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Review of the dissertation thesis of mgr Marta Grosiak entitled „Can heat dissipation limit (HDL) theory explain reproductive ageing? Insights from experimental evolution in bank voles"

The thesis of Ms Marta Grosiak is a collective dissertation that contains one published peer-reviewed article and two articles intended to be published later. The thesis consists of six chapters, including a general introduction presenting the theoretical background and research objectives, an overview of the animal model and the selection experiment, three chapters testing the main hypothesis of the dissertation and a final chapter summarizing all results. The dissertation consists of 121 pages.

The doctoral thesis has been completed under the supervision of Prof. Dr. hab. Paweł Koteja and Dr Edyta T. Sadowska in Evolutionary Physiology Department, Institute of Environmental Sciences, Jagiellonian University in Kraków.

In her dissertation, Ms Grosiak was testing the heat dissipation limit (HDL) theory, one of the most discussed ideas aiming to explain the limits to sustainable energy expenditures. According to this theory, the ability of organisms to increase long-term energy expenditures is constrained by the capacity to dissipate excess heat. Recently HDL is drawing more attention in the context of global warming as it provides a relatively simple and attractive explanation for the limits to the organism's performance.

The HDL theory originates from the concept that the maternal capacity to dissipate body heat during the peak lactation in mice might impose a limitation on the reproductive output as it affects milk production rates. Ms Grosiak aimed to test a novel hypothesis that the mammalian age-related reproductive performance decline is a result of an age-related decline in heat dissipation efficiency. Her study was performed on bank voles from lines selected for the maximum rate of oxygen consumption and randomly bred voles from the control lines. Animals from the selection lines were also characterized by higher basal metabolic rates and reduced capacity to thermoregulate at higher ambient temperatures, which makes them an ideal animal model for testing HDL theory.

In the first part of her research, Ms Grosiak asked how the thermoregulatory performance in voles changes with age. To answer this question Ms Grosiak measured resting metabolic rates, evaporative water loss, and body temperature in young adult and old voles at different ambient temperatures. The cold tolerance was lower in the old voles from both selection groups, but maximum cold-induced oxygen consumption in old voles from selection lines was similar to young voles from control lines, which implies that selection for high aerobic performance reduces the adverse effects of ageing on cold tolerance. On the other hand, this advantage is compromised by the poor ability to cope with hot conditions. These findings are in line with some predictions of the HDL theory but also indicate that the capacity for cold and hot tolerance is related both to age and the level of metabolic rate.

In the next part of the dissertation, Ms Grosiak hypothesized that heat dissipation capacity will decrease with age and affect reproductive performance. To test this hypothesis she measured the peak-lactation reproductive and metabolic traits in females from three age classes, whereas half of the females of each group were shaved to relieve them from the heat dissipation limitation. The results of this experiment provided some support for HDL theory as average daily metabolic rates and milk production were higher in shaved than in unshaved voles, however, there were no significant differences in litter growth rates. These differences were more profound in older females but they were characterized by smaller litter size, body mass, and lower growth rate in juveniles. Moreover, the age-related reproductive performance decrease was not affected by the selection. Thus these results did not provide support for the main hypotheses attributing the differences in reproductive performance to the age-related decrease in thermoregulatory capacities.

Discussing the results of this experiment Ms Grosiak declares that they are not providing support for the HDL theory but in my opinion, one has to be careful making such a bold statement as she rightly pointed out in the summary there is only mixed support for the HDL theory. Thus when publishing results I will advise being more careful in formulating conclusions.

I also think that the lack of a link between milk production and litter growth rate requires some more attention as in this case predictions of HDL theory can apply to the mother's performance only but in the juveniles, the central or peripheral limitations could play a more important role.

Before publishing the results of this experiment I will also explore one more possible explanation for the smaller-than-expected differences in heat dissipation between old and

young voles. According to my observations, the fur cover in old rodents (voles, mice) is quite poor due to the lower density of hairs and this feature could be seen as a kind of adaptation increasing heat dissipation abilities in older individuals. Moreover, there was a significant variation in body mass among the treatment groups and lines (e.g. the difference in body mass between the shaved young voles was almost two times bigger than between shaved old voles), making the differences in the breeding performance smaller than predicted.

Finally, in the last part of her dissertation, Ms Grosiak investigated the long-term effects of HDL manipulations during lactation in females on their adult offspring performance. To do this she measured body mass, thermogenic capacity, swim- and run-induced maximum metabolic rate, maximum sprint speed, and endurance running time in weaned pups from litters of mothers from both selection directions, three age classes, and shaving groups. The results of this experiment did not provide support for the hypothesis that adult offspring performance traits decrease due to the age-related decline in the mother's thermoregulatory capacity. In contrast to the predictions neither body mass nor the thermoregulatory performance of the offspring was related to maternal age and this effect was not affected by the mother's fur removal. The lack of age effect in this experiment does not exclude the possibility that such an effect exists under different conditions. As Ms Grosiak rightly pointed out in the discussion, the laboratory conditions and unlimited access to the food could potentially mask the effect on the mother's performance of the weaning offspring. I am happy to see this explanation, which doesn't undermine the findings of the experiment but demonstrates some limitations of this approach.

One more thing worthy of consideration in the context of breeding performance is the ability of mothers from the selected lines to increase further their investment in the offspring as they are probably operating anyway close to their physiological limits. Even if the limitations of the heat dissipation ability had been relaxed by the fur removal the costs of self-maintenance are high and fewer resources could be provided for the offspring. Moreover, from the evolutionary point of view, it makes more sense if there are no differences in adult offspring quality between juvenile and adult mothers. One should rather expect that if adult mothers have smaller litters the quality of offspring should be even higher to secure higher survival rates. Probably under natural conditions, the relationship between maternal age and breeding performance is even more complicated as the mother's condition is also affected by the previous breeding attempts, whereas in this experiment voles produced offspring only once but at different ages.

I have to admit that it was my enormous pleasure to read and review this dissertation. Ms Grosiak's work deserves special recognition in the context of the experimental approach for testing the novel hypothesis and application of advanced research techniques. Presenting results of her studies Ms Grosiak has also proved her excellent analytical skills and very extensive knowledge in the area of evolutionary physiology.

Conclusion

In my opinion, the thesis by Marta Grosiak fulfils all requirements for obtaining the PhD degree according to art. 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018 r. poz. 1668 z późn. zm.). I am recommending the dissertation thesis of Ms Marta Grosiak for the defence and an award for the quality of the presented research.

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