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Report on the PhD thesis of Daniel Sánchez Gárcia

Daniel Sánchez Gárcia has written a thesis entitled "Evolutionary trajectories of reintroduced and source *Phengaris teleius* butterflies thirty years after reintroduction" to obtain the title of Doctor of Phylosophy at the Museum and Institute of Zoology, Polish Academy of Science, in Warszaw, Poland, under the supervision of Magdalena Witek (Museum and Institute of Zoology, Polish Academy of Science, Poland) and Luca Pietro Casacci (University of Turin, Italy).

The thesis describes the experimental work he performed to test for the adaptations occurred to a metapopulation of *Phengaris teleius* butterflies reintroduced in The Netherlands from Poland after their local extinction.

Phengaris teleius butterflies are social parasites; their caterpillars, after feeding on Sanguisorba officinalis flowerheads for about three weeks, are collected by Myrmica ants, brought into their nests and reared to adulthood together with ant larvae before their free life as adults. Inside the host ant nests, *P. teleius* caterpillar feed on ant brood. In 1976, *P. teleius* butterflies got extinct in the Netherlands; in 1990, 86 butterflies were reintroduced into the Netherlands from Poland and gave origin to a population which is now considered as stable. As social parasites' phenotypic traits adapt to the specific trait values of their local hosts, they are typically expected to exhibit high level of local adaptation. Therefore, the candidate's interesting scientific question was to test whether and how the butterflies' phenotypic traits have diverged between the re-introduced and the source population; Daniel has focused on both the pre-imaginal life of the butterflies - because this is the moment when caterpillars are totally dependent on their host ants for survival and on adult butterflies.

In particular, the PhD thesis aims at assessing trait divergence between parasitic butterflies and their host ants in: 1) cuticular hydrocarbons (which have a crucial role in the detection of intruders in ant colonies); 2) vibroacoustic signals (for their role in direct communication between ant workers and caterpillars); and 3) behavioral traits expressed by ants and by caterpillars when the latter are collected by ants and brought to the next (as from an experimental setting in the lab). Finally, the candidate also tested for potential divergence in adult butterfly's morphological traits – divergence which might have occurred between the source and the re-introduced populations. A short genetical analysis to test for genetic variation in the reintroduced population is also added. Indeed, the main question has been developed in these main directions, making the work coherent and the flow of arguments through the thesis manuscript logic and easy to follow.

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The manuscript counts 140 pages (including the list of references), all written in English, and is composed of an Introduction and three chapters which address the goals listed above. The three chapters are in the form of scientific manuscripts ready (or almost ready) for submission.

The introduction is a detailed summary of what the manuscripts contain.

Chapter 1 is the main manuscript of the thesis and contains the results of the comparisons of chemical and vibroacoustic communication traits in the source and reintroduced populations. The results suggested that while vibroacoustic signals have diverged - caterpillars in the reintroduced population mimic the signals of their local hosts more than those of the source population – there was no evidence of local adaptation in cuticular hydrocarbons. However, behavioral data suggest that the ants in the source populations are effectively fooled by their local parasitic caterpillars and less so by the those from the re-introduced population.

The next two chapters aimed to test for variation in morphological traits (and genetics) between the two populations, although, as the candidate honestly states, ascribing these changes to local selective pressures is not easy. Chapter 2 contains the results of analyses aimed to test the variation in morphological traits of adult butterflies (size and shape of hindwings, size of thorax) and their metapopulation genetic structure, using microsatellite analyses. In Chapter 3, using wing spots as the target, tests for variations in this trait likely used by butterflies for mate recognition, predation deterrence, etc.

The work done finds replies to many scientific questions but, as usual in the scientific process, it also raises plenty of questions. Some of the questions I have come from the general “experimental setting” per se. I would like to know more on the species biology: what are the respective generation time and how could these affect the relative speed of local adaptation in hosts and parasites? I would also like to know more on the re-introduction of animal species in general: how commonly is this procedure used, how often is it successful, are insects and maybe social parasites easier to re-introduce in areas where they got extinct than other animal organisms? Was this reintroduction as successful as others done using the same - or closely related - species? Are there similar studies done on the same or other model systems?

If the coevolutionary process triggers some level of local adaptation, is there an inherent contradiction in our attempts to re-introduce locally extinct social parasitic species by moving individuals from a source population (where they are supposedly locally adapted) to a new population (where they are supposedly not locally adapted at all)? Should we expect that hosts in the re-introduced population are especially effective to reject the alien parasites? Or vice versa, should we expect that hosts in the re-introduced population are especially vulnerable to the alien parasites? Do we have evidence for

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that? Also, if there is some level of concern as for the conservation status of a species, what are the ethical issues in collecting individuals from one site and moving them to another site taking the inherent risk that they will be unable to survive and establish a stable population? Finally, using this as an example of coevolution occurring at a relatively short time scale (30 years), what large conclusions on coevolutionary processes (for example, their speed and effectiveness) could we draw from this study?

I will not detail a few other very minor comments in this written report, as I will ask them as questions during the oral discussion.

Overall, the candidate has used an impressively wide range of different methodologies and techniques to achieve his objectives, switching from chemical to acoustical analyses, from behavioral assays to morphometry and genetics, which indicates he has acquired a solid expertise in a variety of experimental settings (as well as data collection and analyses) and did so using two distinct model systems: ants and butterflies. The data analyses are original and performed properly and with appropriate conclusions. I appreciate the experimental work, data analyses and data interpretation and the whole thesis manuscript, which is relatively easy to read. Finally, the many questions raised by the work highlight the extent at which this investigation involves an interesting and current topic.

In summary, the subject of the doctoral dissertation is an original solution to a scientific problem and Daniel Sánchez Gárcia's skills and general theoretical knowledge in the field of biological sciences presented in the doctoral dissertation are sufficient to independently conduct scientific work. In view of the above, I conclude that the evaluated doctoral dissertation of Daniel Sanchez Garcia fully meets the conditions specified in art. 187 of Act of 20 July 2018, Law on Higher Education and Science (Journal of Laws of 2018, item1668, as amended). I therefore request the Scientific Council of the Museum and Institute of Zoology of the Polish Academy of Sciences to admit Mr Daniel Sanchez Garcia to the further stages of the doctoral dissertation.

Sincerely,

Maria Cristina Lorenzi