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Reviewer report on the PhD thesis of Mgr. Štěpánka Kadochová

“Thermoregulation in ant genus *Formica*, and individual vs. colony conflict”

Charles University, Faculty of Science, Prague

The presented thesis describes thermoregulation in wood ants of the *Formica rufa* group. These wood ants are known as ecosystem engineers, and are dominant ecosystem components and keystone species in many aspects, as e.g. affecting carbon and nutrient cycling in many forest ecosystems by building large and long-lived above-ground mound nests of organic matter, such as needles, twigs, as well as resin and other material. They also affect the insect fauna by preying on numerous insect species and by tending canopy-dwelling aphids. One characteristic feature of wood ants is the elevated and stable temperature inside their mounds during summer, i.e. wood ants are capable of an active thermoregulation of their nests, which is a vital factor for brood development, and thus could be one key factor for their overall success.

The candidate introduces three main objectives in her doctoral thesis: (1) timing of active thermoregulation in red wood ant nests, (2) one specific aspect of thermoregulation, the sun basking, and (3) testing a hypothesis under laboratory conditions, arisen from field experiments. The thesis consists of a summary part and three papers, each of one dealing with one main objective.

The work for this thesis was conducted both under field and laboratory conditions and made use both of traditional scientific methods, like temperature measurements or individual ant marking, as well as modern techniques, like video recordings, infrared thermography or respiration measurements of individual ants.

The methodology used in this thesis is described in detail and is relevant to the presented research questions and the overall theme of the thesis. The structure of the dissertation is coherent and flows logically from the major questions and presented hypotheses through a general introduction of wood ant thermoregulation and specifications of its mechanisms to the presentations of the major findings of the thesis.

One key aspect of this thesis is the so-called sun basking, also referred to as “Sonnung”, which, despite it has been known already for a longer time, had not been taken into more detailed research so far. One important merit of this thesis is definitely the exploration of the sun-basking behaviour of wood ants and how it is correlated with environmental factors. Another important outcome is the knowledge that there seems to exist a sun basking caste in wood ants, that are more willing to bask even under an artificial heat source, in this case an

infrared lamp, and that sun basking did not result in any long-term changes in the metabolic rates of sun-basking ants. However, sun basking could affect the ant life span negatively, and thus there seems to exist a trade-off between colony needs for elevated nest temperatures and individual needs for avoiding lethal temperatures during sun basking. Consistently, the candidate suggests that sun basking could be an important trigger for the early spring temperature increase in wood ant nests, even when still covered by snow.

After critical reading of the thesis, I have several comments and questions to the PhD candidate.

Abstract:

First paragraph: “some researchers” believe... I guess this active regulation of nest temperature is more or less “accepted knowledge” among ant research nowadays.

Second paragraph: the candidate could have mentioned the hypothesis to be tested in the last paper.

Third paragraph: “older authors” sounds odd, maybe better writing “older research” or “earlier research”.

Major questions and hypotheses:

Detailed measurements and discussions concerning elevated and constant temperatures in wood ant nest mounds were published by Steiner already in 1925 (*Zeitschrift für Vergleichende Physiologie*). Connected to this, the first question to find out the seasonal pattern of ant nest temperatures is not really new, but of course it had to be studied here.

Concerning the thermoregulatory methods for elevated temperatures, I would say that it is today mostly agreed that it is a combination of several mechanisms.

Since the sun basking is such a striking feature of wood ants in spring, it is a bit surprising that no study before focused on this. Does the candidate plan to concentrate more on this topic?

General introduction to wood ant thermoregulation

The basics of red wood ant ecology could have been slightly more elaborative.

Are all RWA species polydomous and polygynous? Compare to page 13: “often more than one queen” (not always so?)

It is mentioned that the nests persist for many years, even decades. But later they are mentioned to be virtually immortal? What could be reasons for nest abandonment?

A small but critical typo appeared in chapter 3.1. (second and third paragraph): young queens probably do not choose “well insulated places for new nest establishment”, but “well insolated”!

Page 9, last paragraph: It is mentioned that the inner nest temperature is significantly affected by ant activity, i.e. by the number of ants coming into the nest. As this ant activity is again dependent by the surrounding air temperature, could it be that air temperature is the actual factor behind this?

Page 10: laboratory experiment; why did the authors not try to imitate field conditions (e.g. surrounding air temperature or temperature of the hot spot)?

Page 11: "Other theories": I feel that this does not really fit into the context.

Does the candidate have any observations concerning insolation intensity and mound shapes? (cf. paper 1).

How about wood ants in extreme (e.g. cold) climates? How about the role of solar insolation and sun basking in the temperature maintenance and thermoregulation there?

Paper 1:

Abstract (also later): it could have been clarified that the lowest temperatures in September were not the lowest on an annual basis, but referred to the ant active season.

Introduction: what could be other outer energy sources?

Were all investigated nests at edges in sunny patches? If yes, how did the shade category vary that much? What was nest shape = "other" (Table 1).

Table 4 & 5: is the use of moisture as a variable useful? It was measured only once during the ant active season? Were there any signs that it varied during the season?

Table 5: for sure there might be still multicollinearity left between the remaining variables?

Page 303 Discussion: the fast decrease of nest temperature, the explanation about air temperatures is not really convincing. Is the decrease really faster than the increase in spring?

Does the candidate have any observations or ideas of nest size vs. temperature stability?

Paper 2:

Maximum temperatures of sunning clusters of 44.8°C were observed, and body temperatures of individual ants were similar to the cluster. Did the ants still not avoid such high temperatures?

Modelling ADC: was multicollinearity tested also between all other variables? Were the variables tested also individually?

When testing all variables, the inner nest temperature was overlaid by nest surface temperature. Could air temperature also here be the most important factor, as it affects also nest surface temperatures?

Paper 3:

Introduction:

Ant nest temperatures being higher than air temperatures throughout the year? Also Paper 1 showed that at some point they are rather similar (e.g. March and November).

Material and Methods:

What would the infrared lamp of 150 W correspond to under field conditions (e.g. sunny day? Spring? Air temperature?). Why this lamp, in particular, was chosen?

Could the candidate describe the “group effect” when measuring respiration?

Discussion:

One-third of all workers would participate in sun basking in spring, and later sun basking ceased. Could one conclude from this that the sun-basking would be a general “spring job”, and later these ants have other duties, like aphid tending or hunting?

Evaluation

Overall, I think this thesis is well-written and easy to read. The methodology is clear and appears scientifically sound. Two papers are published in high-quality international journals, and also the third one had been already accepted for major revision. This, and the fact that the candidate contributed significantly to all papers, corroborates the high quality of the work presented in this dissertation. To summarise my report, I am convinced that Mgr. Štěpánka Kadochová’s PhD thesis meets all the standards required for a doctoral degree, and demonstrates her ability to perform creative and high quality research. It is thus my pleasure to recommend its acceptance as a doctoral thesis to the Faculty of Sciences at the Charles University of Prague.

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