Chapter 1

General Introduction

“Evolution happens. What remains open to dispute, especially among scientists, is how evolution happens. Scientific theories themselves evolve, adapting to fit new observations, new discoveries and new interpretations of old discoveries.”
– Terry Pratchett, Ian Stewart & Jack Cohen (Darwin’s Watch, 2005)

Evolution, natural selection and environmental heterogeneity

Evolution involves change in the inherited traits of a population of organisms over generations. These changes are caused by a combination of three main processes: variation, reproduction, and selection (Ridley 1996). When heritable variation in traits is present within populations, the frequency of traits can be altered in the next generation. Individuals with advantageous traits have a higher fitness, i.e. are more likely to reproduce, so that more individuals in the next generation inherit these traits (Darwin 1859). Evolution takes place through a change in the frequency of alleles coding for these traits, so that when alleles become more common or rare, the trait distribution in a population changes. Two of the main mechanisms that drive evolution are genetic drift and natural selection. Genetic drift describes the process of random changes in the frequency of alleles in a population. The second mechanism, natural selection, is a process causing heritable traits that aid survival and reproduction to become more common in a population. Natural selection is not the consequence of heritable variation in fitness. Heritable variation in fitness is natural selection (Stearns 1992). Adaptations to the environment occur through a combination of successive small changes in traits over many generations. The combinations best-suited to their local environment will be selected for, because these will yield the highest fitness. When environments change, selection pressures change and new traits will be selected. Environmental variation is thought to be one of the main mechanisms maintaining genetic variation in population throughout time (Hedrick 1986).

Environmental variation can occur on daily, seasonally and yearly timescales. However, organisms experience these timescales differently. In essence, it matters more whether variation occurs within or between generations than the actual time span of variations. If environmental
change takes place within generations, selection on genes is not effective because the response of
the trait to the changing variable will be long-term and is too slow to provide adaptation to the
fast environmental changes. It is also important how predictable the variation is. For example,
seasonal variation in precipitation is predictable on an annual scale, but it is still practically
impossible to accurately predict the weather five days ahead. Especially the temperate regions
experience much non-cyclic variation in many aspects of the environment, e.g. humidity, light
conditions, food availability, predator abundance, etc. If the environment itself changes
unpredictably, the adaptive strategy might be to have flexible traits that can change in
concordance to these changes. Many theoretical and empirical studies have investigated the
evolution and adjustments in response to environmental variability (Huey & Kingsolver 1989;
Gilchrist 1995). Such studies describe how environmental variability within-generations selects
for a broad thermal response in organisms. Likewise, tolerance zones and acclimation capacities
are generally smaller for organisms from thermally stable environments, i.e. in tropical species,
than for their temperate-zone relatives. There are therefore differences in the flexibility of the
response of organisms to their environment that are dependent on how variable this
environment is. This brings us to the fundamental question that motivated a renewed and
increasing interest in the adaptive nature of flexibility of traits in evolutionary biology, which also
shaped the basis of this thesis: What are the selective forces driving the evolution of flexible,
variable traits? And what are the evolutionary and ecological consequences of differences in the
degree of flexibility?

Reaction norms and phenotypic plasticity
The genotype of an organism refers to the inherited instructions it carries within its genetic code.
A phenotype can be any observable characteristic or trait of an organism; such as its
morphology, development, biochemical or physiological properties, or behavior (DeWitt &
Scheiner 2004). Phenotypic traits result from the expression of an organism's genes as well as the
influence of environmental factors, and possibly the interactions between the two. The influence
of an environmental variable on the phenotype of a specific genotype can be described by a
reaction norm (de Jong 1990). A schematic example of a reaction norm is given in figure 1.1;
represented on the x-axis is the range of the environmental variable (temperature, humidity, food
availability) and on the y-axis the phenotypic response of a trait. The reaction norm can be linear
or curved; in the latter case, parts of the reaction norm can be linear (de Jong 1990). There are
two main aspects of a reaction norm that give information on the genotype-phenotype
relationship: the elevation and the slope of the reaction norm. The elevation of the reaction
norm is indicative of the mean value of the traits across environments. When elevation changes, the mean response changes, so if the line in figure 1.1 would represent growth rate as a function of temperature, the higher reaction norm describes faster growth at all temperatures. The slope of the reaction norm describes the strength of the trait response to the environmental variable. If the slope changes, it means that changes in the environment have a stronger, or weaker, effect on the phenotype. The steepness of the slope therefore describes the level of phenotypic plasticity (de Jong 1990; DeWitt & Scheiner 2004).

Figure 1.1 Variation in reaction norms describes different phenotypic responses to an environmental variable. Left figure; change in mean response (elevation). Right figure; change in phenotypic sensitivity to an environmental variable. Note that lines do not necessarily have to cross and that a reaction norm can also change in both mean and slope.

Phenotypic plasticity exists when a trait is affected by environmental conditions and the same genotype can express different phenotypes (Scheiner 1993). Phenotypic plasticity was reviewed by Bradshaw (1965) and defined as follows; ‘Plasticity is … shown by a genotype when its expression is able to be altered by environmental influences’. For a while, plasticity was ignored in studies on adaptive evolution and the variation in the phenotype was even considered a ‘problem’ because they interfered with the natural and artificial selection of a given trait (Falconer 1952). But as we find striking differences in phenotypes across natural environments, the understanding of how phenotypic plasticity affects performance in heterogeneous environments became an important focus of evolutionary ecology. It is now thought to be one of the major immediate responses to environmental change (Pigliucci 2005). For example, a major part of the effects of climate change is explicable as variable expressions of plastic traits (Stillman 2003).
The fact that strong natural selection exists for performance of organisms under variable conditions, and the fact that heritable variation exists for plasticity, makes it possible for plasticity to evolve (Pigliucci 2005). Any form of plasticity that allows an organism to increase its fitness under a particular circumstance compared to organism that are not plastic, will be beneficial (Ghalambor et al. 2007). Yet, this does not mean that the reaction norm for that trait was necessarily shaped by natural selection, i.e. is an actual adaptation (Gotthard & Nylin 1995). For example, the observation that Daphnia produces small bodies in the presence of fish, and that small body size reduces predation risk (Dodson 1988), does not automatically lead to the conclusion that the plasticity in body size is adaptive. Perhaps small size is also favored in the absence of fish, or plasticity in evasive behavior is so costly that it results in a smaller body size. Proof of active adaptive plasticity requires analysis of fitness in multiple environments and documentation of functional trade-offs. This is a rather strict definition of how adaptive plasticity should be assessed, and studies that describe an induced plasticity in response to an environmental variable are still informative of the adaptive nature of the response. Studies that describe e.g. an increase in fin size in response to predators (Van Buskirk & McCollum 2000), plastic responses in leaf characteristics to light (Balaguer et al. 2001), and altered reproductive allocation in response to food availability (Hassall et al. 2005) are strong examples of adaptive plasticity. These studies demonstrate that temporal and spatial habitat heterogeneity selects for specific levels of phenotypic plasticity in life-history traits.

In 1995 a consortium of researchers published a paper on the two main views on how phenotypic plasticity could evolve (Via et al. 1995); (i) the slope of the reaction norm is a trait in itself and can be selected upon, therefore there can be selection on plasticity itself, or (ii) phenotypic plasticity evolves as a by-product of natural selection on the phenotypic values of the character states within the environment or in environmental extremes. The discussion raised in the paper has been superseded by recent empirical research. Although single or very few genes can influence plasticity of a trait, like the switch between wet and dry season morphs in the butterfly Bicyclus anynana (Brakefield et al. 1996), it is thought that often many genes interact to produce a plastic response. Examples of the polygenic influence on plasticity include the activity of the enzyme juvenile hormone esterase in insects (Zera & Harshman 2001), the genetic basis for wing polymorphism (Roff & Fairbairn 1991) and phytochrome-mediated shade avoidance responses in plants (Smith 2000; Casal 2002). This thesis focuses on the ecological consequences of phenotypic plasticity and does not explore the genetic architecture of this plasticity. It is however important to recognize the extensive genetic machinery that underlies the phenotypic responses to prevent simplification of the mechanism behind the reaction norm slope.
If we consider the benefit of plasticity, i.e. the ability to produce a better phenotypic match with different environments than would be possible by producing a single phenotype, then why are not all traits plastic in heterogeneous or fluctuating environments? It is assumed that there are certain costs involved in the maintenance and expression of plasticity that prevents generality. Such costs depend on the reliability of the environmental cue, maintaining a sensory detection mechanism for environmental fluctuations, time lags and interactive genetic effects (DeWitt et al. 1998; Callahan et al. 2008). If costs of maintaining plasticity are high (Edelaar et al. 2005), selection should not only be quick to remove it in an environment where it is of little use, but also from an environment that poses strong demands on resource allocation other than maintaining plasticity levels (DeWitt et al. 1998). In this context, costs of plasticity have been incorporated in many modeling studies (e.g. Moran 1992; Sultan & Spencer 2002). These models predict that costs can have a substantial effect on the evolution of plasticity, for example through a reduction of the slope of the reaction norm (van Tienderen 1997). But when testing these predictions in natural systems, the results are frustratingly diverse; some studies find a significant effect of costs (Krebs & Feder 1998; Kristensen et al. 2008), while many studies either failed to find costs or the costs were very small (DeWitt et al. 1998; Scheiner & Berrigan 1998; Donohue et al. 2000; Relyea 2002).

It is suggested that detecting such costs is difficult because genotypes burdened by high costs of plasticity have been removed from natural populations by natural selection (DeWitt et al. 1998). Therefore, recent studies have tested costs of plasticity in recombinant inbred lines with promising results (van Kleunen & Fischer 2007). The recent studies using recombinant lines found more evidence of costs of plasticity than studies using natural genotypes, which suggest that genetic costs were indeed purged from natural populations (Callahan et al. 2005; Dechaine et al. 2007). The lines used in these studies were derived from only two parental genotypes with limited genetic variation; the observed costs therefore constitute a minimum estimate of the costs that could occur in natural populations (van Kleunen & Fischer 2007). Although theory on costs and constraints of plasticity is still in its infancy, these new insights provide us with a recalibrated starting point for future research.

Plasticity of life history traits
Life history traits are traits concerning maturation and reproductive characteristics that define the life course of an organism. Typical life history traits are fecundity, age at maturity, adult weight, number of offspring per litter. The resources available to an organism, like time and energy, are limited, and trade-offs in the allocation of essential resources exist, causing variations in growth,
body maintenance, and reproduction (Stearns 1992). Therefore, the allocation of these resources to different life functions should be directly linked to fitness and under strong selective pressures. How do such traits respond to environmental variation? There is tension between the need to be buffered from the environment vs. the ability to track and adaptively respond to environmental differences (Nylin & Gotthard 1998). It is expected that traits that are very tightly linked to fitness (like most life history traits) should be more strongly buffered against environmental variation (Richards et al. 2006). A study on several Arctic mammals and birds found support for selection of life history traits that reduce variation in fitness in response to increased environmental variation (Forcada et al. 2008).

Environmental canalization can result from selection in favor of reaction norms that counteract environmental effects. This phenomenon, selection against environmental variability, is referred to as counter-gradient selection (Conover & Schultz 1995). Morphological traits on the other hand, describe traits like bristle number, wing shape, and color, and have a less direct relationship with fitness. For many traits that are not directly related to fitness, such as morphological or physiological traits, predictable environmental variation is known to select for increased phenotypic plasticity (Bradshaw 1965; de Jong 1995; Roff 2002). Under different environmental states, the optimal state of a morphological character can differ (e.g. eye spots in wings of *Bicyclus*), while fitness traits should always be maximized independent of environmental variation (e.g. fecundity). We can therefore expect different levels of plasticity within an organism for these two types of traits, depending on the environmental variation.

**Temperature as an abiotic factor**

Ambient temperature affects many aspects of an organisms’ life, like metabolic rate and physiology (Dahlhoff et al. 2008); enzyme kinetics (van der Have & de Jong 1996); growth rate and body size (Angilletta et al. 2004) and gene expression (Podrabsky & Somero 2004; Ellers et al. 2008). Temperature is considered one of the most important mediators of phenotypic plasticity in ectothermic organisms, resulting in predictable changes in e.g. timing of reproduction, egg size and body size (Angilletta et al. 2004; Steigenga & Fischer 2007a). For example, body size increases as temperature decreases and vice versa, conform the "temperature-size rule" (Atkinson 1994). Size differences are observed over latitudinal and altitudinal clines with generally smaller organisms in tropical regions (Capy et al. 1993; Hoffmann & Weeks 2007), suggesting that geographic variation in temperature drives evolution in size that is concordant with patterns of phenotypic plasticity in size. A similar pattern of thermal plasticity has often been described in egg size. Several studies demonstrate that egg size is plastic in response to
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oviposition temperature, with larger eggs laid at lower temperatures (Ernsting & Isaaks 1997; Fox & Czesak 2000).

Because of this strong link between physiological-chemical properties and temperature, many authors point out that temperature-mediated responses are shaped by physiological constraints (van der Have & de Jong 1996; Van Voorhies 1996). Caution is needed in interpreting temperature interactions from a purely adaptive view. However, these interactions can still be the subject of selection, leading to adaptive thermal responses. Evidence for adaptive explanations is provided by the existence of heritable, genetic variation of trait plasticity, including complete reversal of some responses, e.g. the egg size-temperature relationship (Steigenga et al. 2005; Seko & Nakasuji 2006).

Responses of organisms to changing environmental temperatures are often rapid, for example differences in gene expression for heatshock proteins are measurable within hours of the temperature change (Dahlgaard et al. 1998). Genotypes with a high expression of this protein were found to have a higher thermal tolerance, allowing populations to respond effectively to environmental change (Dahlhoff et al. 2008). These effects are most clearly demonstrated in extremely heterogeneous thermal environments. Because the effects of temperature are so strong, global patterns in temperature also define spatial patterns of species occurrence and habitat boundaries. It is therefore not surprising that changes in temperature due to global climate change have an effect on species. Recent studies report of possible mismatching of species interactions and adjustments in species life history to buffer these effects. Disrupted synchrony in offspring needs and food abundance due to changing patterns of climate has been observed in a passerine (Visser et al. 2006) and a loss of life history buffering in fur seals to increasing environmental variability (Forcada et al. 2008). A commonly shared concern is that species with less flexible life history traits are threatened by increased fluctuations in their habitat, especially in extreme environments. Is it therefore of vital importance to understand patterns in flexibility of traits and to be able to make predictions. A better understanding of selection on phenotypic plasticity in habitats with much thermal variation is not only of interest to evolutionary ecology, but can also aid predictions on thermal variance on a global scale.

Plastic responses to temperature are traditionally studied in plants (Bradshaw 1965) and model organisms like Drosophila (Hoffmann et al. 2003). Plants are thought to be highly plastic in many traits because they are sessile and cannot avoid environmental change by moving away. The focus has never been on soil organisms, which is remarkable because these organisms share exactly that particular characteristic with plants. Collembola and isopods are a globally significant group of soil organisms that play a major role in ecosystem functioning (Rusek 1998). Soil
organisms are exposed to quite different but well-defined and commonly more stable thermal conditions compared to other model organisms, such as *D. melanogaster*. Because of the different nature of the experienced thermal regime, coldhardening capacity (Bahrndorff *et al.* 2009*a*) and production of heatshock protein (Bahrndorff *et al.* 2009*b*) differ between soil-organisms and above ground species. Including soil organisms in studies on evolution of plasticity is therefore a valuable addition to the more commonly used model species.

**Outline and aim of thesis**

This thesis explores how differences in the level of phenotypic plasticity can evolve between habitats with different thermal conditions. The aim was to test the hypotheses that (i) the selective advantage of such differentiation in the level of plasticity is large enough that it can occur between populations on a small spatial scale, (ii) patterns in plasticity are different for life history traits and morphological traits, and (iii) the patterns in phenotypic plasticity can be adaptive.

The main study organism in this thesis is the springtail *Orchesella cincta*, which inhabits the litter layer of the soil. Thermal sensitivity of springtails is closely related to fitness (Hopkin 1997), and the response to temperature varies among populations occupying thermally stable and fluctuating environments (Bahrndorff *et al.* 2006). *Orchesella cincta* occurs in a variety of thermally distinct habitats and shows significant variation in thermal response of life history traits (Driessen *et al.* 2007; Ellers *et al.* 2008). For the study on differences in phenotypic plasticity on a larger geographic scale *Drosophila serrata* was used. *Drosophila* *spp.* are widely used model organisms with strong and well-known responses to temperature. *Drosophila serrata* is endemic to Australia and occurs along a natural gradient, creating the opportunity to test responses in plasticity to local thermal regimes in its natural habitat.

When populations experience substantial variation in environmental conditions, they may evolve phenotypic plasticity in response to varying local selection pressures. To test this, Chapter 2 explores the differences in level of phenotypic plasticity in juvenile growth rate of *O. cincta* from habitats with distinct thermal variation, i.e. forest and heath. These habitats are found in close proximity of each other and differ strongly in amplitude of temperature fluctuations but not in mean temperature. Juvenile growth rate was found to be more buffered in the highly variable habitat, i.e. heath.

To test whether this is a general trend for life history traits, Chapter 3 addressed other life history traits, i.e. development time of eggs, age at maturity and weight at maturity of the same populations. As the link between age and size at maturity often differs between males and
females, their optimal life history response may not be the same (De Block & Stoks 2003). Reaction norms were therefore measured separately for the sexes to reveal possible sex-specific responses. Genetic differences in frequency of neutral markers were also assessed in this chapter to measure level of gene flow between the habitats. The patterns for adult female weight were in agreement with the results of Chapter 2.

Because patterns in level of plasticity are expected to differ between life history traits and morphological traits, a combination of these traits were measured in the subsequent experiment. Chapter 4 describes this experiment in which two morphological traits and one life history trait were measured in eight Drosophila serrata populations along a natural gradient in Australia.

‘Only model organisms live in a world of endless summer’ (Bradshaw et al. 2004). Therefore the question whether the observed ecotype-specific reaction norms are adaptive was addressed in Chapter 5. Growth of O. cincta populations from different habitats was tested in a climate room set-up with two diurnally fluctuating temperature regimes; a temperature regime with low fluctuations simulating an average summer day for the forest, and a more extreme temperature cycle with high fluctuations for an exposed terrain like heath.

Egg size is also plastic in response to temperature; larger eggs are laid at lower temperatures. In Chapter 6 I investigate whether thermal plasticity in egg size results in a better resistance against thermal extremes. The hypothesis was that larger eggs have an advantage under extreme temperature shocks and the results are discussed in the light of a conservative bet-hedging strategy. Finally, I will discuss the results in Chapter 7.