

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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Ants help us understand how animal societies function. Knowing how development occurs in specific stages is a key prerequisite for experiments, yet descriptive staging systems for ants are rare. The carpenter ant *Camponotus floridanus* is an emerging model for eco-evo-devo studies. This paper provides a comprehensive staging system for the development of embryos, larvae, and pupae. The authors collected a large amount of data and present detailed text descriptions accompanied by high-quality images and videos. This study is a great primary source of information for anybody conducting experiments on this species, as well as for anyone interested in comparative studies of ants or insects in general.

Below are my suggestions that might help to improve the manuscript:

p = page

The text is well-written; however, I think that shortening it would be beneficial, such as making it purely descriptive, more concise, and removing redundant words.

Language: I am not a native English speaker, but I think that it would be helpful to have someone proficient in English read the text.

Abstract:

ecological evolutionary developmental Biology – ... biology

Introduction:

p2: influence of development on evolution – I think it should be “evolution influences development.”

p2: comparing features ... of embryonic features – Rewording (“features” repeated): e.g., morphological traits, characters

Results and Discussion:

Please prepare summary tables describing the key developmental characteristics of each stage of the embryo, larva, and pupa.

I suggest giving each embryonic stage also a name, rather than just a number. This would be the main feature of the stage. E.g., “stage 1” could be “stage 1 = the 4-nuclei stage.”

I suggest shortening the text to make it purely descriptive, which would make it clearer, more concise, and easier (= faster) to read. E.g., remove the statements about what was done and how (transfer to Methods). “Stage 2: 8-256 nuclei at the anterior pole (similar to stage 2 in *Drosophila*...)”.

If the events in the stages are similar to those in *Drosophila* and *M. pharaonis* (as you mention at the beginning of the description of the particular stages: “Similar to *Drosophila* and *M. pharaonis*...”), I suggest not repeating this at the beginning of each stage’s description, but making a table highlighting the differences in development at the particular stage between the species.

p6: embryonic rudiment, germdisc, germband – What do these terms mean? Unite them.

p9: “a hole in the centre of the head” – “an opening...”?

p9: segmentation – This term typically refers to the molecular process of segment formation (gene expression), which you did not observe here. I think “formation of morphological segments” is seen here.

p11: “pre-larva”: What exactly does it mean here? A fully formed pharate first instar larva? At hatching (when leaving the chorion), insects also shed their embryonic cuticles. Did you observe these cuticles?

p11-12: Duration – For the experiments, it is important that the embryos that were collected and incubated during the project develop at the same speed. Thus, the incubation conditions need to be described very precisely (see my note for the Methods section). Also, please provide details about the incubations without workers, such as how they were incubated and the duration of embryogenesis.

p14: Larval morphology – Please shorten the text and make it concise (pure description).

p15: galena – Correctly galea.

p17: successful pupation into an adult – Pupation is the transformation of the final instar larva into the pupa (the larva-pupa moult).

Conclusions:

It would be beneficial to briefly summarize the key characteristics, commonalities, and peculiarities of *C. floridanus* development; also the main differences between *C. floridanus* and *M. pharaonis* development.

Materials and Methods

p20: Photo (or schematic drawing) of the lab culture.

p20: embryo collection – Since the presence of workers affects the length of embryogenesis, how many workers were placed together with how many eggs? How large were the boxes? Could you show a photo of the box with eggs during incubation?

p22: heptane glue – Please describe how the glue is prepared.

p23: SEM of larval instars – Please provide details about sample preparation, including fixative, length of fixation, dehydration, drying, and metal coating.

p23: leg disc development – Please provide images of how leg discs develop and include them in the Results section.

p23: culturing pupae – I think “incubation” would be more suitable.

p24: Could you provide an image of the cocoon?

Figures:

Please add scale bars to all images. Please significantly enlarge arrowheads in all Figures.

p26: germband straightening – Please use the commonly used terms “germband elongation” and “retraction”.

p34: Figure 7 – Please enlarge the description of the axes.

p37, p41 (panel a): – The use of the term “instar” – The instar refers to a period between two moults. There are embryonic instars; the pupa is also an instar. Thus, I think that labelling larval instars as “instar” only (especially in Figure 13a) may look strange. E.g., “larva 1”, “larva 2”, “L1”, “L2”, etc. would be more suitable.

p41: The description for panel (a) is unclear. Please consider rewording it.

p48: measuring size – Should it be “body length”?

p49: pupation – Please replace with “Pupa”.

Movies:

Please enlarge the existing labels (descriptions and arrows). Please add more labels (text and arrows) abundantly throughout the videos to make them easier to follow.

Declarations

Potential competing interests: No potential competing interests to declare.