

Review of: "Comparing the appetitive learning performance of six European honeybee subspecies in a common apiary"

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This manuscript analyzes the sucrose responsiveness and the olfactory learning of six subspecies of honey bees from different parts of Europe: *A. m. iberiensis* from Portugal, *A. m. mellifera* from Belgium, *A. m. macedonica* from Greece, *A.m. ligustica* from Italy, *A. m. ruttneri* from Malta and *A.m. carnica* from Germany. The six subspecies were established within a common apiary in Würzburg, Germany, and their performance in these two standard tests (sucrose responsiveness and olfactory learning) was quantified to determine whether they differ in these behavioral traits and whether the positive correlation previously established for European bees (*ligustica* and *carnica*) between sucrose responsiveness scores and learning scores holds for all subspecies considered.

The results show that all bees had high sucrose responsiveness scores (SRS) following the handling and starvation conditions that were common to all subspecies (bees captured at the hive entrance, immobilized in individual metal tubes during 1h and fed afterwards with 5µl of 30% sucrose solution). No significant differences were found for this variable (Fig. 3A). Notably, although the authors did not elaborate on this point, the data dispersion of *A.m. iberiensis* seems to be considerably higher than that of the other subspecies. This being said, there is no explanation about the fact that SRS exceeded the value of 6; 6 sucrose concentrations were tested so that the maximum SRS possible should have been 6, not 7 as shown in fig. 3A. Corrections or explanations are required for this discrepancy.

The learning performance of the different subspecies is shown twice (which is redundant as sucrose responsiveness is just shown as an individual score per bee and not as a population response, which would be equivalent to Fig. 3C): as a learning score and as an acquisition score (number of CS trials responded). For both variables, *A.m iberiensis* showed poorer learning performances compared to the other five subspecies.

Finally, a general trend confirming the positive correlation between learning scores and sucrose responsiveness scores was found, yet, as already mentioned it was not so clear for *A.m. Iberiensis*. This, however, does not take into consideration the variability in the SRS data found for this species.

There are two general comments that arise after reading this interesting work:

1. Was the genetic “purity” of the subspecies analyzed controlled? Of course, the efforts of the authors to import mated queens from their original countries and establish them in a common apiary is remarkable, and it is rare to see such an initiative promoted to achieve a comparative study. Yet, can one be sure that the imported queens were not, themselves, inbred and crossed with other common subspecies such as *A.m. ligustica* or *A.m. carnica* (or any other)? If such a crossing existed, then of course similarities in the behavioral traits quantified may increase. Is there a way in which the authors can ensure/quantify the genetic origins of their different colonies?
2. One possible reason – a simple one – to account for a deficit in the performances exhibited by *A.m. iberiensis* could be that this subspecies is more sensitive to the stress imposed by the confinement within the metal tubes compared to the other subspecies. This possibility has not been considered. The authors elaborated on another form of stress, namely possible differences in temperature-related stress. It is said that “Iberian bees might have a higher energy demand to cope with heat stress and / or higher foraging activity compared to honeybees from Central Europe”. A greater foraging activity, in turn, would lead to accumulation of oxidative stress and to a reduction of learning performance. This is of course plausible, but the simpler argument of differences in confinement-based stress would also help understanding the deficits observed for *A.m. iberiensis*. From this perspective, **adding a simple experiment would yield significant information and could enrich the paper: measuring survival rates (Kaplan-Meier curves¹) of these subspecies, with individuals confined in the tubes, could report whether these subspecies differ in their sensitivity (i.e. mortality) to the confinement conditions.**

This being said, the paper is a very nice comparative study that achieves and provides a valuable comparison between subspecies in controlled laboratory conditions. The authors have to be congratulated for their efforts, which required significant logistics for importing and setting the colonies in a common apiary. In any comparative study, a basic assumption is that individuals from different species (or subspecies) may not react in the same way to the constraints imposed by the same experimental design. Perhaps this is an important point explaining the differences reported in this work.

1 Bewick, V., Cheek, L. & Ball, J. Statistics review 12: survival analysis. *Crit Care* **8**, 389–394. (2004).

