The impact of social complexity on the efficacy of natural selection in termites

Camille Roux[1], Alice Ha[2], Arthur Weyna[2], Morgan Lodé[1], Jonathan Romiguier[2]

[1] Univ. Lille, CNRS, UMR 8198 - Evo-Eco-Paleo, F-59000, Lille, France
[2] Institut des Sciences de l'Evolution (UMR 5554) University of Montpellier, CNRS Montpellier 34000 France
Correspondence: camille.roux@univ-lille.fr and jonathan.romiguier@umontpellier.fr

Abstract

In eusocial species, reproduction is monopolized by a few reproductive individuals. From the perspective of population genetics, this implies that the effective population size (*Ne*) of these organisms is likely to be smaller compared to solitary species, as has been proposed in the literature for eusocial hymenoptera. In this study, we investigate the genomic consequences of eusociality in termites (Isoptera) on two different timescales. First, by analyzing transcriptome data from 66 Blattodea species, we focus on the ratio of non-synonymous to synonymous mutations (d_N/d_S) as a marker of natural selection efficiency and effective population size. Our results demonstrate an elevated d_N/d_S ratio in termites compared to other members of Blattodea, further generalizing the idea that convergent evolution toward eusociality strongly reduces the effective population size and the genome-wide efficiency of natural selection. Then, by comparing 68 termite transcriptomes, we show that this decrease in natural selection efficiency is even more pronounced in termites displaying high levels of social complexity. This study contributes to understanding the complex interplay between social structures and natural selection patterns, highlighting the genetic footprint of eusociality in shaping the evolution of termites.

Keywords: Eusociality; Genome Evolution; Natural Selection; Termites; Blattodea

Introduction

Eusociality represents the ~~pinnacle~~ highest level of social organization within the animal kingdom, characterized by a clear division of reproductive labor among different castes, cooperative care for young and the presence of overlapping generations within a colony (Keller and Chapuisat, 2001). Various forms of social organizations have ~~independently emerged~~ emerged independently in several taxa, such as Coleoptera, Thysanoptera (Choe and Crespi, 1997), crustaceans (Duffy, 1996), spiders (Lubin and Bilde, 2007), or mammals (Jarvis et al., 1994), but the most complex forms of eusociality appeared only within Hymenoptera (e.g. ~~,~~ bees, wasps, ants) and Isoptera (termites; Howard and Thorne, 2011). In social insects such as ants and termites, a caste is defined as a subset of individuals within a colony that perform specialized roles, often with distinct morphological traits. Both ants and termites display sharp contrasts in terms of levels of social complexity, ranging from small colonies of ~~hundred~~ hundreds of individuals with low caste polymorphism ~~,~~ to large colonies of several millions of individuals and extreme caste differentiations.

For a given number of individuals ~~making~~ that make up a population, the shift from solitary living to eusociality corresponds ~~with~~ to a notable decrease in the effective population size *Ne*, mainly because reproduction becomes ~~confined to a limited~~ limited to a reduced number of individuals within a colony (Romiguier, Lourenco, et al., 2014). Such reduction of *Ne* has important consequences ~~on genome evolution because of the interplay between the efficiency of natural selection~~ for genome evolution due to the interaction between natural selection efficiency and random genetic drift (Kimura, 1983). The potency of selection ~~in either promoting~~ to promote the fixation of advantageous mutations or ~~removing deleterious ones hinges~~ to remove deleterious ones depends on the selection coefficient *vis-à-vis* the influence of genetic drift, whose intensity is increased for lower N_e values. When the influence of selection weakens in comparison to genetic drift ($|N_e.s| < 1$, s being the selective coefficient), alleles behave as if they are effectively neutral. Thus, genomes of species with a large effective population size are characterized by a higher relative accumulation rate of slightly advantageous mutations compared to species with a small N_e (Galtier, 2016). In the same direction, weakly deleterious mutations are not effectively eliminated from the genomes of low N_e species because genetic drift is stronger than purifying selection for them (Galtier, 2016).

This phenomenon sets a theoretical threshold dubbed the “drift barrier” (Lynch and Walsh, 2007), delineating the boundary where random genetic drift limits the ability of selection to forestall the fixation of suboptimal alleles (Kimura et al., 1963; Ohta, 1973). The empirical demonstration of the drift barrier has established it as a key factor shaping the genetic architectures of living organisms. The central involvement of the N_e effect has since been empirically demonstrated to explain the evolution of different genomic components: mutation rate (Lynch, Ackerman, et al., 2016), intron emergence (Lynch, 2002), precision of intron splicing (Benitiere et al., 2022), optimization of codon usage (Galtier et al., 2018), complexity of protein-protein interactions (Lynch, 2012), protein optimization (Huber et al., 2017) and rate of adaptive evolution (Galtier, 2016).

The evolution of coding regions themselves is shaped by the mutation-selection-drift equilibrium, with life history traits significantly impacting the rates of nonsynonymous substitutions relative to synonymous substitutions (d_N/d_S; Botero-Castro et al., 2017; Figuet et al., 2016; Nikolaev et al., 2007; Popadin et al., 2007; Rolland et al., 2020; Romiguier, Ranwez, et al., 2013; Weyna and Romiguier, 2021). At synonymous positions, the majority of mutations are presumed to be neutral, although some exceptions exist (Galtier et al., 2018). This implies that the substitution rate d_S is a result of the number of mutations emerging per generation in the population ($2.N_e.\mu$, where μ represents the mutation rate per nucleotide per generation) multiplied by the probability of fixing a new neutral mutation ($\frac{1}{2.N_e}$). Hence, in absence of selective pressure on synonymous positions, the evolution of d_S is ~~solely~~ predominantly driven by the mutation rate. Conversely, the fixation probability of new nonsynonymous mutations is directly reliant on the $N_e.s$ product: the smaller the N_e, the greater the accumulation of weakly deleterious nonsynonymous mutations in genomes. Consequently, any life-history trait impacting N_e is likely to increase the relative amount of deleterious nonsynonymous mutations within genomes by intensifying the role of genetic drift

Eusociality in ants, bees, wasps~~and termites~~, termites, shrimps and spiders appears to be such a life-history trait by restricting reproduction to few individuals. ~~However, a recent study using an extensive dataset~~ In spiders of the genus *Stegodyphus*, where only a small fraction of females lay eggs, whole-genome analyses have shown that the shift towards higher sociality is linked to a continuous reduction in effective population size and an increase in d_N/d_S (Ma et al., 2024). However, the analysis of 169 Hymenopteran species provide a ~~more~~ slightly nuanced view of the link between eusociality and effective population size (Weyna and Romiguier, 2021). While social Hymenoptera featured an increase of d_N/d_S compared to solitary species, the results highlighted the notable exception of solitary bees displaying the same level of d_N/d_S than their social counterparts. ~~This~~ Also, another recent study suggests that low division of reproductive labour (i.e. worker reproduction) may not necessarily translate into lower d_N/d_S (i.e increased strength of natural selection, Barkdull and Moreau (2023)). These results highlighted that strong decreases of effective population size is not necessarily a direct consequence of ~~the emergence of eusociality~~division of reproductive labour, but might ~~also~~ as well predate it.

In this study, we aim to ascertain whether the negative impact of eusociality on the efficacy of natural selection applies to termites. Similarly to ants despite ≈ 383 my of divergence (Kumar et al., 2017), termites features various level of eusociality, the most complex forms being characterized by particularly large colonies (up to $> 10^6$ individuals; Porter and Hawkins, 2001) and high caste specialization. We thus hypothesize further that termite species with the highest levels of social organization might feature the lowest effective population size~~, in a similar way than in~~. This would mean that large superorganisms (i.e. large colonies) are analogous in terms of *Ne* with large organisms, reflecting previous works in mammals or birds where species with large body size feature ~~lower effective population size than small body size species~~ high dN/dS ratio (Botero-Castro et al., 2017; Romiguier, Ranwez, et al., 2013).

To explore this inquiry, we leverage two publicly accessible sequencing datasets delineating phylogenetic information at two different evolutionary levels:

1. across Blattodea, allowing for the comparison between 6 species of termites and 60 non-termites species (Evangelista et al., 2019).
2. across 68 species of termites, to study the effect of variation in levels of social complexity on d_N/d_S (Bucek et al., 2019).

Our findings reveal that termites (*i.e.* eusocial Blattodea) exhibit higher d_N/d_S ratios than solitary Blattodea species~~.~~, interpreted as a relaxation of purifying selection. This finding aligns with a recently published study that compared three termite species with five cockroach species (Ewart et al., 2024). Furthermore, among termites, species displaying the highest levels of social complexity tend to exhibit higher d_N/d_S ratio in comparison to those considered to exhibit simpler social organization.

Material and methods

The analysis presented here focuses on two phylogenetic scales: within the Blattodea in first, then a zoom on the Isoptera. For both scales, all the data including molecular alignments, phylogenetic trees and life history traits were taken from the literature. d_N/d_S by branch was then inferred in this study, based on the available aligned transcriptomes and the reconstructed phylogenetic trees.

Blattodea dataset

The molecular data (sequence alignments + phylogenetic tree) for the Blattodea come entirely from the study by Evangelista et al. (2019). This dataset corresponds to 3,244 coding genes aligned in 66 species, and is from the Genbank Umbrella Bioproject (ID PRJNA183205). The 66 Blattodea species had been chosen by the authors to represent the major lineages (except the Anaplectidae). Evangelista et al. (2019) sequenced the transcriptomes of 31 Blattodea species, and complemented this dataset by transcriptomic data coming from Misof et al. (2014) and Wipfler et al. (2019), consisting of 14 additional Blattodea and 21 outgroups. RNA extraction, library construction and paired-end sequencing (Illumina HiSeq 2000) are part of the 1KITE project (www.1kite.org). The used phylogenetic tree corresponds to the file Blattodea_FigTree_95_aa_run1_clean.treefile (Supplementary Material), was produced by Evangelista et al. (2019) using a maximum-likelihood approach implemented in IQ-TREE (Haeseler et al., 2014; Kalyaanamoorthy et al., 2017). The final dataset we used for the d_N/d_S analysis is composed over the 66 surveyed species by a median number of 2,102 coding genes per species, ranging from 1,243 in *Nyctibora* to 2,573 in *Zootermopsis nevadensis*.

Before carrying out an ANOVA analysis to test an effect of social organization on d_N/d_S, we checked whether this ratio followed a normal distribution in each social group using a Shapiro-Wilk test (P-values ranging from 0.077 in the gregarious group to 0.838 in the eusocial group). Then we checked that variances of the d_N/d_S ratios are homogeneous between the different social systems using a Levene's test ($P = 0.3$). Once the normality of the d_N/d_S and the homogeneity of the variances between groups had been confirmed, we performed an ANOVA test to check an effect of sociality. To identify specific differences between groups, we used a Tukey-HSD (Honest Significant Difference) test, which tests the differences in means between each pair of groups

Isoptera dataset

The Isoptera dataset comes from the study carried out by Bucek et al. (2019). They sequenced the transcriptomes of 53 termite species and two entire genomes. These data were used to complete publicly available genomes and transcriptomes for 13 termite species and 7 dictyopteran outgroups (*Blaberus atropos*, *Blattella germanica*, *Cryptocercus wrighti*, *Empusa pennata*, *Mantis religiosa*, *Metallyticus splendidus*, *Periplaneta americana*; Bourguignon, Šobotník, et al., 2016; Dedeine et al., 2015; Harrison et al., 2018; Hayashi et al., 2013; Huang et al., 2012; Misof et al., 2014; Mitaka et al., 2016; Poulsen et al., 2014; Terrapon et al., 2014; Wu et al., 2016). Transcriptomes of workers were sequenced by Bucek et al. (2019) using the Illumina HiSeq 4000 platform. The authors then aligned the assembled orthologous genes using MAFFTv7.305 (Katoh and Standley, 2013). They inferred the phylogenetic tree using IQ-TREE 1.6.7 (Nguyen et al., 2015). The used phylogenetic tree corresponds to the file Isoptera_Bucek.treefile (Supplementary Material). The final dataset we used to estimate d_N/d_S corresponds to a median number of 3,604 coding genes per species, ranging from 2,391 (*Reticulitermes grassei*) to 3,927 (*Blattella germanica*).

Phylogenetic estimation of the d_N/d_S ratio

d_N/d_S values were computed using the mapnh program within the TestNH suite (J Dutheil and Boussau, 2008; JY Dutheil et al., 2012; Guéguen and Duret, 2018; Romiguier, Figuet, et al., 2012; https://github.com/BioPP/testnh) from the publicly available cleaned alignments, and from the tree topologies available for both the Blattodea and Isoptera datasets. Each ~~loci~~ locus which had sequences with over 90% non-informative sites were removed. mapnh estimates d_N and d_S for four substitution categories ($W \rightarrow S$, $S \rightarrow W$, $W \rightarrow W$, $S \rightarrow S$, referring to nucleotides affected by substitutions, AT = W for Weak and CG = S for Strong). ~~These estimates were then associated with each terminal branch of the available phylogenies.~~ Non-synonymous and synonymous substitution counts are estimated for each branch in the phylogeny. However, for downstream analyses, we use the d_N/d_S ratio estimated from the terminal branches as representative for each species. Alignments with fewer than 30 species were discarded.

Testing for relaxation of natural selection

To assess the relaxation of selective pressure, we employed the RELAX model within the HyPhy v.2.5 software (Schrader et al., 2021; Wertheim et al., 2015). The RELAX model estimates the selection intensity parameter

(k), allowing us to compare a null model ($k = 1$) with an alternative model, thereby evaluating the strength of natural selection.

A k value greater than 1 indicates intensified natural selection, while a k value less than 1 suggests relaxed natural selection in the test branches (six termite species) relative to the reference branches (60 non-termite species) using the dataset from Evangelista et al. (2019). We assessed the statistical significance of the k value (p<0.05) using a Bonferroni correction.

Social traits

The data from Evangelista et al. (2019) were utilized to investigate potential variations in d_N/d_S within Blattodea associated with eusociality. Each species was correlated with its social category following Michener's classification in 1969 Michener (1969) and average individual size. Sociality data were compiled from various bibliographic (Bohn, 1989; Cabrera and Scheffrahn, 2001; Legendre, 2007; Lihoreau, 2009; Picker, 2012; Roth, 1986, 2000) and online (Bugguide; Cockroachcare; Collections.museumvictoria; Jasa; Ozanimals; Padil; Roachcrossing; Virginiacheeseman; Wikipedia) sources. When species-level information was lacking, we utilized the average observation from within genera or subfamilies.

The data sourced from Bucek et al. (2019) were employed to identify potential variations in d_N/d_S within Isoptera linked to varying degrees of sociality. Each species was detailed with various characteristics, including category (Neoisoptera ~~or~~ which is a clade grouping most families with high level of sociality vs Basal Isoptera), taxonomic family, queen(s) egg-laying count, approximate colony size, colony type (monogynous, polygynous, or both), polygyny type, worker type ("true workers" than are completely sterile vs "pseudergates" that can become reproductives), dietary habits, geographical distribution, soldier and queen morphometrics, and invasive status. Notably, the Termitidae and Rhinotermitidae families, encompassing several paraphyletic clades in the dataset, were arbitrarily divided into Termitidae A and B and Rhinotermitidae A to E to study monophyletic groups. However, this division doesn't align with established subfamilies in taxonomy. Data on species characteristics were collected from various literature sources (Ahmad, 1963; Atkinson and ES Adams, 1997; Barbosa and Constantino, 2017; Bourguignon, Scheffrahn, et al., 2010; Chhotani, 1975; Constantino and De Souza, 1997; Constantino, 1990; De Saeger, 1954; Dejean and Fénéron, 1996; Dupont et al., 2009; Emerson and Banks, 1965; Evans, 2011; Florian et al., 2019; Fougeyrollas et al., 2015; F Gay, 1974; F Gay and Barrett, 1983; FJ Gay and JA Watson, 1982; Ghesini and Marini, 2013; Goodisman and Crozier, 2002; Hanus et al., 2006; Hellemans et al., 2017; Husseneder et al., 1998; Krishna, 1963; Krishna and CL Adams, 1982; Krishna and Araujo, 1968; Lewis, 2009; Liang et al., 2017; Maistrello and Sbrenna, 1996; Martius and Ribeiro, 1996; Matsuura, 2002; Miller, 1994; Miura et al., 2003; Noirot, 1959; Noirot, 1989; Parmentier and Roisin, 2003; Rasib and Saeed Akhtar, 2012; Scheffrahn and Krecek, 1999; Scheffrahn, Su, et al., 1999; Snyder, 1924; Soleymaninejadian et al., 2014; Thorne, 1984; Vargo, 2019; Vargo et al., 2006; J Watson, Brown, et al., 1989; J Watson, Metcalf, et al., 1977; Williams, 1966). Similar to the dataset in ~~Evangelista et al. (2019)~~Evangelista et al. (2019) , the search scope was extended to include closely related genera and subfamilies because of shared characteristics among closely related species.

Results and discussion

d_N/d_S evolution in Blattodea

We estimated the d_N/d_S ratio as a proxy of natural selection efficiency across a phylogenetic tree of 66 Blattodea species, including 6 termites (Isoptera). The dataset and phylogenetic tree employed in this investigation are derived from the study by Evangelista et al. (2019) encompassing 3,244 coding genes (Fig. 1).

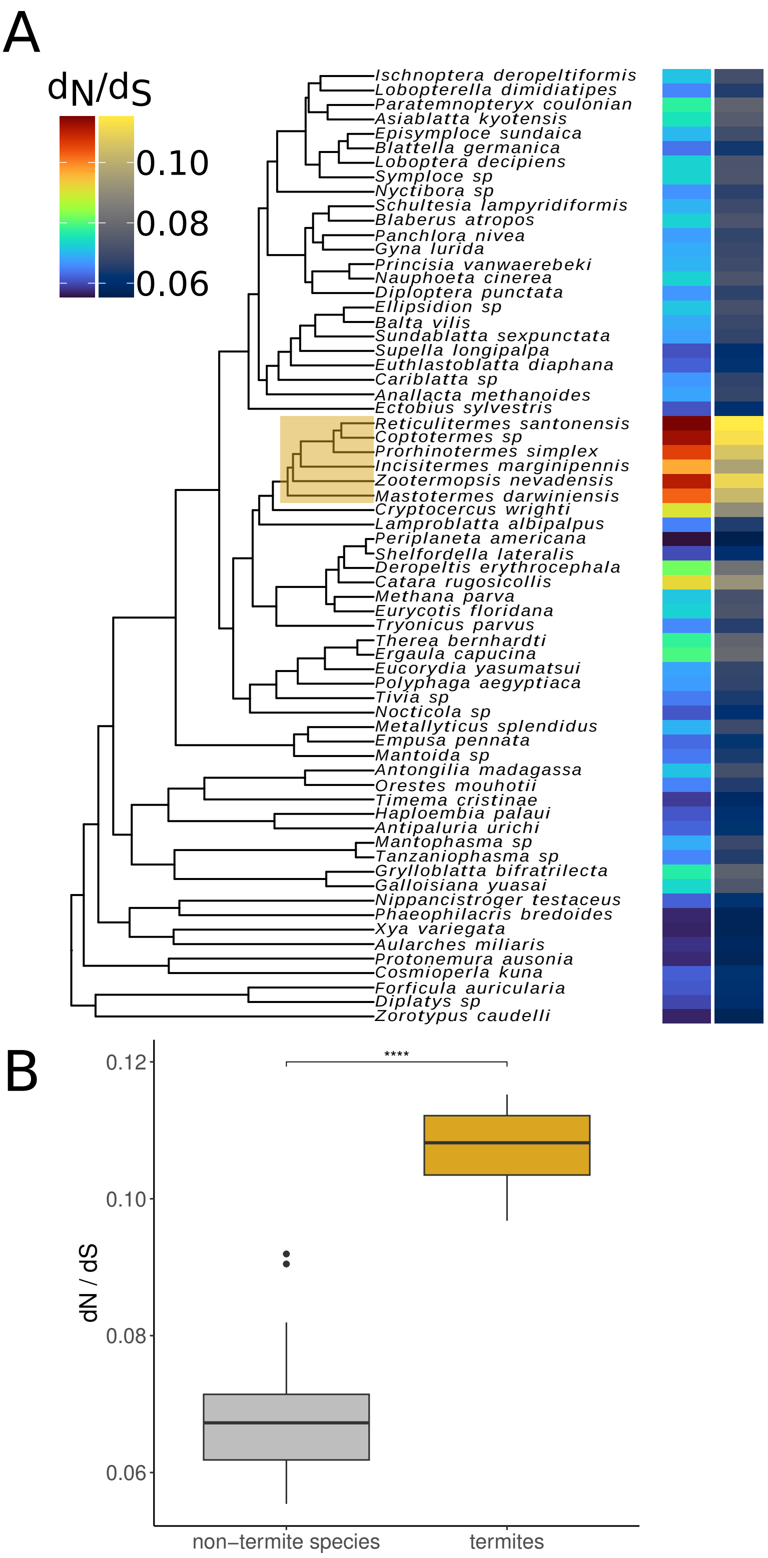

Figure 1. Distribution of the d_N/d_S ratio in Blattodea.

A. The phylogenetic tree employed in this study was produced by Evangelista et al. (2019), constructed from sequenced transcriptomes. Utilizing the ~~identical~~ same set of coding genes, we estimated the d_N/d_S ratios along each phylogenetic branch by mapnh. In this study, we exclusively report the d_N/d_S values corresponding to the terminal branches, represented here by a gradient of colours between 0.055 ~~(dark blue)~~ and 0.115~~(dark red)~~. Two color palettes are used here to include the diversity of color perception. The clade encompassing termites is highlighted by a yellow rectangle. **B.** Boxplot representing the distribution of d_N/d_S ratios in the terminal branches of ~~Blattodae~~Blattodea. In grey: estimated values for non-termite species. In yellow: estimated values for termites. Horizontal lines indicate median values. Statistical significance is denoted as follows: *ns* ($P > 0.05$), * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), **** ($P \leq 0.0001$).

Upon comparing the mean genomic d_N/d_S ratios calculated for the external branches leading to the 66 species under study, we observe a notably higher d_N/d_S ratio that can be interpreted as lower efficiency of natural selection within the clade that includes termites (mean=0.107; standard deviation=0.00687) as opposed to outside this group (mean=0.0675; standard deviation=0.00756). This disparity is statistically significant, as confirmed by a Wilcoxon signed-sum test (~~$V = 2211; P = 1.679 \times 10^{-12}$~~$W = 0; P = 6.235 \times 10^{-5}$). The two distributions do not overlap, with the highest d_N/d_S for non-termites (0.092 in *Catara rugosicollis*) being lower than the lowest d_N/d_S for termites (0.097 in *Incisitermes marginipennis*).

In mammals, body size is known to strongly influence the d_N/d_S ratio because of its negative relationship with Ne. Thus, large and long-lived species display on average a higher d_N/d_S ratio than small and short-lived mammals (Nikolaev et al., 2007). Here we test whether a similar relationship is found in ~~Blattodae~~ Blattodea (Fig. 2-A; Table S1).

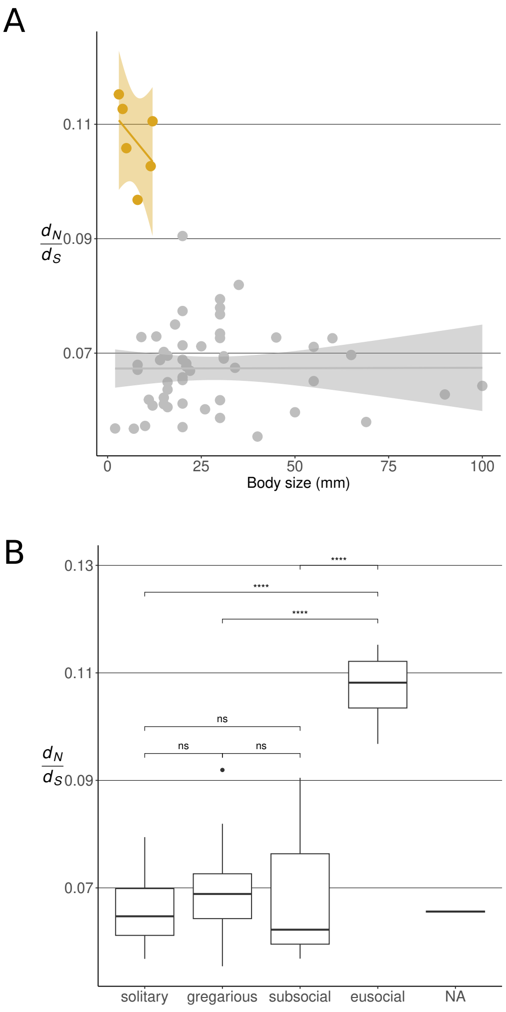

Figure 2. Comparative analysis of body size and social system influences on d_N/d_S ratios
Panel A: relationship between organism body size reported for adults (in mm) and d_N/d_S. Termite data points are shown in orange, while non-termite points are displayed in grey.
Panel B: distribution of d_N/d_S ratios within different social systems according to Michener (1969). Bonferroni-adjusted statistical significance is denoted as follows: *ns* ($P > 0.05$), * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), **** ($P \leq 0.0001$).

To do this, we take advantage of the information available on adult body length in ~~Blattodae~~ Blattodea and test its impact on ~~the effectiveness~~ d_N/d_S which is interpreted as a measure of the efficiency of purifying selection. Contrary to mammals, where an equivalent relationship is strong, we found no relationship between body size and d_N/d_S in either termites (Spearman's $\rho = -0.6$, $P = 0.2417$) or non-termite ~~Blattodae~~ Blattodea (Spearman's $\rho = 0.1645$, $P = 0.2439$). This result converges with what has been observed in another insect clade where eusociality has evolved, the Hymenoptera, whose d_N/d_S is explained by colony size (Rubin, 2022) and not affected by body size (Benitiere et al., 2022; Weyna and Romiguier, 2021).

We then test the hypothesis that an increase in social organization within the ~~Blattodae~~ Blattodea would reduce the effectiveness of natural selection by reducing the number of reproductive individuals. To do this, we assigned each of the 66 species studied to one of the social categories defined by Michener (1969).

- Solitary = no social behaviour, individuals live separately from each other.
- Gregarious = individuals live together but do not co-operate or care for young.
- Subsocial = individuals take care of their young for a certain period of time.
- Communal = individuals of the same generation cooperate in nest building but not in the care of young.
- Quasisocial = individuals from the same generation use the same nest and cooperate in the caring for the young.
- Semisocial = individuals from the same generation cooperate in caring for young and sharing reproductive tasks.
- Eusocial = individuals from several generations cooperate in caring for the young and where reproductive tasks are shared.

Throughout the explored dataset, we found 24 solitary species, 31 gregarious, 3 subsocial and 6 eusocial ~~. Social~~ (social organization was not assigned for species of the genus *Tanzaniophasma* and for *Tryonicus parvus*~~. Before carrying out an ANOVA analysis to test an effect of social organization on d_N/d_S, we checked whether this ratio followed a normal distribution in each social group using a Shapiro-Wilk test (P-values ranging from 0.077 in the gregarious group to 0.838 in the eusocial group). Then we checked that variances of the d_N/d_S ratios are homogeneous between the different social systems using a Levene's test ($P = 0.3$). Once the normality of the d_N/d_S and the homogeneity of the variances between groups had been confirmed, we performed an ANOVA test highlighting a~~). Statistical analyses highlighted a strong effect of the social system (ANOVA test, $P < 2.66 \times 10^{-16}$~~). To identify specific differences between groups, we used a Tukey-HSD (Honest Significant Difference) test, which tests the differences in means between each pair of groups (~~; Fig. 2-B; Table 1).

Table 1. Tukey multiple comparisons of means for d_N/d_S ratios across different social systems. The table displays the pairwise comparisons between each social system. The *difference* column shows the difference in mean d_N/d_S ratios between the two groups being compared. The *lower* and *upper* columns represent the lower and upper bounds of the 95% confidence interval for these mean differences, respectively. The *adjusted p-values* indicate the significance of these differences after adjusting for multiple comparisons. Significant p-values (p < 0.05) are highlighted in bold.

~~comparison~~ Comparison	~~difference~~ Difference in Means	~~lower~~ Lower Bound	~~upper~~ Upper Bound	~~adjusted~~ Adjust
Subsocial - Gregarious	0.0011	-0.0110	0.0133	0.9948
Solitary - Gregarious	-0.0028	-0.0083	0.0027	0.5327
Eusocial - Gregarious	0.0386	0.0296	0.0475	**<0.0001**
Eusocial - Subsocial	0.0374	0.0232	0.0517	**<0.0001**
Eusocial - Solitary	0.0414	0.0322	0.0506	**<0.0001**
Solitary - Subsocial	-0.0039	-0.0163	0.0084	0.8338

The results of ~~the~~ a Tukey-HSD test (Table 1) suggest that the eusocial social system is associated with a higher d_N/d_S than the other groups (gregarious, subsocial, and solitary). The other comparisons (subsocial *versus* gregarious, solitary *versus* gregarious, and solitary *versus* subsocial) did not show statistically significant differences, indicating that d_N/d_S is only increased for eusocial species. At this phylogenetic scale, our analysis is potentially influenced by a pronounced phylogenetic bias, given the fact that only termites are classified as eusocial in Blattodea. We therefore note:

1. d_N/d_S is a rate along a branch and is estimated in a phylogenetic framework, which means that technically two neighbouring branches are independent and should not have similar values due to phylogenetic inertia in the same way than two neighbouring nodes for other statistics (such as $\%GC$ or body mass).
2. When adding this result with those obtained in a very phylogenetically distant group, Hymenoptera (Weyna and Romiguier, 2021), this is the fourth observation of strongly increased d_N/d_S associated to the emergence of eusociality.

Our results ~~reinforce the notion~~ further support the idea that eusociality is ~~associated with~~ linked to a genome-wide reduction in ~~natural selection efficiency, an effect not exclusive to eusocial Hymenoptera.~~ the efficiency of natural selection. This is evidenced by the RELAX analysis, which indicates that approximately 32% of the 3,166 coding genes studied are strongly consistent with a model of relaxed selection, whereas only about 0.72% of the genes are strongly supported by a model of positive selection (Fig. S1).

Interestingly, we note that the closest relative of termites, *Cryptocercus wrighti*, displays an intermediate d_N/d_S ratio ~~between other~~ highter to non-social species and relatively close to eusocial ones ($d_N/d_S = 0.0905$ compared to an average of 0.0675 in non-social Blattodea and 0.107 in eusocial ones). The *Cryptocercus* genus is characterized by biparental parental care of the brood (Evangelista et al., 2019), which likely precede the evolution of eusociality where parental care is extended to sibling workers. This is reminiscent of the situation in *Anthophila* bees, where solitary bees features high parental care (nest building and pollen collection for few larvae) and display elevated d_N/d_S, suggesting low effective population size preceding multiple evolution of eusociality in this group (Weyna and Romiguier, 2021). ~~Our~~ Demographic inferences using PSMC-like approaches (H Li and Durbin, 2011; Lynch, Haubold, et al., 2020; Terhorst et al., 2017) in non-social species with high d_N/d_S ratios, such as *Cryptocercus* and solitary bees, as well as in closely related eusocial groups, could provide a clearer understanding of the demographic context in which eusociality evolved. This would be particularly valuable for pinpointing the timing of *Ne* variations associated with the emergence of eusociality. This approach has already been applied to social spiders of the genus *Stegodyphus*, where it revealed a reduction in *Ne* associated with increasing social complexity (Ma et al., 2024). Moreover, it could help determine whether the reduction in *Ne* preceded the emergence of eusociality in bees (from solitary species) and termites (from *Cryptocercus*), or if it resulted from the evolution toward more complex social structures.

These results reinforce the idea that high parental care and low effective population size might facilitate evolution towards eusociality. As eusociality is a reproductive strategy characterized by ~~parental care~~ care of offspring shared by many ~~sibling individuals~~siblings, high parental care is likely to be a first pre-requisite to its evolution. High parental care has also been identified as the best ecological predictor of ~~low genetic diversity (Romiguier, Lourenco, et al., 2014), which theoretically increases inbreeding and the relative advantages~~ species with low effective population sizes (Romiguier, Lourenco, et al., 2014). As low effective population size increases the probability of sharing common gene ancestry (Wang et al., 2016), it is expected to increase genetic relatedness among individuals and thus amplify the benefits of kin selection ~~(Tabadkani et al., 2012). Together with the example of bees~~(Epplen et al., 1999; Tabadkani et al., 2012). In this regard, the evolution of eusociality in termites might have been facilitated by the combination of high parental care and reduced effective population size. Whether these are universal pre-requisites for eusociality emergence is still open to question~~, as~~: while low effective population size seems to precede sociality in termite and bees, the same pattern ~~is not observed for~~ has not been observed in ants and social wasps (Weyna and Romiguier, 2021). This can either mean that eusociality can emerge without initial drop of effective population size, or just that we did not detect it because of a lack of genomic data for closely related non-social species for ants and social wasps.

To further understand the influence of sociality on the effectiveness of natural selection in termites, we analyzed an additional dataset derived from transcriptome sequencing (Bucek et al., 2019). This dataset encompasses 68 termite species as well as 7 dictyopteran outgroups, allowing us to test whether the diversity in levels of eusociality among termites correlates with d_N/d_S variations.

Contrasting d_N/d_S ratios among eusocial Blattodea (Isoptera)

In the second part of our study, we investigated the evolution of the d_N/d_S ratio within Isoptera using transcriptome data from Bucek et al. Similarly to the approach adopted for Blattodea, we computed the median genomic d_N/d_S for all branches of the Isopteran phylogenetic tree. The reported d_N/d_S per species corresponds to their respective terminal branch value (Fig. 3).

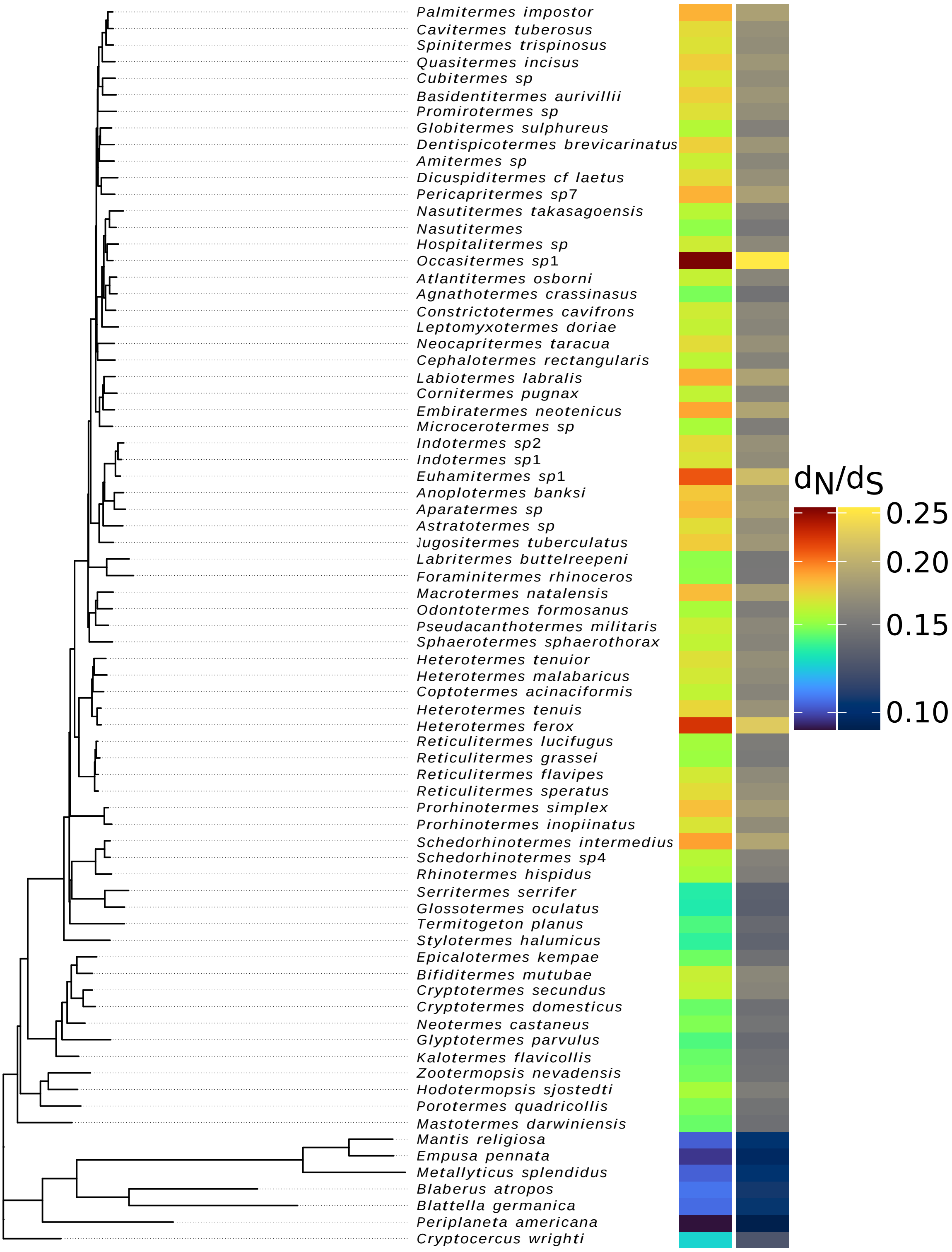

Figure 3. Distribution of the d_N/d_S ratio in Isoptera (and outgroup species). The phylogenetic tree employed in this study was produced by Bucek et al. (2019), constructed from sequenced transcriptomes. Utilizing the identical coding genes, we estimated the d_N/d_S ratios along each phylogenetic branch by mapnh. In this study, we exclusively report the d_N/d_S values corresponding to the terminal branches, represented here by a gradient of colours between 0.0925 (~~dark blue~~ for the outgroup *Periplaneta americana*) and 0.256 (~~dark red for~~ *Occasitermes*). Two color palettes are used here to include the diversity of color perception.

The transcriptomes were available for a total of 75 species. Of these, 68 belong to the Isoptera, exhibiting a d_N/d_S variation range from 0.132 (*Glossotermes oculatus*) to 0.256 (*Occasitermes*). The remaining seven species constitute the outgroup, showing a d_N/d_S variation range from 0.0925 (*Periplaneta americana*) to 0.125 (*Cryptocercus wrighti*). This second dataset thus corroborates the overall trend we previously identified using the first dataset (Evangelista et al., 2019), also indicating an increased d_N/d_S in Isoptera, with values that do not overlap with the range found in non-eusocial Blattodea.

We then tried to explain the large variations of d_N/d_S across termites. More particularly, we hypothesized that the effect of eusociality on d_N/d_S could be modulated by the level of social complexity (Table S2). As in ants (Romiguier, Borowiec, et al., 2022), termite societies can be more or less complex, with some species featuring extreme forms of eusociality with highly specialized division of labour, large and long-lived colonies. To test whether social complexity increase the effect of eusociality on effective population size (*Ne*), we tested the effect on d_N/d_S of two social traits recognized as two good proxies for social complexity in the literature: nesting strategies and developmental pathways (Mizumoto and Bourguignon, 2021). Nesting strategies are typically divided in three categories following Abe (1987): OP (One Piece), MP (~~Multiple Pieces~~Multiple-Piece), and SP (~~Separated Pieces~~Separated-Piece). In the ~~One Piece~~ One-Piece strategy, termites ~~live in~~ inhabit a single piece of wood ~~used as both~~ that serves as both their nest and food source, ~~which means that colonies are typically smalland~~ resulting in typically small, short-lived ~~. Multiple Piece nests are typically larger~~colonies. Multiple-Piece nests involve colonies that occupy and connect several pieces of wood via excavated tunnels or constructed shelter tubes, allowing safe travel between different nest sites. In contrast, Separated-Piece nests are physically detached from the food sources, often featuring complex internal structures like chambers and corridors, and are commonly found in subterranean, mound, or arboreal forms (Mizumoto and Bourguignon, 2020) . MP and SP colonies are typically larger than OP, but social complexity is at its highest in Separated Piece strategies, with large nests distinct from food source, meaning that they do not reduce in size over time and can growth in size over extended period of time. We conducted a Mann-Whitney U test between each pair of nesting strategies, and always found significant differences except for the MP *versus* SP comparison (W=211; adjusted P=1; Table S3). Although termites, collectively, exhibit a higher d_N/d_S ratio compared to non-social ~~Blattodae~~ Blattodea species, the variations within termites are strongly associated with the complexity of their eusocial structures. Larger colonies, characterized by multiple pieces (MP) and separated pieces (SP) strategies, display higher d_N/d_S values than the smaller, single-piece (OP) colonies (Fig. 4-A). Within the same termite family, different species tend to exhibit identical nesting strategies, complicating the attribution of a direct correlation between nesting strategy and d_N/d_S. Nonetheless, a minimum level of variation in nesting strategies is at least observed within two families: Archotermopsidae, with two OP species and one MP species, and Rhinotermitidae B, comprising two OP species and three MP species. Despite the limited number of data points, we observed that within these families, the d_N/d_S ratio is consistently higher in MP species compared to OP (Fig. ~~S1~~S2). This preponderance of species with less complex sociality in the Archotermopsidae could explain why it is the termite family with the lowest d_N/d_S (median=0.149) over the 11 surveyed families (Table S4).

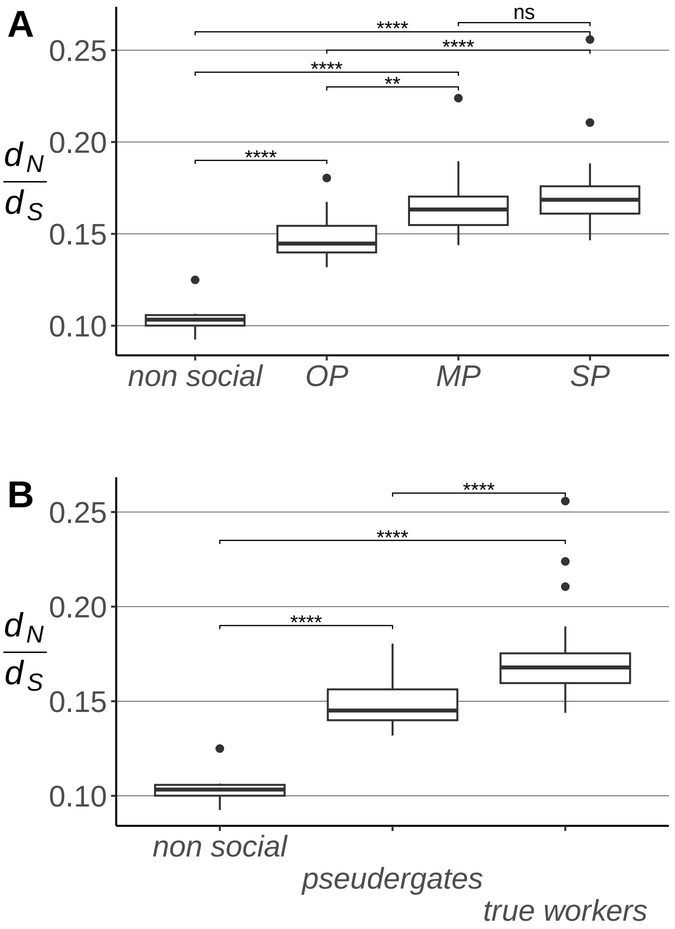

Figure 4. Relationship between social complexity in termites and d_N/d_S ratio.
A. Social complexity, as approximated by nesting strategy, following Mizumoto and Bourguignon (2021). Non-social species are represented solely by the outgroup. OP denotes One-Piece nesters, MP signifies Multiple-Pieces nesters, and SP refers to Separate-Pieces nesters. **B.** Social complexity, as approximated by the working castes, following Mizumoto and Bourguignon (2021). Pseudergates are workers with the plasticity to transition between castes, including reproductive roles, whereas true workers lack this ability to change castes. Bonferroni-adjusted statistical significance is denoted as follows: *ns* ($P > 0.05$), * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), **** ($P \leq 0.0001$).

This intricate social structure likely leads to higher d_N/d_S values due to the formation of larger colonies, which in turn reduces the number of breeding individuals, significantly affecting *Ne* and thus natural selection efficiency. Such association between advanced sociality and high d_N/d_S ratio has been retrieved in two empirical studies (Romiguier, Gayral, et al., 2014; Rubin, 2022) but not in a recent study in ants (Barkdull and Moreau, 2023) . In contrast, colonies that inhabit a single piece of wood, referred to as OP (One Piece strategy; see Fig. 4-A), are typically smaller. Consequently, these are anticipated to exhibit lower d_N/d_S values, stemming from decreased reproductive variance among females. ~~Here, we classify the nesting strategies of termites as OP (One Piece), MP (Multiple Pieces), and SP (Separated Pieces) following the categorization in the termite literature (Mizumoto and Bourguignon, 2021).~~

Mizumoto and Bourguignon (2021) suggests an alternative method for gauging the complexity of eusociality in termites by classifying species based on the developmental pathways of their worker caste, which are either pseudergates or true workers. Pseudergates are developmentally immature reproductive individuals, and can perform the colony's labor tasks but also differentiate later into either soldiers or reproductives. In contrast, true workers lack such developmental plasticity and are highly specialized to the colony's tasks. This specialization of true workers, as opposed to pseudergates, results in a higher division of labor, thereby indicating increased social complexity. Both species possessing pseudergates or true workers exhibit higher d_N/d_S ratios compared to non-social species (Mann-Whitney U test: W=0 in both comparisons; Adjusted P-value of ~~1.22×10^{-3} and 2.15×10^{-4} respectively). Interestingly, species with true workers display significantly higher d_N/d_S ratios than those with pseudergates (W=117; Adjusted P-value=1.87×10^{-5}~~6.26×10^{-4} and 6.29×10^{-5} respectively; Fig. 4-B). This reinforces the idea that effective population size is not only affected by eusociality, but also at a more finer scale by the different levels of social complexity ~~.~~ that affect effective population size. One potential effect increasing further d_N/d_S in "true workers" species is that worker being sterile, worker-specific genes can not be directly selected but only *via* kin selection. Such an indirect selection is predicted to be reduced compared to direct selection, an idea that has been supported by previous work in the pharaoh ant (Warner et al., 2017). To better quantify the effect of indirect selection in termites and disentangle it from the effect of Ne, an interesting perspective would be to replicate our study on queen-biased genes *vs.* worker-biased genes.

~~Our results suggest with~~ An effect of worker reproduction has also been tested in a recent study in ants but led to different results (Barkdull and Moreau, 2023). We suggest that this difference between ant and termites can be explained by differences in turnover between these two groups. In termites, species with true workers are highly represented in one clade which suggests that this trait is highly stable throughout their evolution. In contrast, worker polymorphism in ant species may be less stable as suggested by Barkdull and Moreau (2023) . We therefore propose that worker polymorphism in ants might not be maintained over long enough periods to have as clear effects as in termites on d_N/d_S ratio.

Overall, our results suggest with two different proxies of eusociality level that there is a molecular cost to social complexity. Such a finding have been first suggested in ants, where a correlation between d_N/d_S ratios and another proxy of social complexity (queen/worker dimorphism) have been reported (Romiguier, Lourenco, et al., 2014). ~~This finding was therefore based on only 7 species. Here, we confirmed this idea on a larger dataset of the other major eusocial taxa.~~ Taken independently, ~~both results are therefore~~ these results in ants and termites are sensitive to correction for phylogenetic inertia, as species with high social complexity tend to be clustered in few related clades~~within both ants and termites~~. Taken collectively, both results therefore reinforce the conclusion of the other, as it seems unlikely that the association between high d_N/d_S and high social complexity is due to chance in ~~the two taxa. Supplementary analyses on more species, including other eusocial clades such as beesor wasps will be useful to further confirm our conclusion.~~both distant taxa. Further reinforcing this conclusion, an association between advanced sociality and a high d_N/d_S ratio has also been observed in bees (Rubin, 2022), indicating that this pattern is present across all three major eusocial taxa. A similar trend has been identified in social *Synalpheus* shrimps compared to their non-social counterparts (Chak et al., 2021). These findings provide yet another example of the negative impact of eusociality on the efficiency of natural selection.

~~Overall, this study confirms~~ We therefore note that less efficient purifying selection at low *Ne* does not necessarily translate into less positive selection events. In bees, eusocial lineages appear to exhibit higher d_N/d_S ratio (likely due to less efficient purifying selection), but also more positive selection events (Shell et al., 2021) . In a similar way, a positive selection hotspot is associated to the origin of advanced eusociality in ants (Romiguier, Borowiec, et al., 2022). Such an apparent paradox can be explained because smaller populations are predicted to have a larger proportion of beneficial mutations due to the increased fixation of deleterious mutations in such populations, which in turn creates more opportunities for new beneficial back-mutations (i.e, compensatory mutations; Weissman and Barton, 2012). This theoretical expectation of saturation in adaptive rates over the long term for species with large *Ne* has been empirically demonstrated across various animal groups (Rousselle et al., 2020), including insects, mollusks, annelids, birds, and mammals. In this study, the relationship between effective population size and ω_A, the rate of adaptive amino-acid substitution is indeed positive only in taxa with low *Ne*.

To conclude, our study supports that eusociality is associated to significant decreases in terms of ~~effective population size~~natural selection efficiency, which validates population genetic theory and highlights that euso-

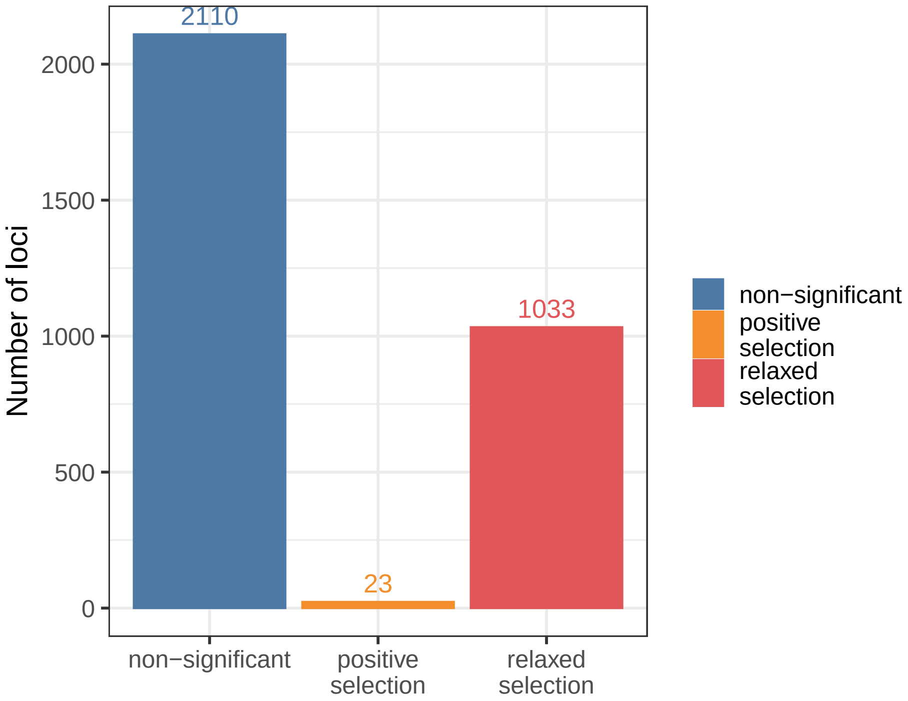

Figure S1. Test for the relaxation of purifying selection ($k < 1$) and positive selection ($k > 1$) against a null model for 3,166 orthologous codon-aligned genes in the Evangelista et al. (2019) dataset. Blue: loci that do not reject the null model (p-value > 0.05 after Bonferroni correction). Orange: loci supporting a positive selection model in termites ($k > 1$, p-value < 0.05 after Bonferroni correction). Red: loci supporting a relaxation of purifying selection in termites ($k < 1$, p-value < 0.05 after Bonferroni correction).

cial insects undergo molecular evolution that is closer to large ~~vertabrates~~ vertebrates than other insects. This support the view that social insects should be considered as superorganisms (Boomsma and Gawne, 2018), and that their population size vary depending on their social complexity in the same way than population size vary depending on life history traits for non-social species (Romiguier, Gayral, et al., 2014).

Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article.

Data, script, code, and supplementary information availability

Data and scripts are available online from a Zenodo depository: https://doi.org/10.5281/zenodo.11057544

Table S1. Relationship between d_N/d_S ~~values for termite families~~ratios, social traits and adult body size in the Blattodea dataset (Evangelista et al., 2019)

Species	~~mean d_N/d_S~~ **Order**	~~min d_N/d_S~~ **Nb Genes**	~~max d_N/d_S~~ **dN/dS**	**Social System**
Anallacta_methanoides	Blattellinae	1562	0.0682	gregarious
Antipaluria_urichi	Embioptera	2114	0.0622	subsocial

Continued on

Table S1 continued from previous page

Species	Order	Nb Genes	dN/dS	Social System
Antongilia_madagassa	Phasmatodea	1602	0.0711	solitary
Asiablatta_kyotensis	Blattellinae	1714	0.0750	gregarious
Aularches_miliaris	Orthoptera	2171	0.0580	gregarious
Balta_vilis	Pseudophyllodromiinae	2362	0.0688	gregarious
Blaberus_atropos	Blaberidae	2010	0.0726	gregarious
Blattella_germanica	Blattellinae	2409	0.0636	gregarious
Cariblatta_sp	Pseudophyllodromiinae	1850	0.0671	solitary
Catara_rugosicollis	Blattidae	2039	0.0919	gregarious
Coptotermes_sp	Isoptera	1819	0.113	eusocial
Cosmioperla_kuna	Plecoptera	1679	0.0618	solitary
Cryptocercus_wrighti	Cryptocercidae	2505	0.0905	subsocial
Deropeltis_erythrocephala	Blattidae	2105	0.0819	gregarious
Diplatys_sp	Dermaptera	2222	0.0597	gregarious
Diploptera_punctata	Blaberidae	1537	0.0669	gregarious
Ectobius_sylvestris	Ectobiinae	1770	0.0608	solitary
Ellipsidion_sp	Pseudophyllodromiinae	2426	0.0714	solitary
Empusa_pennata	Mantodea	2144	0.0628	solitary
Episymploce_sundaica	Blattellinae	1690	0.0702	gregarious
Ergaula_capucina	Corydiidae	1991	0.0794	solitary
Eucorydia_yasumatsui	Corydiidae	2071	0.0682	solitary
Eurycotis_floridana	Blattidae	1812	0.0727	gregarious
Euthlastoblatta_diaphana	Pseudophyllodromiinae	2034	0.0619	gregarious
Forficula_auricularia	Dermaptera	2281	0.0612	solitary
Galloisiana_yuasai	Grylloblattodea	2339	0.0734	solitary
Grylloblatta_bifratrilecta	Grylloblattodea	2096	0.0768	solitary
Gyna_lurida	Blaberidae	1601	0.0690	gregarious
Haploembia_palaui	Embioptera	2225	0.0611	solitary
Incisitermes_marginipennis	Isoptera	2010	0.0968	eusocial
Ischnoptera_deropeltiformis	Blattellinae	2099	0.0712	gregarious
Lamproblatta_albipalpus	Lamproblattidae	2439	0.0649	gregarious
Loboptera_decipiens	Blattellinae	2396	0.0728	gregarious
Lobopterella_dimidiatipes	Blattellinae	1918	0.0653	gregarious
Mantoida_sp	Mantodea	1861	0.0643	solitary
Mantophasma_sp	Mantophasmatodea	1769	0.0689	gregarious
Mastotermes_darwiniensis	Isoptera	2398	0.103	eusocial
Metallyticus_splendidus	Mantodea	2180	0.0695	solitary
Methana_parva	Blattidae	1893	0.0716	gregarious
Nauphoeta_cinerea	Blaberidae	2068	0.0726	gregarious
Nippancistroger_testaceus	Orthoptera	2379	0.0619	solitary
Nocticola_sp	Nocticolidae	2350	0.0611	gregarious
Nyctibora_sp	Nyctiborinae	1349	0.0666	solitary
Orestes_mouhotii	Phasmatodea	2192	0.0651	solitary
Panchlora_nivea	Blaberidae	1942	0.0677	gregarious
Paratemnopteryx_couloniana	Blattellinae	1909	0.0774	gregarious
Periplaneta_americana	Blattidae	2194	0.0554	gregarious
Phaeophilacris_bredoides	Orthoptera	2283	0.0571	solitary

Continued on

Table S1 continued from previous page

Species	Order	Nb Genes	dN/dS	Social System
Polyphaga_aegyptiaca	Corydiidae	1847	0.0674	solitary
Princisia_vanwaerebeki	Blaberidae	1793	0.0697	gregarious
Prorhinotermes_simplex	Isoptera	2507	0.106	eusocial
Protonemura_ausonia	Plecoptera	2295	0.0573	solitary
Reticulitermes_santonensis	Isoptera	1892	0.115	eusocial
Schultesia_lampyridiformis	Blaberidae	2466	0.0696	gregarious
Shelfordella_lateralis	Blattidae	2317	0.0602	gregarious
Sundablatta_sexpunctata	Pseudophyllodromiinae	2516	0.0680	gregarious
Supella_longipalpa	Pseudophyllodromiinae	2431	0.0606	gregarious
Symploce_sp	Blattellinae	2464	0.0729	gregarious
Tanzaniophasma_sp	Mantophasmatodea	2127	0.0654	NA
Therea_bernhardti	Corydiidae	1778	0.0779	solitary
Timema_cristinae	Phasmatodea	1958	0.0587	solitary
Tivia_sp	Corydiidae	1592	0.0643	solitary
Tryonicus_parvus	Tryonicidae	2149	0.0658	NA
Xya_variegata	Orthoptera	2423	0.0568	solitary
Zootermopsis_nevadensis	Isoptera	2573	0.111	eusocial
Zorotypus_caudelli	Zoraptera	2298	0.0568	subsocial

Table S2. Relationship between d_N/d_S ratios and social traits in the Isoptera dataset (Bucek et al., 2019)

Species	Family	Nb Genes	dN/dS	Abe Nestir
Agnathotermes_crassinasus	Nasutitermitinae	3481	0.147	SP
Amitermes_sp	Termitinae	3613	0.163	SP
Anoplotermes_banksi	Apicotermitinae	~~0.179~~ 3732	0.178	SP
Aparatermes_sp	Apicotermitinae	3768	0.182	SP
Astratotermes_sp	Apicotermitinae	3648	0.170	SP
Atlantitermes_osborni	Nasutitermitinae	3727	0.162	SP
Basidentitermes_aurivillii	Termitidae_B	3745	0.175	SP
Bifiditermes_mutubae	Kalotermitidae	3397	0.163	OP
Blaberus_atropos	outgroup	2483	0.106	non_social
Blattella_germanica	outgroup	3927	0.105	non_social
Cavitermes_tuberosus	Termitidae_B	3711	0.171	SP
Cephalotermes_rectangularis	Termitidae_A	3479	0.160	SP
Constrictotermes_cavifrons	Nasutitermitinae	3701	0.164	SP
Coptotermes_acinaciformis	Rhinotermitidae_E	3803	0.161	MP
Cornitermes_pugnax	Syntermitinae	3721	0.161	SP
Cryptocercus_wrighti	outgroup	3491	0.125	non_social
Cryptotermes_domesticus	Kalotermitidae	3800	0.144	OP
Cryptotermes_secundus	Kalotermitidae	3707	0.161	OP
Cubitermes_sp	Termitidae_B	3616	0.168	~~0.211~~ SP
~~Archotermopsidae~~ Dentispicotermes_brevicarinatus	~~0.149~~ Termitinae	3805	0.175	SP
Dicuspiditermes_cf_laetus	Termitinae	3510	0.171	SP
Embiratermes_neotenicus	Syntermitinae	3787	0.188	SP
Empusa_pennata	outgroup	2690	0.0971	non_social

Cont

Table S2 continued from previous page

Species	**Family**	**Nb Genes**	**dN/dS**	**Abe Nestin**
Epicalotermes_kempae	Kalotermitidae	3505	0.145	~~0.155~~ OP
Euhamitermes_sp1	Apicotermitinae	3451	0.211	SP
Foraminitermes_rhinoceros	Foraminitermitinae	2394	0.151	SP
Globitermes_sulphureus	Termitinae	3719	0.158	SP
Glossotermes_oculatus	Rhinotermitidae_B	3824	0.132	OP
Glyptotermes_parvulus	Kalotermitidae	~~0.149~~ 3616	0.140	~~0.163~~ OP
Heterotermes_ferox	Rhinotermitidae_E	3483	0.224	MP
Heterotermes_malabaricus	Rhinotermitidae_E	3391	0.165	MP
Heterotermes_tenuior	Rhinotermitidae_E	3629	0.169	MP
Heterotermes_tenuis	Rhinotermitidae_E	3569	0.173	MP
Hodotermopsis_sjostedti	Archotermopsidae	2927	0.155	MP
Hospitalitermes_sp	Nasutitermitinae	3563	0.164	SP
Indotermes_sp1	Apicotermitinae	3107	0.168	SP
Indotermes_sp2	Apicotermitinae	2947	0.171	SP
Jugositermes_tuberculatus	Apicotermitinae	3590	0.176	SP
Kalotermes_flavicollis	Kalotermitidae	3812	0.144	OP
Labiotermes_labralis	Syntermitinae	3753	0.187	SP
Labritermes_buttelreepeni	Foraminitermitinae	2559	0.150	SP
Leptomyxotermes_doriae	Nasutitermitinae	3522	0.162	SP
Macrotermes_natalensis	Macrotermitinae	~~0.167~~ 3863	0.181	SP
Mantis_religiosa	outgroup	2936	0.103	non_social
Mastotermes_darwiniensis	Mastotermitidae	3266	0.144	MP
Metallyticus_splendidus	outgroup	2878	0.103	non_social
Microcerotermes_sp	Syntermitinae	3804	0.156	~~0.181~~ SP
Nasutitermes	Nasutitermitinae	~~0.170~~ 3465	~~0.147~~ 0.150	SP
Nasutitermes_takasagoensis	Nasutitermitinae	2588	0.159	SP
Neocapritermes_taracua	Termitidae_A	3770	0.171	SP
Neotermes_castaneus	Kalotermitidae	3757	0.148	OP
Occasitermes_sp1	Nasutitermitinae	3604	0.256	SP
~~Rhinotermitidae~~ Odontotermes_formosanus	Macrotermitinae	3713	0.156	SP
Palmitermes_impostor	Termitidae_B	3680	0.185	SP
Pericapritermes_sp7	Termitinae	3804	0.185	SP
Periplaneta_americana	outgroup	3085	0.0925	non_social
Porotermes_quadricollis	Archotermopsidae	3842	0.147	OP
Promirotermes_sp	Termitidae_B	3516	0.169	SP
Prorhinotermes_inopiinatus	Rhinotermitidae_C	3370	0.167	OP
Prorhinotermes_simplex	Rhinotermitidae_C	3262	0.180	OP
Pseudacanthotermes_militaris	Macrotermitinae	3450	0.164	SP
Quasitermes_incisus	Termitidae_B	~~0.154~~ 3572	~~0.132~~ 0.176	~~0.190~~ SP
Reticulitermes_flavipes	Rhinotermitidae_D	~~0.161~~ 3276	0.166	MP
Reticulitermes_grassei	Rhinotermitidae_D	2391	0.152	MP
Reticulitermes_lucifugus	Rhinotermitidae_D	2599	0.154	MP
Reticulitermes_speratus	Rhinotermitidae_D	2825	0.171	MP
~~Rhinotermitidae_E~~ Rhinotermes_hispidus	Rhinotermitidae_B	~~0.178~~ 3849	~~0.161~~ 0.155	~~0.224~~ MP
~~Syntermitinae~~ Schedorhinotermes_intermedius	~~0.173~~ Rhinotermitidae_B	~~0.156~~ 3653	~~0.188~~ 0.190	MP
Schedorhinotermes_sp4	Rhinotermitidae_B	3702	0.158	MP

Cont

Table S2 continued from previous page

Species	**Family**	**Nb Genes**	**dN/dS**	**Abe Nestir**
Serritermes_serrifer	Rhinotermitidae_B	3786	0.133	OP
Sphaerotermes_sphaerothorax	Sphaerotermitinae	3315	0.161	SP
Spinitermes_trispinosus	Termitidae_B	~~0.173~~ 3783	~~0.168~~ 0.169	~~0.185~~ SP
~~Termitinae~~ Stylotermes_halumicus	~~0.170~~ Stylotermitidae	~~0.158~~ 3855	~~0.185~~ 0.135	OP
Termitogeton_planus	Rhinotermitidae_A	3517	0.140	OP
Zootermopsis_nevadensis	Archotermopsidae	3786	0.145	OP

Table S3. Pairwise comparisons of differences in ~~dNdS~~ d_N/d_S between alternative nesting strategies

Pair	*W*	Adjusted P-value
Non-social *vs.* OP	0	~~2.77×10^{-3}~~ 1.48×10^{-3}
Non-social *vs.* MP	0	~~3.72×10^{-3}~~ 1.78×10^{-3}
Non-social *vs.* SP	0	~~6.02×10^{-4}~~ 1.91×10^{-4}
OP *vs.* MP	40	~~1.77×10^{-2}~~ 2.93×10^{-2}
OP *vs.* SP	74	~~6.46×10^{-5}~~ 1.53×10^{-4}
MP *vs.* SP	211	1.00

Adjusted *p*-values from Mann-Whitney U tests comparing the d_N/d_S values between different nesting strategies of termite species. Adjustments were made using the Bonferroni method to correct for multiple comparisons.

Table S4. d_N/d_S values for termite families

Family	mean d_N/d_S	min d_N/d_S	max d_N/d_S
Apicotermitinae	0.179	0.168	0.211
Archotermopsidae	0.149	0.145	0.155
Kalotermitidae	0.149	0.140	0.163
Macrotermitinae	0.167	0.156	0.181
Nasutitermitinae	0.170	0.147	0.256
Rhinotermitidae_B	0.154	0.132	0.190
Rhinotermitidae_D	0.161	0.152	0.171
Rhinotermitidae_E	0.178	0.161	0.224
Syntermitinae	0.173	0.156	0.188
Termitidae_B	0.173	0.168	0.185
Termitinae	0.170	0.158	0.185

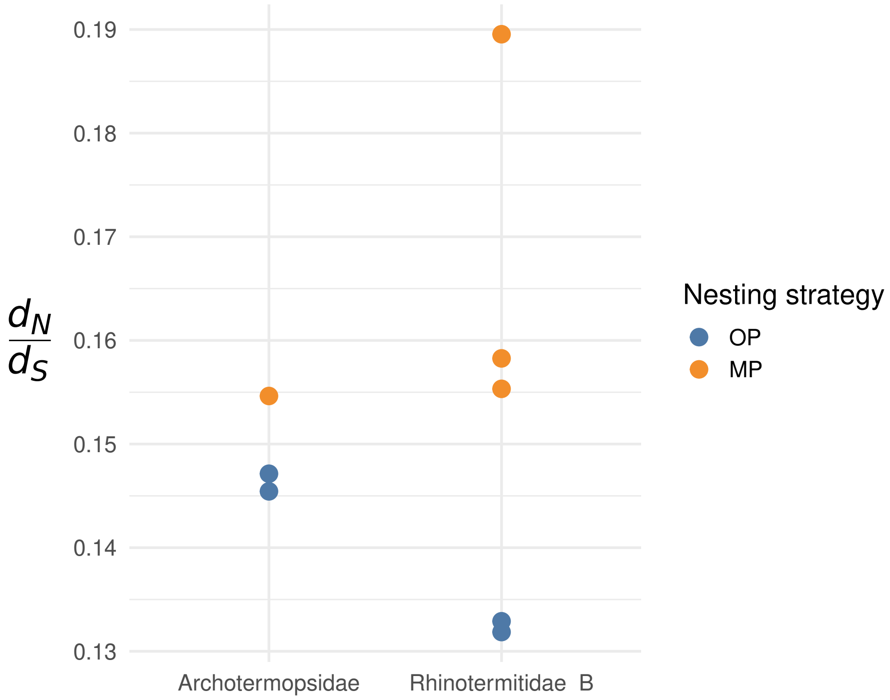

Figure S2. Association between social complexity and d_N/d_S ratios in Archotermopsidae and Rhinotermitidae B using the nesting strategy as proxy.

References

Abe T (1987). Evolution of life types in termites. *Evolution and coadaptation in biotic communities*.

Ahmad M (1963). On the phylogenetic position of Indotermes, with description of a new species (Isoptera, Termitidae). *Journal of Natural History* 6, 395–399.

Atkinson L and ES Adams (1997). The origins and relatedness of multiple reproductives in colonies of the termite Nasutitermes corniger. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 264, 1131–1136.

Barbosa JRC and R Constantino (2017). Polymorphism in the neotropical termite S erritermes serrifer. *Entomologia Experimentalis et Applicata* 163, 43–50.

Barkdull M and CS Moreau (2023). Worker reproduction and caste polymorphism impact genome evolution and social genes across the ants. *Genome Biology and Evolution* 15, evad095.

Benitiere F, A Necsulea, and L Duret (2022). Random genetic drift sets an upper limit on mRNA splicing accuracy in metazoans. *bioRxiv*, 2022–12.

Bohn H (1989). Revision of the sylvestris group of Ectobius Stephens in Europe (Blattaria: Blattellidae). *Insect Systematics & Evolution* 20, 317–342.

Boomsma JJ and R Gawne (2018). Superorganismality and caste differentiation as points of no return: how the major evolutionary transitions were lost in translation. *Biological Reviews* 93, 28–54.

Botero-Castro F, E Figuet, MK Tilak, B Nabholz, and N Galtier (2017). Avian genomes revisited: hidden genes uncovered and the rates versus traits paradox in birds. *Molecular biology and evolution* 34, 3123–3131.

Bourguignon T, RH Scheffrahn, J Křeček, ZT Nagy, G Sonet, and Y Roisin (2010). Towards a revision of the Neotropical soldierless termites (Isoptera: Termitidae): redescription of the genus Anoplotermes and description of Longustitermes, gen. nov. *Invertebrate Systematics* 24, 357–370.

Bourguignon T, J Šobotník, J Brabcová, D Sillam-Dussès, A Buček, J Krasulová, B Vytisková, Z Demianová, M Mareš, Y Roisin, et al. (2016). Molecular mechanism of the two-component suicidal weapon of Neocapritermes taracua old workers. *Molecular biology and evolution* 33, 809–819.

Bucek A, J Šobotník, S He, M Shi, DP McMahon, EC Holmes, Y Roisin, N Lo, and T Bourguignon (2019). Evolution of termite symbiosis informed by transcriptome-based phylogenies. *Current Biology* 29, 3728–3734.

Cabrera BJ and RH Scheffrahn (2001). Western drywood termite, Incisitermes minor (hagen)(insecta: Isoptera: Kalotermitidae). *UF/IFAS Extension EENY* 248, 1–7.

Chak ST, JA Baeza, and P Barden (2021). Eusociality shapes convergent patterns of molecular evolution across mitochondrial genomes of snapping shrimps. *Molecular Biology and Evolution* 38, 1372–1383.

Chhotani O (1975). A new species of Euhamitermes and the imago of Nasutitermes garoensis from Arunachal Pradesh (Isoptera: Termtidae). *Oriental Insects* 9, 149–155.

Choe JC and BJ Crespi (1997). *The evolution of social behaviour in insects and arachnids*. Cambridge University Press.

Constantino R and O De Souza (1997). Key to the soldiers of Atlantitermes Fontes 1979, with a new species from Brazil (Isoptera Termitidae Nasutitermitinae). *Tropical Zoology* 10, 205–213.

Constantino R (1990). Agnathotermes crassinasus, new species of termite from the Amazon basin (Isoptera: Termitidae: Nasutitermitinae).

De Saeger MH (1954). Exploration du Parc national de la Garamba. *Introduction. Brussels: Institut des parcs nationaux du Congo Belge*.

Dedeine F, LA Weinert, D Bigot, T Josse, M Ballenghien, V Cahais, N Galtier, and P Gayral (2015). Comparative analysis of transcriptomes from secondary reproductives of three Reticulitermes termite species. *PLoS One* 10, e0145596.

Dejean A and R Fénéron (1996). Polymorphism and oligogyny in the ponerine ant Centromyrmex bequaerti (Formicidae: Ponerinae). *Insectes Sociaux* 43, 87–99.

Duffy JE (1996). Eusociality in a coral-reef shrimp. *Nature* 381, 512–514.

Dupont L, V Roy, A Bakkali, M Harry, et al. (2009). Genetic variability of the soil-feeding termite Labiotermes labralis (Termitidae, Nasutitermitinae) in the Amazonian primary forest and remnant patches. *Insect Conservation and Diversity* 2, 53–61.

Dutheil J and B Boussau (2008). Non-homogeneous models of sequence evolution in the Bio++ suite of libraries and programs. *BMC evolutionary biology* 8, 1–12.

Dutheil JY, N Galtier, J Romiguier, EJ Douzery, V Ranwez, and B Boussau (2012). Efficient selection of branch-specific models of sequence evolution. *Molecular biology and evolution* 29, 1861–1874.

Emerson AE and FA Banks (1965). The neotropical genus Labiotermes (Holmgren): its phylogeny, distribution, and ecology (Isoptera, Termitidae, Nasutitermitinae). American Museum novitates; no. 2208.

Epplen J et al. (1999). Within-colony relatedness in a termite species: genetic roads to eusociality? *Behaviour* 136, 1045–1063.

Evangelista DA, B Wipfler, O Béthoux, A Donath, M Fujita, MK Kohli, F Legendre, S Liu, R Machida, B Misof, et al. (2019). An integrative phylogenomic approach illuminates the evolutionary history of cockroaches and termites (Blattodea). *Proceedings of the Royal Society B* 286, 20182076.

Evans TA (2011). Invasive termites. *Biology of termites: a modern synthesis*, 519–562.

Ewart KM, SY Ho, AA Chowdhury, FR Jaya, Y Kinjo, J Bennett, T Bourguignon, HA Rose, and N Lo (2024). Pervasive relaxed selection in termite genomes. *Proceedings of the Royal Society B* 291, 20232439.

Figuet E, B Nabholz, M Bonneau, E Mas Carrio, K Nadachowska-Brzyska, H Ellegren, and N Galtier (2016). Life history traits, protein evolution, and the nearly neutral theory in amniotes. *Molecular biology and evolution* 33, 1517–1527.

Florian OP, RH Scheffrahn, and TF Carrijo (2019). Aparatermes thornatus (Isoptera: Termitidae: Apicotermitinae), a new species of soldierless termite from northern Amazonia. *Florida Entomologist* 102, 141–146.

Fougeyrollas R, K Dolejšová, D Sillam-Dussès, V Roy, C Poteaux, R Hanus, and Y Roisin (2015). Asexual queen succession in the higher termite Embiratermes neotenicus. *Proceedings of the Royal Society B: Biological Sciences* 282, 20150260.

Galtier N (2016). Adaptive protein evolution in animals and the effective population size hypothesis. *PLoS genetics* 12, e1005774.

Galtier N, C Roux, M Rousselle, J Romiguier, E Figuet, S Glémin, N Bierne, and L Duret (2018). Codon usage bias in animals: disentangling the effects of natural selection, effective population size, and GC-biased gene conversion. *Molecular biology and evolution* 35, 1092–1103.

Gay F (1974). The Australian termite genus Occasitermes (Isoptera: Termitidae: Nasutitermitinae). *Australian Journal of Entomology* 13, 275–283.

Gay F and R Barrett (1983). The occurrence in Australia of Prorhinotermes inopinatus Silvestri (Isoptera: Rhinotermitidae). *Australian Journal of Entomology* 22, 75–77.

Gay FJ and JA Watson (1982). The genus Cryptotermes in Australia (Isoptera: Kalotermitidae). *Australian Journal of Zoology Supplementary Series* 30, 1–64.

Ghesini S and M Marini (2013). A dArk-necked drywood terMite (isopterA: kAloterMitidAe) in itAly: description of KalotErmEs italicus SP. NOV. *Florida Entomologist*, 200–211.

Goodisman MA and RH Crozier (2002). Population and colony genetic structure of the primitive termite Mastotermes darwiniensis. *Evolution* 56, 70–83.

Guéguen L and L Duret (2018). Unbiased estimate of synonymous and nonsynonymous substitution rates with nonstationary base composition. *Molecular Biology and Evolution* 35, 734–742.

Haeseler A von, HA Schmidt, MQ Bui, and LT Nguyen (2014). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies.

Hanus R, J Šobotník, I Valterová, and J Lukáš (2006). The ontogeny of soldiers in Prorhinotermes simplex (Isoptera, Rhinotermitidae). *Insectes Sociaux* 53, 249–257.

Harrison MC, E Jongepier, HM Robertson, N Arning, T Bitard-Feildel, H Chao, CP Childers, H Dinh, H Doddapaneni, S Dugan, et al. (2018). Hemimetabolous genomes reveal molecular basis of termite eusociality. *Nature ecology & evolution* 2, 557–566.

Hayashi Y, S Shigenobu, D Watanabe, K Toga, R Saiki, K Shimada, T Bourguignon, N Lo, M Hojo, K Maekawa, et al. (2013). Construction and characterization of normalized cDNA libraries by 454 pyrosequencing and estimation of DNA methylation levels in three distantly related termite species. *PLoS One* 8, e76678.

Hellemans S, D Fournier, R Hanus, and Y Roisin (2017). Secondary queens in the parthenogenetic termite Cavitermes tuberosus develop through a transitional helper stage. *Evolution & development* 19, 253–262.

Howard KJ and BL Thorne (2011). Eusocial evolution in termites and Hymenoptera. *Biology of termites: a modern synthesis*, 97–132.

Huang Q, P Sun, X Zhou, and C Lei (2012). Characterization of head transcriptome and analysis of gene expression involved in caste differentiation and aggression in Odontotermes formosanus (Shiraki). *PLoS One* 7, e50383.

Huber CD, BY Kim, CD Marsden, and KE Lohmueller (2017). Determining the factors driving selective effects of new nonsynonymous mutations. *Proceedings of the National Academy of Sciences* 114, 4465–4470.

Husseneder C, R Brandl, C Epplen, JT Epplen, and M Kaib (1998). Variation between and within colonies in the termite: morphology, genomic DNA, and behaviour. *Molecular Ecology* 7, 983–990.

Jarvis JU, MJ O'Riain, NC Bennett, and PW Sherman (1994). Mammalian eusociality: a family affair. *Trends in Ecology & Evolution* 9, 47–51.

Kalyaanamoorthy S, BQ Minh, TK Wong, A Von Haeseler, and LS Jermiin (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature methods* 14, 587–589.

Katoh K and DM Standley (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30, 772–780.

Keller L and M Chapuisat (2001). Eusociality and cooperation. *Encyclopedia of life sciences*, 1–9.

Kimura M (1983). *The neutral theory of molecular evolution*. Cambridge University Press.

Kimura M, T Maruyama, and JF Crow (1963). The mutation load in small populations. *Genetics* 48, 1303.

Krishna K (1963). The African genus Foraminitermes Holmgren (Isoptera, Termitidae, Termitinae). American Museum novitates; no. 2161.

Krishna K and CL Adams (1982). The Oriental termite genus Labritermes Holmgren (Isoptera, Termitidae, Termitinae). American Museum novitates; no. 2735.

Krishna K and R Araujo (1968). A revision of the neotropical termite genus Neocapritermes (Isoptera, Termitidae, Termitinae). Bulletin of the AMNH; v. 138, article 3.

Kumar S, G Stecher, M Suleski, and SB Hedges (2017). TimeTree: a resource for timelines, timetrees, and divergence times. *Molecular biology and evolution* 34, 1812–1819.

Legendre F (2007). Phylogénie et Évolution du Comportement social chez les Blattes et les Termites. PhD thesis. Paris 6.

Lewis VR (2009). Isoptera:(Termites). In: *Encyclopedia of insects*. Elsevier, pp. 535–538.

Li H and R Durbin (2011). Inference of human population history from individual whole-genome sequences. *Nature* 475, 493–496.

Liang WR, CC Wu, and HF Li (2017). Discovery of a cryptic termite genus, Stylotermes (Isoptera: Stylotermitidae), in Taiwan, with the description of a new species. *Annals of the Entomological Society of America* 110, 360–373.

Lihoreau M (2009). Organisation et fonctionnement des groupes sociaux chez une blatte grégaire: un autre modèle de société d'insectes. PhD thesis. Université de Rennes 1.

Lubin Y and T Bilde (2007). The evolution of sociality in spiders. *Advances in the Study of Behavior* 37, 83–145.

Lynch M (2002). Intron evolution as a population-genetic process. *Proceedings of the National Academy of Sciences* 99, 6118–6123.

— (2012). The evolution of multimeric protein assemblages. *Molecular biology and evolution* 29, 1353–1366.

Lynch M, MS Ackerman, JF Gout, H Long, W Sung, WK Thomas, and PL Foster (2016). Genetic drift, selection and the evolution of the mutation rate. *Nature Reviews Genetics* 17, 704–714.

Lynch M, B Haubold, P Pfaffelhuber, and T Maruki (2020). Inference of historical population-size changes with allele-frequency data. *G3: Genes, Genomes, Genetics* 10, 211–223.

Lynch M and B Walsh (2007). *The origins of genome architecture*. Vol. 98. Sinauer associates Sunderland, MA.

Ma J, J Bechsgaard, A Aagaard, P Villesen, T Bilde, and M Schierup (2024). Sociality in spiders is an evolutionary dead-end. *bioRxiv*, 2024–04.

Maistrello L and G Sbrenna (1996). Frequency of some behavioural patterns in colonies of Kalotermes flavicollis (Isoptera Kalotermitidae): the importance of social interactions and vibratory movements as mechanisms for social integration. *Ethology Ecology & Evolution* 8, 365–375.

Martius C and J d'Arc Ribeiro (1996). Colony populations and biomass in nests of the Amazonian forest termite Anoplotermes banksi Emerson (Isoptera: Termitidae). *Studies on Neotropical Fauna and Environment* 31, 82–86.

Matsuura K (2002). Colony-level stabilization of soldier head width for head-plug defense in the termite Reticulitermes speratus (Isoptera: Rhinotermitidae). *Behavioral Ecology and Sociobiology* 51, 172–179.

Michener CD (1969). Comparative social behavior of bees. *Annual review of entomology* 14, 299–342.

Miller L (1994). Dimorphic minor soldiers in Schedorhinotermes breinli (Hill)(Isoptera: Rhinotermitidae). *Australian Journal of Entomology* 33, 319–320.

Misof B, S Liu, K Meusemann, RS Peters, A Donath, C Mayer, PB Frandsen, J Ware, T Flouri, RG Beutel, et al. (2014). Phylogenomics resolves the timing and pattern of insect evolution. *Science* 346, 763–767.

Mitaka Y, K Kobayashi, A Mikheyev, MM Tin, Y Watanabe, and K Matsuura (2016). Caste-specific and sex-specific expression of chemoreceptor genes in a termite. *PLoS One* 11, e0146125.

Miura T, S Koshikawa, and T Matsumoto (2003). Winged presoldiers induced by a juvenile hormone analog in Zootermopsis nevadensis: implications for plasticity and evolution of caste differentiation in termites. *Journal of Morphology* 257, 22–32.

Mizumoto N and T Bourguignon (2020). Modern termites inherited the potential of collective construction from their common ancestor. *Ecology and Evolution* 10, 6775–6784.

— (2021). The evolution of body size in termites. *Proceedings of the Royal Society B* 288, 20211458.

Nguyen LT, HA Schmidt, A Von Haeseler, and BQ Minh (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular biology and evolution* 32, 268–274.

Nikolaev SI, JI Montoya-Burgos, K Popadin, L Parand, EH Margulies, NI of Health Intramural Sequencing Center Comparative Sequencing Program, and SE Antonarakis (2007). Life-history traits drive the evolutionary rates of mammalian coding and noncoding genomic elements. *Proceedings of the National Academy of Sciences* 104, 20443–20448.

Noirot C (1959). Les nids de Globitermes sulphureus Haviland au Cambodge. *Insectes Sociaux* 6, 259–268.

Noirot C (1989). Social structure in termite societies. *Ethology ecology & evolution* 1, 1–17.

Ohta T (1973). Slightly deleterious mutant substitutions in evolution. *Nature* 246, 96–98.

Parmentier D and Y Roisin (2003). Caste morphology and development in Termitogeton nr. planus (Insecta, Isoptera, Rhinotermitidae). *Journal of Morphology* 255, 69–79.

Picker M (2012). *Field guide to insects of South Africa*. Penguin Random House South Africa.

Popadin K, LV Polishchuk, L Mamirova, D Knorre, and K Gunbin (2007). Accumulation of slightly deleterious mutations in mitochondrial protein-coding genes of large versus small mammals. *Proceedings of the National Academy of Sciences* 104, 13390–13395.

Porter EE and BA Hawkins (2001). Latitudinal gradients in colony size for social insects: termites and ants show different patterns. *The American Naturalist* 157, 97–106.

Poulsen M, H Hu, C Li, Z Chen, L Xu, S Otani, S Nygaard, T Nobre, S Klaubauf, PM Schindler, et al. (2014). Complementary symbiont contributions to plant decomposition in a fungus-farming termite. *Proceedings of the National Academy of Sciences* 111, 14500–14505.

Rasib KZ and M Saeed Akhtar (2012). Caste Developmental Pathways in Populations of Microcerotermes championi (Isoptera: Termitidae, Microcerotermitinae). *International Journal of Agriculture & Biology* 14.

Rolland J, D Schluter, and J Romiguier (2020). Vulnerability to fishing and life history traits correlate with the load of deleterious mutations in teleosts. *Molecular Biology and Evolution* 37, 2192–2196.

Romiguier J, ML Borowiec, A Weyna, Q Helleu, E Loire, C La Mendola, C Rabeling, BL Fisher, PS Ward, and L Keller (2022). Ant phylogenomics reveals a natural selection hotspot preceding the origin of complex eusociality. *Current Biology* 32, 2942–2947.

Romiguier J, E Figuet, N Galtier, EJ Douzery, B Boussau, JY Dutheil, and V Ranwez (2012). Fast and robust characterization of time-heterogeneous sequence evolutionary processes using substitution mapping. *PLoS One* 7, e33852.

Romiguier J, P Gayral, M Ballenghien, A Bernard, V Cahais, A Chenuil, Y Chiari, R Dernat, L Duret, N Faivre, et al. (2014). Comparative population genomics in animals uncovers the determinants of genetic diversity. *Nature* 515, 261–263.

Romiguier J, J Lourenco, P Gayral, N Faivre, LA Weinert, S Ravel, M Ballenghien, V Cahais, A Bernard, E Loire, et al. (2014). Population genomics of eusocial insects: the costs of a vertebrate-like effective population size. *Journal of Evolutionary Biology* 27, 593–603.

Romiguier J, V Ranwez, EJ Douzery, and N Galtier (2013). Genomic evidence for large, long-lived ancestors to placental mammals. *Molecular biology and evolution* 30, 5–13.

Roth LM (1986). The genus Symploce Hebard. VII. African species (continued).(Dictyoptera: Blattaria, Blattellidae). *Insect Systematics & Evolution* 17, 433–454.

— (2000). Episymploce sundaica australiensis, a new subspecies from Australia and Papua New Guinea (Blattellidae: Blattellinae). *Oriental Insects* 34, 239–243.

Rousselle M, P Simion, MK Tilak, E Figuet, B Nabholz, and N Galtier (2020). Is adaptation limited by mutation? A timescale-dependent effect of genetic diversity on the adaptive substitution rate in animals. *PLoS Genetics* 16, e1008668.

Rubin B (2022). Social insect colony size is correlated with rates of molecular evolution. *Insectes Sociaux* 69, 147–157.

Scheffrahn RH and J Krecek (1999). Termites of the genus Cryptotermes Banks (Isoptera: Kalotermitidae) from the West Indies.

Scheffrahn RH, NY Su, and TG Myles (1999). Amitermes amicki, a new subterranean termite (Isoptera: Termitidae: Termitinae) from Aruba. *Florida Entomologist*, 7–14.

Schrader L, H Pan, M Bollazzi, M Schiøtt, FJ Larabee, X Bi, Y Deng, G Zhang, JJ Boomsma, and C Rabeling (2021). Relaxed selection underlies genome erosion in socially parasitic ant species. *Nature Communications* 12, 2918.

Shell WA, MA Steffen, HK Pare, AS Seetharam, AJ Severin, AL Toth, and SM Rehan (2021). Sociality sculpts similar patterns of molecular evolution in two independently evolved lineages of eusocial bees. *Communications Biology* 4, 253.

Snyder TE (1924). Descriptions of new species and hitherto unknown castes of termites from America and Hawaii. *Proceedings of the United States National Museum*.

Soleymaninejadian E, B Ji, S Liu, J Yang, and X Zhang (2014). Foraging polyethism in Odontotermes formosanus Shiraki. *Journal of Advanced Agricultural Technologies Vol* 1.

Tabadkani SM, J Nozari, and M Lihoreau (2012). Inbreeding and the evolution of sociality in arthropods. *Naturwissenschaften* 99, 779–788.

Terhorst J, JA Kamm, and YS Song (2017). Robust and scalable inference of population history from hundreds of unphased whole genomes. *Nature genetics* 49, 303–309.

Terrapon N, C Li, HM Robertson, L Ji, X Meng, W Booth, Z Chen, CP Childers, KM Glastad, K Gokhale, et al. (2014). Molecular traces of alternative social organization in a termite genome. *Nature communications* 5, 3636.

Thorne BL (1984). Polygyny in the Neotropical termite Nasutitermes corniger: life history consequences of queen mutualism. *Behavioral Ecology and Sociobiology* 14, 117–136.

Vargo EL (2019). Diversity of termite breeding systems. *Insects* 10, 52.

Vargo EL, TR Juba, and CJ Deheer (2006). Relative abundance and comparative breeding structure of subterranean termite colonies (Reticulitermes flavipes, Reticulitermes hageni, Reticulitermes virginicus, and Coptotermes formosanus) in a South Carolina lowcountry site as revealed by molecular markers. *Annals of the Entomological Society of America* 99, 1101–1109.

Wang J, E Santiago, and A Caballero (2016). Prediction and estimation of effective population size. *Heredity* 117, 193–206.

Warner MR, AS Mikheyev, and TA Linksvayer (2017). Genomic signature of kin selection in an ant with obligately sterile workers. *Molecular Biology and Evolution* 34, 1780–1787.

Watson J, W Brown, LR Miller, FL Carter, and MJ Lacey (1989). Taxonomy of Heterotermes (Isoptera: Rhinotermitidae) in south-eastern Australia: cuticular hydrocarbons of workers, and soldier and alate morphology. *Systematic Entomology* 14, 299–325.

Watson J, E Metcalf, and J Sewell (1977). A re-examination of the development of castes in Mastotermes darwiniensis Froggatt (Isoptera). *Australian Journal of Zoology* 25, 25–42.

Weissman DB and NH Barton (2012). Limits to the rate of adaptive substitution in sexual populations. *PLoS genetics* 8, e1002740.

Wertheim JO, B Murrell, MD Smith, SL Kosakovsky Pond, and K Scheffler (2015). RELAX: detecting relaxed selection in a phylogenetic framework. *Molecular biology and evolution* 32, 820–832.

Weyna A and J Romiguier (2021). Relaxation of purifying selection suggests low effective population size in eusocial Hymenoptera and solitary pollinating bees. *Peer Community Journal* 1.

Williams R (1966). The east African termites of the genus Cubitermes (Isoptera: Termitidae). *Transactions of the Royal Entomological Society of London* 118, 73–118.

Wipfler B, H Letsch, PB Frandsen, P Kapli, C Mayer, D Bartel, TR Buckley, A Donath, JS Edgerly-Rooks, M Fujita, et al. (2019). Evolutionary history of Polyneoptera and its implications for our understanding of early winged insects. *Proceedings of the National Academy of Sciences* 116, 3024–3029.

Wu W, Z Huang, Z Li, S Zhang, X Liu, and D Gu (2016). De novo transcriptome sequencing of Cryptotermes domesticus and comparative analysis of gene expression in response to different wood species. *Gene* 575, 655–666.