

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 study delivers significant new insights into developmental mechanisms underlying the wing polyphenism and eusocial evolution in ants based on an impressively sampled comparative data set. Premises and conclusions are clearly laid out and supported.

Adding a section to the discussion about how the role of PCD in the ant wing discs compares to other wing regression phenotypes, most significantly the dipteran halteres, which are well studied in *Drosophila*, might make for an interesting addition for the broader evodevo community.

Minor issues:

1.

“For example, in *Dictyostellium*, PCD facilitates the formation of two alternative cell types under stressful environments, in which one cell type undergoes programmed cell death becoming stalk cells, thereby helping the other cell type to disperse their spores[10].”

Dictyostellium = the slime mold *Dictyostelium discoideum*

2.

“Wing polyphenism is the ability of an egg to develop either into a winged queen or wingless worker in response to environmental cues.”

My understanding is that wing polyphenism describes a variable character state within a species. Its expression is contingent on differentially initiated developmental pathways during embryonic or

postembryonic development. Note that an egg is the composite of an embryo, nutritive compounds, and multiple protective layers.

3.

“The origin of eusociality and wing polyphenism in ants appeared during the same time period and are now universal traits (excepting for a few secondary losses)[15][13][16][19][20].”

Consider revising to:

“Eusociality and wing polyphenism in ants appeared during the same time period and are now universal traits (excepting for a few secondary losses)[15][13][16][19][20].”

Otherwise, the singular of “origin” and the plural of “are” clash.

4.

“Therefore, the main goal of our study is to test whether apoptosis played a role during the major transition to eusociality by testing its role in the origin of wing polyphenism in ants.”

Consider revising to past tense, consistent with the rest of the results part.

5.

“Therefore, we first tested for the presence of apoptosis in the winged males and wingless workers and across several developmental stages of a species belonging to the poneroid clade *Harpegnathos saltator*, which is thought to have ancestral (plesiomorphic) characteristics.”

This sentence was difficult to read for me. Consider breaking it up into two?

6.

“Ant colonies were obtained from the following locals. *Stigmatomma pallipes* were collected at McGill Gault Nature Reserve (Quebec, Canada). *Lasius niger*, *Tetramorium immigrans*, *Aphaenogaster rudis*, and *Aphaenogaster picea* were collected at Mont Royal Park and McGill Gault Nature Reserve (Quebec, Canada). *Odontomachus brunneus* and *Camponotus floridanus* were collected at Gainesville (Florida, USA). *Pheidole dentata* were collected at Gainesville (Florida, USA) and Austin (Texas, USA). *Pheidole hyatti* were collected from Tempe (Arizona, USA). *Pheidole pallidula* were collected from Lyon (France). *Pheidole noda*, *Tetraponera rufonigra*, and *Polyrhachis rastellata* were collected from Mae Tang (Chiang Mai, Thailand). *Atta colombica* and *Acromyrmex echinator* were collected from Gamboa (Panama). *Harpegnathos saltator* colonies were propagated in the laboratory since the original collection in 1999 from different locations in India.”

Consider condensing this into a table.

Did any of the species require collecting permits?

7.

“and remove any obstructing fat tissue”

Fat body?

8.

“Hybridization Chain Reaction”

Which probes were used/generated? Provide primer sequences in the supplementary data.

9.

Figure 1

A more comprehensive annotation of panels c and d would be helpful. Are these ventral views showing both wing and leg discs of all thoracic segments?

10.

Figure 2

“All wing discs are oriented with the distal axis on the left and the proximal axis on the right (see Fig. 1b).”

Consider revising into

“All wing discs are oriented with the proximodistal axis oriented from the right to the left (see Fig. 1b).”

11.

“Figure 4. Programmed cell death is carried out via the apoptosis pathway. Expression of DAPI (blue) and cleaved-caspase 3 (pink) during mid (left) and late (right) terminal larval stages in *H. saltator* (a–a’), and *P. dentata* (c–c’), and soldier larvae of *C. floridanus* (e–e’). Expression of DAPI (blue) and autophagy marker atg8 or LC3A/B (red) in rudimentary wing discs of *H. saltator* (b–b’) and soldier larvae of *P. dentata* (d–d’).”

DAPI is a nucleic acid marker. Replace “expression” with “detection”?

12.

Figure 5

“Branch length do not indicate evolutionary time.”

Revise into:

“Branch lengths do not indicate evolutionary time.”

Declarations

Potential competing interests: No potential competing interests to declare.