

## 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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Wing polymorphism is a key evolutionary innovation in ants, which facilitated the emergence of distinct female castes with specialized roles (winged reproductive queens and wingless workers). However, how wing polymorphism arose and is regulated remains poorly understood. In this manuscript, Hanna and colleagues examine how programmed cell death (PCD) influences the evolution of wing polyphenism in ants. Using TUNEL and anti-cleaved caspase-3 staining on 16 ant species sampled from across the ant phylogeny, the authors demonstrate that programmed cell death in the wing primordia of future workers is widespread and likely ancestral.

The manuscript is clearly written, and the data support its main conclusions. I do not have the expertise to determine whether some of the weaker TUNEL signals in certain species are conclusive. Still, the presence of stronger signals in other species scattered across the phylogeny provides a strong case for PCD being broadly involved in ants. Moreover, the anti-cleaved caspase-3 staining in three species from different subfamilies is particularly convincing.

My only potential concern is that, without data from Leptanillinae and Martialinae, concluding with over 95% confidence (using which program or estimation method?) that PCD is ancestral to all ants seems premature. This may be a statistical outcome of having no outgroups where PCD was tested. Placing the origin of PCD at the base of the Poneroid/Formicoid clades should be equally parsimonious.

Regarding the absence of PCD in *Tetramorium immigrans*, this species does not appear unusual in terms of its phylogenetic position, external morphology, or social organization. It is mentioned that *T.*

*immigrans* possesses very small, rudimentary wing discs. It would be helpful to clarify whether this is a unique trait of this species/genus.

I assumed that the positive controls in Supplemental Figure 3 represent other tissues from the same larvae featured in Figure 3, but additional clarification would be helpful.

Overall, the study provides great insights into the role of PCD in ant wing polyphenism. I am looking forward to seeing the final version of this work.

Hugo

## **Declarations**

**Potential competing interests:** No potential competing interests to declare.