General introduction 1

Dealing with the unknown

Temporal variation in abiotic and biotic variables such as temperature, rainfall, food availability or predation pressure profoundly affects the abilities of organisms to survive and reproduce successfully. Most organisms are remarkably flexible in the face of such heterogeneity in the quality of their habitat, and have evolved a range of behavioural, physiological or morphological responses (Beldade *et al.* 2011; Piersma & van Gils 2010; West-Eberhard 2003; Whitman & Ananthakrishnan 2009). Such flexibility is can be regarded as adaptive phenotypic plasticity, which can formally be defined as 'the property of a given genotype to produce different phenotypes in response to distinct environmental conditions' (Schlichting & Pigliucci 1998). Some notable examples of phenotypic plasticity are briefly described in Box 1.

When environments are seasonal—and thus predictable—there is scope for anticipating and preparing for changes in the environment before they occur. Seasonal plasticity is a widespread feature of animal life, and includes diverse adaptations including migration, diapause and plumage moult (Denlinger 2002; Piersma & van Gils 2010). Studying how organisms have adapted to these annual cycles of ecological opportunity and threat is a key topic for evolutionary ecology (Visser *et al.* 2010). In addition, understanding how animals cope with environmental challenges is an important requirement for predicting biotic responses to climate change (Hofmann & Todgham 2010; Meylan *et al.* 2012).

A special case of phenotypic plasticity is developmental plasticity, where the phenotypic changes induced by the environment originate during the course of development (Beldade *et al.* 2011). A striking example is environmental sex determination in many reptile species, in which the sex of the developing embryo is determined by the temperature at which the egg is incubated (Sarre *et al.* 2004). Studying developmental plasticity provides a unique window into the developmental processes that translate genotypes into phenotypes, and reveals how environmental modulation of these processes can be a source of phenotypic variation (Gilbert 2012; West-Eberhard 2003).

A powerful concept in studies of plastic responses is the reaction norm (Schlichting & Pigliucci 1998). Here, the phenotypic value for a trait is plotted as a function of environmental variation. Genotypes responding readily to environmental variation have steep reaction norms, whereas those less sensitive to the environment (i.e. more canalised) have flatter reaction norms (Fig. 1*a*). Measuring phenotypic variation for several genotypes across environments can reveal the extent of genetic variation for environmental responses (gene-by-environment interaction), a prerequisite for evolutionary change towards increased or decreased plasticity. If genotypes differ only in the intercepts of their reaction norms and not in the slopes, evolutionary change in plasticity is constrained within that population, at least in the short term (Debat & David 2001; Schlichting & Pigliucci 1998; Stearns 1989). In addition, reaction norms can also be compared among different traits to gain some insight into the extent of integration between these traits (Fig 1*b*). If traits have similarly shaped reaction norms they might also share the underlying developmental mechanisms generating the environment-sensitive responses. Such phenotypic integration can be driven

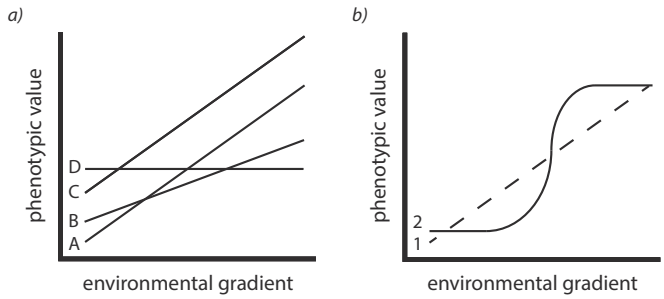

Figure 1. Reaction norms and phenotypic plasticity. *a)* Four genotypes illustrating different responses to the environmental gradient. Genotype A and B show different degree of phenotypic plasticity, as indicated by a different slope for the reaction norms, and express a different phenotype in some but not all environments. Genotype A and C show the same degree of plasticity, as the reaction norms have the same slope. The different intercepts for the reaction norms indicate that in each environment the genotypes express a different phenotype. Genotype D is a canalised genotype, showing no plasticity and expressing the same genotype across the environmental gradient. *b)* Two phenotypically plastic traits in the same organism. Trait 1 (dashed line) responds in a linear manner to the environment, expressing intermediate phenotypes across along the whole environmental gradient. Trait 2 (solid line) responds in a threshold-like manner to the environment, expressing a single phenotypic value over the lower part of the environmental gradient and switching to a different single phenotypic value over the higher part of the gradient. Note that trait 2 is canalised within each of the two parts of the environmental range, but the plasticity lies in the ability to switch to the other phenotypic state. If the environment is discrete, and individuals only experience one of two extremes, both traits display a polyphenism but produced from differing underlying mechanisms (Nijhout 2003).

by correlated selective pressures, for example due to variation between seasons affecting many organismal traits simultaneously (Ketterson *et al.* 2009; Nijhout 2003; Pigliucci 2003). Correlated selection pressures do not necessarily lead to shared mechanisms regulating the phenotypic reaction norms. Nevertheless, comparing reaction norms among traits can be a useful first step in revealing potentially shared underlying regulatory mechanisms of plasticity, and eventually understanding how such mechanistic integration has evolved.

Mechanisms of plasticity in animals

Phenotypic plasticity entails the production of different, alternative phenotypes from a single genotype, dependent on the experienced environment. Strikingly, the genetic information needed to produce those phenotypes is thus encoded in a single genome. This places the study of environment-dependent regulation of gene expression at the heart of a mechanistic understanding of phenotypic plasticity (Beldade *et al.* 2011; Bossdorf *et al.* 2008; Evans & Wheeler 2001; Gilbert 2005). In the simplest model, environmental conditions induce the expression of particular genes, leading to expression of a particular phenotype in that environment. Alternative environmental conditions induce expression

Box 1. Examples of phenotypic plasticity in the animal kingdom

In several groups of **horned beetles** (e.g. the dung beetle *Onthophagus taurus*), male larvae reared under high nutritional conditions develop into dominant males, with large horns that are used in male-male competition. Males reared on poor food not only fail to develop these anatomical structures but also employ a sneaker male reproductive tactic (Emlen & Nijhout 2001). Female larvae in **social insects** (e.g. the honey bee *Apis mellifera*, and the fire ant *Solenopsis invicta*) also respond to nutrition, and develop into fertile and long-lived queens when fed rich nutrients during development (Ross & Keller 1995; Smith *et al.* 2008). Larvae fed normal food develop into short-lived, less reproductive or sterile adult workers. **Wing dimorphic insects** such as crickets (e.g. *Gryllus spp.*) and aphids (e.g. *Acyrthosiphon pisum*) develop into a winged dispersive or into a flightless reproductive morph, dependent on a variation in density, photoperiod, temperature or food quality during development (Brisson 2010; Zera 2009). A special case of this is **phase polyphenism in locusts**, where solitary locust nymphs (e.g. *Schistocerca gregaria*) can switch to a gregarious phase upon crowding, forming devastating swarms that can last several generations (Simpson *et al.* 2011). **Diapause** is a ubiquitous feature of animal life in seasonal environments. When the growing season ends, animals switch to a metabolically dormant 'waiting mode' characterised by arrested development or delayed initiation of reproduction (Hahn & Denlinger 2011). In insects, there is a huge diversity in the life cycle stage in which diapause occurs, including in embryos (e.g. the Asian tiger mosquito *Aedes albopictus*; Lounibos *et al.* 2003), larvae (e.g. the Indian meal moth *Plodia interpunctella*; Bell *et al.* 1979), pupae (e.g. the green-veined white butterfly *Pieris napi*; Friberg *et al.* 2012), or adults (e.g. the fruit fly *Drosophila melanogaster*; Schmidt 2011). In temperate regions, diapause is typically triggered by changes in photoperiod, although temperature and other factors also often play a role (Bradshaw & Holzapfel 2010). The **nematode dauer stage** is also a form of diapause, when instead of continuing development to adulthood, larvae in poor conditions arrest development as stress-resistant, long-lived 'dauer larvae' (e.g. in *Caenorhabditis elegans*; Fielenbach & Antebi 2008). Many animals respond to **dietary restriction** with an extension in lifespan and a reduction in reproductive investment, as a presumably adaptive response to temporal reductions in environmental quality, reminiscent of a mild diapause syndrome (Nakagawa *et al.* 2012; Shanley & Kirkwood 2000). This form of plasticity has been described in nematodes (Sutphin & Kaeberlein 2008), fruit flies (Tatar 2011), and rodents (Swindell 2012) and likely provides health benefits in primates as well (Colman *et al.* 2009; Mattison *et al.* 2012).

Some notable and well-studied examples of phenotypic plasticity in insects. *a)* Horned and hornless male morphs of the dung beetle *Onthophagus nigriventris*. Photo courtesy of Doug Emlen. *b)* Queen, workers and larvae of the black garden ant *Lasius niger*. Photo courtesy of Romain Libbrecht. *c)* Solitary (left) and gregarious (right) *Locusta migratoria* locusts. Photo courtesy of Gabriel Miller, reprinted from Simpson *et al.* (2011) with permission from Elsevier.

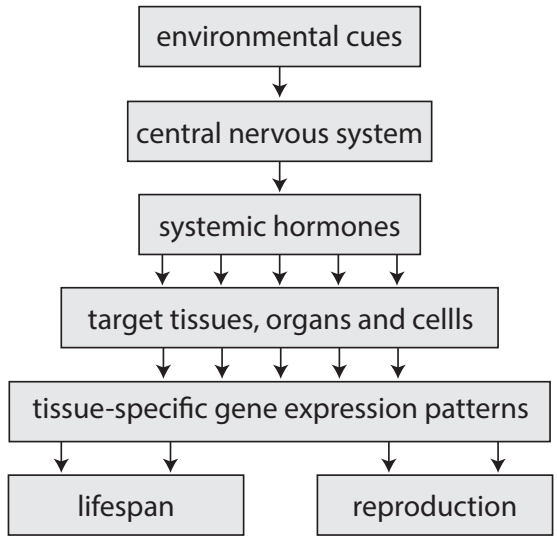

Figure 2. Potential mechanisms regulating phenotypic plasticity in life histories. Environmental variation, for example a reduction in nutritional levels, is processed by the central nervous system. Allocation decisions are translated via systemic hormone signalling to target tissues, which can respond in a tissue- or trait-specific manner, dependent on the local sensitivity to the hormone. This results in altered gene expression patterns in these tissues in response to the hormone signal, which affects the phenotype. Together, these phenotypic changes contribute to an adjusted life history strategy, for example a thriftier phenotype with reduced investment in reproduction and increased investment in somatic maintenance. This framework allows a centrally regulated, coordinated response to the environment as well as local trait-specific fine tuning of the response. Adapted from Tatar *et al.* (2003).

of other genes, leading to a different phenotype in the other environment. A pivotal mechanism linking centrally processed environmental cues with regulation of gene expression in the various tissues and cells responsible for the phenotypic changes, are hormones (Evans & Wheeler 2001; Finch & Rose 1995; Gilbert 2005; Zera *et al.* 2007). On the one hand, systemic hormone titres are centrally regulated from the central nervous system in response to signals sensed from the environment. This allows the organism to mount a systemic, integrated and coordinated response to environmental variation. On the other hand, how the tissues and cells that ultimately bring about the phenotypic changes respond to the hormone is a local property of those tissues (Fig 2). Hormone sensitivity can be regulated via variation in expression of hormone receptors, intracellular activity and localisation of those receptors, and chromatin state of the genomic transcriptional targets of the hormone. Whichever way it is achieved, local hormone sensitivity allows for a cell-, tissue- or trait-dependent differentiated response to the circulating hormone (Klowden 2007).

In almost all cases of adaptive plasticity studied in animals, hormones play a crucial role in translating an environmental stimulus into expression of alternative phenotypes (Beldade *et al.* 2011; Finch & Rose 1995; Nijhout 2003). One important hormonal pathway that has been studied in this context is Insulin signalling in *Drosophila melanogaster*. The circulating hormones are a class of Insulin-like peptides (ILPs) that are produced mainly in the brain and whose expression is determined by the organism's nutritional state. Under high nutritional conditions, ILPs bind to Insulin Receptors in the target tissues, where they activate an intracellular phosphorylation cascade that leads to cytoplasmic localisation and deactivation of the growth-inhibiting transcription factor FoxO. Disruption of this nutrient-sensing pathway by genetic mutation in some of its components (e.g. the Insulin Receptor Substrate) results in lifespan extension and often a reduction in fecundity (Partridge 2010; Tatar *et al.* 2003). FoxO has many transcriptional targets, which are presumably responsible for the observed phenotypic effects, either directly or via additional layers of hormonal and transcriptional regulation (see Alic *et al.* 2011).

Ecdysteroids are another important class of insect hormones. Their canonical function is that of a developmental timer in the larval stage, with short pulses of high concentrations signalling the transition to the next larval moult or to pupation. Unlike ILPs they are not peptide but steroid hormones. In the cell, Ecdysteroids bind to nuclear hormone receptor complexes that also act as transcription factors, containing both ligand binding and DNA binding domains (Klowden 2007). The genomic transcriptional targets of Ecdysteroids are numerous, and include many other transcription factors (Gauhar *et al.* 2009). In addition to their role as developmental timers in the larval stage, Ecdysteroids have also been implicated in diapause regulation (Denlinger 2002) and in adult female reproduction (Schwedes & Carney 2012).

Plasticity, development, and ageing: a life history perspective

Life history theory aims to explain the diversity in life history traits such as growth rate, size at maturity, reproductive investment and lifespan in relation to spatial and temporal variation in ecological opportunities. A key component of life history theory is understanding observed positive and negative correlations between life history traits (e.g. trade-offs) as a result of nutrient acquisition and their allocation to competing physiological functions. Life history strategies can thus be interpreted as optimal allocation decisions, maximising fitness given a particular environment (Boggs 2009; de Jong & Noordwijk van 1992; Roff 1992; Stearns 1992). One typical prediction of such environment-dependent variation in life history strategies is a trade-off between reproduction and lifespan. When resources are abundant, rapid reproduction is favoured over investment in a durable body, resulting in a short lifespan. Facing (temporarily) harsh conditions, a strategy of delayed reproduction and increased investment in somatic repair is instead favoured, resulting in increased lifespan.

Life history theory thus sets the stage for an evolutionary understanding of ageing, including in humans. Termed the disposable soma theory, ageing is understood to evolve in this context as a by-product of selection for investment in early reproduction at the cost of investment in a durable soma and late survival (Kirkwood 1977; Kirkwood & Rose 1991). This theory links evolutionary theories of ageing, focusing on life history trade-offs, with a mechanistic perspective, focusing on molecular pathways affecting lifespan. It can make predictions of what classes of genes might be involved in variation in ageing, such as those involved in somatic repair. The disposable soma theory therefore provides a powerful integrative framework for a mechanistic and evolutionary understanding of ageing (Zwaan 1999).

Two hypotheses have been proposed to try to understand human ageing and age-related morbidity from an evolutionary perspective. The 'thrifty genotype hypothesis' aims to explain the dramatic increase in prevalence of obesity and metabolic syndrome among affluent populations (Neel 1962). Briefly, it assumes that our life history strategy has evolved in a feast-famine environment. Such an environment would favour genotypes that maximise glucose intake and rapid storage of any excess resources into fat reserves, providing energy in recurring times of food scarcity. In the modern food-rich environment these 'thrifty genotypes' are hypothesised to have become detrimental because resource accumulation when energy-rich foods are constantly available can lead to obesity, insulin resistance and other related health problems (Neel 1962). However, this hypothesis makes many assumptions regarding ancestral diets and selective forces which are very hard to test (Bouchard 2007; Zwaan 2003). In addition, the few genes that have been found to have pleiotropic effects on fitness between pre-industrial and modern environments are not related to metabolism, but to immunity (Kuningas *et al.* 2009).

The second hypothesis aimed at understanding human ageing patterns from a life history perspective is the 'thrifty phenotype' or Barker hypothesis, which is strongly focused on development. It stems from the observation that fetal malnutrition, especially during early gestation, has a wide range of adverse effects on health at middle and old age. It is proposed that altered Insulin signalling mediates this developmental response (Hales & Barker 1992, 2001). Moreover, it has been hypothesised that it is an adaptive response to variation in nutrition (Gluckman *et al.* 2005), a hypothesis known as the 'predictive adaptive response'. This hypothesis states that the developmental nutritional response evolved as an adaptation to fluctuations in food resources. In this scenario, the physiological imprint of malnutrition during embryonic development would be predictive for future conditions of scarcity and prepare offspring for said conditions (Gluckman & Hanson 2004; Gluckman *et al.* 2005). The observed adverse health outcomes would then be a result of a mismatch between developmental and adult food conditions. However, this adaptive explanation has been heavily criticised by evolutionary biologists (Rickard & Lummaa 2007; Wells 2007), most notably because it would require conditions during gestation to be predictive for those during middle age.

Although the observations are real, a more likely scenario would be that the developmental response is not adaptive for adult life, but rather for early childhood survival. As this period is closer in time to embryonic development, nutrition levels experienced during gestation

might have some predictive value here. In this scenario, the benefits of a thrifty phenotype during early life would have detrimental consequences later in life, and the developmental response would thus represent a trade-off between early and late life survival. Alternatively, fetal malnutrition in humans may always adversely affect the offspring's juvenile and adult health, irrespective of adult nutritional conditions (i.e. 'scarring', see Brakefield *et al.* 2005; Rickard & Lummaa 2007). These theories may sound plausible, but lack clear or convincing hypotheses for genetic and physiological mechanisms involved. Using model organisms to study these hypotheses unleashes the power of manipulative experimentation to uncover mechanistic and evolutionary links between development and adult health span. For example, distinguishing between adaptive (mismatch) and non-adaptive (scarring) explanations for the detrimental effects of fetal malnutrition requires evaluating fitness in response to variation in juvenile and adult environments separately. Extending such an approach with an ecological component is particularly important for understanding human ageing and ageing-related morbidity.

Mechanisms of life history plasticity: towards an ecological approach

It is hard to overstate the importance of model organisms in contributing to our mechanistic understanding of ageing. The powerful genetic methods available for *C. elegans* and *D. melanogaster* have allowed an unprecedented dissection of the physiological and genetic regulation of ageing and of plastic food responses (Fontana *et al.* 2010; Toivonen & Partridge 2009). Of particular relevance here is the nutrient-sensitive plastic reallocation known as the dietary restriction (DR) response. In many, although not all, animals studied so far, moderate DR results in an increased lifespan, usually accompanied by a decreased reproductive output (Nakagawa *et al.* 2012). This can be interpreted as an adaptive response that evolved to cope with temporary reductions in nutrient availability. Animals respond by activating physiological processes that promote lifespan extension, such as somatic repair, and repressing reproductive processes while retaining the ability to reproduce later when conditions improve (Kirkwood & Shanley 2005; Shanley & Kirkwood 2000). However, adaptive explanations are often hard to test in traditional model organisms. For most of these, little is known about the natural ecological situation, where the mechanisms underlying lifespan extension in response to DR presumably evolved. This is especially problematic for species where the average lifespan in the field is much shorter than in the laboratory, as is the case for *D. melanogaster*. Genetic manipulation studies may reveal molecular pathways with major effects on lifespan and reproduction under laboratory conditions, but this does not mean that such effects are relevant under ecologically realistic conditions. These challenges highlight an important limitation of the traditional model organisms. To understand how and where mechanisms contributing to life history variation evolved, it is necessary to look beyond the laboratory models and supplement them with organisms more amenable to ecological studies (Partridge & Gems 2006).

1

The study of phenotypic plasticity has a rich and productive history in the evolutionary and ecological literature, and plasticity of life history traits has been widely recognised as highly relevant for fitness in variable environments (Nylin & Gotthard 1998; Stearns 1989). This provides an excellent resource for studying, in an evolutionary context, the mechanisms underlying environmental responses that contribute to variation in ageing (Flatt & Schmidt 2009). Impressive progress has been made in connecting the traditions of evolutionary biology with molecular genetics and physiology towards an integrative understanding of mechanisms of life history evolution, including phenotypic plasticity (Flatt & Heyland 2011b; Zera & Harshman 2001; Zera *et al.* 2007). In particular, the emerging field of ecological and evolutionary genomics holds great promise for using high-throughput DNA sequence data to probe how the environment and the genome interact to produce ecologically relevant phenotypes. Despite the complex nature of life history phenotypes, comprising the interactive effects of many physiological, morphological and behavioural traits (Flatt & Heyland 2011a), considerable advances are being made (Aubin-Horth & Renn 2009).

Ecological and evolutionary genomics aims to understand how genes function in the real world outside the laboratory. It proposes to develop an Ecological Association Ontology, conceptually similar to the existing Gene Ontology framework but instead aimed at an ecological annotation of genes (Pavey *et al.* 2012). An exciting development in this light is the more than exponential decrease in DNA sequencing costs over the last decade, and the corresponding explosion of available analysis tools. This has allowed ecologists and evolutionary biologists to develop and deploy a variety of genomic tools to organisms studied for their ecological and evolutionary relevance (Ekblom & Galindo 2011; Orsini *et al.* 2013).

The obvious drawback of using non-model organisms is the lack of available resources, in particular genetic tools. Although genomics has now become accessible for ecologists, other tools such as manipulation of gene function are still largely lacking for many non-model species (Sommer 2009). Developing such tools for these organisms requires substantial investment in time and resources, and this is especially difficult for organisms that are only being studied by a handful of research groups. For example, RNA interference (RNAi) has revolutionised the study of gene function and expanded it into systems where this was not previously possible (Boutros & Ahringer 2008; Tomoyasu *et al.* 2008). This has allowed not only to test involvement of candidate genes in phenotypes of interest (e.g. Emlen *et al.* 2012) but also the application of genome-wide functional screens (e.g. the iBeetle project in *Tribolium castaneum*, see http://ibeetle.uni-goettingen.de/). However, in Lepidoptera RNAi has not lived up to its promise. Results in this group of insects have been mixed and it is unclear whether this is solely due to technical issues or whether Lepidopteran biology might make this group less responsive to RNAi (Terenius *et al.* 2011).

Despite the technical challenges involved, adding an ecological component to the study of mechanisms underlying life history variation will also contribute to a better understanding of human ageing and ageing-related morbidity. As the knowledge of genetic

factors underlying human ageing increases, it is becoming clearer that the interaction between genes and the environment is critical in determining a healthy lifespan. Our genetic makeup was shaped by very different selective forces than those in place today. Using laboratory models for which knowledge on natural life histories is at best anecdotal will only get us so far in understanding how our old genes produce healthy and unhealthy phenotypes in the new modern environment. Combining extensive ecological knowledge with the ability to perform manipulative experiments in the laboratory, the butterfly *Bicyclus anynana* provides an excellent model system to study mechanistic and evolutionary links between development and ageing.

Study system: the seasonal butterfly *Bicyclus anynana*

In this thesis, I use a captive laboratory population of an ecologically well characterised butterfly to try to understand mechanisms of developmental plasticity in life histories. The Nymphalid butterflies belonging to the genus *Bicyclus* comprise *ca.* 90 species distributed throughout Africa, including Madagascar. *Bicyclus anynana* inhabits the seasonal grassland savannahs and open woodland ecosystems of tropical and subtropical East Africa, roughly from South Africa to Ethiopia. *B. anynana* adults feed on fermenting fruit fallen from the trees, and the larvae are grass feeders. The savannah habitat is characterised by strong seasonality. In Malawi, where the laboratory population originates, the dry season is relatively cool and the wet season is warm. During the dry season, grasses dry out completely, precluding survival for any caterpillars. Adult butterflies do get by, feeding on the limited fruit that is still occasionally available. After bad times come good times, and the rains of the wet season bring larval food in the form of fresh grasses.

To cope with these seasonal fluctuations in ecological opportunities, *B. anynana* is able to express a distinct life history strategy in each season. Adult butterflies bridge the harsh dry season by being relatively inactive and temporarily relinquishing reproduction. In contrast, wet season adults are active and reproduce readily. Another striking difference between the seasons is in the ventral wing pattern. Dry season adults have uniformly brown, cryptic wings, allowing them to rest inconspicuously among the dead leaf litter. In contrast, butterflies of the wet season display prominent eyespots along the margin of the wing, hypothesised to deflect predator attacks away from the vulnerable adult body when attached at rest (Fig. 3). Phenotypic plasticity is widespread in the *Bicyclus* genus and related genera, but this has only systematically been studied for wing pattern, not life history (Brakefield & Frankino 2009; Brakefield & Reitsma 1991; Brakefield & Zwaan 2011). Bringing this species into the laboratory and establishing a captive population has allowed a detailed examination of how *B. anynana* is able to express these two distinct phenotypes in the two seasonal conditions.

Developmental plasticity has turned out to be crucial. Larvae developing at cooler temperatures, which in the field occur over the transition from the wet to the dry season before host plants completely dry out, eclose with a dry season phenotype. When larvae experience high temperatures during development, which in the field indicate the

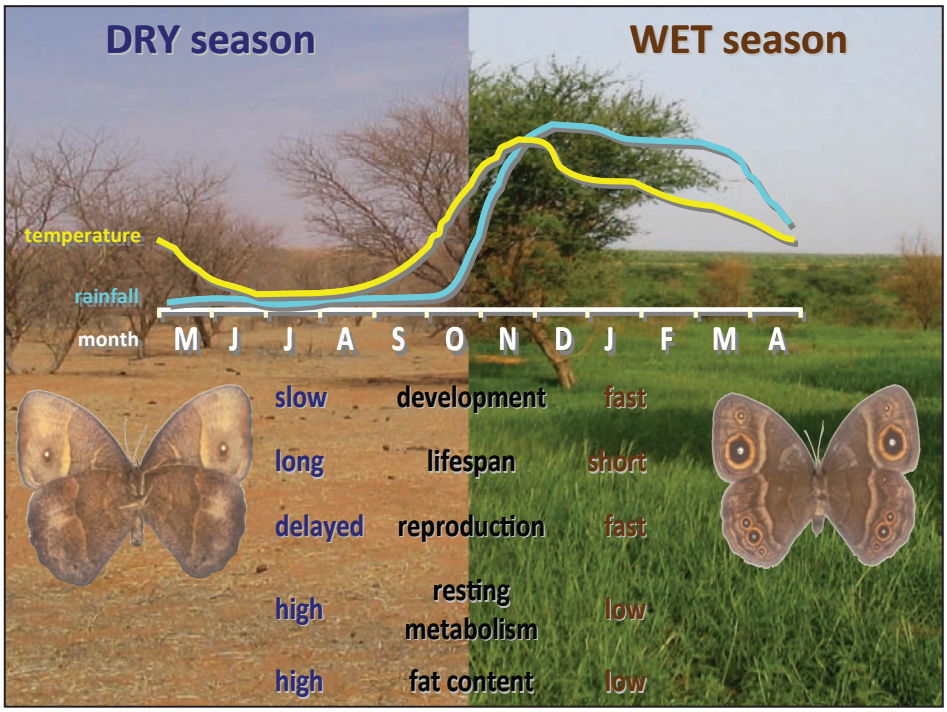

Figure 3. Seasonal phenotypic plasticity in *Bicyclus anynana*. Seasonal climate and alternative phenotypic morphs in *B. anynana* for the dry (left) and wet (right) season. The graphs at the top depict rainfall (yellow) and temperature (turquoise) throughout the year (horizontal axis), redrawn from Brakefield & Reitsma (1991). The table and photos of adult butterflies indicate phenotypic differences between the seasonal morphs. The photos in the background were taken at the same location on the savannah in Sudan during the dry and wet season, respectively, and serve to illustrate the seasonally fluctuating habitat quality (photos courtesy of Jonas Ardö, adapted from Ardö *et al.* (2008), used with permission).

wet season, they develop into wet season adults. Thus temperatures in the field act as a predictive cue for the forthcoming environment the adults will experience. Developing individuals are particularly sensitive to temperature during the last (fifth) larval instar and the early pupal period, although this has only been established for wing pattern plasticity. Young adults developed at alternative temperatures differ in a variety of traits related to life history, including egg laying rate, resting metabolic rate (RMR), size and fat content (Fig 3). However, temperature does not induce the full extent of life history plasticity as observed in the field. In particular, lifespans of up to six months as observed in the dry season in the field have never been observed in the laboratory. Wing pattern pigmentation is an irreversible process completed during the pupal stage, and young adults eclose with a dry or wet season wing pattern fixed for life. Life history on the other hand is more malleable. Adult butterflies are able to acclimate after a certain adjustment period when conditions change in the adult

stage from those experienced during the larval stage. Most notably, female egg laying rates in dry season-reared adults can increase from their initially low values if the females are placed in warm wet season condition as adult (Brakefield *et al.* 2007; Fischer *et al.* 2003). However, this acclimatisation does take some time and thus developmental plasticity is an important mechanism that allows adults to emerge with the best matched phenotype from the very start of adult life. This gives them a time advantage compared to if they had to rely solely on adult acclimation (Brakefield & Frankino 2009; Brakefield & Zwaan 2011).

Hormonal systems have emerged as crucial mediators of developmental plasticity in *B. anynana*. Individuals reared at alternative temperature conditions differ in dynamics of Ecdysteroid signalling during pupal development, with an early hormone peak in the wet season morph and a late peak in the dry season morph. Lines artificially selected to diverge in plasticity for ventral wing pattern recapitulate this difference in hormone dynamics when reared at a single temperature. Furthermore, exogenous Ecdysteroids applied to young pupae reared at dry season conditions are able to induce a wet season-like wing pattern (Brakefield & Frankino 2009; Brakefield & Zwaan 2011; Koch *et al.* 1996; see also Chapters 2 and 3 of this thesis).

An important and successful genetic tool that has allowed *B. anynana* to rise to prominence in evolutionary studies of wing pattern, seasonal adaptation and life history is artificial selection. Using an outbred laboratory population derived from the wild, it has been possible to obtain phenotypically divergent selection lines for a wide variety of traits, including body size, development time, wing pattern, starvation resistance and lifespan (Brakefield 2003; Brakefield *et al.* 2003; Pijpe *et al.* 2007; Pijpe *et al.* 2006; J. Pijpe unpubl. data). Antagonistic selection experiments on different traits or sexes have yielded important insights into the role of developmental and hormonal constraints in short term evolutionary change (Allen *et al.* 2008; Beldade *et al.* 2002; Zijlstra *et al.* 2002; Zijlstra *et al.* 2004).

Box 2. LifeSpan: integrating research into development and ageing

The work described in this thesis was carried out in the context of the **Network of Excellence LifeSpan**, a collaborative and integrative research programme aimed at studying mechanistic links between development and ageing. Central to LifeSpan was the realisation that ageing cannot be understood without considering the whole life history, including development. LifeSpan (January 2007 – December 2011) comprised of seventeen research groups from ten European countries, working on different aspects of ageing research. This provided the opportunity to interact between observational studies in humans and manipulative studies in model organisms. For example, genetic, epigenetic, transcriptomic and immunological aspects of human longevity were studied in several cohorts of long-lived people and their families, as well as in monozygotic twins. At the same time, model organisms (including. rodents, fruit flies, worms, butterflies, ants, worms) were used to identify novel ageing and longevity genes, as well as to functionally test involvement of candidate genes. Model organisms were also used to examine mechanisms underlying environmental responses, in particular how variation in juvenile conditions affects adult health and lifespan. In addition to generating and disseminating scientific knowledge, LifeSpan also provided a valuable platform for initiating and maintaining interdisciplinary collaborations (see for details http://www.lifespannetwork.nl/).

Another field where *B. anynana* has become an important model is evo-devo (Brakefield & French 1999). The marginal eyespots on the four wings have proved a fruitful system to study the genetics of morphological diversity, the role of modularity in evolutionary change, and the origin of evolutionary novelties (Beldade & Brakefield 2002; Brakefield *et al.* 2009; Saenko *et al.* 2008). The evo-devo studies in particular have pushed forward the development of genomic tools for this species. Genomic tools available today include several expressed sequence tag (EST) data bases, microsatellite and AFLP markers, a linkage map, BAC libraries and a custom designed microarray (Beldade *et al.* 2006; Beldade *et al.* 2009a; Beldade *et al.* 2009b; Conceição *et al.* 2011; Van 't Hof *et al.* 2008). Although originally developed for studies of wing pattern development, these tools have also started to be used for genomic analyses of life history variation (e.g. de Jong *et al.* 2013; Pijpe *et al.* 2011; see also Chapters 4 and 5 of this thesis). With several on-going sequencing projects, the genomic toolbox for *B. anynana* is only increasing.

Aims and outline of this thesis

The general aim of this thesis is to gain insight into the hormonal and transcriptional patterns that underlie developmental plasticity of life history in the seasonal butterfly *B. anynana*. An additional goal is to use these findings to enhance mechanistic and evolutionary understanding of variation in human health and ageing.

Using complementary approaches, the thesis deals with two main aspects of the seasonal adaptation in *B. anynana*. The first question is how the environment experienced during larval and pupal development induces the two adult seasonal forms via conserved hormonal pathways. In particular, I investigate the extent to which Ecdysteroids, known to be involved in wing pattern plasticity in this species, are also instrumental in mediating the response of adult life history to developmental conditions (**CHAPTERS 2** and **3**). The second major question covered in this thesis is what transcriptional changes in the adult are associated with the seasonal forms. Using a candidate gene approach (**CHAPTER 4**) in parallel to an unbiased screen (**CHAPTER 5**), I analyse gene expression variation in adults that have developed as larvae under alternative seasonal conditions. Finally, **CHAPTER 6** provides a broader evolutionary perspective and asks to what extent developmental plasticity, as measured in the laboratory, is retained in the rainforest species *Bicyclus martius* under relaxed selection.

In **CHAPTER 2**, I use a fine gradient of developmental temperatures to study the precise thermal response of adult life history traits involved in the seasonal adaptation. The extent to which the adult traits show similarly shaped reaction norms—from linear to threshold-like—may indicate whether the regulatory mechanisms underlying the thermal responses are shared among traits. Crucially, within the same experiment I also study thermal reaction norms for pupal Ecdysteroids and Juvenile Hormones, the mechanisms hypothesised to regulate the environmental response.

CHAPTER 3 provides a functional test of the involvement of Ecdysteroids in mediating developmental plasticity of the adult life history syndrome. First, it is established

whether application of exogenous Ecdysteroids during pupal development can induce the phenotypic changes normally induced by developmental temperature. Hormones are injected during the pupal stage, at four time points that represent different parts of natural Ecdysteroid titre dynamics. This is done for three different seasonal temperatures, allowing to test environment-specific windows of sensitivity. Within the same experiment, but not published in this thesis, adult wings of the injected individuals have been analysed to assess the effect of Ecdysteroids on wing pattern (R. Mateus *et al.*, unpubl. data). Second, in a follow-up experiment it is assessed to what extent the adult phenotypic changes induced by exogenous Ecdysteroids during development affect reproductive output, lifespan and starvation resistance. Together, these experiments establish whether Ecdysteroids play a functional role in translating predictive information on environmental quality during development into adaptive alterations in a suite of adult traits.

Next, **CHAPTER 4** examines how the seasonal forms differ in the expression of selected life history-related genes. As both adult seasonal forms develop from the same genetic background, environmentally induced phenotypic differences ultimately depend on transcriptional regulation. Young adults start their life expressing distinct life history strategies as end points of divergent developmental pathways. These pathways are in turn induced by the alternative seasonal conditions experienced as larvae and pupae, mediated by hormone signalling. I use qPCR to examine expression in young adults of 27 candidate life history-related genes, as putative molecular effectors of the two strategies. The genes are related to reproduction, immune defence, carbohydrate metabolism and lipid metabolism. Thus, a first goal is to characterise the adult seasonal morphs at the molecular level. A second goal is to understand whether genes known to be responsible for life history adaptation in other organisms are also involved in the seasonal adaptation in *B. anynana*. I analyse whether the selected genes are up- or downregulated in young adults differing in their developmental history.

In **CHAPTER 5**, microarrays are used to analyse how ageing affects the global transcriptional profile, and how this is modulated by the adults' seasonal developmental history. The first aim is to characterise the whole-genome transcriptional signature of ageing for this organism which has not traditionally used as a model in ageing studies. Special emphasis is placed on sex-specificity in ageing-related expression changes. The second goal is to analyse to what extent seasonal conditions experienced during juvenile development leave a life-long transcriptional imprint in middle-aged and old adults, when those conditions are no longer experienced. Finally, I compare the transcriptional response to ageing among cohorts reared under the alternative conditions. This allows assessing which transcriptional changes contribute to the alternative seasonal life histories, including lifespan.

Taking a broader evolutionary perspective, **CHAPTER 6** examines the fate of seasonally plastic traits in a butterfly under relaxed natural selection. *Bicyclus martius* inhabits the West-African rainforest, a generally wet season-like habitat with limited fluctuation in temperature, larval food availability or reproductive opportunities. The lack of seasonal exposure to harsh dry season conditions likely reflects a situation of relaxed selective

pressures, both on dry season-specific adaptations and on plasticity itself. This chapter aims to establish the extent to which *B. martius* has retained the ability to express alternative phenotypes when exposed in the laboratory to a range of temperatures not normally encountered in the field. In the savannah butterfly *B. anynana*, these temperatures induce plasticity for a variety of traits, some of which share a hormonal regulator (e.g. Chapters 2 and 3 of this thesis). Analysing variation between traits in environmental responses in *B. martius* allows determining the extent to which hormonal regulation precludes independent evolution of plastic responses among traits.

Finally, in **CHAPTER 7**, I summarise the findings presented in the previous chapters and provide a perspective on how these data contribute to a better mechanistic understanding of plastic responses as adaptation to environmental variation. In addition, I discuss how these findings can increase our knowledge on mechanisms linking development and ageing in humans, and how events during early development can affect health and lifespan.

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