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Posudek vedoucího práce na doktorskou dizertaci Mony Awad: Reproductive biology of ladybirds

Mona Awad asked me to be her supervisor in the time I had a grant for investigation the biology of the alien invasive ladybird beetle, *Harmonia axyridis*, provided by the Ministry of Agriculture, which corresponded well with her education in agricultural entomology. She chose the topic on reproductive aspects of ladybirds among several topics included in the project mentioned. Reproductive biology of ladybird beetles is attractive because of the high mating activity and promiscuity known in this family. We wanted to compare the alien species to several native ones and to other exotic species used in biological control of pests but there was enough work to do with the invasive species, and there was low abundance of the other species in the wild, partially because the competitive pressure and intraguild predation by *Harmonia*. Thus, all studies included in the thesis deal only with the single species and we might make only indirect comparisons with the others according to older literature data.

Among these literature sources, there were a few articles on the fertilization rate of ladybird females of several species before overwintering. We found rather high fertilization rate in *Harmonia* females migrating to overwintering sites, comparable to the common European seven spot ladybird *Coccinella septempunctata*, and higher than in other three native species. This was found by two simple methods, dissection of females and rearing the females in suitable conditions. Moreover, we looked for the number of fathers that sired the offspring of these females by analysing eight microsatellite loci in the larvae and found that the females were mated two to three times before overwintering. This information was not available for any other ladybird species. It probably contributes to the invasive success of the species.

Subsequently we answered the long-time asked question whether this frequent insemination in autumn will really result in frequent fertilisation of eggs in spring. It does. The fecundity and fertility of females that spend several months in cold storage and were subsequently reared in suitable conditions without any further access to mating was very high, comparable to parameters found in mated females and in the common *Coccinella septempunctata*, higher than in other native species. High fertility without mating in spring was again unknown in other ladybird species before.

As the next step, we sampled the same generation of ladybirds in spring to estimate the level of promiscuity by microsatellite analysis of progeny and we found about five males per one female contributing to the next generation. This was higher than in the native *Adalia bipunctata*, the only ladybird species studied for such data before.

Next, we studied the sperm competition between the males that mated with a single female, and found that the first male had the advantage of higher reproductive fitness, which is contradictory to the older results found in the native *Adalia bipunctata*, and that the males of the colour forms that were more common in the wild had higher fitness in laboratory experiments.



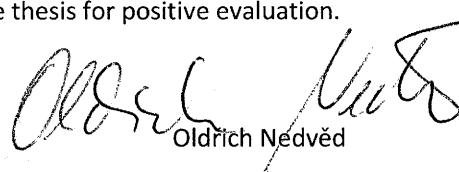
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The thesis consists of one article published in a journal rated as the 13th of 86 entomological journals in Web of Science. The second article was once rejected, and we do not have the decision on the third one. Article 4 contains preliminary data on the study 1 which is already published, article 5 contains preliminary data on the subject of article 3, but data not used in the final full version, article 6 is an independent study which is in press in a journal not included in Web of Science. Besides these, we have other data on reproduction of ladybirds not yet prepared for submission.

The contribution of Mona Awad to the studies included in the thesis ranged from planning the design of experiments, finding and learning diverse methods, insect rearing, field sampling, and writing the draft manuscripts. The only stage she did not contribute much was advanced statistical analysis of data. Remarkable was mainly her interest in learning more and more new methods, some of which I might teach her, like spectrophotometric protein content analysis and staining sperm cells for microscopic evaluation, but for others she asked several specialists around our faculty. She was also very active in searching for literature, and I did not manage to read all articles she considered interesting.

The last but not least positive feature of Mona Awad and her thesis is that it was built up during three years which is rather exceptionally soon. I recommend the thesis for positive evaluation.

In České Budějovice, 22.5.2013



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