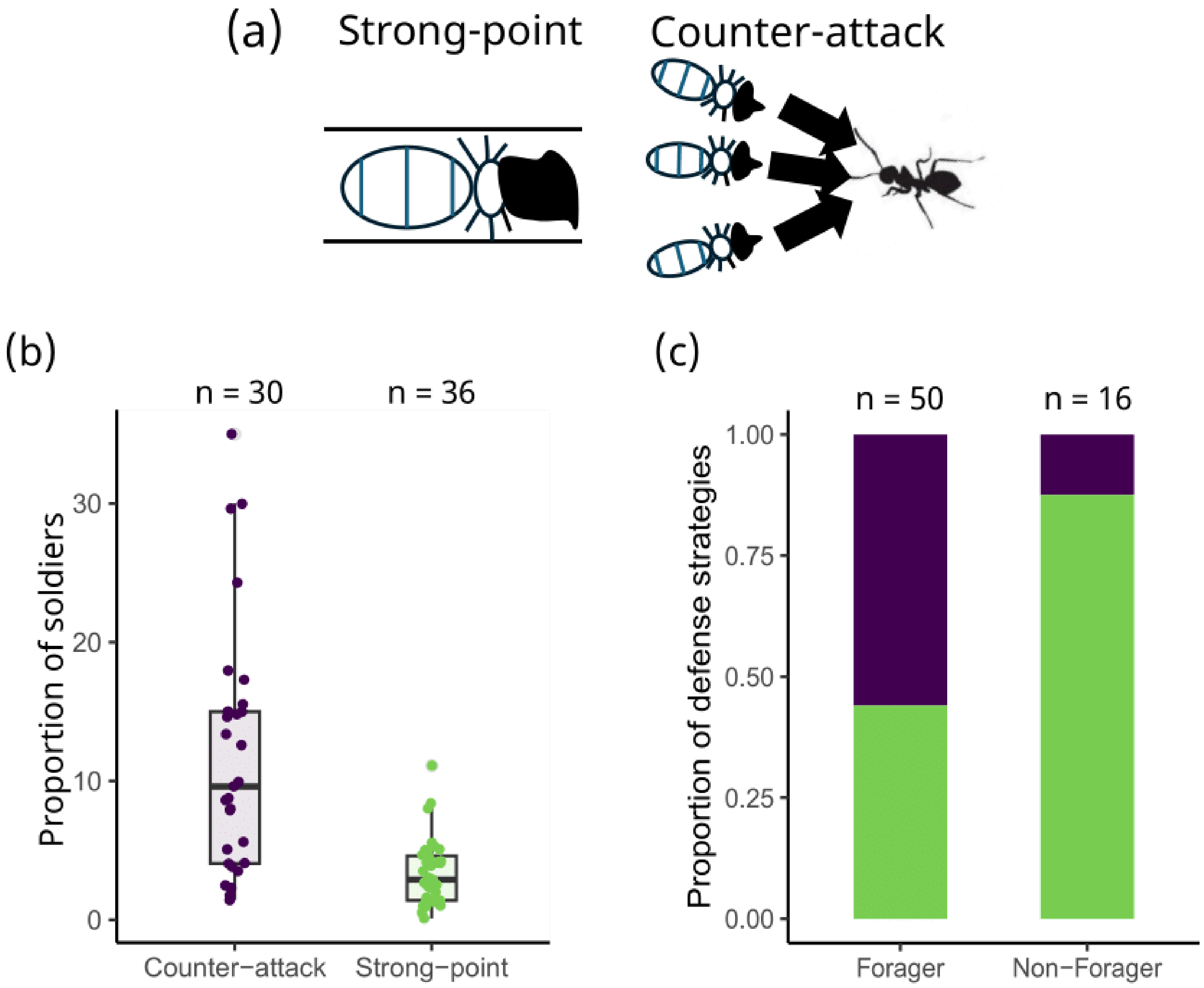

Figure 1

Relationship between termite defense strategy and soldier proportion. (a) Two different defensive strategies of termites. (b) Comparison of soldier proportion between the two defensive strategies. One dot represents one genus. (c) Proportion of defense strategies employed in foraging and non-foraging termites. Purple and green bars indicate defense strategy (green; strong-point, purple; counter-attack strategy).

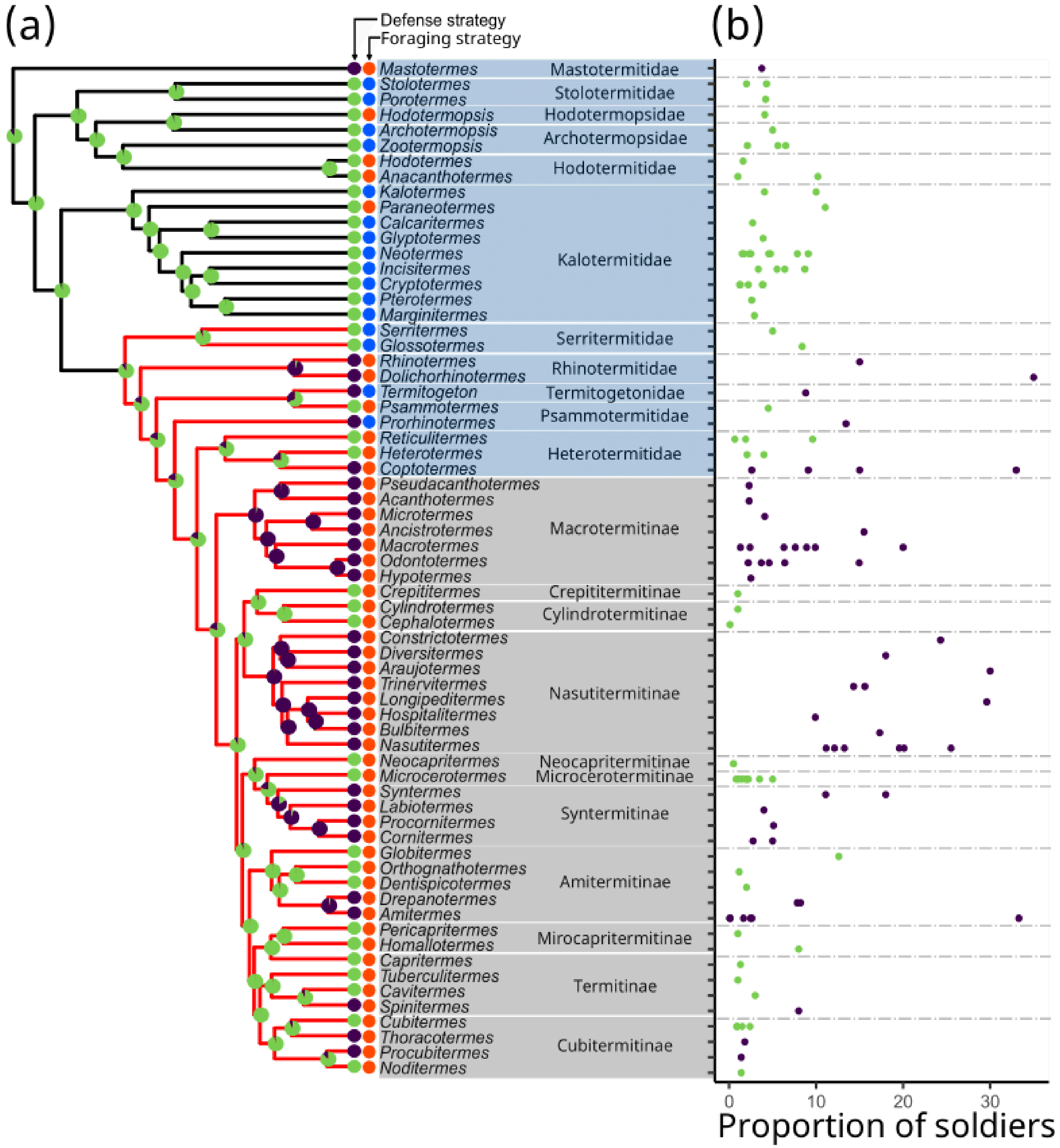

Figure 2

Phylogeny and proportion of soldiers in termites. (a) Phylogeny of termites with a defensive strategy, foraging type, and family name. The relative likelihoods of alternative ancestral defense strategies (strong-point or counter-attack) are shown using pie charts at each node in the phylogeny. (b) Proportion of soldiers for each genus. Purple and green dots indicate defense strategy (purple, counter-attack; green, strong-point), orange and blue dots indicate foraging strategy (orange, foraging; blue, non-foraging). Genera belonging to the family Termitidae are listed by subfamily name on a gray background. Red branches indicate linkage classified as Neoisoptera.

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