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Peer Review

Review of: "Programmed Cell Death and the Origin of Wing Polyphenism in Ants: Implications for Major Evolutionary Transitions in Individuality"

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This manuscript utilizes the phylogenetic comparative method to investigate the role of programmed cell death (PCD) in the evolution of wing polyphenism in ants—a key adaptation that facilitated the emergence of eusocial ant colonies and contributed to one of the major evolutionary transitions in individuality. While it has been hypothesized that the wingless phenotype universally observed in ant workers may be driven by PCD, this hypothesis has not been formally tested across species within a robust phylogenetic framework. The study examines the presence of apoptosis in 16 species of poneroid and fomicoid ants, representing five major ant subfamilies and across different developmental stages. The authors primarily employed TUNEL staining, and then they stained c-caspase-3 to confirm that the positive signals were indeed apoptotic signals. The apoptotic signals were observed in early terminal larvae of both winged and wingless castes. However, apoptosis was found to cease in the winged caste while continuing to spread in the wingless caste in late terminal larvae. Ancestral state reconstruction revealed that apoptosis was present in the last common ancestor of all extant ants. This study is well-designed and rigorously conducted, offering compelling evidence for the pivotal role of programmed cell death (PCD) in the evolution of wing polyphenism in ants—a key adaptation that underpins the major evolutionary transition from solitary organisms to eusocial colonies.

I have a few minor suggestions to further improve the presentation of the study:

1. In previous studies, the authors demonstrated that the wingless phenotypes of workers were the result of interruptions at various points within the gene regulatory network (GRN) across

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different ant species. I'm wondering how the interruption of the GRN and the spread of apoptosis are mechanistically related. Is it possible that disruptions in the GRN might regulate apoptosis pathways by altering the expression of key pro-apoptotic or anti-apoptotic genes, effectively determining whether programmed cell death spreads or is arrested in specific tissues? Alternatively, apoptosis may come first; it could initiate a cascade of downstream signaling events that disrupt the GRN associated with wing development. For instance, apoptotic signaling might degrade or inhibit key transcription factors or structural components necessary for wing formation, thereby halting the developmental process. I suggest the authors expand the discussion to include a proposed model that integrates universal apoptosis with species-specific interruptions of the GRN, providing a comprehensive framework for the emergence of the wingless phenotype in workers.

2. This study undoubtedly encourages further research to elucidate how programmed cell death (PCD) is initiated and differentially propagated in winged and wingless individuals in ants. Notably, the roles of insulin and juvenile hormone (JH) signaling pathways are of particular interest and warrant deeper investigation.
3. Why is there no PCD in a few ant species, such as Tetramorium immigrans ants? What caused the exception?
4. The authors may explain the positive and negative controls in supplemental 3. What treatments were performed and what tissues were stained?
5. To enhance the visual presentation, it would be beneficial to align the left two panels with the right two panels in Figure 3.

Declarations

Potential competing interests: No potential competing interests to declare.