Seasonal timing in a warming world

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Chapter 1

General introduction
“It is not the strongest of the species that survives,  
nor the most intelligent that survives.  
It is the one that is the most adaptable to change.”

L. C. Megginson  
(paraphrase inspired by the “Origin of species” by C. Darwin)

The complexity and function of organisms’ adaptations, which fascinated Darwin as well as many other scientists before and after him, is a central theme in evolutionary biology. Organisms have countless strategies to survive and reproduce (Stearns 1992). Their adaptations can be of many different kinds: from behaviour that allows escaping from too hot temperatures to an anatomic feature to prevent the attack of a predator or a coloration to be camouflaged with the substrate.

Over evolutionary time, this multitude of morphological, physiological and behavioural traits have been continuously shaped by natural selection. Evolution of adaptive traits is the result of the complex interactions between an organism and its current environment. Via the process of natural selection, novel characteristics conferring an advantage to an individual are positively selected. From evolutionary theory we know that a prerequisite for adaptive evolution is the presence of phenotypic variation which is heritable and give rise, in a specific environment, to fitness differences among individuals. When this heritable phenotypic variation is present in a population, selection pressures favour the genotypes of those individuals with higher fitness than the rest of the population and as a result, those favoured genotypes become more common in the population. However, adaptive evolution is not only limited to genetic changes of a given phenotypic trait, but it can also occur via changes in the genetics underlying the plasticity of a trait.

A trait is considered plastic when its value changes in response to environmental conditions. Phenotypic plasticity is defined as the property of a given genotype to express different phenotypes under different environmental conditions (Pigliucci 2001). A well-known example of morphological phenotypic plasticity is the water flea that develops a defensive helmet in presence of predators (Woltereck 1909). In long-lived individuals, traits repeated over lifetime as the number and the weight of offspring produced can also be phenotypically plastic (Albon et al. 1987). The range of phenotypic trait values of a genotype exposed to different environmental...
conditions can be described by a reaction norm which has two important parameters: the slope and the elevation. The slope indicates the degree of plasticity (i.e. the sensitivity of a phenotypic trait to an environmental variable) while the elevation is the mean value of the phenotypic trait in the average environment.

Phenotypic plasticity is a key process in adaptation. Adaptation via phenotypic plasticity can occur at different time scales and via different mechanisms. On short term, plasticity of a trait per se allows organisms to respond quickly to changes in their environment and to live in a wider range of environmental conditions. Furthermore, the plasticity of a trait can evolve, thus leading to adaptation on the long term. Evolution of phenotypic plasticity can occur via both changes in the elevation and the slope of the reaction norm. When selection acts on the slope a trait can become more or less plastic while if selection acts on elevation the mean value over all environment can increase or decrease. For example if the trait under study is plant height at different altitudes, when selection acts on increased slope (i.e. plasticity) the differences in plant heights at the extremes of the altitudinal range will increase. On the other hand, if selection acts on the elevation (i.e. trait value in the average environment) plant’s height will increase in all the latitudes studied.

The role of micro-evolution of reaction norms in adaptation to changing conditions is recently gaining growing attention. Selection on the slope of the reaction norm for timing of egg-laying has been shown to exist in a wild population (Nussey et al. 2005). Other studies have also emphasized the role of evolution of phenotypic plasticity on adaptation to changing condition (Charmantier et al. 2008). Overall, the analysis of the phenotypic plasticity and its evolution is of great importance when studying whether a population can respond adaptively to environmental change (Visser 2008; Chevin et al. 2010).

Seasonal adaptations
Among many factors, seasonality is a prime selective factor expected to result in adaptation of life-history characteristics of organisms living at temperate latitudes. In order to maximize their fitness organisms must adapt to the Earth’s 365-day revolution around the sun resulting in cyclical annual changes in light, temperature, rainfall and humidity. Many biological processes at the metabolic, physiological and behavioural level are altered to match the annual environmental variation. The most obvious and well-known examples related to these environmental changes are the migration patterns of birds and large mammals to avoid unfavourable conditions such as harsh winters and dry seasons. Seasonal adaptations are several and include hibernation in rodents, dormancy in plants or diapause in insects. To avoid harsh
conditions, cold in winter or heat in summer, many insects undergo diapause, a state of reduced metabolism during which morphogenesis is reduced, resistance to environmental extremes is increased and behavioural activity is reduced (Tauber et al. 1986).

In a broader perspective, seasonal adaptations such as migration or diapause are not only important for surviving a specific period of the year, but often are fundamental for the overall synchronization of entire life cycles to seasonal availability of different resources. For example, food availability for many species is strongly influenced by seasonality. Most birds’ species living at temperate latitudes time the birth of their offspring in spring when food availability is highest. Traits relative to the temporal dimension, such as timing of reproduction or transition from one life stage to another, can have strong fitness consequences.

Seasonal timing is the key theme of this thesis. In the struggle for survival and reproduction organisms have to get the timing of their biological activities right. Physiological and developmental preparations must be made in advance of the actual seasonal event. Thus, almost all organisms use environmental cues, such as changes in photoperiod, temperature, humidity, population density or food availability to anticipate seasonal changes. Seasonal cues are species-specific and often there is only a restricted period of time during which the species is responsive to the cue. Furthermore, it is essential that the cues provide accurate and reliable information.

The annual change in photoperiod is the most reliable seasonal cue. It is the most precise signal for seasonal change and it is invariant from year to year. Next to photoperiod, temperature also plays a pivotal role as seasonal cue. Temperature becomes important in the immediate environment, fine-tuning the timing and the rate at which biological activities such as growth and reproduction occur. Temperature has direct consequences for metabolic rates, activity patterns and developmental rates, especially in ectotherms. In many insect species, temperature patterns strongly affect timing of emergence, growth or diapause. Temperature patterns also play an important role in timing of reproduction in vertebrates. Although birds use mainly photoperiod as primary cue inducing onset of reproduction (Dawson et al. 2001), temperature has a crucial role in timing of reproduction, in years with high spring temperatures many species reproduce earlier (Visser et al. 2009). However, the causal mechanism between temperature and timing of reproduction is still unclear in birds (Caro et al. 2013b). The regulation of seasonal timing mechanism is complex and that is why to make progress toward a complete understanding of them, the collaboration between physiologist, ecologist and chronobiologists has been advocated (Visser et al. 2010).
Box 1.1: Endogenous Biological Clocks

Nearly all organisms, from eubacteria to humans, possess endogenous timing systems responding to external cues to anticipate the daily and annual changes in the environment. We can identify at least two types of clocks: a circadian clock that has a periodicity of approximately 24-h and a circannual clock that ticks around the 365-days annual cycle. In natural conditions, the clocks are synchronized with the environment, a process referred to as entrainment. Cyclical annual variations in photoperiod, temperature or rainfall are the prominent environmental factors entraining the clocks. In absence of external stimuli (Zeitgebers), as in constant darkness, the rhythm free-runs according to the organism own endogenous circadian system. Experiments in animals kept under constant conditions of photoperiod, temperature and food availability showed that both circadian and circannual rhythms persist for long times. Extensive work on avian clocks by Gwinner showed that circannual clocks exists in birds and that they regulate timing of activity, reproduction, migration and many other life history traits (Gwinner 1986; Gwinner 2003). The circannual clock also determines how sensitive an animal is to external cues depending on the period of the year (Helm et al. 2009).

Effects of climate change on seasonal timing

The Earth’s temperature is currently increasing at an unprecedented rate and this trend is expected to continue (IPCC 2013). In the past decades many studies have shown evidence that the recent changes in climate have already affected a variety of biological systems (McCarty 2001; Walther et al. 2002). Ecological and evolutionary consequences of climate change on the timing of seasonal events in plants, insects, and birds are well documented (Parmesan et al. 2003; Parmesan 2006). In insects, increased ambient temperatures have direct consequences for metabolic rates, activity patterns and developmental rates. Consequently, in many species both an earlier termination of diapause and prolongation of growing season occurred in parallel with global warming. In multivoltine butterflies and moth long-term studies have shown changes in voltinism with an increased number of generations (Altermatt 2010).

Effects of climate change on phenological traits have been shown to be disruptive in many species leading to extinction in some populations (Both et al. 2006). Despite its negative consequences, climate change can be seen as a unique opportunity to
study adaptive evolution as the environment that the organisms experience is rapidly changing. In fact, nowadays selection pressures are changing and many life-history traits are currently under directional selection (Visser et al. 1998; Nussey et al. 2005; van Asch et al. 2007a). However, so far, experimental evidences of genetic adaptation to climate change are still scarce (Gienapp et al. 2008; Merilä 2012).

A pressing question is whether organisms will be able to cope with the changes caused by the increasing temperatures. The answer to this question is complex and requires a complete understanding of the interaction between the species studied and its environment. First, it is fundamental to identify which traits are under selection and which are the environmental factors underlying the selection pressures. Next, it is necessary to know how the environmental factors affect fitness. Most phenological traits are phenotypically plastic in response to environmental conditions. At the individual level, plastic responses can increase fitness, allowing adaptation on short-term, but when the environment is directionally and steadily changing, plasticity alone is likely to be not enough to adapt to the changing conditions and microevolution is needed (Visser 2008). Thus, to forecast the rate of adaptation to environmental change, standing genetic variation and selection pressures need to be estimated. Long-term field studies coupled with experiments in controlled conditions are a great opportunity to study how species respond evolutionary to changing conditions. Long-term field studies on phenology can provide measurements of fitness and selection pressures. In long-lived species it is possible to measure plasticity of phenological traits in different environments (e.g. egg-laying date of the same individual across several years) and record environmental variables such as temperature, precipitations or snow cover.

In the traditional quantitative genetic analysis, to predict adaptation, heritability of the trait under selection is estimated and selection pressures are quantified. Recently, a novel approach also aiming to forecast species’ response to changing conditions is emerging. In the traditional approach, standing genetic variation is calculated under the present conditions but used to forecast response which will take place under different conditions (i.e. the changed environment). There is however evidence that both genetic variation and selection pressure estimates can differ under distinct environments (Husby et al. 2011). Husby and colleagues show that for a phenological trait, timing of egg-laying, the amount of additive genetic variation ($V_a$) differs depending on spring temperatures and suggest these differences could influence the speed of micro-evolution. In addition, other studies have shown that heritability of the trait studied is lower under stressful conditions, although less is known about $V_a$ (Hoffmann et al. 1999; Charmantier et al. 2005). Ignoring this covariance between genetic variation in the trait itself and environment in which the trait is expressed is likely to introduce a bias when making evolutionary predictions.
for future scenarios. Thus, the study of the genetic variation in the mechanisms underlying a phenotypic trait, rather than the genetic variation underlying the trait itself, has been suggested to provide more accurate predictions (Sultan 2007; Visser et al. 2010).

To measure the genetic variation in the components of the underlying mechanism, the first step is the precise understanding of the mechanisms underlying the trait of interest. In the context of this work, what I refer to as “mechanisms” can be intended as for example how external and internal environmental cues, physiological pathways or genetic expression are translated in a given phenotypic trait. A (physiological) mechanism can be for example a specific part of the physiological cascade preceding avian reproduction or the influence on the rate of enzymatic reactions in insects. Once the necessary information on the proximate causes of a given phenotypic trait under selection is provided, the next step is to estimate the additive genetic variation present in these underlying mechanisms. With this knowledge we can then predict upon which “wheels” selection can act.

To address this question I used in this thesis a simplified food chain of a plant-insect-bird as a study system. An important feature of this system is the phenological match between the herbivore insect and its host plant and the bird’s offspring highest food demand and the peak in caterpillar biomass.

**Box 1.2: The study system: the simplified food chain of the oak-winter moth-great tit.**

In this thesis, I investigated seasonal timing mechanisms in the simplified food chain of oak (*Quercus robur*), winter moth (*Operophtera brumata*) and great tit (*Parus major*). At each trophic level, the phenological synchrony with the lower trophic level is crucial for fitness. As the oak’s buds burst in the spring, the winter moth’s eggs hatch as the newly hatched larvae can only feed on the young leaves. Later in spring, great tits need to feed their chicks and the caterpillars, rich in proteins, represent a high-quality diet. The phenological mismatch between the bud burst and the winter moth egg-hatching has strong fitness consequences on the newly hatched caterpillars (van Asch et al. 2007b). Fresh oak’s leaves are available only for a restricted amount of time, thus the timing of egg-hatching is of fundamental importance for the survival and growth of the winter moth newly hatched caterpillars.
Temperature plays an important role in this system as both oak bud burst and egg-hatching are temperature-dependent and in the last decades, due to the increased temperatures, both phenologies of bud burst and egg-hatching have advanced. However, winter moth egg-hatching has responded more strongly to temperature leading to a decreased synchrony with the oak bud burst. Previous studies by van Asch and co-authors have shown that timing of egg-hatching is under directional selection for later egg-hatching (van Asch et al. 2007a) and given that genetic variation in the egg-hatching reaction norm also exists, timing of egg-hatching is expected to evolve.

Timing has also major fitness consequences higher up in the food chain. The seasonal caterpillars’ peak in biomass lasts only for a few weeks, thus, great tits have a very restricted period of high food availability. Great tits must synchronize their nestling’s feeding period with the caterpillar phenology in order to fully exploit their primary food resource. Across years, there is a large variation in the date of the caterpillars’ peak in biomass and, thereby also in the optimal egg-laying date for great tits. Birds are plastic in their laying date and they make use of environmental cues and internal timing mechanisms to time the onset and termination of the reproduction. There is evidence that temperature directly affects time of reproduction (Schaper et al. 2011; Visser et al. 2011) although the mechanisms is not yet clearly elucidated (Caro et al. 2013b). In warm years, when the caterpillars’ peak in biomass is earlier, the mean population egg-laying date is also earlier. As in the oak and the winter moth system, also great tits are advancing their breeding phenology forward in time, but at a slower rate. Over the past decades, caterpillars’ peak in biomass has advanced more than bird’s egg-laying date. As a result, the time interval between the food peak and the date that the chicks’ needs are highest is increasing, which can have severe consequences on both the number of fledglings and their body mass at fledging (Visser et al. 2006). However, although the fitness consequences of the phenological mismatch are severe on individual birds, at the population level the negative impact of climate change is mitigated by density-dependent dynamics (Reed et al. 2013).
General introduction

Aim of this study and thesis outline

The focus of this thesis is the study of seasonal timing in the food chain of the oak-winter moth-great tit. As temperature increased over the last decades, both phenologies of the host plant, the oak, and the herbivorous insect, the winter moth, have advanced. However, timing of egg-hatching in the winter moth has advanced more than the oak bud burst leading to the phenological mismatch and increased selection for later egg-hatching. As a result of the earlier phenology of the insects, at the higher trophic level of the food chain, a phenological mismatch between the great tit offspring’s food demand and the peak in caterpillars’ biomass has occurred, leading to enhanced selection for earlier egg-laying on the birds.

In the beginning of this thesis, I present the evidence of adaptation of the winter moth to warmer temperatures via microevolutionary shift in timing of egg-hatching. Next, I describe how different environmental cues, in particular temperature and photoperiod, influence the phenology of the entire life-cycle in the winter moth and the timing of reproduction in the great tit. Then, I describe how the underlying mechanism translates the environmental factors in the phenotypic expression and, finally, I introduce a preliminary analysis of the additive genetic variation in the mechanisms underlying seasonal timing. I conducted this research using experimental and theoretical approaches in combination with observational data collected in the field. The work presented here represents the first step to the understanding of the genetic variation underlying the mechanisms of seasonal timing. My overall aim was to deepen our understanding of proximate mechanisms underlying seasonal timing and ultimately investigate the genetic variation present in the mechanisms themselves in order to forecast the rate of adaptation to changing conditions. From different angles, all research presented in this thesis was aimed to answer the unifying question: how will organisms respond to climate change?

In Chapter 2 I report the evolutionary response of the winter moth egg-hatching to warmer temperatures using both long-term field data and an experimental approach. In this chapter we present experimental evidence that winter moth eggs have become less sensitive to temperature, as predicted from a genetic model. Using long-term field data we also show that, as a result of the changes in temperature sensitivity, the phenological match between oak bud burst and egg-hatching has been restored.

In Chapter 3 we explore into detail the role of temperature on winter moth egg development. Experimental evidence shows that the effect of temperature on egg developmental rate is not linear during development. At later developmental stages, the effect of temperature on developmental rate becomes larger. Furthermore, in this chapter we present a novel physiological model to predict winter moth egg-hatching date, that incorporates the interaction between developmental rate and
developmental stage.

Chapter 4 and 5 examine the role of photoperiodic cues on the winter moth seasonal timing. In Chapter 4 we investigate the influence of the photoperiod on timing of egg-hatching. We show that eggs do not use the photoperiodic information to time their hatching. However, despite the lack of a photoperiodic effect, we still find large variation in timing of egg-hatching. Thus, we suggest that the micro-evolutionary change in egg-hatching (described in Chapter 2) is likely to involve a change in the mechanisms underlying winter moth response to temperature, and not to photoperiod.

In Chapter 5 we ask the question whether in the winter moth the photoperiodic information received during the larval stage (i.e. as caterpillar) is used to synchronize its life-stages with the annual variation in environmental conditions. Our results show that winter moths can use photoperiodic information acquired during the larval stage to set the timing of their annual program by adjusting the duration of subsequent life-cycle stages.

Chapter 6 deals with the single experiment on the great tit in this thesis. In this chapter we asked the question whether the response to selection for earlier egg-laying may be hampered by the elevated energetic costs of earlier breeding. To test this hypothesis we attempted to advance timing of reproduction in a wild great tit population in order to measure the fitness consequences of earlier egg-laying by modifying the bird’s hypothalamic photoperiodic perception. The manipulation lead to an increase in gonadal size but no effect was found on egg-laying date. These results open up to further questions concerning other possible environmental or social factors playing a role in regulating egg-laying decisions in the great tit.

Finally, a summarizing discussion of the results and proposed future research directions are presented in Chapter 7.