Physiology of thermal phenotypic plasticity
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Summary

The world in which plants and animals live changes continuously. Day-night rhythm, daily or seasonal weather patterns, or climate change causes environmental conditions to vary on different time scales. In addition to abiotic factors, biotic factors change as well (e.g. community composition). To prevent extinction of a species, individuals must constantly adapt to this changing environment, but the question is whether they are able to? The question is important, because differences in adaptability between species can lead to disruption of the functioning of an ecosystem due to abolition of certain functions that organisms occupy within the ecosystem. Organisms have evolved short term (physiological) and long-term (genetic) mechanisms to cope with environment change. The phenotype of an organism is the result of interactions between its genotype and environment conditions. The capabilities and flexibility an organism has to adjust its phenotype to the environment, is called phenotypic plasticity.

Ectotherms are organisms whose body temperature depends on environmental heat sources. Because almost all biological processes depend on body temperature, environmental temperature is very important for the performance of ectotherms. In this thesis, I studied how ectotherms physiologically adjust to changing environmental temperatures. I have looked at their thermal phenotypic plasticity and I have chosen specifically to look at their (body) lipid adaptation. Cell membranes mainly consist of lipids: membranes keep the body together on a micro scale and protect it against influences of the outside. In addition, lipid storage is an important source of energy for the body, but in order to metabolize it, it must be in an accessible form for enzymes, which cannot cope with lipids that are too solid due to low temperature. Thermal adaptation of lipids is therefore very important for the functioning of the whole body.

Springtails were chosen as the model system, because they occur in a wide variety of habitats: they occur from the poles to the tropics and from forest to beach or even water. As a consequence, they have evolved strategies to cope with a large range of environmental conditions, and both genetic and physiological adaptations have been described. Springtails are small soil arthropods that graze especially on fungi and algae. The function of their grazing for the ecosystem is enhancement of the nutrient recycling.

The main research aim of this thesis was to find out how springtails adapt their lipid composition to altered temperature. What are the differences in thermal adaptation between species, and which fatty acids increase or decrease in response to temperature? Subsequently, I wanted to know whether differences in thermal phenotypic plasticity relate to variation in extreme temperature tolerance. The fatty acid response was not as straightforward as
predicted from literature. Theory assumes that when the temperature decreases, fatty acids become more unsaturated, and by increasing the temperature they become more saturated. Without adaptation, lipids solidify at low temperature and liquefy at high temperature. By adjusting the degree of the fatty acid saturation, the viscosity (fluidity) of body lipids remains in equilibrium (this is called homeoviscosity). This thesis showed that springtails indeed changed their lipid composition in response to temperature; hence, they showed a plastic response. For example, the proportion of the fatty acid C\textsubscript{16:0} often increased with temperature, while C\textsubscript{20:5n3} mostly decreased. There was however a difference in the response of the membrane lipids compared to the storage lipids: the response of the storage lipids fitted better with the expectation from the literature. The most likely cause of this difference was that storage lipids have fewer alternative avenues available for lipid adaptation. Membrane lipids can, besides adjusting the fatty acid saturation, change the shape of the phosphoheads and the position of the head on the molecule, while storage lipids can only adjust the fatty acid saturation.

I started by determining the best way to measure lipids in springtails. It matters how the biomaterial is processed in the laboratory before the lipids are measured. Especially the polyunsaturated fatty acids are very sensitive to certain treatments: they oxidize easily after which they are no longer detectable. I found that slow saponification and flushing the headspace before saponification and methylation with nitrogen gas, maximize preservation of these polyunsaturated fatty acids, resulting in more reliably measurements of the lipid composition.

The fatty acid adaptation in a springtail species (*Orchesella cincta*) during lipid adaptation was determined in response to moderate temperature change in juvenile and adult springtails. Adult springtails showed more unsaturated fatty acids than juveniles, but both groups increased the proportion of unsaturated fatty acids in response to a temperature decrease. In storage lipids, this response was reversed during a temperature increase, but not in membrane lipids. A plausible cause for this difference between membrane and storage lipids could be the alternative avenues for adaptation, as explained above.

Subsequently, I looked at thermal reaction norms for specific fatty acids over five different temperatures. Which fatty acids change in proportion and which do not? The fatty acid composition of *O. cincta* after four weeks of acclimation changed largely in line with the previous experiment. Again the fatty acids showed a high degree of plasticity, but we found some striking deviations from expectations. Two specific fatty acids (C\textsubscript{18:2\textsuperscript{o6}} and C\textsubscript{18:3n3}) increased in quantity at a higher temperature, while an overall decline of these polyunsaturated fatty acids would be expected. These fatty acids are probably essential and can therefore only be obtained through the diet. Only two insect species have been found to produce these fatty acids themselves, other species probably do not. If these fatty acids are essential for *O. cincta*, then preservation of these fatty acids for future processes can have a
higher priority than the thermal response. There is then a trade-off between optimal temperature adaptability and future reproduction, for which these essential fatty acids are needed, which can lead to a sub-optimal temperature adaptation.

In addition to the physiological aspects of thermal adaptation of lipid composition, I looked at the RNA expression of several genes encoding desaturase enzymes in *O. cincta*. An organism gets a large proportion of the fatty acids from its diet or produces them *de novo*. If the ingested fatty acids do not have the required (un)saturation, organisms can reduce the saturation by use of specific desaturase enzymes. In *O. cincta*, indeed no homologues of $\Delta 12$ or $\Delta 15$ desaturases were found, confirming that $C_{18:2n6}$ and $C_{18:3n3}$ for *O. cincta* probably are essential fatty acids. In this experiment, I also observed that most of the desaturase genes showed much higher expression in animals that were adapted to heat than to cold; the opposite of what I expected. Increased activity due to warmer temperatures possibly resulted in other priorities than temperature adaptation, e.g. increased feed intake and reproduction.

Laboratory experiments investigating thermal adaptation often simulate only a single temperature change, but in a natural environment, the temperature fluctuates more often. To examine how *O. cincta* responds to repeated temperature changes, I exposed the animals to a repeated two-day temperature fluctuation, and a warm and cold constant temperature. In the early cycles of this regime, *O. cincta* adapted its fatty acids to the new temperature, but later in the experiment there was an attenuation of this response. Instead, their fatty acid composition was stable and similar to the fatty acid composition of animals that were adapted to constantly warm temperature, even during the cold part of the temperature cycle. To relate adjustment of lipid composition to functioning of *O. cincta*, I also determined extreme temperature tolerance after exposure to the three temperature regimes. *O. cincta* exposed to constant cold was better able to withstand cold shock, while individuals exposed to constant warmth were able to withstand a heat shock. *O. cincta* exposed to fluctuating temperature resisted a heat shock just as well and cold shock a little better than individuals adapted to constant warmth. Although the fatty acid composition was consistent with that of individuals exposed to constant warmth, the animals that were exposed to fluctuating temperature had a higher thermal tolerance.

Differences in temperature fluctuations in the soil are mostly found along a vertical gradient. Springtail species that live deep in the soil differ in microhabitat with species living more on the soil surface, because the environmental conditions are very constant deeper in the soil. Soil dwelling species have had less (evolutionary) selection pressure on adaptation to changing temperatures than surface dwelling species. Consequently, I expected surface dwelling species (e.g. *O. cincta*) to be physiologically better able to adapt to changing temperatures than soil dwelling species. Surface dwelling species indeed showed larger physiological plasticity than soil dwelling species. Also, their temperature tolerance was higher: surface dwelling species could better withstand extreme heat than soil dwelling
species. Unless soil dwelling species are able to behaviorally protect themselves to temperature, this group of springtails renders more vulnerable to climate change, despite the fact that surface dwelling species will be exposed to larger temperature changes.

There are many factors that can explain observed variation in the responses to temperature. This thesis shows that age of the individual, species, period of adaptation, rate of temperature change, and direction of temperature change affected the degree of body lipid adaptation. From the literature possible effects of diet, air or soil moisture, and the interaction with enzymes were added to the list of possible causes of variation in the thermal lipid response. Still membrane and storage lipids showed a high degree of plasticity in response to temperature, from which I conclude that fatty acid modifications are probably essential for the physical characteristics of body lipids under different environmental temperatures. My results have highlighted adaptive changes in body lipids that can increase the thermal resistance during the temperature changes to which these animals are exposed.