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Effects of climate change are occurring at a time when many populations are already under pressure from increased fragmented natural environments due to habitat destruction and increased species invasion due to species that are inadvertently moved around the globe (Hoffmann and Sgro, 2011). To prevent extinction of a species, individuals of the species must continuously adapt to this changing environment. The challenge for ecologists is to predict the winners as well as the losers, based on their adaptive capacity. It is important to understand this issue 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 (Hooper et al., 2005).

**Temperature-induced phenotypic plasticity**

The world’s ecosystems are exposed to slow increases in global temperature and more frequent occurrence of intense and longer lasting heat waves (Meehl and Tebaldi, 2004; IPCC, 2007; Smith et al., 2009) or cold winter extremes (Petoukhov and Semenov, 2010). In the field, organisms are exposed to fluctuations that differ in time scale as well as in magnitude. For example, temperature can fluctuate on a daily, seasonal, or even a year-to-year basis. The magnitude of the fluctuations generally increases towards higher latitudes and altitudes. Even at a single location one can find variation in fluctuations across the soil profile, as deeper soils tend to attain less extreme temperatures than the top layer of soils.
Ectotherms are especially vulnerable to thermal change, because their basic physiological functions are strongly influenced by ambient temperature (Haywards et al., 2007). Moreover, various ectotherms try to maintain their body temperature within a narrow, species-specific range, at least while active (Martin and Huey, 2008). Most often, an ectotherm can behaviorally escape from a change in temperature, e.g. by hiding in the shade during the hottest part of the day, by diurnal vertical migrations, or by migrating to different parts of the world. As modern landscapes are highly fragmented, they provide little flexibility for ecological communities to adjust rapidly to temperature changes; species with low dispersal capacity will either go extinct or they need to adapt to their physiology to the new conditions (Walther et al., 2002; Berg et al., 2010). Many biotic shifts, such as shifts in geographical ranges (Bale et al., 2002), seasonal phenology (Visser and Holleman, 2001), or extinction (Umina et al., 2005) have been detected for arthropods with varying dispersal capabilities (Deutsch et al., 2008).

To cope with the different thermal regimes to which it is exposed within a lifetime, an individual (i.e. a genotype) can express different phenotypes depending on the environmental conditions, i.e. phenotypic plasticity (Bradshaw, 1965). Phenotypic plasticity can be measured as a reaction norm (De Jong, 1990). A thermal reaction norm reflects the phenotype expressed by a given genotype when exposed to a range of environmental temperatures (or body temperature). Thermal reaction norms can take the form of thermal performance curves, which describe the thermal sensitivities of behavioral or physiological rates, or thermal tolerance curves, which describe thermal sensitivities of fitness parameters (Angilletta et al., 2006). The thermal reaction norm for a particular trait consists of an optimal temperature at which the relative performance is maximal for that trait, and a lower and upper critical limit at which temperature the performance is zero (Figure 1; Huey and Stevenson, 1979). The range of body temperatures in which an individual can perform (e.g. survive, grow, or reproduce) is called the thermal tolerance range (Angilletta et al., 2002; Van der Have, 2002), and the range of ambient temperatures in which an individual can perform is called the thermal niche. Thermal reaction norms have been particularly well characterized for many morphological and life history traits (Nylin and Gotthard, 1998; Scharf et al., 2008), as well as for several physiological and biochemical traits (Nylin and Gotthard, 1998; Stillwell et al., 2010).

**Thermal acclimation**

Prosser (1955) defined physiological adaptation as “any functional property of an individual which favors continuous successful living in an altered environment”, and used the term acclimation for the phenotypic adaptive alterations of individual organisms (as in Lagerspetz, 2006). In order to alter the phenotype in response to changed thermal conditions, an organism needs mechanisms to sense and respond to the environmental temperature change (Bicego et
Most organisms acclimate to thermal change through behavior (Stevenson, 1985; Ellers and Boggs, 2004), morphology (Fischer et al., 2003; Liefting et al., 2009), or physiology (Huey and Stevenson, 1979; Hochachka and Somero, 2002).

Restoring thermal homeostasis of the body temperature by using behavioral, morphological or physiological acclimation strategies is called thermoregulation (Angilletta et al., 2006). Which acclimation strategy is preferred depends on the costs and benefits associated. Behavioral changes can be applied relatively fast, although they can also be very costly, i.e. increased predation (Dreisig, 1981), lower food quality (Stamp and Bowers, 1990), or high costs of movement (e.g. migration, dispersal, locomotion, flight) (Schmidt-Nielsen, 1972; Rankin and Burchsted, 1992). If temperature changes are long-lived compared to the life span of an organism, physiological or morphological changes are potentially more beneficial than behavioral strategies. Especially when individuals are exposed to temperatures outside their thermal niche, behavior alone cannot prevent the organism from dying; hence physiological acclimation is needed to increase the chance of survival. Particularly well known are the short-term effects of extreme temperatures on cellular responses, such as the expression of protective heat shock proteins at high temperatures (Feder and Hofmann, 1999;
Bahrndorff et al., 2009a), and late embryogenesis abundant proteins (Thomashow, 1998) or cryoprotectants (Storey and Storey, 1988) when exposed to freezing conditions. In environments where these thermal extremes occur often or for longer periods, species have evolved specialized behavioral, morphological and/or physiological mechanisms to counteract these harsh conditions, for example by hibernation (Zachariassen, 1985), diapause (Tauber and Tauber, 1976; Hodkova et al., 1999), aestivation (Masaki, 1980), daily torpor (Johnston and Bennett, 1996), or morphological change (different life stage; Storey and Storey, 1986).

The impact of ambient temperature on an organism’s functioning depends on the thermal sensitivity of this organism (Angilletta et al., 2002; Deutsch et al., 2008; Angilletta, 2009), expressed by the slope of the reaction norm (Angilletta et al., 2006). For example, in terrestrial ectotherms a slow and small increase of the mean ambient temperature or thermal fluctuation is likely to have more deleterious fitness consequences for tropical species than for species at higher latitudes, because species at higher latitudes, 1) have broader thermal tolerance (Williams et al., 2003; Deutsch et al., 2008), and 2) live in climates that are currently cooler than their physiological optima, and a relatively small warming may even enhance their fitness. High performance over a broad range of temperatures is supposed to increase the chances of success of a genotype or species in a highly variable thermal environment compared to species with a smaller thermal range (Huey and Stevenson, 1979; Bale et al., 2002; Williams et al., 2003; Angilletta, 2009). It has been convincingly argued that species experiencing a more variable environment have broader thermal range (and therefore relatively insensitive to temperature) due to higher selection pressure on physiological capabilities to meet this variation, compared to species experiencing less variability (Stevens, 1989; Stevens, 1992; Bahrndorff et al., 2006; Bahrndorff et al., 2007; Terblanche et al., 2007; Terblanche and Chown, 2007). Differences in thermal sensitivity are observed with latitude (Stevens, 1989; Hallas et al. 2002; Williams et al., 2003; Bahrndorff et al., 2006; Bahrndorff et al., 2007), altitude (Sorensen et al., 2001; Sorensen et al., 2005; Karl et al, 2008), and between local habitats (Liefting and Ellers, 2008; Berg et al., 2010). Highly plastic physiological machinery to maintain high performance over a broad range of temperatures is hypothesized to come at a cost (Figure 2; Dewitt et al., 1998; Pigliucci, 2005; Van Kleunen and Fischer, 2005), although empirical evidence for this hypothesis is still biased and largely lacking (Gaston and Spicer, 2001; Caley and Munday, 2003; Loeschke and Hoffmann, 2007).

At a smaller spatial scale, differences in thermal variability are found along a vertical stratification gradient in the soil (Bahrndorff et al., 2009b). Deep-soil dwelling species habituate relatively stable thermal environments (Willmer, 1982), whereas surface dwelling or near surface dwelling species, experience an increased range of thermal fluctuations with much more rapid shifts between extremes. A group of organisms very common in and on top of the soil are the springtails (Collemboila). Collemboila occur along the entire soil gradient
(Berg et al., 1998; Krab et al., 2010) and show diverse physiological adaptations to the environment depending on the geographic or local habitat in which they live (Joosse and Verhoef 1987; Holmstrup et al. 2002; Kærsgaard et al. 2004; Liefting and Ellers, 2008). Plasticity of several physiological traits and extreme temperature tolerance in *Orchesella cincta* (Bahrndorff et al., 2006; Bahrndorff et al., 2009b) and *Megaphorura artica* (Bahrndorff et al., 2007) were shown to be higher in geographic areas that were more variable in temperature. The effect of temperature on growth rate in *Orchesella cincta* was smaller in heath habitats, suggesting lower sensitivity to thermal change than in the thermally more stable forest (Liefting and Ellers, 2008).

![Figure 2. Illustration of a cost of plasticity. The fixed genotype 1 and the plastic genotype 2 have the same phenotype in environment B. The cost of plasticity is indicated by a lower fitness of the plastic genotype 2 than the fixed genotype 1 in this environment (From Van Kleunen and Fischer, 2005).](image)

**Physiology of temperature acclimation**

The core assumptions of thermal biology are (1) temperature is one of the most pervasive state variables affecting biological processes, and (2) the laws of thermodynamics define the direction and rate of biochemical processes that underlie the performance of whole organisms (Brown et al., 2004; Angilletta et al., 2006). The thermal environment then exerts strong selective pressures on all organisms and is thus a key parameter explaining many physiological, ecological, and evolutionary patterns. Adaptive responses to thermal heterogeneity involve all levels of biological organization from the expression of genes to the behavior of the organism, but these responses often occur on different temporal scales (Figure 3). The interactions among levels of organization link these responses into a mechanistic cascade flowing from the biochemical to the organismal levels. The challenge for biologists is
to define the mechanistic links between thermal responses at different levels and to identify their impact on fitness – i.e. to understand the evolution of the mechanistic cascade (Angilletta et al., 2006).

Temperature affects essentially every aspect of an organism’s physiology, from the basic structure of macromolecules that are responsible for catalysis and information processing, to the rate at which reactions occur (Hochachka and Somero, 2002). As temperature has such a profound effect on performance (Pörtner, 2002), it is not surprising that ectotherms have the ability to respond to a changing thermal environment in several ways. One important physiological adaptation mechanisms in response to temperature involves chances in lipids. The concept that lipids alter their physical properties in response to cold exposure originated over a century ago, when Henriques and Hansen (1901) noted the higher melting point of subcutaneous fats in pigs raised wearing clothing, which resulted in increased tissue temperature. Considerable evidence now indicates that the physical properties of membrane lipids contribute to the definition of thermal limits for e.g. growth, lifespan and survival of these ectotherms (Hazel 1995; Crockett and Hazel, 1997; Hulbert and Else, 2000; Hochachka and Somero, 2002; Hulbert and Else, 2005; Overgaard et al., 2006; Murray et al., 2007; Clark and Worland, 2008; Angilletta, 2009).

Temperature determines the movement and the conformation of the membranes (Hazel and Williams, 1990). Low temperatures make the membrane gel-like and high temperatures make the membrane too fluid or even disrupt the laminar structure (Figure 4). No or partial acclimation of membrane lipids is costly, as lipids solidify at low temperatures and liquefy at high temperatures, disturbing enzyme-membrane functions such as physical barrier, transport, bioenergetics, or cell signalling (Cossins et al., 1977; Brand et al., 1991; Crockett and Hazel, 1997; Brookes et al 1998; Else and Wu, 1999; Wu et al, 2001; Hochachka and Somero, 2002; Kostal et al., 2004; Hulbert and Else, 2005). For example, proton leak due to liquid membranes dissipates the trans-membrane proton gradient generated by the electron transport chain in mitochondria, and thus leads to oxygen consumption without ATP synthesis (Brand et al., 1991; Hochachka and Somero, 2002; Hulbert and Else, 2005). Accordingly at low temperatures, chill injury in arthropods is probably associated with loss of membrane function as cold shock causes dissipation of transmembrane gradients of $\text{Na}^+$ and $\text{K}^+$ and a depolarisation of the cell membrane (Crockett and Hazel, 1997; Else and Wu, 1999; Kostal et al., 2004).
Figure 3. A cascade of mechanisms operating from cellular to organismal levels is responsible for adaptive responses to thermal heterogeneity. These mechanisms alter commonly measured properties of organisms from gene expression to behavior, on different temporal scales (From Angilletta et al., 2006).

Lipid composition as part of homeoviscous adaptation

From the somewhat bizarre beginnings in 1901, the lipid hypothesis of temperature adaptation has developed to encompass the idea of an optimal physical condition of cellular and subcellular membranes for supporting critical membrane-associated functions, and a homeostatic mechanism for preserving this property in the face of environmental disturbance (Sinensky, 1974; Cossins, 1983). The process of conserving membrane physical state under fluctuating environmental conditions is called homeoviscous adaptation (HVA) and is commonly used by ectotherms to avoid detrimental effects of temperature (Sinensky, 1974; Hazel and Landrey, 1988a,b; Hochachka and Somero, 2002). HVA involves modifications of the lipid composition, such as unsaturation of fatty acids, composition of the phospholipid head group, amount of sterols incorporated in the membrane bilayer, or the placement of fatty acids on the sn1 or sn2 position of the glycerol backbone (Hazel and Williams, 1990; Hochachka and Somero, 2002). One of the predicted ways to achieve HVA is that membrane fatty acids become more unsaturated during a cold response to compensate the negative effect of
membrane solidification (Hochachka and Somero, 2002). An increase in the degree of unsaturation is mainly achieved by incorporating a higher proportion of mono- or poly-unsaturated fatty acids in the phospholipids that constitute the membrane. These mono- or poly-unsaturated fatty acids can be synthesized from other fatty acids or acquired via the food (Hochachka and Somero, 2002, Perreia et al, 2003; Leonard et al., 2004). Changes in lipid unsaturation have been shown in short-term acclimation (Overgaard et al., 2005) and long-term acclimation, such as seasonal adaptation (Ohtsu et al., 1993; Bennett et al., 1997).

Many studies have shown a thermal response in membrane lipid composition that is consistent with HVA (Sinensky, 1974; Cossins et al., 1977; Bly et al., 1986; Hazel and Landrey, 1988b; Kostal and Simek, 1998; Brodte et al., 2008). On the other hand, there is much variation in the nature of temperature-induced changes in lipid composition, e.g. which fatty acids are responsible for the change in saturation, or in the rate of change. It has been suggested that a variety of factors may contribute to variation in membrane lipid response, including interactions with enzymes (Owicki and McConnell, 1979; Hochachka and Somero, 2002), exposure to thermal fluctuations (Williams and Somero, 1996; Bennett et al., 1997; Pernet et al., 2007), preservation of certain fatty acids for reproduction (Stanley, 2006; Stanley, 2009), drought (Holmstrup et al., 2002) diet (Haubert et al., 2004), and age (Ogg and Stanley-Samuelson, 1992). For example, higher concentrations of membrane-associated proteins change the phase transition temperature and weaken the phase transition (Owicki and McConnell, 1979). Additionally, the fatty acid composition may be limited in thermal response to provide protein functioning within membrane lipid rafts (Stulnig et al., 2001).

Recent studies have suggested that temperature change makes storage lipids worse substrates for enzymes such as lipases (Kostal and Simek, 1998), resulting in a potential need for thermal adaptation in storage lipids as well (Kostal and Simek, 1998; Brodte et al., 2008; Haubert et al., 2008). Storage lipids (triacylglycerol fatty acids) differ in function from membrane lipids as they represent the major fuel storage in fat bodies for organisms, for example during overwintering (Ohtsu et al., 1993) or in flight (Beenakker et al., 1985; Zera et al., 1999). Although several environmental conditions are known to affect the storage lipid composition, for example drought (Holmstrup et al., 2002) and diet (Canavoso et al., 1998; Haubert et al., 2004; Chamberlain et al., 2005), the thermal response of storage lipids has received relatively little consideration (Haubert et al., 2008). Some studies have indeed shown that ectotherms under cold conditions show increased unsaturation of storage lipids (Ohtsu et al., 1993; Bennett et al., 1997; Kostal and Simek, 1998; Brodte et al., 2008).
Besides being structural components and energy storage of membranes, fatty acids have a number of other functions such as precursors in biosynthesis of waxes, pheromones and defensive secretions (Stanley-Samuelson et al., 1988), and precursor for biosynthesis of eicosanoids, which are involved in reproduction (Stanley, 2006). These additional functions may constrain insects in adjusting fatty acid composition to temperature, or may cause changes in fatty acid composition over the lifetime, due to distinct requirements of different life stages.

![Diagram showing phase behavior and molecular geometry of membrane phospholipids](image)

*Figure 4.* Solid arrows indicate the effects of either a rise or drop in temperature on the phase behavior and molecular geometry of membrane phospholipids. The physiological temperature refers to the temperature at which an organism is either adapted or acclimated. The dashed arrow illustrates the presumed involvement of the inverted hexagonal phase in membrane fusion (From Hazel, 1995).

**Model organism**

The organism that is used for most of this thesis is the springtail *Orchesella cincta* (Collembola). *O. cincta* is a surface-dwelling arthropod species distributed across Europe. It lives in the top litter layer of a wide variety of seasonal habitats (Timmermans et al., 2005), where it shows local adaptation to temperature regimes (Bahndorff et al., 2006; Liefting and Ellers, 2008), and variation in thermal responses of life history traits (Bahndorff et al., 2007;
Driessen et al., 2007; Ellers et al., 2008). Previous experiments show that *O. cincta* is metabolically active over a large thermal range, for example, it has a linear growth rate between 10°C and 24°C (unpublished data G. Driessen), continues to molt even down to temperatures of 3.1°C (Joosse and Veltkamp, 1970), and lays eggs at temperatures as low as 5°C (Van Straalen and Joosse, 1985).

The average annual density of *O. cincta* may reach up to 1500 per m², but densities are variable (Van Straalen, 1989). *O. cincta* lives in the litter layer of the soil where it is feeding on fungi and algae, and occurs in a wide variety of habitats, including dense and open forest, heath, and coastal shrub. Hence, considerable intraspecific variation exists in temperature range as well as thermal fluctuations encountered. Some of the thermal variations they encounter are e.g. the occurrence of sunny spells that can repeatedly raise the temperature by more 10°C even on an hourly basis (Overgaard and Sorensen, 2008) or warmer and colder periods at the timescale of several days (Klein Tank et al., 2002).

**Outline thesis**

In this thesis, I focus on thermal physiology in springtails, its plasticity, and its effect on the temperature tolerance of individuals. Evolutionary processes have put all organisms into a particular thermal niche, within which they may function well, but outside of which they may not survive. No single species spans the whole global thermal range (from –80°C in terrestrial habitats to 100°C in geothermal hot springs). Nothing escapes its control and thus temperature shapes the phenotype of an organism. Still, the same change in temperature will not affect all organisms equally. Moreover, temperature does not affect the same organism equally at all stages of life. These phenomena raise serious questions for biologists. How fast and to what extend are organisms able to adapt to temperature? What are the underlying physiological mechanisms to thermal adaptation? How flexible are these physiological mechanisms and are they somehow limited in their response? Moreover, what are the subsequent consequences for life? This thesis is built around three main hypotheses. First, the fatty acids will respond to temperature as predicted by the HVA theory: more unsaturation of the fatty acids in response to cold than in response warmer temperatures. Besides insight in an underlying mechanism of temperature acclimation, investigating this hypothesis will give insight in the rate and the plasticity of the fatty acid response and potential limitations. Second, HVA will also be applicable to storage lipids or body lipids in general, instead of only being applicable to membrane lipids. Storage lipids are an important resource for energy in insects and could profit from being plastic. Third, there will be differences in physiological plasticity between species and these differences will lead to higher thermal insensitivity. Investigating this hypothesis will help us understand the evolution of plastic responses in general, by elucidating potential mechanisms and constraints shaping thermal response curves.
Chapter 2 investigates which preparation method of the samples best preserves the poly-unsaturated fatty acids in ecological experiments. Furthermore, I applied the preparation method with the highest preservation of poly-unsaturated fatty acids in an ecological experiment, to get a rough idea of the fatty acid response of *O. cincta* in response to two acclimation temperatures.

The Chapters 3 to 5 deal with the two first main questions in more detail. Each of these chapters tests the lipid composition in response to temperature in both storage and membrane lipids of *O. cincta*. In more detail, Chapter 3 deals with the change in lipid composition in both juveniles and adults during cold and warm temperature adaptation. In Chapter 4 the effect of magnitude and direction of temperature change were investigated by determining the reaction norms for lipid composition in response to five different temperatures. Chapter 5 deals with the change in lipid composition in response to constant and fluctuating temperature. Does *O. cincta* repeatedly change its lipid composition in response to fluctuating temperature and what are the consequences for their thermal tolerance?

Chapter 6 deals with third main question on differences in HVA between species. It shows the plasticity of the lipid response for surface dwelling and soil dwelling Collembola and the effect on the thermal sensitivity for some of these species to extreme temperatures. To investigate potentials for future research Chapter 7 briefly deals with the gene expression for desaturases in *O. cincta* in relation to different acclimation temperatures; an attempt to link the thermal response of the fatty acid composition to the control mechanism of fatty acid unsaturation. This thesis is concluded with a summarizing discussion (Chapter 8) of all previous chapters, extended with some overall conclusions.