

Peer Review

Review of: "A Developmental Table for the Florida Carpenter Ant *Camponotus floridanus*: Establishing Foundations for Mechanistic Studies of Development and Evolution in Ants"

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1. "The discovery of the phylotypic stage, which is a stage in embryos within a phylum that show the highest level of morphological similarity, was one of the breakthroughs that made comparison across species easier." – The "Developmental staging tables" were used not only in insects, but also in animals with lower complexity (nematode [<https://doi.org/10.1163/156854107781487288>] [<https://doi.org/10.1038/s41467-020-19863-x>]) and higher complexity (mammalian [<https://doi.org/10.1016/bs.ctdb.2019.10.002>] [<http://doi.org/10.1016/j.ydbio.2012.12.008>]). Would it be better to raise this concept as generally as possible at the beginning?
2. "However, the early development of ants remains poorly studied, and a clear picture of developmental timing and standardized staging is entirely lacking." – Why does the early development of ants remain poorly studied? The development of small animals such as nematodes and fruit flies (as mentioned by the authors) has been studied for at least half a century. Please explain a bit more.
3. "We analyzed embryonic development using DIC microscopy (Figure 2) and fluorescent microscopy (Figure 3) of fixed embryos as well as movies of live embryos (Embedded Video 1)." – Should it be "fluorescence microscopy" instead of "fluorescent microscopy"?
4. "Figure 3. Wide field epifluorescence images of the stages of embryonic development." – The arrowheads are too small. They look like dirt, not cells.

5. “In the posterior region the cells called bacteriocytes containing endosymbionts are now clearly visible. Each bacteriocyte contains a few zygotic nuclei and is about 40–50 micron in diameter. The cytoplasmic halos observed at the posterior pole in stage 4 (white arrowhead in Figure 3d) are now absent or hidden underneath the mass of bacteriocytes (Figure 3e).” – What is the known biological function of “bacteriocyte”?
6. “Our live imaging data suggests that they may eventually be consumed by the embryo at a later stage (stage 17; Embedded Video 1: 4 minutes 50 seconds onwards). We therefore propose to call these trophocytes, but this proposal requires to be further tested.” – If the authors have no sufficient confidence, it is inappropriate to propose anything. It could be presented in a suspicious tone (like being discussed).
7. Scale bars are missing in many figures and videos.
8. As the authors used *D. melanogaster* and *M. pharaonis* as benchmarks, it would be more intuitive to add cartoon schematics showing the critical difference (or similarity) between them and *C. floridanus*.
9. “In *C. floridanus*, we also noted that embryos that were allowed to develop in the absence of workers on water agar plates developed much faster than the embryos that were accompanied by the workers (unpublished observations). This phenomenon may be attributed to the founding of colonies where the initial eggs are accelerated in development to establish a colony as fast as possible to supply the colony with foragers.” – There are two experimental-computational papers demonstrating that nematode and mouse development is also accelerated [<https://doi.org/10.1103/PhysRevE.104.054409>] [<https://doi.org/10.1016/j.cell.2011.12.025>], which could support the authors' observation.
10. “Figure 7. Relative duration of embryonic stages. *C. floridanus* (black bars) in comparison to *M. pharaonis* (blue bars) and *D. melanogaster* (gray bars).” – It is interesting that the percentages of total duration for each stage are quite conserved. How about their absolute duration lengths? Does the larger organism take a longer or shorter time for development?
11. “3rd instar larvae were first observed on the 10th day after hatching and continued to be observed for a minimum of 4 days.” – “th” should be written as superscript just like the others. There are many grammar errors and typos throughout the paper. Please proofread all texts carefully.
12. “Because *C. floridanus* minor and major worker larvae vary continuously in size, the duration of larval instars is highly varied between individual larvae.” – Is there a quantitative measurement of larvae sizes?

13. *“Although, C. floridanus larvae are able to undergo successful pupation into an adult ant without a cocoon the survival of these pupae is compromised due to multiple factors including exposure to infections.”* - Should it be *“Although C. floridanus larvae are able to undergo successful pupation into an adult ant without a cocoon, the survival of these pupae is compromised due to multiple factors including exposure to infections.”*? Please check all texts carefully.
14. *“Confocal images were taken using a Leica SP8 inverted microscope.”* - Are there any time-lapse 3D images taken using the Leica SP8 inverted microscope? Or only for fixed samples?
15. *“Z-series consisting of 2 positions surface and center of embryo were captured for each well at 5-minute intervals beginning at the collection time. Imaging was continued for 14 days.”* - Absolute time should be shown in the videos.

Declarations

Potential competing interests: No potential competing interests to declare.