Temperature effects on genetic and physiological regulation of adaptive plasticity

Mateus, A.R.

CHAPTER 1. EVOLUTION AND MOLECULAR MECHANISMS OF ADAPTIVE DEVELOPMENTAL PLASTICITY

ARA Mateus[1,2] & P Beldade[1,2]

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1-Instituto Gulbenkian de Ciência, Portugal
2-Institute of Biology, Leiden University, The Netherlands

DEVELOPMENTAL PLASTICITY

It has become clear that more than a filter of phenotypic variation during the trans-generational process of natural selection, the environment also plays a key role in generating variation during organismal development. In fact, some degree of an effect of the external environment on phenotype seems pervasive in nature, and is accounted for in classical evolutionary genetics by the environment and the genetic-by-environment components of phenotypic variation. However, until recently environmentally-induced variation, or variation altogether, was seen more as a nuisance in developmental biology. Research in that field typically focused on single (often inbred) laboratory strains of one of a handful of model organisms kept in constant (often very unnatural) laboratory environments. This situation is rapidly changing as new disciplines are emerging and growing. Evolutionary developmental biology (evo-devo) brought the focus to intra- and inter-specific (morphological) variation and its genetic basis (see Stern 2000). More recently, ecological developmental biology (eco-devo, or eco-evo-devo) has started to bring the focus to how the external environment affects organismal development and how this impacts evolutionary change (see Gilbert & Epel 2009).

Phenotypic plasticity is the property whereby a single genotype produces distinct phenotypes in distinct environments. Organisms have different ways of adjusting to the environmental conditions they live in, including alterations in behavior and/or physiology and/or morphology leading to a better match between phenotype and selective environment (examples in Table 1.1). The term

developmental plasticity is used to refer to those cases where the environmentally-induced variation is the product of changes in pre-adult development (e.g. coat color variation in laboratory mice that depends on maternal diet, Waterland & Jirtle 2003). This thesis will focus on adaptive developmental plasticity linked to changes in development affecting morphological traits, with emphasis on the physiological and molecular mechanisms involved in the environmental-regulation of development and in the evolution of this phenomenon.

Traditionally, studies of developmental plasticity have focused on the phenotypic responses to environmental variation and on its ecological role and underlying physiological mechanisms. Researchers have also explicitly addressed the evolution of plasticity and its contribution to adaptive evolution. A detailed analysis of those topics has been covered in a number of insightful books and reviews (e.g. Callahan *et al.* 1997, Nijhout 2003, Pigliucci 2001, Schlichting & Pigliucci 1998, West-Eberhard 2003). New technological and conceptual advances are now being recruited to unravel the molecular mechanisms of developmental plasticity (e.g. Aubin-Horth & Renn 2009, Gilbert & Epel 2009, Minelli & Fusco 2010). This has precipitated a tremendous expansion of information on these mechanisms and their relationship to evolution justifying the pertinence of new synthetic efforts.

Some key concepts in developmental plasticity

Developmental plasticity refers to the property by which the same genotype can produce different phenotypes through environmental regulation of development (see main text). At the other end of the spectrum (Braendle & Felix 2009), *canalization* (or, *robustness*) is used to describe those situations where development produces the same phenotype despite environmental (and/or genetic) perturbation (e.g. blue solid line in Figure 1.1, Flatt 2005). Both plasticity and canalization are not absolute properties of a developmental program: the development of a particular trait might show environmental-sensitivity during a specific time window and be highly robust outside of that. Reversible changes in adult phenotypes, often in behavior or physiology, correspond to a form of phenotypic plasticity sometimes referred to as *acclimation* (e.g. Brakefield *et al.* 2007, Wilson & Franklin 2002) to distinguish from effects on development.

Table 1.1 - Examples of developmental plasticity for selected animal systems.

Biological system and plastic trait	Examples of inductive cues	Ecological relevance	References
Wings in female pea aphids	Crowding Nutrition Photoperiod Temperature	Dispersion	Braendle *et al.* 2006
Wing polyphenism in locusts	Crowding	Solitary versus gregarious and migratory morphs	Pener 1991, Simpson *et al.* 2001
Horns in dung beetles	Nutrition	Mating strategies	Moczek & Emlen 2000
Castes in social insects	Nutrition Pheromones	Division of labour	Korb & Hartfelder 2008
Teeth-like denticles in diplogastrid nematodes	Nutrition	Alternative diets	Bento *et al.* 2010
Seasonal polyphenism in butterflies	Temperature Photoperiod Nutrition	Anti-predator strategy Thermoregulation	Beldade & Brakefield 2002, Nijhout 1999
Gender determination in vertebrates (e.g. reptiles, fishes, amphibians)	Temperature	Optimal sex ratio	Janzen & Paukstis 1991, Ospina-Álvarez & Piferrer 2008, Nakamura 2008
Gender determination in invertebrates (e.g. Daphnia magna)	Photoperiod Crowding Temperature pH Nutrition Salinity	Optimal sex ratio	Hobaek & Larsson 1990; Cook 2002
Morphological defenses in planktonic crustaceans (Daphnia spp)	Density of predators (assessed via kairomones)	Defense	Dodson 1974, Stabell *et al.* 2003, Stibor & Lampert 2000
Head-size in spadefoot toad tadpoles	Density of conspecifics (assessed via food levels)	Food resources	Pfennig 1992, Pfennig *et al.* 2006

Reaction norms are graphical representations of the environmental dependence of the phenotype. Developmental plasticity can manifest itself in the form of graded variation in phenotype or in discrete switches between alternative developmental trajectories. A reaction norm displays phenotypic variation across an environmental gradient (see Schlichting & Pigliucci 1998). It is often used for situations where this environmental gradient corresponds to a more or less linear grading in phenotype (e.g. yellow line in Figure 1.1), but it can also describe situations of (nearly) discrete alternative phenotypes (e.g. non-linear relationship as in the orange line in Figure 1.1). Importantly, reaction norms can be obtained for different "end phenotypes" (morphology, life-history, behavior) but also for "intermediate phenotypes" such as hormone titers, methylation patterns and levels of gene expression during development (e.g. Aubin-Horth & Renn 2009). The reaction norms for such different phases do not necessarily need to have the same shape (dotted versus solid lines in Figure 1.1). In fact, even invariant phenotypes (i.e. flat reaction norm represented by the solid blue line in Figure 1.1) can result from cellular and molecular processes that are plastic (e.g. dotted blue line in Figure 1.1) (see Braendle & Felix 2008). Reaction norms drawn for different genetic backgrounds allow an assessment of genotype-by-environment interactions (e.g. Debat *et al.* 2009, Ostrowski *et al.* 2000, Sarkar & Fuller 2003). The genetic-by-environment component of phenotypic variation translates into reaction norms of different shapes for different genotypes, while the environment component corresponds to non-flat reaction norms.

Polyphenism describes a situation where inter-individual variation in phenotype does not result from differences in genotype, but rather from differences in the environment (e.g. wing development in pea aphid females influenced by different environmental cues, Braendle *et al.* 2006). The term polyphenism is used for situations where alternative phenotypes are discrete (e.g. orange line in Figure 1.1) – even if, in some cases, intermediate phenotypes can be produced (e.g. intercastes in ants). To contrast with polyphenism, the term *polymorphism* is used for those cases where inter-individual variation in phenotype is due to differences in genotype, often single or few alleles of large effect (e.g. wing development in pea aphid males influenced by allelic variation at the *aphicarus* locus, Braendle *et al.* 2006).

Genetic assimilation describes an evolutionary process by which an environmentally-induced phenotype becomes genetically fixed, so that the environmental cue is no longer necessary for the expression of that phenotype (see

Pigliucci *et al.* 2006). The term *genetic accommodation*, on the other hand, is a broad term referring to evolutionary mechanisms whereby selection acting on quantitative genetic variation moulds a novel phenotype, environmentally-induced (but also one arising by mutation), into an adaptive phenotype (e.g. Suzuki & Nijhout 2006). The concept of genetic accommodation describes trans-generational mechanisms of (quantitative) genetic change that can both fine tune developmental plasticity or canalize development. In contrast, the term *phenotypic accommodation* has been used to refer to intra-generational adjustment between developmental variables that does not depend on genetic change (see West-Eberhard 2003).

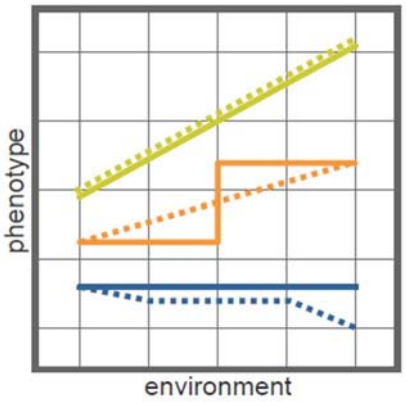

Figure 1.1 - Different shapes of reaction norms describing the environmental dependence of phenotypes produced from the same genotype. The lines can represent either end phenotype (solid) or some intermediate step such as gene expression (dotted), with different colors corresponding to different types of developmental-sensitivity to the environment. The blue example illustrates robust development, where even despite variation in underlying gene expression (non-flat dotted line), development always results in the same end phenotype across environments (flat solid line). Both the orange and yellow examples correspond to plastic development, where the same genotype will produce different phenotypes in different environments. The yellow is an example of a linear relationship between environmental and phenotypic gradient, and the orange to a non-linear relationship with discrete alternative phenotypes (i.e. polyphenism). Note that we intended to illustrate qualitatively different types of shapes of reaction norms; the heights and quantitative values being irrelevant here.

EVOLUTION OF AND VIA DEVELOPMENTAL PLASTICITY

Natural selection acting on genetic variation has led to differences between species (e.g. Scheiner 1993) and between populations of the same species (e.g. Crispo & Chapman 2010) in the degree and types of plastic responses. Analyses of those populations/species provide insights into the ecological conditions and biological properties that favor plastic versus non-plastic development, and into the mechanisms underlying evolutionary transitions between the two.

Evolutionary transitions to and from plastic development

Recent theoretical models have advanced our understanding of factors that favor the evolution of plasticity, including the predictability of environmental fluctuations (e.g. Leimar *et al.* 2006, Reed *et al.* 2010) and the costs of plasticity (see Snell-Rood *et al.* 2010). Transitions between plastic and robust development, as well as between environmentally and genetically determined alternative phenotypes (i.e. polyphenism and polymorphism, respectively) have been documented at different phylogenetic levels. For example, post-colonization erosion of plasticity of head-size was reported for snakes (Aubret & Shine 2009), the evolution of different degrees of genetic caste determination for ants (reviewed in Schwander *et al.* 2010), and back-and-forth transitions between genetic and environmental sex determination for vertebrates (see Stelkens & Wedekind 2010). Environmental sensitivity of developmental processes is probably the ancestral condition in most cases, with selection then working for the ability to buffer environmental effects (see Newman & Müller 2000, Nijhout 2003). This has been suggested, for example, for caste determination in ants (Anderson *et al.* 2006) and sex-determination in reptiles (Janzen & Paukstis 1991).

Beside studies of natural populations such as those mentioned above, there are also revealing studies where changes in plasticity resulted from artificial selection in laboratory populations. Temperature-dependent coloration in butterflies and moths offers some of the most compelling examples of these studies. Artificial selection on adult wing patterns in *Bicyclus anynana* butterflies and on larval coloration in *Manduca sexta* moths (Suzuki & Nijhout 2006) produced changes in the height and/or shape of the reaction norms that describe the relationship between environmental and phenotypic change. In both cases, these changes were associated with changes in hormone titer dynamics and were of polygenic nature. In contrast, the importance of single genes has also been documented; for instance, by analyses of mutants which loose or gain environmental sensitivity. Examples include loss of sensitivity to the hormone that mediates diet-associated mouth morphology in *daf-12* mutants of *Pristionchus pacificus* nematodes (Bento *et al.* 2010), and exposure of hidden temperature-sensitivity for larval coloration in *black* mutants of *Manduca sexta* (Suzuki & Nijhout 2006).

In recent years, sophisticated analyses have started to highlight specific developmental and genetic mechanisms that presumably confer robustness or

plasticity to development. Robustness may be enhanced by redundancy in cell precursors (e.g. Braendle & Felix 2008), in gene enhancers (e.g. Frankel *et al.* 2010), and in regulatory microRNAs (e.g. Brenner *et al.* 2010), as well as the action of particular gene families such as heat-shock proteins (e.g. Takahashi *et al.* 2010). Modularity in developmental genetic networks, in turn, has been proposed to have an important role in enabling phenotypic plasticity; decreased pleiotropy between networks may facilitate the induction of different modules under different environmental conditions (Snell-Rood *et al.* 2010). By acting on all those types of mechanisms, natural selection can presumably adaptively adjust the likelihood and/or the extent of plasticity in trait development. Through a process that has been referred to as genetic accommodation, natural selection can also fine-tune this plasticity, including its degree (e.g. Lind & Johansson 2007), which environmental cue triggers it (e.g. Edgell & Neufeld 2008), and the sensitivity thresholds for that cue (e.g. Moczek & Nijhout 2003).

Impact of developmental plasticity on adaptive evolution

The relevance of developmental plasticity to adaptive evolution is receiving increasing attention, despite the fact that developmental plasticity is characterized by phenotypic changes without changes in gene sequence, while adaptive evolution is specifically characterized by changes in allele frequencies. Phenotypic plasticity was often seen as being irrelevant or even a deterrent for adaptive evolution (see discussion in Pfenning *et al.* 2010): 1) irrelevant because the raw material for evolution by natural selection is heritable phenotypic variation, and not environmentally-induced phenotypes not transmitted from parents to progeny; and 2) deterrent because plasticity can shield genetic variation from natural selection, either because alternative genotypes can end up producing the same phenotype or because environment-specific genes (i.e. those expressed only in one environment) will be under relaxed selection in the non-inducing environment. However, this view has changed and increasing attention is now being given to the contribution of developmental plasticity to adaptive evolution and the mechanisms whereby this contribution can occur. Studies on different systems illustrate the impact of plasticity on phenotypic diversification (e.g. West-Eberhard 2003), including the origin of novel traits (e.g. Moczek 2010), and on speciation, including adaptive radiations (e.g. Wund

et al. 2008). The arguments and empirical evidence for these effects were reviewed recently by Pfennig *et al.* 2010.

Different types of non-mutually-exclusive mechanisms account for the potential positive impact of plasticity on adaptive evolution. Clearly, by providing the means by which organisms can cope with new environmental challenges (Yeh & Price 2004), plasticity can play an important role for the immediate survival of populations exposed to change in external environment. Then, exactly because phenotypic plasticity can shield genetic variation from natural selection, it can presumably promote the accumulation of cryptic variation (i.e. genetic variation which does not result in phenotypic variation). When released, this heritable variation can provide raw material for adaptive evolution and be important for phenotypic diversification (reviewed in Schlichting 2008). Under some circumstances, environmentally-induced phenotypes can become fixed through a process called genetic assimilation. It has been argued that plasticity can, in fact, accelerate adaptive evolution. For example, studies of melanogenesis in *Daphnia* have suggested that the developmental mechanism underlying ancestral plasticity was repeatedly co-opted to facilitate rapid adaptation (Scoville & Pfrender 2010).

Insights into the evolutionary transitions between environmentally-sensitive and environmentally-insensitive development, and into the contribution of plasticity to evolutionary diversification, require an understanding of both the ecological relevance of plasticity and the mechanisms by which the environment regulates development.

ECOLOGY AND DEVELOPMENT IN PHENOTYPIC PLASTICITY

Development translates genotypes into phenotypes in a process that is influenced by the external environment. Aside providing some basic building blocks, particular variables of the external environment, in some cases, function as cues that trigger switches in development and lead to the production of alternative phenotypes to face different types of ecological challenges (examples in Table 1.1). This section focuses on the ecological significance of developmental plasticity, and on the types of effects that external environmental cues can have on organismal development.

Ecological significance of environmentally-induced phenotypic variation

Developmental plasticity is adaptive when the environmentally-induced changes result in a better match between the adult phenotype and its selective environment. The induced alternative phenotypes typically correspond to different ecological tactics, such as alternative tactics to achieve copulation in horned (guarding of nest) versus hornless (sneaky copulations) males of *Onthophagus taurus* dung beetles (Moczek & Emlen 2000); alternative tactics to escape predation in cryptic versus conspicuous *Bicyclus anynana* butterflies; and presumably alternative foraging tactics in "toothless" (bacteriovorous) versus "toothed" (predatory) *Pristionchus pacificus* nematodes (Bento *et al.* 2010).

A good match between phenotype and ecological conditions is achieved when the environmental cue that triggers changes in development is a reliable predictor of the future selective environment (but not necessarily the same). Such external cues can be of different types, both abiotic (e.g. temperature and photoperiod) or biotic (e.g. presence of other species and density of conspecifics), and they typically reflect environmental heterogeneity in time and/or in space. For example, temperature fluctuations predict alternating seasons relating to many cases of seasonal polyphenims including coloration in butterflies; fish kairomone concentration reflects high predation environments that leads *Daphnia* crustaceans to develop morphological defenses; and leg rubbing in locusts reflects high population densities that result in the production of the winged migratory morph (see Table 1.1, also for references). The environment can also be manipulated by conspecific individuals. In most ants, for example, the high-nutrition diet that determines that a juvenile will develop into a queen is the result of feeding by adult workers. In this case, there is micro-environmental heterogeneity within which the different morphs co-occur and can carry out the division of labor within the colony.

Environmental cues and developmental sensitivity

The environmentally-induced phenotypic variation can be more or less continuous (e.g. larger or smaller wings in *Drosophila*, Powell *et al.* 2010) or discrete (e.g. presence or absence of wings in queens versus workers in some social insects). Both gradual or "switch-like" changes in development can be triggered by different types

of environmental cues, often in combination (e.g. Braendle *et al.* 2006), and result in simultaneous changes in different traits.

There is rarely, if ever, a "one cue to one trait" relationship. Plasticity often involves changes in multiple traits in the same organism. For example, environmentally-induced wing development in ants, locusts and pea aphids (references in Table 1.1) is associated with changes in other morphological traits (e.g. body mass and ovary development in ants, body pigmentation in locusts, antennae and eye development in aphids) and with changes in life-history traits (e.g. longevity and fertility in ants, gregarious versus solitary life-styles in locusts, mode of reproduction in aphids). On the other hand, there is also a substantial degree of cue specificity in determining how the development of particular traits is altered. For example, different species of predators induce different types of anti-predator morphologies in *Daphnia* (e.g. Beckerman *et al.* 2010, Laforsch & Tollrian 2004) as well as in frogs (Vonesh & Warkentin 2006). The same cue can affect different developmental switches at different developmental stages (e.g. low food availability determines formation of teeth and production of dauer larvae in some nematodes, Bento *et al.* 2010). Also, different cues can induce developmental switches at multiple stages. In ants with strong caste dimorphism, for example, queen-worker determination depends on hormones deposited by the queen during oogenesis (Passera & Suzzoni 1979), and the differentiation of subcastes (such as minor and major workers or soldiers) depends on nutrition during larval development (Wheeler & Nijhout 1983). These multiple environmentally-sensitive switch points along the developmental trajectory allow diversification of adult morphs specialized for different roles.

The effect of change in a particular environmental cue on phenotype, characteristically represented as a reaction norm, is highly dependent on developmental sensitivities. These sensitivities exist in relation to thresholds of the values of the inductive environmental cue beyond which there is change in development and in phenotype (Ostrowski *et al.* 2000). They also exist in relation to restricted time-windows of the development during which the external environment can influence the outcome (Ostrowski *et al.* 2002); development being quite robust outside these sensitive periods (Braendle & Felix 2008). Both sensitivity thresholds and sensitivity periods can evolve and might differ between populations.

Effects of the external environment on developmental timing and trajectories

The effects of the environment on developmental timing can be of different types; with the environmental cue more or less uniformly extending or reducing the total duration of development, affecting specifically particular developmental stages, or leading to arrested development altogether. For example, temperature (e.g. Bochdanovits *et al.* 2003), nutrition (e.g. Brian 1975), and presence of predators (e.g. Beckerman *et al.* 2010) often affect development time and lead to differences in body size and correlated life-history traits. In some arthropods, the duration but also the actual number of instars can vary across environments (e.g. Beckerman *et al.* 2010, Esperk *et al.* 2007). Furthermore, some organisms, typically in unfavorable environments, have environmentally-induced arrested development at different stages: embryonic diapause (Moriyama & Numata 2008), larval diapause (Golden & Riddle 1984), and pupal diapause (Belozerov *et al.* 2002). While it is clear that diapause represents an adaptive plastic response, the same is probably not true for many cases where developmental rates (and correlated body size) are affected by availability of energy resources (such as temperature or food) (see examples in Gotthard & Nylin 1995).

The environmental control of developmental rates can also affect body structure and result in the production of not just larger or smaller, but distinct adult morphologies. For example, if the rates of development of different traits are not affected in the same manner, environmental-sensitivity can modify the correlation between traits and generate novel trait combinations. A role for this type of heterochrony has been proposed in relation to differences between castes and body parts in ants (Miyazaki *et al.* 2010). Differential rates in association to different body structures have also been suggested to explain changes in allometry (i.e. characteristic patterns of relative organ size; see Stern & Emlen 1999) in environmentally-dependent omnivore versus carnivore morphs of spadefoot toad tadpoles (Storz & Travis 2007).

Aside from the global or local effects on developmental timing, the environmental cue can also trigger a switch between alternative developmental trajectories that result in drastically different morphologies. Studies of the actual process of development of different organisms are adding to a detailed characterization of the formation of alternative environmentally-induced

morphologies. These include some classic examples of adaptive developmental plasticity such as *Daphnia* anti-predator morphologies (Laforsch & Tollrian 2004, Miyakawa *et al.* 2010), beetle horns (Moczek 2007, Moczek & Nijhout 2002, Tomkins & Moczek 2009), pea aphid wings (Braendle *et al.* 2006, Brisson 2010, Legeai *et al.* 2010), and social insect castes (Abouheif & Wray 2002, Miura 2005). The way by which external environmental cues control patterns of gene expression that result in alternative phenotypes is now being elucidated for these and other examples of plastic development and is discussed in more detail below.

MOLECULAR MECHANISMS OF DEVELOPMENTAL PLASTICITY

Current research in adaptive developmental plasticity is characterizing the molecular mechanisms that link variation in external environmental cues to the changes in organismal development that result in the production of different phenotypes. For a long time, the external environment and plasticity were disregarded in studies of developmental biology. This is despite the fact that organismal development itself, with its characteristic tissue-by-stage specific gene expression, is perhaps the most compelling example of cellular plasticity. During organismal development, cell differentiation and pattern formation is the result of intrinsic signals that provide cells of developing organisms with information about their position. In developmental plasticity, the choice of alternative developmental trajectories is also fixed genetically, while the decision between those paths depends on different mechanisms that control gene expression.

Gene content and gene expression

Despite the fact that phenotypic plasticity is defined as environmentally-induced phenotypic variation produced from one single genotype (thus leaving out consideration of genetic variation), there are many revealing examples of a clear correlation between genetic composition and plasticity. This can be seen both in terms of allelic variation at specific loci and the extent of plasticity in different populations, as well as in the gene content on the genomes of species characterized by very plastic development.

Whatever the allelic or gene composition of an organism is, it is clear that environmentally-induced changes in development ultimately result from environmentally-induced changes in gene expression. The latter can have an effect on

which and to what level particular genes are expressed, and probably also particular alternative transcripts or alleles. An emblematic example of genes whose expression, and thus effect, depends on the environment is that of heat shock protein (Hsp) encoding genes. Their expression is characteristically influenced by temperature or other types of environmental stress to buffer perturbations to development and ensure the production of predictable phenotypes (e.g. Takahashi *et al.* 2010).

Analysis of plasticity in gene expression has also been carried out for groups of candidate genes or pathways involved in particular environmentally-sensitive developmental switches. Examples include analysis of wing development genes in queen versus worker ants (Abouheif & Wray 2002), of key body-plan and hormone-related genes in *Daphnia*'s induced defenses (Miyakawa *et al.* 2010), and of sex determining genes in species with environmental sex determination (Shoemaker *et al.* 2007). New analytical tools such as microarrays and RNA-Seq now make it possible to move from (necessarily biased) candidate gene approaches, to less biased (but of more challenging interpretation) whole transcriptome scans.

Environmental regulation of gene expression

Different mechanisms are known that act interactively to regulate gene expression, keeping it in tune with physiological adjustments to the environment. Among these, the role of endocrine hormones has received, and is receiving, special attention in the context of developmental plasticity (see Gilbert & Epel 2009).

The sensitivity of hormones to the environment, together with their widespread role as regulators of post-embryonic development, underscores their role as intermediaries in linking external environmental information with developmental switches (Nijhout 1998). In fact, a hormonal regulation has been characterized for most, if not all, well described examples of developmental plasticity (see Gilbert & Epel 2009, Nijhout 2003). Insect juvenile hormone and ecdysteroids, in particular, have been implicated in many cases of plastic development, including that of seasonal polyphenism in butterfly wing patterns and of castes in social hymenoptera. In many cases, the same hormone influences multiple developmental decisions and different traits during the development of one same organism; often associated to different sensitivity thresholds (Bento *et al.* 2010) and/or different sensitivity periods (Moczek & Nijhout 2002, Oostra *et al.* 2011). The environmental cues can induce changes in titers and/or dynamics of hormone production, and the hormones can then affect gene

expression. This can happen, for example, via their nuclear receptor proteins which, when activated by the hormone signal, have transcription regulator activity (Baniahmad & Tsai 1993) or possibly also via hormone-related changes in chromatin (Lu *et al.* 1998).

CHALLENGES AND TRENDS

In the section above we provided a broad overview of some of the best studied molecular mechanisms underlying developmental plasticity: changes in gene expression and its regulation by hormones. These mechanisms interact in complex ways whereby they regulate and are regulated reciprocally. For example, steroid hormones can influence gene expression by affecting chromatin states (Lu *et al.* 1998), and, conversely, their biosynthesis and action can itself be under epigenetic regulation (e.g. Martinez-Arguelles & Papadopoulos 2010).

A complete understanding of adaptive developmental plasticity will require knowing the different sensory and regulatory mechanisms, but also how these, in turn, affect development to produce changes in phenotype that result in differences in individual fitness in natural populations. In nature, the integration of all levels of information is complicated by the fact that the developmental environment is more complex than one single changing cue, the phenotype is more than one particular trait, and the selective environment presents more than one ecological challenge. Also, typically, there is extensive genetic variation in natural populations and different genotypes do not necessarily respond to environmental variation in the same manner. Current studies are starting to specifically address variation in nature also at the molecular level, including for gene expression (e.g. Scott *et al.* 2009), hormone dynamics (e.g. Zera 2007), and epigenetics (see Bossdorf *et al.* 2008 and Richards 2008). The integration of these different studies of the proximal mechanisms of the environmental-sensitivity of development will need to be done within an evolutionary framework, including the evolutionary history of the regulating mechanisms and their interactions (Johnson & Tricker 2010), as well as the origin and diversification of (plastic) developmental networks (Minelli & Fusco 2010). It is clear that environmentally-induced variation will need to continue to be studied in multiple systems (representing different types of cues, developmental and phenotypic changes, and ecological situations), at different levels of biological organization (changes in molecular processes, organismal development, and impact in natural populations) and

bringing together different disciplines (genetics, developmental biology, ecology and evolutionary biology).

Environmentally-induced variation is at the heart of new trends in biological and biomedical research. The new discipline of eco-(evo-)devo is perhaps the most emblematic example of this. It unites fields such as epigenetics and evo-devo (see Gilbert & Epel 2009) around the study of developmental plasticity. It takes explicit account of the environment in generating inter-individual variation in phenotype through changes in development, and in contributing to evolutionary diversification (see also West-Eberhard 2003). In fact, plasticity has been highlighted as one of the major themes for an extended evolutionary synthesis (Müller 2007, Pigliucci 2007). Aside its obvious place at the center of an effort to unite ecology and developmental biology and its contribution to evolutionary biology, the influence of the developmental environment on phenotype can also have important implications for biomedicine and biodiversity. First, both the *in utero* environment (including maternal stress and nutrition, e.g. Burdge & Lillycrop 2010), and trans-generational environmental effects carried in parental gamete epigenomes (including in the sperm; Puri *et al.* 2010) have been implicated in the developmental origin of adult disease (examples in Gilbert & Epel 2009, Gluckman *et al.* 2009). Second, the study of developmental plasticity can also be of relevance for appropriately assessing the biodiversity consequences of anthropogenic environmental change. Natural populations have different mechanisms for dealing with environmental change, including global change in climate (see Figure 1.2). While demographic and genetic mechanisms have received considerable attention in this context, the role of developmental mechanisms (Chevin *et al.* 2010, Reed *et al.* 2010) is lagging behind. Clearly, plasticity can help organisms exploit novel environments (e.g. Ghalambor *et al.* 2007, Yeh & Price 2004) and provides a means of rapidly adjusting to external change, but it might also pose problems. For example, in organisms with temperature-dependent sex determination, dramatic climate change can potentially lead to extremely biased sex ratios with serious demographic consequences (Janzen 1994, Miller *et al.* 2004).

It is clear that developmental plasticity will continue to be an active area of research, and will greatly profit from the availability of sophisticated methods of molecular analysis (which traditionally were a privilege of only a handful of classical laboratory models) for multiple systems with interesting ecology and/or unique

biological properties (see Abzhanov *et al.* 2008, Aubin-Horth & Renn 2009, Milinkovitch & Tzika 2007). It is also clear that a complete understanding of natural variation will gain from including the study of development, and it will continue to bring genetic models out of the laboratory, and ecological systems into the laboratory. These are certainly exciting times when different disciplines are joining efforts to understand what is arguably one of the most fascinating, and until recently largely ignored, properties of biological systems; that of variation.

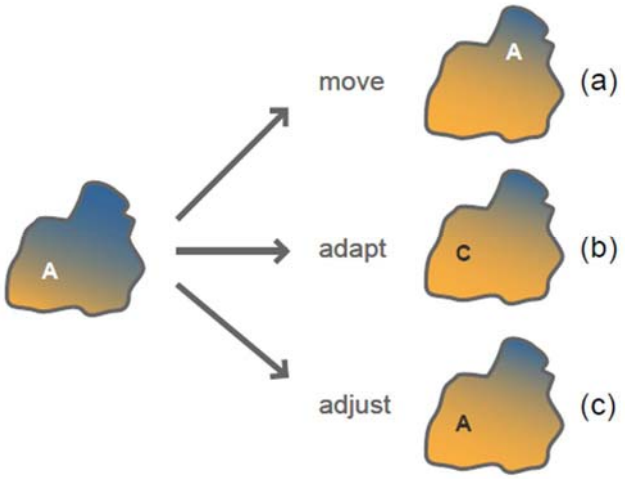

Figure 1.2 - Coping with changing environments. In nature, populations can deal with climate change in different ways: (a) through habitat tracking, individuals move to different places and this can result in changes in species distributions, (b) through natural selection on segregating genetic variation, allele frequencies change across generations as populations adapt to novel environmental situations, and (c) through phenotypic plasticity, individuals can adjust without changes in genetic composition. Background color shading represents an environmental gradient (e.g. temperature), and characters represent populations with letters (A or C) corresponding to different genotypes and colors (white or grey) to different phenotypes. Figure adapted from Beldade *et al.* 2011.

Here we will use an emerging model in evolutionary and ecological genomics, the tropical Nymphalid *Bicyclus anynana* butterflies, for which existing knowledge of the adaptive value of plasticity in natural populations (Brakefield *et al.* 2009) can be complemented with an understanding of its underlying mechanisms. *B. anynana* has been established as a laboratory model for research on the evolution and development of adaptive traits and it is an exceptional modem to address some of the current trends (e.g. Beldade & Brakefield 2002, Brakefield *et al.* 2009). It is small enough that large

laboratory populations can be maintained (essential for population-level analysis), but large enough that individuals can be easily manipulated (necessary for organismal-level analysis). More recently, genomic tools (Beldade *et al.* 2008) have been developed for this species allowing modern molecular-level approaches.

SEASONAL POLYPHENISM IN BICYCLUS ANYNANA BUTTERFLIES

Ecological and evolutionary context of B. anynana developmental plasticity

Like many butterflies from seasonal environments (examples in Beldade & Brakefield 2002), *B. anynana,* exhibits clear seasonal polyphenism in wing pattern and various life-history traits (Brakefield *et al.* 2007, Brakefield & Frankino 2009). In sub-Saharan Africa, where they occur naturally, larvae that develop during the wet season produce adults with conspicuous wing patterns that include large marginal eyespots, while those that develop during the dry season produce adults with dull brown colors and very small eyespots (Figure 1.3a). These alternative wing patterns correspond to alternative strategies to avoid predation. While the marginal large eyespots of the wet-season butterflies are thought to attract the predator's attention to the wing margin and away from the vulnerable body, the all-brown dry-season butterflies are thought to be cryptic against a background of dry leaves in the florest floor (Brakefield & Frankino 2009, Olofsson *et al.* 2010). Laboratory studies showed that the temperature during development, which predicts the natural seasonal fluctuations in precipitation, determines the production of the alternative wing pattern phenotypes (Brakefield & Frankino 2009). Curiously, only the pattern on the ventral side of the wings (the surface exposed at rest) shows plasticity in relation to developmental temperature (Brakefield *et al.* 1998) and has been associated to predator avoidance. Despite correlations between wing surfaces (e.g. Beldade & Brakefield 2003), the patterns on the dorsal side (exposed only during flight or courtship) are largely not plastic and have been implicated in mate choice (Robertson & Monteiro 2005). Examination of this contrast in a phylogenetic context suggested that ventral patterns, shaped by natural selection, evolved at a lower rate than dorsal patterns, shaped by sexual selection, during *Bicyclus* diversification (Oliver *et al.* 2009).

Phisiologycal underpininings

Like many polyphenisms, in *B. anynana* ecdysteroids are involved in the regulation of the differences in the wing pattern and life-history traits between the wet and the dry seasonal forms (Koch *et al.* 1996, Brakefield *et al.* 1998, Zijlstra *et al.* 2004, Oostra *et al.* 2011). Titers of ecdysone and 20-hydroxyecdysone peak relatively earlier at the higher temperature that typically leads to the production of large eyespots (Figure 1.3b). Furthermore, artificial manipulation of hormone titers can affect ventral eyespot size. Microinjections or infusions of 20-hydroxyecdysone into pupae resulted in the development of individuals reared at low temperatures into adults with wing patterns characteristic of the wet season form (Koch *et al.* 1996, Brakefield *et al.* 1998, Zijlstra *et al.* 2004). It is not yet known how precisely ecdysteroid dynamics regulates eyespot development, but, the ecdysone receptor, which has transcription factor function, possibly directly or indirectly regulates eyespot genes (Koch *et al.* 2003).

Correlated responses are regularly observed in artificial experiments which can be explained in part by the fact that ecdysone is involved in the regulation of multiple traits (Oostra *et al.* 2011). Lines that have been selected for short or long pupal development time show larger or small ventral eyespots, respectively (e.g. Zijlstra *et al.* 2004). Fast-developing butterflies have higher levels of ecdysone shortly after pupation in comparison with slow-developing individuals (Zijlstra *et al.* 2004). In addition, the slow-selected butterflies show a decreased response to ecdysone injections in the pupal stage relative to fast-selected butterflies.

The genetics of developmental plasticity in B. anynana

Previous studies of the genetic basis of developmental plasticity in *B. anynana* have used artificial selection to derive butterflies expressing wet or dry-like phenotypes across temperatures, changing the height of reaction norms but failing to significantly change their shape (Brakefield *et al.* 1996, Wijngaarden & Brakefield 2001, Wijngaarden *et al.* 2002). Butterflies from these lines, as well as from unselected laboratory populations reared at different temperatures, characterized the physiological and gene expression changes associated with the development of alternative wing patterns The eyespot gene *Distal-less*, proposed to contribute to variation in dorsal eyespot size (Beldade *et al.* 2002), has a larger area of expression in larval wings of individuals that develop into the wet-season-like phenotype with

larger eyespots (Brakefield *et al.* 1996). Further studies will be necessary to link hormone dynamics to the regulation of genes and processes involved in eyespot formation (Beldade & Brakefield 2002), as well as to investigate the involvement of other regulatory and sensory mechanisms in environmentally-sensitive wing pattern development. Genomic tools available today including e. g. expressed sequence tag (EST) data bases, microsatellite, linkage map and a custom designed microarray will be of extreme relevance in these studies (Beldade *et al.* 2006, Beldade *et al.* 2009a, Beldade *et al.* 2009b, Conceição *et al.* 2011).

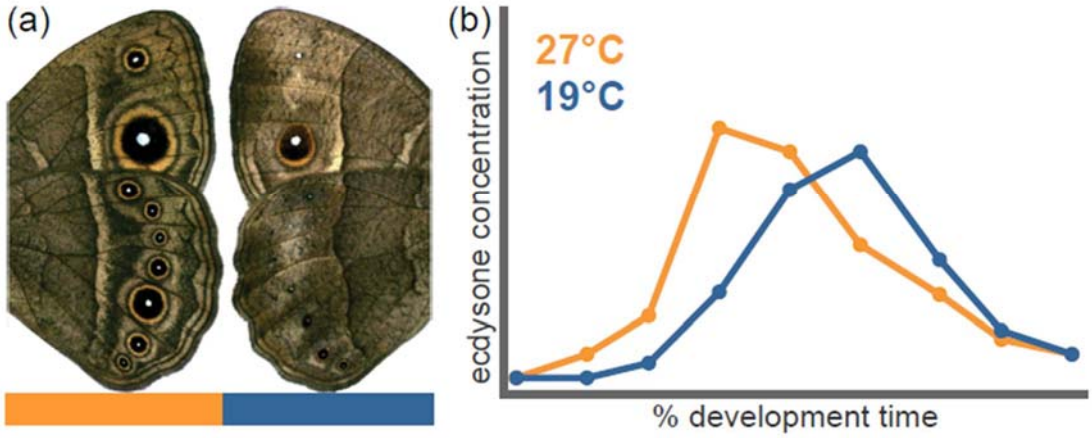

Figure 1.3 - Seasonal phenotypic plasticity in *B. anynana*. (a) B. anynana wet- (left) and dry-season-like (right) phenotypes obtained by rearing larvae at different temperatures. Note that the larger eyespot on the forewing is typically hidden behind the hindwing in resting butterflies (the posture relevant for the anti-predatory strategies described). Also, note that wing size (typically larger in dry-season phenotypes) was adjusted to emphasize comparison of color patterns. (b) Differences in hormone titer dynamics (adapted from Brakefield *et al.* 1998, Oostra *et al.* 2011) during pupal development, when patterning and pigment biosynthesis (cf. Wittkopp & Beldade 2009) genes are expressed. Figure adapted from Beldade *et al.* 2011.

OUTLINE OF THIS THESIS

Developmental plasticity is an important strategy for adaptation to fluctuating environments (reviewed in this CHAPTER 1). Such plasticity has one of its most compelling examples in seasonal polyphenism in butterflies; individuals can have different wing patterns and life-histories in alternating seasons. Previous studies have shown that the mechanism that mediates seasonal polyphenism involve ecdysteroids

hormones; with alternative seasonal forms being characterized by differences in the timing of hormone increase after pupation. This thesis will contribute to a broader understanding of the genetic, developmental and physiological mechanisms that regulate developmental plasticity represented by temperature-regulated variation in butterfly wing color patterns. We will focus on a lab model for the study of adaptive phenotypic plasticity: color patterns on the wings of *Bicyclus anynana* butterflies. The adaptive value of the alternative seasonal phenotypes in this species is well documented, and their underlying physiological underpinnings have started to be explored, however how animals perceive and assess temperature and how that influences development is still a black box.

In CHAPTERS 2 AND 3 we explored the coordination of responses of different plastic traits to temperature and hormone manipulations. Both in CHAPTER 2 AND 3 we studied the integration of response of different traits by combining the analysis of changes induced by temperature in hormone physiology and traits development that lead to changes in phenotype. For that purpose, we explored the effects of manipulating external temperature, and internal levels of the active form of ecdysone and analyze phenotypic effects on different wing pattern (CHAPTER 2) and life-history traits (CHAPTER 3). In CHAPTER 2 we also explored the mechanism for local sensitivities to systemic levels of ecdysone by testing the hypothesis that groups of cells that responded differently to ecdysone manipulations would differ in expression of ecdysone receptor. In CHAPTER 3 we additionally tested the ecological consequences of any hormone-induced changes in morphology and physiology observed by manipulating ecdysteroid at a single temperature and injection time point, and monitoring the effects on multiple aspects of adult fitness.

Genotypes can differ in many properties of reaction norms such as height, slope, or shape. In CHAPTERS 4 AND 5 we explored the genetic basis of variation in developmental plasticity. In CHAPTER 4, we explored the effect of alleles of large effect on wing pattern on plasticity therein. To achieve this goal, we characterized thermal reaction norms for the size of eyespot color rings for *B. anynana* mutants with altered eyespot size and/or color composition. In CHAPTER 5 we explored standing genetic variation for alternative plastic phenotypes. To explore genotype (G), temperature (T), and GxT effects on *B. anynana* development, we derived artificial selection lines expressing extreme wet season-like or dry-season-like phenotypes at intermediary temperatures and, we characterized thermal reaction norms for several

traits for a wider range of temperatures than is usually explored in this species to characterize the shape of reaction norms.

In CHAPTER 6, I summarized the conclusions from the previous chapters and provide ideas for future research to deeper our understanding of developmental plasticity.

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