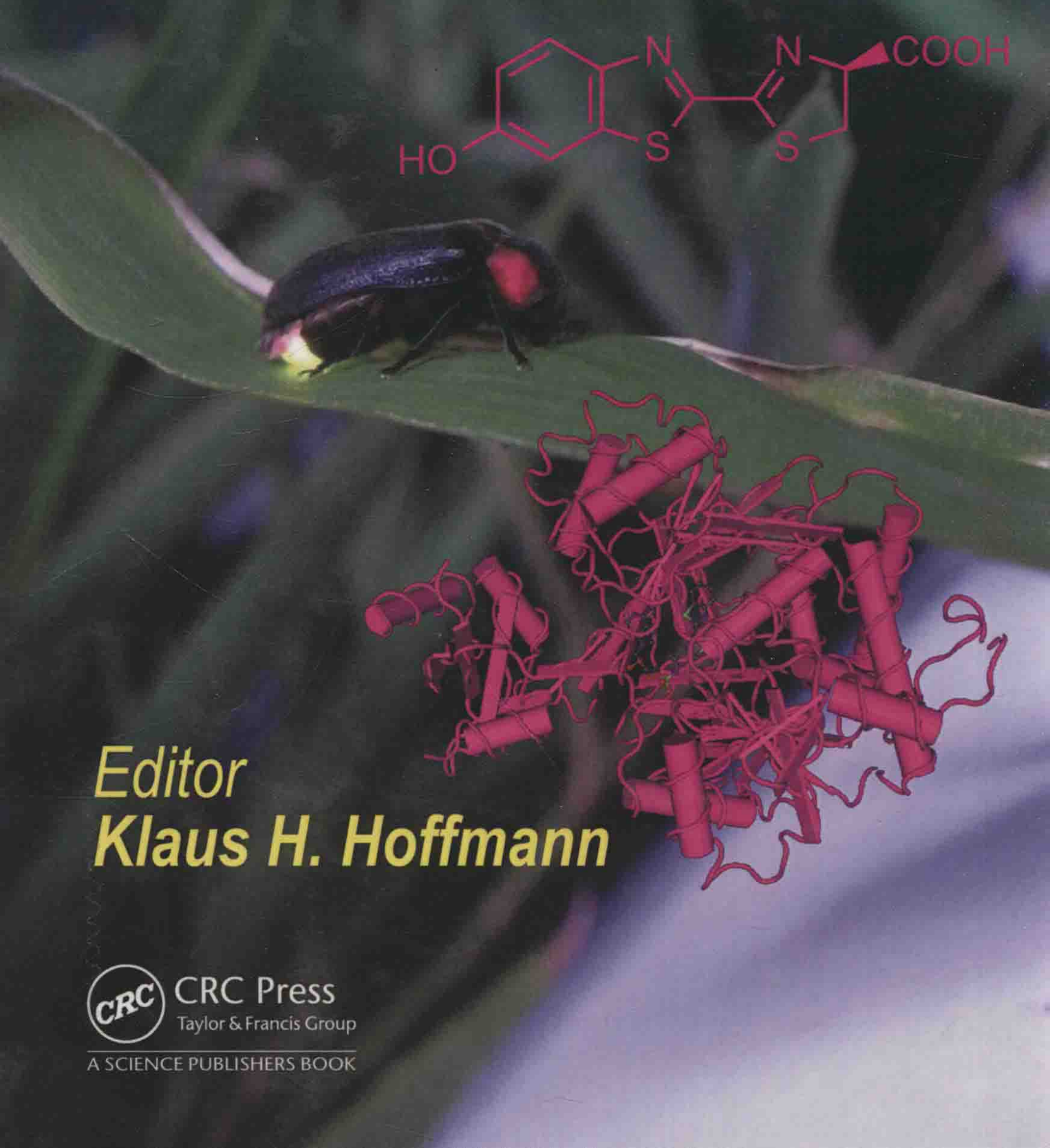
Insect Molecular Biology and Ecology



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Insect Molecular Biology and Ecology

Dedication

Dedicated to my grand daughters

Preface

A major challenge in current entomology is to integrate different levels of organization, from cellular mechanism to function in ecosystem. In the postgenomic era of the 21st century various fields of study have become possible, which use the information of fully sequenced insect genomes (https://www.hgsc.bcm.edu/arthropods/i5k-pilot-project-summary). However, the rapid development of molecular techniques for studying gene functions will revolutionize entomology not only for the insect model organisms, but in general. The majority of these techniques can also be applied if only partial sequence information is available. With these tools, entomologists are able to answer questions in insect biochemistry, physiology, and endocrinology, but also illuminate very complex behavioral and ecological aspects.

When I edited a book on "Environmental Physiology and Biochemistry of Insects" in 1985 for the Springer-Verlag, Berlin, mechanisms of environmental adaptation in growth and development, energy metabolism, or respiration to temperature, oxygen tension, food supply or salt concentrations were in the focus of interest. It was at the time of "Physiological Ecology". About 30 years later, the omics era gives us the opportunity to gain deeper insight into different aspects of insect physiology and environmental adaptation, for example, by overexpression or silencing of candidate genes of interest. When we understand, how physiological processes are regulated and at what time, we will be able to manipulate them, hereby providing attractive potential for practical application, for example, in an ecologically friendly insect pest control.

In 2008, we started with a Master program in "Molecular Ecology" at our University of Bayreuth, which has become very successful during the last 6 years. The Master's program was designed to play a special role in the synergistic cross-linking of the two focal points at our University, "Ecology and Environmental Sciences" and "Molecular Biosciences". The focus of interest is the functions of organisms—and especially of insects—in their environment and the analysis of (bio)chemical interactions in complex ecosystems. "Molecular" should mean not only to study the function of macromolecular compounds such as proteins and nucleic acids, but to analyze also the structure and capacity of low molecular weight substances like signal molecules, toxins or drugs. This Master program inspired me to edit the present book on Insect Molecular Biology and Ecology.

The book provides a mix of topical review articles and current research work. In several chapters previously unpublished data are presented showing novel applications for the use of omics technologies in the postgenomic era. The book should prove useful not only to researchers of the Insecta, but also to teachers and graduate students who are interested in understanding the molecular basis of insect functioning in their natural environment.

I acknowledge the support received from the authors who accepted the invitation to write an article on their area of expertise and for delivering the manuscripts in due time. Any success this book may achieve has to be attributed to their efforts.

Bayreuth, July 17, 2014

Contents

1

Mechanisms of Polyphenism in Insects

Stephen M. Rogers

Introduction: What is Polyphenism?

Polyphenism, broadly defined, is where two or more distinct phenotypes can be produced by the same genotype. Woltereck (1909) coined the term 'reaction norm' (Reaktionsnorm) to describe how the phenotype of an individual depends on the interaction between its particular genotype and environmental cues. In practice many or most genes with pleiotropic effects have context-dependent expression or action during development, i.e., the internal environment in which they are expressed. Phenotypic plasticity is therefore usually defined as a change in phenotype driven by cues in the external environment, which may be abiotic, such as temperature or photoperiod, or biological in origin, deriving from other species or even members of the same species. Phenotypic plasticity can result from variation in developmental, physiological, biochemical and behavioral processes that are sensitive to these environmental variables (Nijhout and Davidowitz 2009).

Polyphenism, in one sense, occurs very widely in insects. In holometabolous insects, larvae and pupal stages often differ radically in appearance and function from the adults; this is a clear example of very different phenotypes being generated from the same genotype during the normal course of post-embryonic development. Even in the

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hemimetabolous insects where the distinction between larval and adult forms is usually less dramatic, there is typically a clear distinction between specializations for feeding in larvae/nymphs and for dispersal and reproduction in the winged adults, with clear differences in phenotype to accomplish these tasks. Polyphenism in insects, however, is usually taken to designate the occurrence of alternate developmental pathways leading to an endpoint of two or more distinct phenotypes, rather than successive stages of post-embryonic growth (Fig. 1A). These may be discrete mutually exclusive alternatives, or range across a continuum between two extreme phenotypic outcomes. In practice, environmental variables may tend to canalize the development towards discrete and distinctive final phenotypes, even though the underlying developmental and physiological processes may allow for more variety in outcome.

Polyphenisms are a major reason for the success of the insects (Simpson et al. 2011), allowing them to exploit the advantages of being both specialists and generalists without the disadvantages of either strategy. The advantages of specialism is that morphology, physiology, biochemistry and behavior can be tailored to the demands of a particular environment, allowing an insect to efficiently exploit resources whilst being protected from predators (for example, camouflage coloration or exploitation of secondary plant defenses); but the disadvantage is that particular environments may change or only be periodically available (for example, seasonal, or host plant abundance). Generalists are more likely to find an environment that will support them, but they may be inefficient or conspicuous in any one environment and find themselves outcompeted by specialists.

Polyphenism may therefore allow insects to adopt phenotypes that best suit predictable and regular environmental changes, or respond to 'predictably unpredictable' transformations in the environments such as exploiting the increase in plant abundance in deserts after rains, or conversely escaping the degradation of an environment following overcrowding (Simpson et al. 2011). Eusocial insects, bees, ants, wasps and termites are some of the most abundant and successful insect species, and polyphenism has been central to this success allowing them to partition labor between individuals within social groups.

The expression of alternative phenotypes suited to different environments requires exposure to salient trigger stimuli related to or anticipatory of those environments during a critical window, which then sets the insect onto an alternate developmental pathway (Brakefield and Frankino 2009; Fig. 1A). This may be during an early period of development, or may even derive from the experience of a parent who then transmits an epigenetic change to their offspring. These critical windows may occur during a fixed period in ontogeny where an animal is only

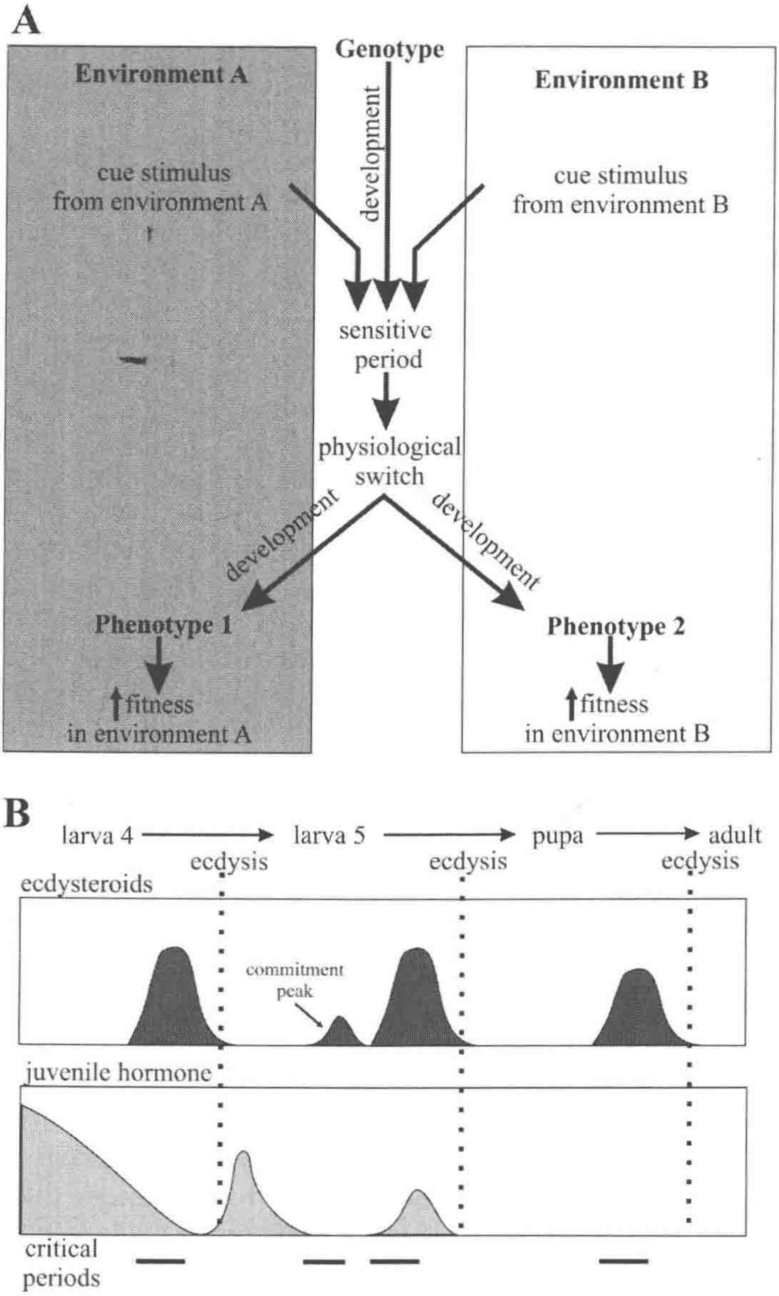

Figure 1. A. Schematic showing how different environments can redirect development towards two or more phenotypic outcomes depending upon suitable stimuli being received at a critical point in development. The environmental cue stimuli must reliably anticipate the environment to be faced by the organism after the induction of plasticity in order to increase fitness within that environment. B. A simplified diagram showing variation in the titers of ecdysteroids and juvenile hormone during molting events (ecdysis) across normal postembryonic development in a holometabolous insect, such as *Manduca sexta*. See text for more details. B. Derived from Reynolds (2013).

susceptible to appropriate stimuli for a small period or developmental stage. Alternatively an animal may be susceptible to these stimuli at any time during their life but the extent of phenotypic change depends on the longevity of their exposure to appropriate stimuli.

The way in which post-embryonic development occurs in insects perhaps makes them particularly suited to the expression of alternate phenotypes as pronounced phenotypic change is part of their normal experience during growth. A second reason is that insects change in size through periodic molting events, therefore the occurrence of discrete periods during which intense physiological activity occurs are a frequent occurrence. During these events epidermal tissues divide and grow, new cuticle is laid down, old cuticle reabsorbed and imaginal discs may proliferate and generate new adult structures (Reynolds 2013). This process is under the control of an extensive network of morphogenetic hormones but two, juvenile hormone and ecdysone, have an executive role in controlling the process. The structures of these two hormones and the precise nature of their control over moulting and development varies between insect taxa (hence the use of the more generic 'ecdysteroid') but the broad principles of their actions are shared across the insects (Nijhout and Davidowitz 2009; see chapters Orchard and Lange, this book and Hoffmann et al., this book). Molting is induced and regulated by ecdysteroids: hemolymph titers of this hormone increase in the period immediately before a molt and it initiates an extensive process of gene transcription and cell division (Fig. 1B). The type of cuticle produced, whether larval, pupal or adult depends on juvenile hormone. In hemimetabolous insects high titers of circulating juvenile hormone ensure that the next instar will be larval in character; the absence of juvenile hormone triggers the molt to adulthood and the expression of adult characters. In holometabolous insects, such as the moth *Manduca sexta*, a small peak of ecdysteroid called the commitment peak occurs before the main surge that initiates molting (Fig. 1B). If no juvenile hormone is present during this commitment peak the insect is set irretrievably on a course of metamorphosis and as part of this process juvenile hormone receptors are lost from most larval tissues. The main ecdysteroid peak now occurs, which induces the molting process, and this time is accompanied by a surge in juvenile hormone as well. Most tissues are unaffected by juvenile hormone as the receptors have been silenced, but it blocks the development of imaginal discs and causes the larva to molt into a pupa. The next molt to adulthood then follows the same pattern as in hemimetabolous insects; ecdysteroids in the absence of juvenile hormone. The key point about these hormones is that they are physiologically potent and have executive roles in the control of morphogenesis, and it therefore unsurprising that these two hormones have often been the first target of analysis for those looking to understand the physiological basis of polyphenism.

Behavior is a manifestation of the phenotype, and learning, defined as an experience-dependent change in behavior, is one of the most universal and basic expressions of phenotypic plasticity. Some form of learning has been found in all insect orders where it has been looked for (Jermy 1987). Learning and memory operate over many different timescales from seconds to lifetimes, and may or may not be reversible. Mechanistically, it also operates at many levels, from phosphorylation or other short-term modification of existing neuronal structures through to new gene expression and extensive remodeling and growth of neuronal tissues. The mechanisms underlying learning and memory have been extensively studied in insects and other organisms, and there is a good understanding of some of the molecular pathways involved linking initial stimulus through to altered gene expression (Kandel 2001). Some forms of phenotypic plasticity leading to polyphenism resemble learning. This understanding has also been a useful starting point for those wishing to analyze the mechanistic basis of polyphenism. In this chapter I review progress in understanding the mechanistic basis of polyphenism in several classic insect systems: seasonal polyphenism in butterflies and moths; phase change in locusts; flight/flightless polyphenism in aphids and crickets, and caste determination in two very different groups of social insect—the termites and bees.

Why study mechanisms underlying polyphenism? The case was succinctly put by West-Eberhard (2003): 'For evolutionary biology, proximate mechanisms represent more than just different levels of analysis or research styles. They are *the* causes of variation upon which selection acts'. She goes on to say that 'Among the consequences of the neglect of mechanisms in modern evolutionary biology are the problems that arise when the black box of mechanism is filled with imaginary devices'. This is a survey of the state of current attempts to open that black box.

Seasonal Morphs in Lepidoptera

Seasonal morphs are a frequent occurrence in Lepidoptera with two or more generations per year, in larvae (e.g., Greene 1989, Hazel 2002), pupae (e.g., Angersbach 1975, Hazel and West 1979) and as adults (e.g., Brakefield and Reitsma 1991, Kingsolver 1995, 1996). One of the best characterized examples is that shown by the African Satyrine butterfly, *Bicyclus anynana* (Fig. 2A). This tropical woodland species experiences two distinct seasons: a warm wet season when food is abundant and during which egg laying and larval growth can occur, and a cooler dry season when vegetation is withered and through which the butterflies

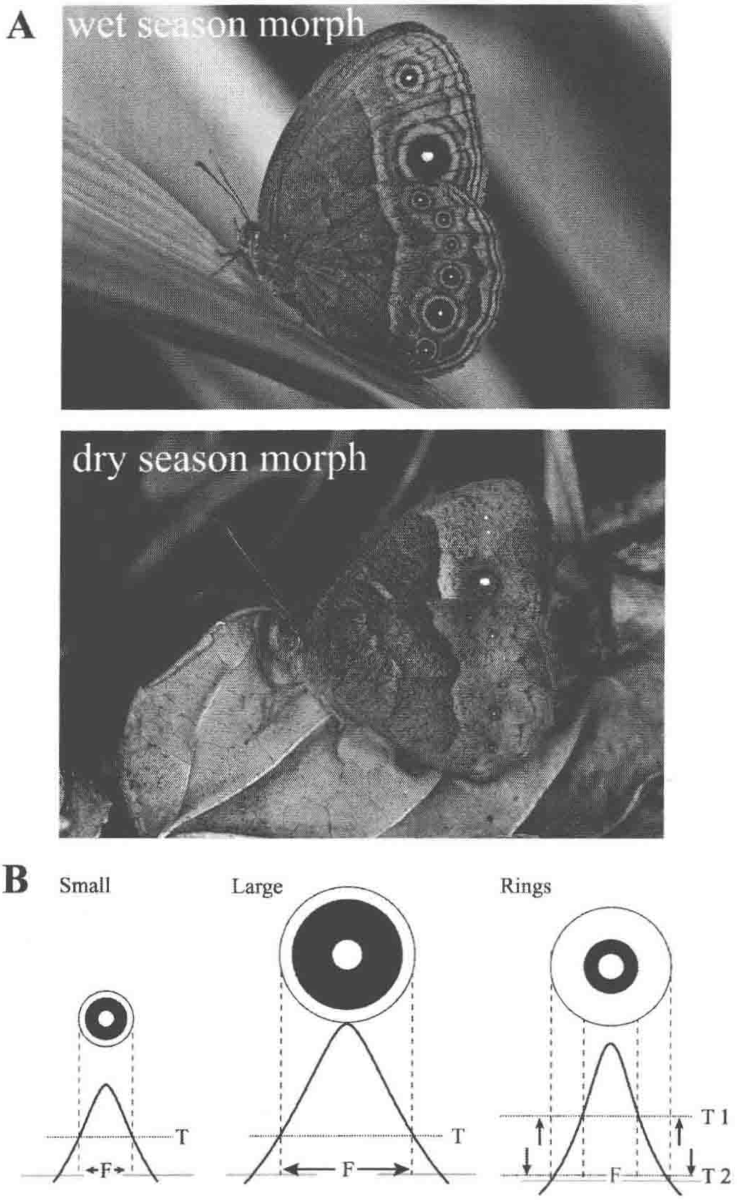

Figure 2. A. The wet season (upper panel) and dry season morphs (lower panel) of the Satyrine butterfly *Bicyclus anynana*. B. Developmental control of eyespot size and features on the wing of a developing butterfly. Chemical signals diffuse out down a concentration gradient (shown as the curves) from an organizing focus (F) on the developing wing. Large quantities diffuse further and induce larger eyespots. Ring structures are produced according to the threshold (T) of induction in the surrounding tissue. If the threshold is high (T1) then large amounts a chemical signal near the point of origin are necessary in order to induce a pattern. Chemical signals to which the tissue has a low threshold (T2) will still induce a phenotypic effect when they have diffused far from the focus and are at a low concentration. Photographs are courtesy of Oskar Brattström and Andre Coetzer. B. Redrawn from Beldade and Brakefield (2002).

must survive until the onset of the next rains several months later (Shapiro 1976, Brakefield and Reitsma 1991, Brakefield and Mazzotta 1995). Each season is associated with a distinct morph. The wet season morph has a distinctive pale medial stripe and rows of conspicuous 'eyespot' patterns on the ventral surfaces of its wings. The dry season morph is much less conspicuous with highly reduced markings and is mostly a drab brown color. There are also differences in behavior and physiology: the wet season form is highly active, reproduces quickly and has a short lifespan; the dry season is relatively long-lived and fairly inactive, sitting out the dry season and only reproducing once the wet season recommences (Brakefield et al. 2007). Typically, two rapid generations of the wet season morph are followed by a single generation of the dry season morph that survives the rest of the year.

The dry season form has been suggested to be a stress-tolerant form (Brakefield et al. 2007), and various field experiments have attempted to establish the adaptive value of each morph. Although in nature the two morphs are distinctive, in the laboratory a continuum of forms can be generated between the two extreme morphs by adjusting rearing conditions (Brakefield and Reitsma 1991). Releasing these lab-reared forms into the field in different seasons allow the relative success of each morph to be assessed. From these, and other experiments with direct experimental alteration of the wing patterns (Brakefield and Frankino 2009), it is clear that the conspicuous wet season morphs have much lower survivorship in the dry season than the cryptic dry season morph, which benefits from its enhanced camouflage. The converse fitness effect, increased survivorship of the dry season morph in the wet season, is much weaker. There is some evidence that the wing eyespots confuse naïve predators and enhance the chance of escape (Lyytinen et al. 2003), but it may be that the conspicuous patterns serve another purpose entirely, for example making the butterflies more obvious to potential mates in a form where rapid reproduction is prioritized over prolonged survival (Brakefield and Frankino 2009).

Temperature is the major environmental determinant of adult morphology (Brakefield and Reitsma 1991). The second generation of wet season morphs develops through a period of declining ambient temperature as the wet season gives way to the dry season, which induces the larvae to metamorphose into the dry season form. Conversely, the larvae of dry season morph butterflies develop in the uniformly warm conditions of the wet season and develop into the wet season morph. Adults of either morph can be generated in the laboratory by altering the developmental temperature (Brakefield and Reitsma 1991). Other factors that retard or accelerate development can also influence the final form of the adult. For example, caterpillars reared on a low-quality artificial diet that slowed the rate of development developed into adults similar to the dry season morph, despite being kept at temperatures that would normally induce the wet season morph (Holloway et al. 1992). Artificial selection experiments with the aim of producing separate rapidly and slowly developing lineages induced corresponding changes in adult morphology; those of the slowly developing lineage had smaller eyespots than wild type butterflies reared at the same temperature whereas the rapidly developing lineage had a hypertrophied wet season phenotype (Zijlstra et al. 2003, 2004). So, even though ambient temperature is the normal driver of plasticity, the underlying arbiter is the rate of development.

The wing pattern is determined during late larval and pupal development and is controlled by morphogenetic hormones, especially ecdysteroids and these same hormones also appear to control seasonal polyphenism (Koch 1992, Koch et al. 1996). There is a surge of circulating ecdysone in the hemolymph after molting to the pupal stage which cues the development of adult structures. The timing of this surge relative to the start of pupation appears to be critical for cueing which form the adult will assume in a number of different butterfly species (Koch and Bückmann 1987, Rountree and Nijhout 1995, Koch et al. 1996). Thus, seasonal differences are represented internally by circulating levels of hormones (Koch 1992).

Ecdysteroid titer appears to have a key role in determining whether a *B. anynana* larva develops to have the wet or dry season morph as an adult (Koch et al. 1996), with lower rearing temperatures prolonging pupal development so that an increase in circulating ecdysone titer occurs later in development, which in turn induces the dry season morph. Microinjecting ecdysone into early stage pupae reared at low temperatures led to the emerging adults having larger eyespots and markings akin to that of the wet season form. In an artificial selection experiment designed to canalize larvae into consistently producing wing patterns with either a wet or dry season morph at a constant rearing temperature of 20°C, larvae selected for the wet season form also had faster pupal development times and higher ecdysteroid titers shortly after pupation (Koch et al. 1996). In other butterfly species other signaling mechanisms may act in conjunction with ecdysone. For example, neuropeptides may also alter the sensitivity of different tissues to ecdysteroids in some species and therefore have a role in determining the final adult morph (Endo and Kamata 1985).

How do ecdysteroids and other hormones determine wing pattern in butterflies? Butterfly wings are covered in minute scales, each of which has a single color (Nijhout 1985). The regulation of color pattern was investigated by Sawada et al. (2002) who examined the expression of guanosine triphosphate cyclohydrolase I (GTP-CH I), an enzyme with a key role in the biosynthesis of white pteridine pigments during