

Review of PhD Thesis

Author: Mona A. Awad, MSc.

Title: Reproductive biology of ladybirds

The submitted PhD Thesis „Reproductive biology of ladybirds“ (99 p.) concerns selected problems of reproduction biology of an invasive coccinellid species *Harmonia axyridis* (Pallas). The topics included in the study are many: characteristics of hibernating populations, effect of hibernation on fecundity and fertility, sperm survival during hibernation, etc. The Thesis consists of six papers one of which is in pre-print stage (Biocontrol, if=1.927). Two others are submitted (Journal of Ecology and Evolution, Animal Behaviour), three papers are in press (all IOBC Bulletin). All papers are authored by a collective of 2-5 members where the dissertant is the first author in 5 cases, the second one in one case. The contribution of the dissertant to each of the papers is specified in a „List of papers“ preceding the main text of the Thesis. The Thesis is started with a General Introduction (13 pp) and finished with a Summary of Results and Discussion (2 pp) and General Conclusions (1p).

The scientific content of the Thesis is abundant and its original contribution to knowledge of *Harmonia axyridis* biology is significant. I have few questions. In my comments I follow the line of exposition of the subject as presented by the dissertant in the Summary of results: (1) In some samples of *H. axyridis* adults collected in the autumn when flying to hibernation sites, sex ratio was significantly biased in favour of females. The dissertant explains this deviation through operation of male-killing factors. This might be of course so if the flying population was a representative sample of a local interbreeding population. Was it? Had samples caught at the same place in successive days similarly biased sex ratio? (2) Dissertant means that mean weight of individuals that decreased during autumn indicates progressive shortage of food (this is my interpretation because nutrient content of food was not analysed) for late developing beetles. Were the late flying beetles really the late developing ones? Timing of flight in handicapped individuals might be delayed by tendency to feed more (for longer time) before hibernation. (3) Females mated in the previous year after hibernation have high fertility and fecundity so that „without access to repetitive mating may found a large new colony“. Does exist any indication that this possibility becomes actual in the open? The idea is seductive, particularly when proposed in context of life history of an „invasive“ species, attributing it demonic capacities. But is it also true? Particularly with respect to high polyandry observed in spring female populations. Please, comment.

The main body of the Thesis text has been published or is under review process, therefore not only its content but also its form are above standard. Consequently, I have few comments on form. The first is similarity of papers 1 and 5, and papers 3 and 4. This is easy to understand because the later papers in both above mentioned pairs of papers (published in IOBC Bulletin) are clearly conference proceedings publishing preliminary data. The dissertant then performed additional work before writing the more developed texts – which is clearly seen from description of the authors contribution in the „List of papers“. My query here is the small difference in Figs. 1 and 2 in papers 1 and 5 (the numbers of figures in both papers are

identical). Otherwise the text of the Thesis is impeccable, perhaps except for two strange in-text citations on page 2 (lines 6 and 15).

The papers, introductory and concluding texts are all well written, demonstrating a good knowledge of investigated problems and a hard work of the dissertant. The scientific contribution of the results is indisputable. After successful defence of the Thesis I consider the work a strong basis for providing the PhD title to the dissertant, Mona A. Awad, MSc.

Prague, June 18, 2013



doc. RNDr Alois Honěk, CSc.

Mona Awad PhD thesis: “Reproductive Biology of Ladybirds”

Opponent’s Review: John Sloggett (Maastricht University, the Netherlands)

I found this thesis to be an interesting read on a highly topical subject, the invasive ladybird *Harmonia axyridis*. The thesis builds on earlier research on ladybird mating behaviour using creditable variety of different methodological approaches including studies of behaviour and morphological and physiological attributes such as size and protein content and both older and newer genetic approaches in the form of colour pattern polymorphism and microsatellite DNA analysis. This is certainly one of the strengths of this thesis.

Although the thesis draws on a wide body of literature, both on *H. axyridis* and mating behaviour, there were some weaknesses that could have been improved by more careful reading of the literature. Thus a list of the reasons why *H. axyridis* dominates aphidophagous guilds originates with a paper from 2006 contains no subsequent literature, in spite of the fact that there has been abundant work on this subject in the last seven years. A paper by Ueno (1994), cited here, tested a variety of models of sperm displacement in *H. axyridis* and concluded that a flushing mechanism operated. This was relevant to all papers on patterns of paternity, suggesting as it does that the contributions of individual males will never be equal and that the dominance of individual males is controlled by ejaculate size and order. However sperm displacement used by *H. axyridis* is nowhere discussed. The approach to literature, is for me, the weakest element of this thesis by far.

There other theoretical elements or assumptions, I would also have liked expanded on. The link postulated between the mating behaviour of *H. axyridis* and its capacity as an invasive species is a central plank of this thesis. However there is no real satisfactory demonstration of this link. Observations on promiscuity in other ladybird species suggest that many elements of *H. axyridis*’ mating behaviour occur widely across the Coccinellidae, but virtually no other introduced ladybird has become invasive in the same manner when introduced elsewhere. What therefore is it about *H. axyridis*’ mating behaviour that makes it special? In another more minor example, there is no real evidence that multiple mating in ladybirds is an evolved bet hedging strategy, although this is frequently mentioned. Indeed a recent paper concluded that the whole idea of the evolution of bet hedging generally was a concept “mired in conceptual and practical difficulties”.

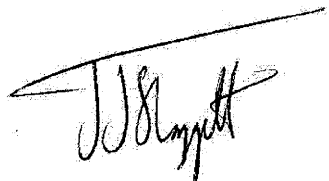
Following on from this, I found the introduction to need further fleshing out on the subject of the invasiveness-mating link and a clearer focus on the specific of *H. axyridis* biology. The first paper (and the fifth which was an abbreviated symposium version) was a nice physiological study on sperm storage, overwintering and reproduction in the species, which I believe goes some way to expanding our knowledge on these subjects generally. I found the protein analytical work especially novel and interesting and the observations on trophic eggs were a pertinent contribution to an already long debate over ladybird parental provisioning. My one question arising from this paper is why, given its absolute numerical dominance over much of the planet, would anybody now wish to store this species for biocontrol these days?

The second paper was an up-to-date molecular approach to paternity analyses of ladybird clutches from field collected individuals. It provided an advance over earlier colour pattern based studies, but suffered from the problem outlined above, that no link to earlier work on sperm displacement mechanisms was provided. In fact consideration of that work would have helped this paper along as many of its conclusions are in agreement with such a mechanism, or flow from it. There was however some nice results and discussion here, for example on numbers of fathers pre- and post-winter.

The third paper (and the associated fourth paper symposium abstract) looks like it must have been a huge amount of work to carry out and must have kept the author busy for a long time! However, notwithstanding this, there are some statistical issues that make the conclusions of the paper open to question. Most specifically the proportion of eggs fertilised by particular colour morphs (Table 2) are calculated using frequencies of individuals reared from the 10 pairs, i.e. the unit of analysis is the individual offspring. In fact the unit of analysis should be the individual mother. Thus, for example, the proportions of *spectabilis* should be the mean of the individual proportions of *spectabilis* from each female. It would be quite possible to produce some of the deviations from 1:1 observed if some individual males were sterile, leading some females to produce only one type of offspring. We know nothing about individual variation from the data presented here. Elsewhere in this paper, other data is also pooled in a similar manner.

The last paper, VI, though short, was an account of the overwintering biology of *H. axyridis*. The results from this paper provide useful data for future researcher and the analysis and discussion are wholly appropriate to the data involved.

The written style of the thesis is generally good, although there are some oddities of phraseology and the odd typo. There are elements of this thesis that could yet be improved, but at the same time the author is to be commended for the broad diversity of approaches she used in pursuit of this particular problem and on her evident hard work.

A handwritten signature in black ink, appearing to read 'J. J. Sloggett', with a long horizontal stroke above it.

Dr. John J. Sloggett

Maastricht, 15th June 2013

Reproductive biology of ladybirds

by

Mona A. Awad, MSc.

The thesis comprehensively studies a very important topic: the reproductive biology of the invasive alien population of one of the most important coccinellid species in Europe and North America, *Harmonia axyridis*, by demonstrating several aspects such as reproductive success of the hibernating females, verifying of the level of polyandry in the wild for the overwintering and summer populations and inspecting female choice and sperm competition phenomena. The species studied has a long history of use as a classical biological control agent of aphids and coccids. However, the worrying aspect is its invasiveness: in a risk assessment of 31 exotic natural enemies of pest species used in biological control in Europe, *H. axyridis* had the second highest environmental risk index. Its establishment is sometimes associated with the decline of native coccinellid populations in urban, agricultural, and natural habitats in Europe.

The results presented here are based on six papers. One of them was already published in a very good and relevant journal: *Biocontrol* (IF₂₀₁₂ = 1.927) and three are in press in a non-impacted journal (*IOBC Bulletin*), which is a sufficient result for a PhD thesis, I think.

I have several points to be addressed:

1. The yet unpublished papers will have to be more focused in parts, I am afraid, to become publishable – I feel that sometimes they are too detailed and maybe too long for a journal. This is especially true for Paper II, where the Introduction is too long and - in my eyes - many statements are irrelevant to the main message the papers aims to convey to the reader.
2. The results presented in this thesis are certainly academically interesting. Their practical importance for management and biological control can, however, be a topic for an interesting discussion during the defense.
3. The author states in the General Introduction that "*H. axyridis* has succeeded to control aphid species on crops, including apples and citrus fruits". I wonder how this "success" was measured. It is generally accepted that ladybirds are not very efficient in controlling their aphid prey because of the large generation time ratio. If the statement of the author is correct, then it would be an argument against. Is it really? The author should defend this.
4. In paper III, the dependence in Fig. 2 might be fitted by a negative exponential and that in Fig. 3 maybe by a Gaussian curve. This might give the results a bit more "general" aspect.
5. In the "general conclusions" the author says "the investigation of the reproductive biology of ... *Harmonia axyridis* can open the door to understand the rapid spread of its invasive alien population through Europe". My question is: how can it explain the rapid spread of this species specifically? E.g., the study demonstrates persistence of high fecundity and fertility of the hibernating females of *H. axyridis* over a long storage - but is this relevant to the situation in nature? How does polyandry affect the success of *Harmonia axyridis* in

the field? What do the results on hatchability vs. multiple mating tell us about invasiveness of this species? As I already stated above, these results are certainly academically interesting, but their relevance to invasiveness of *H. axyridis* is rather doubtful, or else I am missing something. This is not to criticize the results, it is only a small memento suggesting that maybe the applicability of the results is sometimes overstated in the thesis.

Despite all the comments above I strongly feel the thesis is very good, its results may potentially have a very important practical use, and suggest it can be defended.



Pavel Kindlmann

Prague, June 18, 2013