

## Recommend major revisions

### Major comments:

I have two major comments for the authors. First, this paper is premised on using dN/dS ratios as a proxy for effective population size and as an indicator of the efficacy of natural selection, where higher dN/dS is assumed to result from accumulation of weakly deleterious mutations as a result of low effective population size and reduced selective efficacy. However, elevated dN/dS can, of course, also result from positive selection, which is not mentioned anywhere in this manuscript (discussed in Wertheim et al. 2015). I think that the hypothesis of positive selection leading to eusociality is as plausible as the hypothesis that eusociality increases the strength of genetic drift, and as it currently stands, this paper is not able to distinguish the two hypotheses.

Either the manuscript should be substantially revised to include the possibility that the patterns recovered stem from positive selection, or I would encourage the authors to include additional analyses using methods explicitly aimed at measuring the strength of natural selection, distinct from positive selection and in correlation with trait presence, such as RELAX from the HyPhy family of methods (Wertheim et al. 2015). Likewise, as currently phrased the title of the paper is making a strong causal claim that cannot be supported by this approach, and needs to be reworded.

Second, overall this paper would benefit from more extensive engagement with the literature on sociality and molecular evolution (e.g. Chak 2020, Shell et al. 2021, Rubin 2022, Barkdull and Moreau 2023). Many of these studies find somewhat contrasting patterns to those you report, and this paper would benefit from exploring these contrasts. I discuss several specific instances where additional engagement is needed under minor comments, below.

### Minor comments:

“Eusociality represents the pinnacle of social organization”- what does this mean? I am uncomfortable with how this implies a goal-directed kind of evolution; consider rephrasing or removing.

“Both ants and termites display sharp contrasts in terms of levels of social complexity, ranging from small colonies of hundred individuals with low caste polymorphism, to large colonies of several millions of individuals and extreme caste differentiations.”- haven't defined caste, so consider adding a definition prior to this sentence or change caste polymorphism/differentiation to simply physical differentiation.

“Hence, the evolution of *ds* is solely driven by the mutation rate.” This contradicts your prior statement that synonymous substitutions can be under selection, consider rephrasing to something like “Hence, the evolution of *ds* is *usually assumed to be* solely driven by the mutation rate.”

Paragraph starting with “Eusociality in ants, bees, wasps and termites appears to be such a life-history trait by restricting reproduction to few individuals.” Along with the discussion of Weyna and Romiguier 2021, consider also citing Barkdull and Moreau 2023, who found no relationship between worker reproduction and strength of natural selection across ant genomes.

“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 mammals or birds where species with large body size feature lower effective population size than small body size species (Botero-Castro et al., 2017; Romiguier, Ranwez, et al., 2013).” The justification for this hypothesis based on an analogy to body size in birds is not clear; consider expanding your explanation of this analogy and how it justifies your hypothesis.

“The authors then aligned the assembled orthologous using MAFFT v7.305”- the assembled orthologous what?

“Each loci which had sequences with...” change loci to locus.

“These estimates were then associated with each terminal branch of the available phylogenies.” How?

“Each species was correlated with its social category following Michener’s classification in 1969 Michener (1969) and average individual size.” Where was the size data obtained?

From the sentence starting with “Before carrying out an ANOVA analysis to test an effect of social organization on  $dN/dS$ ...” to “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 (Table 1).”- these are methodological details which belong in Materials and Methods, not in the results.

Statements like “we observe a notably lower efficiency of natural selection within the clade that includes termites” and “To do this, we take advantage of the information available on adult body length in Blattodea and test its impact on the effectiveness of purifying selection.” How can you disentangle the efficacy of natural selection/strength of drift from the effects of positive selection?

“This result converges with what has been observed in another insect clade where eusociality has evolved, the Hymenoptera, whose  $dN/dS$  is not affected by body size (Benitiere et al., 2022; Weyna and Romiguier, 2021).” Consider that individual body size in eusocial insects may not be the most appropriate analog to body size in solitary organisms, and that colony size may be a more appropriate metric. In contrast to the Benitiere and Weyna studies you cite here, Rubin 2022 *did* find a correlation between colony size and rates of molecular evolution in ants and bees. You should discuss this study.

“As eusociality is a reproductive strategy characterized by parental care shared by many sibling individuals...” This does not sound like parental care- perhaps change to “characterized by care of offspring shared by many siblings”.

“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 of kin selection (Tabadkani et al., 2012).” This sentence is confusing. Does high parental care increase inbreeding, or does low genetic diversity increase inbreeding? Either way, the casual relationship is not clearly explained. Please rephrase for clarity.

“Multiple Piece nests are typically larger, but social complexity is at its highest in Separated Piece strategies...” Need a more substantial explanation of the multiple piece nest strategy.

“This intricate social structure likely leads to higher  $dN/ds$  values due to the formation of larger colonies, which in turn reduces the number of breeding individuals, significantly affecting  $N_e$  and thus natural selection efficiency.” Again, this is in contrast to results in ants from Barkdull and Moreau 2023, so it could be interesting to discuss that finding here.

“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).” This sentence feels out of place, since it is the last sentence in your discussion of nesting strategies. Either move this to the beginning of your nesting strategy section, or remove.

“Interestingly, species with true workers display significantly higher  $dN/ds$  ratios than those with pseudergates...” This finding also contrasts with Barkdull and Moreau 2023, who found no effect of worker polymorphism on patterns of molecular evolution. Could you explore/discuss why termites might differ from ants in this regard?

“Such a finding have been suggested in ants, where a correlation between  $dN/ds$  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.” Again, you should cite other work in ants, including Rubin 2022 and Barkdull and Moreau 2023, which used much larger datasets than Romiguier 2014, and who found different patterns to those in Romiguier 2014.

“Supplementary analyses on more species, including other eusocial clades such as bees or wasps will be useful to further confirm our conclusion.” Again, this would be a great place to discuss Rubin 2022, which included 11 bee species. You should also cite and discuss Shell et al. 2021, which analyzes 16 bee genomes and finds that eusocial lineages exhibit stronger positive selection, which contrasts your findings in termites. Consider also including work in social shrimp (e.g. Chak et al. 2020), where analysis of mitochondrial genomes suggests a link between sociality and relaxation of selection.

Figures 1 and 3- rainbow color scales are perceptually misleading (see Borland and Taylor 2007 and Stoelzle and Stein 2021) and cannot be used by people with achromatopsia. Please re-color these figures using a more appropriate color scale.

Figures 1, 2, and 4: please indicate statistical significance on these plots.