

INTRAGENERATIONAL DEVELOPMENTAL PLASTICITY

The central nervous system can integrate information about the animal's internal and external environment and use this information to regulate the secretion of hormones.

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In the course of evolution metazoans have evolved a high potential for adapting within their lifetime to changes and unpredictabilities of the environment, which is known as phenotypic plasticity. They express this potential in the form of a continuum of only quantitatively differing phenotypes, that is the reaction norm, or in the form of sudden discontinuous, discrete qualitative changes in phenotypic characters, that is intragenerational developmental plasticity. This latter form of phenotypic plasticity may be triggered by specific environmental cues (acquired developmental plasticity) or it may appear in the offspring independently of the environmental cues (innate developmental plasticity). As a rule, the intragenerational developmental plasticity is adaptive and involves changes or switches in specific developmental pathways activated by signals originating in the central nervous system. Intragenerational developmental plasticity involves no changes in genes, i.e. it is epigenetically determined and is not transmitted to the offspring.

Developmental Plasticity: Beyond the Reaction Norm

Metazoans are dynamic systems that can change during the lifetime, both *in response* to external or internal stimuli and *as a result* of external influences. This is the *phenotypic plasticity* in the broadest meaning of the term (figure 11.1). Phenotypic plasticity may occur in the form of the *norm of reaction* (Woltereck, 1909), consisting of a continuum of incrementally varying phenotypes involving no qualitative changes, or in the form of *developmental plasticity*, which implies appearance of *discrete* alternative phenotypes.

In describing the phenomenon of the appearance of alternative discrete phenotypes in this work I will use the term developmental plasticity to emphasize the fact that their appearance is determined by switches in developmental pathways.

Both the norm of reaction and developmental plasticity involve no changes in genes or genetic information in general. However, developmental plasticity and reaction norm are two qualitatively

different phenomena as far as the nature of the change they bring about and the underlying mechanisms are concerned.

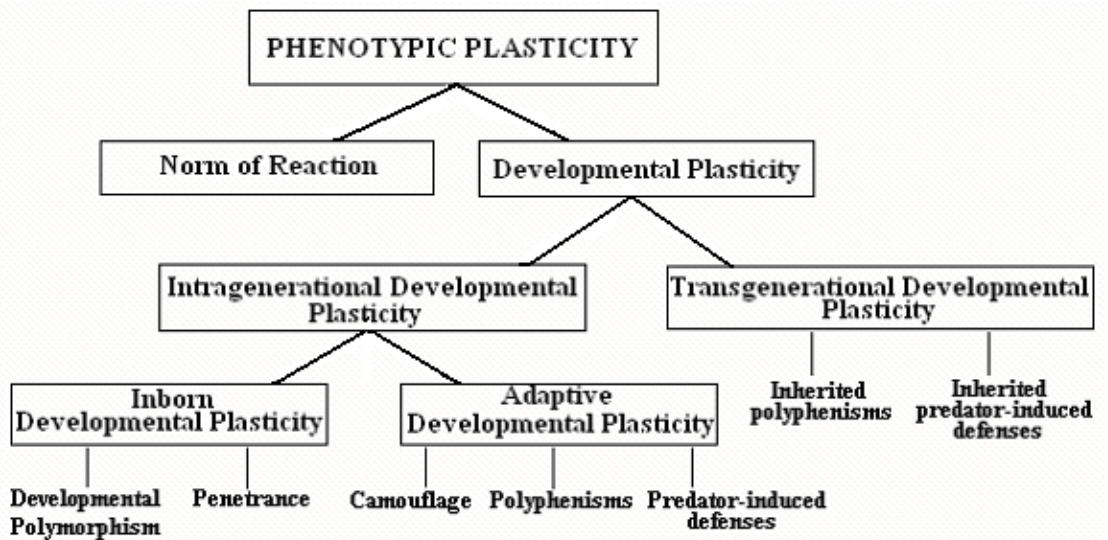


Figure 11.1. Main forms of phenotypic plasticity in metazoans under natural conditions.

First, the norm of reaction, as the term itself indicates, is a *reaction* to changes in the environment, whereas developmental plasticity does not always/necessarily depend on environmental stimuli (developmental polymorphisms, e.g.). In cases when the developmental plasticity is related to/depends on environmental stimuli it is an adaptive *response* rather than a reaction implying that it is not determined by the nature of the stimulus but by the adaptive needs of the organism, which switches to a new developmental pathway for achieving a discrete phenotypic change.

Second, developmental plasticity is related to switches in developmental pathways and mechanisms, while the reaction norm is not.

Third, and as mentioned earlier, the norm of reaction implies existence of a continuum of phenotypes displaying only *quantitative differences* between them, whereas developmental plasticity usually implies *qualitative changes* of the phenotype.

Some authors, however, believe that differences between polyphenisms (herein to be considered under the general term of developmental plasticity) and the norm of reaction are only quantitative differences:

It is likely that polyphenisms originate from continuously plastic phenotypes. Most modern polyphenisms are, in fact, reaction norms, although this is not always easy to detect in nature. In many and perhaps most polyphenisms, the discrete alternative phenotypes develop either because the environment is discontinuous or because the environment-sensing physiology has a threshold. A discontinuous environment is one that would be experienced by a bivoltine insect (an insect that has two generations per year). In such animals, each generation develops in a different season and thus experiences a different combination of photoperiod, temperature, nutrition, and population density. In many cases when such polyphenic insects are exposed to intermediate environmental

conditions, they develop a range of intermediate phenotypes not normally seen in nature.

Examples of threshold environmental sensitivity can be found in the seasonal polyphenisms of multivoltine insects that have a critical photoperiod for the induction of the polyphenism...Such insects develop alternative phenotypes depending on whether the photoperiod they experience is longer or shorter than the critical day length. Even with such thresholds it is possible to obtain a range of intermediate phenotypes, either by manipulating the environment or by manipulating the underlying developmental physiological mechanisms that produce the threshold. (Nijhout, 2003)

Some objections, however, can be raised against this interpretation of the relationship between the norm of reaction and polyphenisms.

Basically, Nijhout argues that

1. In some cases developmental plasticity is related to discontinuation of environments to which the animal is exposed during its life cycle and under experimental intermediate environmental conditions it may develop intermediate phenotypes, and

2. In other cases, the appearance of the alternative phenotypes depends on the level of the threshold of the sensitivity to the environmental stimuli.

The author initially identifies polyphenisms (developmental plasticity) with the norm of reaction but he is cautious to avoid generalizations by stating that this only applies to “Most modern polyphenisms”.

However, and as already pointed out, the appearance of polyphenisms depends on switches in developmental pathways, while the norm of reaction implies no such switches. This is an essential difference and the reason of the differences in the phenotypic results: the norm of reaction generally produces incremental quantitative changes in expression of the same character, whereas polyphenisms, under natural conditions, imply development of new or discrete changes, emergence of qualitatively different characters without intermediate forms that characterize the norm of reaction. The discontinuous changes observed in cases of developmental plasticity often are of the type “All-or-none” as in numerous cases of predator-induced defenses, developmental polymorphisms (animals give birth to offspring of two or more distinct morphs), or the development of different castes with conspicuously different morphology, physiology and behavior in social insects.

It is true that in a *limited* number of cases, when the environmental stimulus responsible for the norm of reaction is experimentally escalated beyond an upper limit, discrete changes of the type of developmental plasticity are produced. But this does not prove that there are no differences between the mechanisms of the norm of reaction and developmental plasticity. To the contrary, it proves that the norm of reaction and developmental plasticity result from clearly different mechanisms, with the later only resulting from activation of a new developmental pathway.

Most of the cases of developmental plasticity (polyphenisms) are related to attainment of a threshold in the sensitivity of the organism to external agents or conditions. But what determines a threshold or a set point? Metazoan organisms are under constant action of numerous external agents, but not all of them are perceived as stimuli. Whether an external agent will be perceived as a stimulus to which the organism responds by a developmental plasticity or not that is neurally determined. An external agent acts as a stimulus only when it is perceived as such in the CNS, i.e. when it attains a certain level of intensity, which represents a threshold or the set point beyond which the organism responds by a specific developmental plasticity. The threshold or the set point is determined by the specific neural circuit that processes the action of that agent or the stimulus in the CNS. When the agent is perceived as a stimulus (the action of intensity is above a neurally determined set point), the CNS activates a developmental pathway that leads to the sudden (not gradual or incremental) development of the new, discrete phenotypic trait, which is characteristic of the “polyphenism” (developmental plasticity) rather than of the norm of reaction. A threshold is not an environmentally imposed but a neurally determined level of intensity of a parameter, whose attainment serves as a stimulus for starting a new or modified developmental pathway. By processing the stimulus in a specific neural circuit the

organism generates an output that switches on a specific developmental pathway leading to the development of an alternative discrete phenotype.

Essential to keep in mind when the norm of reaction and the developmental plasticity are compared is the fact that the developmental plasticity, as a rule and as opposed to the norm of reaction, excludes intermediates between the two alternative forms.

Both the norm of reaction and developmental plasticity are biological concepts intended to categorize the ways an organism reacts to external stimuli: by incremental or by discrete changes in the phenotype. Concepts are created for organizing our information on the material and immaterial world and phenomena. In the case of the norm of reaction and developmental plasticity we have two clearly defined, related but not overlapping concepts. Hence, no change in our concepts and definitions of the norm of reaction and developmental plasticity is necessary. To blur the distinction is possible but hardly useful.

In the majority of described cases of developmental plasticity the discrete phenotypic changes are not passed on to the offspring. Hence they represent *intragenerational developmental plasticity* (polyphenisms, seasonal polyphenisms, predator-induced defenses, camouflage, changes in life history, discrete changes in the body mass, etc.). In cases when discrete phenotypic changes are transmitted to the offspring (or appears first in the offspring) of individuals that have experienced specific environmental stimuli, we speak of *transgenerational developmental plasticity*.

Intragenerational developmental plasticity may be *inborn* (appearance of more than one morph in the same brood), which represents *developmental polymorphism* (also known as genetic polymorphism), or may be acquired during the lifetime, what is known as *adaptive developmental plasticity* (camouflage, seasonal polyphenisms, predator-induced defenses, changes in the life history, changes to the age of maturity, etc).

In developmental plasticity I also include the so-called genetic polymorphisms. This is certainly controversial, hence I have to present the rationale for the inclusion.

What have been considered to be genetic polymorphisms, the presence of two or more distinct morphs in the offspring of the same brood, by definition is not genetically determined for all the individuals of the brood are of the same genotype.

As a validation of the hypothesis that inborn polymorphisms are genetically determined is usually presented the appearance of Mendelian ratios but, as argued by West-Eberhard, such ratios also appear in unambiguous cases of environmentally determined polyphenisms, such as caste determination in insects, suggesting that these ratios may be artifacts of constant experimental conditions (West-Eberhard, 2003f1).

Appearance of Mendelian ratios for specific phenotypic characters in the offspring is not necessarily an indication of existence of specific genes determining the characters. There is no scientific justification for deducing the presence of one or more genes based solely on the appearance of these ratios. In the modern era of genome sequencing it is scientifically very risky and substandard to infer existence of genes for particular phenotypic characters solely based on the experimental ratios of the appearance of phenotypic characters in the offspring.

Mendelian ratios are determined by the fact that genetic factors, genes are transmitted to the offspring via the parental gametes, but now we know that in the same way are transmitted other hereditary non-genetic, epigenetic factors, such as thousands of parental cytoplasmic factors, as well as imprinted genes, whose presence can lead to similar Mendelian ratios.

Let's consider only two example of the fallacy of the genetic approach of inferring presence of genes based solely on the appearance of the Mendelian ratios. Based on the appearance of the 3:1 ratio of worker to queens in the bee *Melipona marginata*, and 7:1 in other *Melipona* species, in 1950 Kerr concluded that these ratios were result of differences in genotypes of larvae that produce workers from those that produce queens:

The development of a diploid larva into a queen or into a worker bee depends in several species of *Melipona* upon the genetic constitution of that larva. In *Melipona marginata* the queens are always

heterozygous for two pairs of genes, *AaBb*. Homozygosis for either of these genes produces a worker. Males are either *AB*, or *Ab*, or *aB*, or *ab*. The mating of any of these males with the *AaBb* females gives segregation in a ratio of 1 queen: 3 workers among the diploid zygotes. In *Melipona quadrifasciata*, *Melipona schencki*, *Melipona fuscata melanoventer*, *M. favosa orbigny*, *Melipona interrupta fasciculata*, *Melipona javipennis* and *Melipona rujivenfris paraensis* females are heterozygous for three pairs of genes (*AaBbCc*), homozygosis for any one of which makes a diploid zygote develop into a worker. In these species the normal ratios of queens and workers in colonies approach 1:7. Deviations from these ratios are, however, observed in winter and in colonies that are attacked by parasites or diseases. These deviations may be accounted for by supposing that under certain conditions the male pronucleus is eliminated from the egg, and the division products of the female pronucleus fuse to give rise to diploid cleavage nuclei. (Kerr, 1950)

Later, after the recognition of the role of the JH (juvenile hormone) in caste determining in *Melipona*, the same investigator put forward the hypothesis that in *Melipona quadrifasciata* there were two sets of sex genes: one set that acts in the embryo and determines ovary or testis and another that acts in the prepupal stage determining the transformation of the imaginal discs and tegument in adult female or adult male structures (Kerr, 1974). Next he and coll. recognized

juvenile hormone as an integral part of the gene control for caste determination in *Melipona* (Kerr et al., 1975)

Then, in order to reconcile the new data on the JH control of caste determination in *Melipona* they had to postulate that JH activates some unidentified “feminizing genes, inducing differentiation of female larvae into queens” (Bonetti, Kerr and Matusita, 1995). It has also been postulated that JH secretion is regulated by hypothetical genes in two loci. However, more than half a century of studies to demonstrate the existence of specific genes that in heterozygous state could regulate secretion of these hormones (Kerr, 1950), have failed.

Now we know that caste determination in *Melipona* is not related to any difference in the genotype (individuals of all the castes are in possession of the same set of genes). It is function of switches in endocrine developmental programs (Pinto et al., 2002) and these JH and ecdysteroid switches are under control of neural epigenetic mechanisms (see chapter 2 on the mechanisms of gene expression in the CNS) rather than under control of any hypothetic “feminizing” or “masculinizing” genes.

Now we know that caste determination in *Melipona* is determined by the amount of food received (van Veen, 2000) rather than any differences in genotypes of gyne-producing and worker-producing eggs. Changes in the diet exert their influence on caste determination via the central nervous system, which regulates secretion of hormones JH (juvenile hormone) and ecdysteroids

Another example of the unfortunate inference of the presence of genes from the observed Mendelian ratios of phenotypes in the offspring comes from studies on the cave dwelling fish. Based on results of crosses between the blind hypogean and eyed epigeal forms of *Astyanax fasciatus mexicanus*, in 1973 P. Sadoglu came to the conclusion that the loss of eyes in cavefish was caused by mutations in genes responsible for eye development (Wilkins, 1971) and the number of degenerative mutations determines the degree of reduction or the loss of eyes (Wilkins, 1971). Now we know that no loss or mutations in genes involved in the loss of eyes has occurred in the blind hypogean form of *A. fasciatus mexicanus* (Jeffery, 2005).

Developmental Plasticity and Possible Evolutionary Implications

The fact that the developmental plasticity produces discrete traits that are not only quantitatively but qualitatively as well, different from the original phenotype raises the issue of the possible evolutionary implications of the developmental plasticity.

Here I will briefly discuss the possible involvement in the evolutionary process of the intragenerational developmental plasticity alone. The transgenerational developmental plasticity virtually represents a special form of evolutionary change and therefore it will be considered as a separate issue in the next chapter.

Discussing the role of developmental plasticity in evolution, West-Eberhard has shown that new phenotypic traits, arising in response to the changed environment or as a result of mutational events, may become evolutionarily relevant via processes of phenotypic and genetic accommodation. Phenotypic accommodation implies adjustments that are necessary for integrating the novel phenotypic trait to the general phenotype, in order “to reduce the amount of functional disruption occasioned by a developmental novelty”, whereas genetic accommodation implies the presence of variation in alleles whose frequency will increase under new selection regime determined by the phenotypic novelty (West-Eberhard, 2003d). Other authors also believe that novel phenotypic traits arise by environmental rather than genetic factors and they become evolutionarily relevant via the process of genetic assimilation (Pigliucci and Murren, 2003; Pigliucci et al., 2006). Both hypotheses hold that genetic variation is necessary for phenotypic/developmental plasticity to be evolutionarily relevant. The difference is that the first hypothesis implies that the genetic variation is present in natural populations and selection enables the evolutionary fixation of the phenotypic novelty, whereas the latter holds that phenotypic plasticity increases the chances of survival under changed conditions and mutations that may occur thereafter (genetic assimilation) enable the evolutionary fixation of the novelty.

However, it is difficult not only to prove but even to argue that mutations in DNA are involved in the evolution of developmental plasticity. Cases of developmental plasticity described so far, generally, show no signs of “functional disruption” that would require any mutations in genes (Pigliucci and Murren, 2003).

Theoretically, it might be argued that metazoans are capable of producing with the same genes not only small evolutionary changes but even radically different Baupläne (recall amphibian metamorphosis: during its lifetime, i.e. with the same genes, a frog sculptures such radically different Baupläne as that of the class of fish (as a tadpole) and amphibians (as an adult organism), and flies, during their lifetime, sequentially develop both worm and insect Baupläne. The fact that in many cases the plasticity is not adaptive, indicates that accumulation of gradual mutational changes under the action of natural selection is not necessary for the evolution of the developmental plasticity. One should always bear in mind that developmental plasticity involves no changes in genes or DNA, hence is essentially a non-genetic, epigenetic phenomenon.

The fact that the developmental plasticity commonly is adaptive to the changed environment in response to which it arises, suggests that it arises not spontaneously or randomly. The plastic response is somehow computed in the meaning that a relation is established between two unrelated elements, the environmental stimulus and the appearance of developmental plasticity. The establishment of this relationship implies as a *sine qua non* use of new information. The origin and nature of that information is essential for understanding the origin and nature of the epigenetic plasticity, the mechanism by which animals translate environmental stimuli into information for the phenotypic change. It is generally admitted that it is a neuroendocrine mechanism:

Whether there are any cases of adaptive phenotypic plasticity in animals in which development of the relevant trait is directly sensitive to the environmental variable or whether all cases are mediated by evolved integrated systemic processes, as in the case of polyphenisms....*Perhaps the most interesting thing about having a hormonal regulation of development is that development*

comes under the control of the central nervous system. This is because the developmental hormones are directly regulated by neurosecretory factors or are themselves neurosecretory hormones. The central nervous system can integrate information about the animal's internal and external environment and use this information to regulate the secretion of hormones (my emphasis - N.C). In this way, development can become responsive to a wide variety of environmental signals, without the need to have developmental processes themselves be sensitive to the environment. (Nijhout, 2003)

All the discrete morphological changes to be presented in this chapter start with, and essentially involve, reception and processing in neural circuits of visual, olfactory, tactile, or interoceptive input, perception or some other sensing by animals of environmental stimuli, including their predators. Since perception, or sensing, in any case takes place in the CNS it is logical to assume that the brain is the place where the causal chain leading from the stimulus to the changed morphology or life history starts (see chapter 2, *The Origin and Nature of Epigenetic Information for Metazoan Morphology*).

Intragenerational Developmental Plasticity

Adaptive Intragenerational Developmental Plasticity

Camouflage (Adaptive coloration, Cryptic Coloration, Crypsis)

Camouflage is generally defined as morphological adaptation “intended” to make an individual less visible in its background. It serves mostly preys to hide from their predators, but predators too may extract some hunting profits by being less visible. The adaptive character of the phenomenon is obvious and its basic mechanisms are known to a considerable extent. The chain of events from the visual perception of the environmental background to generation of a body pattern resembling the background often is very complex but already known in some details.

Let's consider some paradigmatic examples of adaptive coloration in animals and use current knowledge for understanding its mechanism.

Under strong disturbance or provocation, the cuttlefish *Sepia officinalis* undergoes a series of colour-changes as conspicuous and complete as they are rapid. The first of the successive patterns is one in which two large black spots appear on the dorsal surface of the mantle. Then a rapid and complete paling of the rest of the animal follows, while the black spots themselves become still more saturated and intense... At the same time a black crescent forms beneath each eye, the pupil dilates and the edges of the fins become strongly outlined in black - the rest of the body remaining white.

Alternatively, the two-spot pallid phase may be followed by the animal shooting away, the rapid departure being accompanied by simultaneous darkening of the whole body. Further irritation may lead to total paling and the superimposition of four longitudinal black stripes on the upper surface. These lines flicker vividly over the pallid back, and then suddenly disappear, to be followed by a reappearance of the zebra pattern. All this time, the animal darts about rapidly, as if to avoid the irritation, and its final action when can not do so is to eject a cloud of ink. Then it becomes motionless and hides below the black cloud which it has produced. (Cott, 1966a)

The body pattern of a cephalopod, its gross appearance, is determined by skin chromatophores (from Gr. χρωμα (hroma) - color and φορος (foros) - bear, carry). The primary function of chromatophores is

camouflage. Each chromatophore contains pigment granules and is connected with myocytes and neurons.

Neural signals from the brain determine patterns of muscle contraction and, consequently, the dispersion of pigment granules within the chromatophore, so that in their totality the skin chromatophores produce a pattern that matches the background, achieves general resemblance to the substrate, or breaks up the body's outline. Neural control of chromatophores enables a cephalopod to change its appearance almost instantaneously, which is a key feature in some escape behaviours and during agonistic signaling. This rapid body pattern polyphenism is adaptive because it may hinder search-image formation by predators.

The change of color or patterning of the body in cephalopods is accomplished by millions of chromatophores, multicellular organs consisting of a central pigment-containing cell attached to a set of 6-20 radially arrayed chromatophore muscles. These muscles are innervated by motor neurons whose cell bodies in the European cuttlefish, *Sepia officinalis*, are located in the PSEM (posterior subesophageal mass) of the brain (Dubas et al., 1986; Gaston and Tublitz, 2006) (figure 11.2).

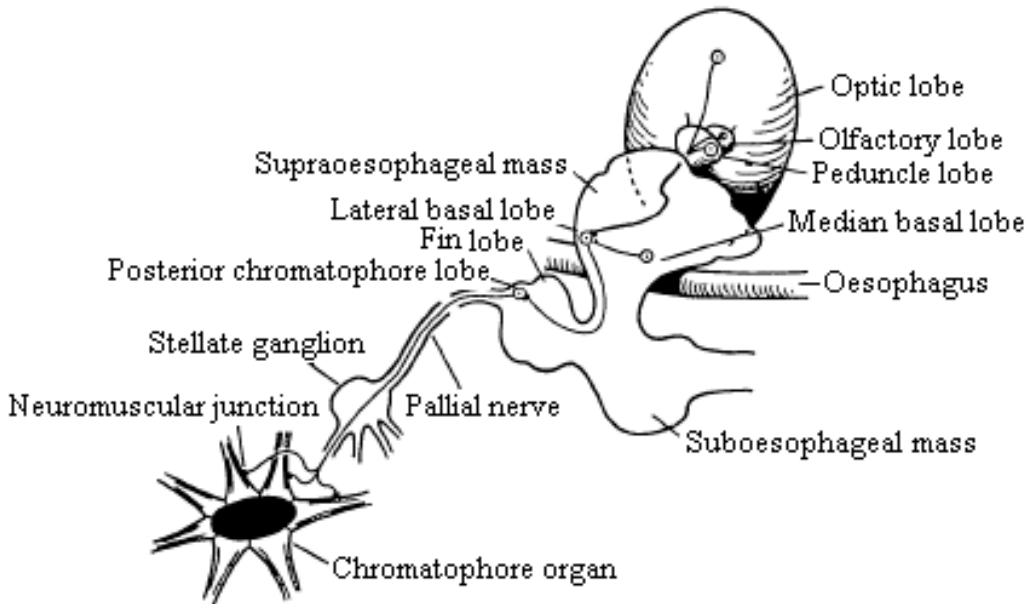


Figure 11.2. Diagrammatic representation of lobes in the cephalopod central nervous system that are thought to control chromatophore patterning. Only one side of the brain is represented and the anterior chromatophore lobe is omitted for the sake of clarity. The main pathway is considered to be: optic lobes to lateral basal lobes to chromatophore lobes to chromatophores (From Dubas et al., 1986).

By releasing neuropeptides of the FaRP (FMRFamide-related peptides) family and glutamate in the chromatophore neuromuscular junction these motor neurons regulate contraction of muscles and the color patterning according to the perception of the background that forms in the cuttlefish's brain (Loi et al., 1996; Loi and Tublitz, 2000). The whole pathway of body patterning is neurally determined:

The chromatophores are controlled by a set of lobes in the brain organized hierarchically. At the highest level, the optic lobes, acting largely on visual information, select specific motor programmes (i.e. body patterns); at the lowest level, motor neurons in the chromatophore lobes

execute the programmes, their activity or inactivity producing the patterning seen in the skin. (Messenger, 2001)

Chromatophore lobes in *Octopus vulgaris* contain over half a million neurons. The fact that those animals change their body pattern to match their background within a very short time of about a second in cuttlefish (Hanlon and Messenger, 1988) and 2-8 sec in fish seems to exclude any inducer molecules (hormones or secreted proteins) as a possible regulator of body patterning:

Thus, a neural rather than humoral, mechanism must be involved. (Ramachandran et al., 1996)

Now we know that specific small molecule- and peptide neurotransmitters released at the cephalopod neuromuscular junction induce chromatophore functions. The first neurotransmitter to be recognized to have a role in the functioning of chromatophores has been the glutamate. Recently, it has been shown that endogenous neuropeptides of FMRFamide family are involved in the body patterning of *Sepia officinalis* (Loi et al., 1996; Hanlon and Messenger, 1988).

And since this form of camouflage results from disruptive coloration, i. e. from combination of a varied number of chromatic units, it has been proposed that a physiological unit in the brain must be responsible for each chromatic-morphological unit in the skin (Packard, 1982).

Thus, *Sepia officinalis* first draws the camouflaging image of its body in the brain. The size, contrast, and the number of white squares in the black background are cues the cuttlefish (order Sepiidae) use to switch from uniformly stippled skin patterns (general resemblance) to disruptive skin patterns (Chuan-Chin and Hanlon, 2001).

The pathway of patterning signals from the brain physiological units to the skin chromatophores in cuttlefish, within the broader schema of information flow along the body patterning circuitry, has been schematized as follows:

visual input → eyes → brain optical lobes → brain lateral basal lobes → brain chromatophore lobes → skin (Chuan-Chin and Hanlon, 2001).

Intraspecific signaling and communication is another function of chromatophores that is well documented in several inshore species, and interspecific signaling, using ancient, highly conserved patterns, is also widespread. Neurally controlled chromatophores lend themselves supremely well to communication, allowing rapid, finely graded and bilateral signaling.

Many *crustaceans* are also able to rapidly modify their color so that it matches the background. Blood-borne factors involved in their phenotypic adaptive coloration belong to two main groups. There is a group of neuropeptides from the X organ-sinus gland complex, which acts directly on chromatophores, causing either dispersion or concentration of the pigment granules. The second group consists partly of neurotransmitters acting within the nervous system by triggering the release of chromatophoretropic neuropeptides. The rest of them, primarily amines, are released in haemolymph from pericardial organs (Gorbman and Davey, 1991). Two main neurohormones involved in adaptive coloration of crustaceans are DRPH (light-adapting distal retinal pigment hormone), with light-adapting function, and its antagonist neurohormone, RPCH (red pigment-concentrating hormone), with the latter being responsible for adaptive coloration of all pigment cells (melanophores, erythrophores, leukophores, and xanthophores) (Josefsson, 1983).

It is demonstrated that electrical stimulation of eyestalks in *Crustacea* results in the release of peptides with activity on specific types of chromatophores (Gorbman and Davey, 1991d). The neuropeptide, RPCH also controls the color changes in shrimps (Strand, 1999j) and modulates the swimmeret activity rhythms in the crayfish.

Neurohormones regulate color changes and movement of pigments during light-and-dark adaptation of eyes in other arthropods (Pearse et al., 1987).

The central nervous system also determines skin color changes in many fish and amphibians. Fish

scales and amphibian skin also contain specialized pigment cells, *melanophores*. The neurohormonal stimulation of these cells, under conditions of stress/alertness, stimulates melanophores to transport (along microtubules) membrane-enclosed pigment granules toward the periphery (producing darkened cell color) or center (producing pale cell color) (Ramachandran et al., 1996; Lodish et al., 1998). In response to artificial changes in the background, fish (Ramachandran et al., 1996), like cuttlefish (Chuan-Chin and Hanlon, 2001), repattern their body to match the background if the backgrounds display “appreciable differences” (Marshall and Messenger, 1996). This clearly implies visual input and perception of the background and since that perception takes place in the brain, the color change and repatterning of the skin is clearly under control of the CNS:

The fish must have independent visual control of each set (or subset) of markings, a possibility that requires verification. There may be ‘feature detectors’ in the fish visual centres that are specialized for detecting different spatial frequencies of textures in the environment and these might exert direct control over the corresponding set of marks on the skin surface. Indeed, there might be a map of effector neurons in (say) the tectum, so that focal electrical stimulation might produce selective contrast enhancement of specific spatial frequency components on the skin. (Ramachandran et al., 1996)

The paradise whiptail, *Pentapodus paradiseus*, is a fish inhabiting the coastal waters of Queensland, Australia. The fish has colored stripes on its body and is able to change its color from blue to red within less than one second (Mäthger et al., 2003). The coloration of this and many other fish is determined by iridophores (light-reflecting cells) in the skin. They contain thin guanine plates, which are multilayer reflectors with refractive index higher than spaces separating them. Plates are connected with each other by microtubular structures. Any change in the distance between plates contained within the reflective cells will produce a change in the reflected color. This adaptive change in the distance between plates is regulated by underlying microtubule structures (Oshima and Fujii, 1987). Changes in the structure or length of microtubules change the distance between guanine plates and this leads to changes in the color reflected by iridophores. It has been found that the sympathetic nervous system regulates the distance between plates and the body color by regulating the length (polymerization/ depolymerization) of microtubules. Under stress conditions, the release of noradrenaline shifts the reflection toward red (longer wavelength), whereas in response to topical application of the neurotransmitter acetylcholine spectral reflections shift towards shorter wavelengths (Mäthger et al., 2004). Fluctuations in intracellular Ca^{2+} also change the structure of microtubules (Mäthger et al., 2003) and, consequently, the distance between plates and the reflected color by iridophores. In experiments it has been found that the neurotransmitter Ach (acetylcholine) increases the Ca^{2+} level (Mäthger et al., 2004). Studies on the color change (from blue to green) in the blue damselfish, *Chrysiptera cyanea*, and in the neon tetra (Nagaishi and Oshima, 1989) have led the investigators to the conclusion that

The motile iridophores are solely under the control of the sympathetic adrenergic system, and that the co-transmitter, adenosine, may function to antagonize quickly the true transmitter-induced colored state of the cells. (Kasukawa et al., 1986)

Monocirrhus polyacanthus is a little Nandid fish in the Lower Amazon Valley. It resembles a dead leaf. Populations of this fish, known as “Peche de folha” by the Brazilian natives, consist of individuals of three main color groups (light gray, golden with only a few mottlings of dark-brown, and brown). All of them are capable of changing within one hour their color to darker or lighter tint according to the tones of the background (Cott, 1966b). At his time, O. v. Frisch, was marvelled: “How the fish brain can command the pigment cells so skillfully challenges comprehension” (Frisch, 1973). When on a white background, the teleost fish medaka, *Oryzias latipes*, gradually acquires a lighter

body color. This is result of two processes: initially, reduction of the size of skin melanophores and later the programmed cell death, apoptosis of dark pigment cells, melanophores (Sugimoto, 2002). The opposite is observed when medaka fish are kept on a dark background: size reduction and apoptosis of leukophores - white pigment cells. Experimental chemical denervation suppresses apoptosis of melanophores, suggesting that the process of melanophore apoptosis is also regulated by skin sympathetic innervation (Sugimoto et al., 2000). In other fish, adaptation to dark background for 2 weeks leads to an increase in the number and size of melanophores in the skin as well as to increased concentration of the pigment in those cells (van Eys and Peters, 1981).

Some teleost fish also change their body color/patterning as a way of communication in their social interactions. In Arctic char, *Salvelinus alpinus*, subordinate individuals, in the presence of dominant individuals, experience constant stress and activation of the hypothalamus-pituitary-interrenal axis via serotonergic neurons. This is associated with a submissive behavior and darkening of the skin as a result of increased secretion of the pituitary α -MSH (α -melanophore-stimulating hormone) and ACTH (adrenocorticotrophic hormone). By contrast, release of neurotransmitters catecholamine, dopamine and norepinephrine, suppresses secretion of the above pituitary hormones and induces aggressive behavior and lighter body color. The darkening of the skin in subordinate Arctic charr fish has been interpreted as being intended to reduce unnecessary fights and energy loss “in an established dominance hierarchy” (Höglund et al., 2002).

The clawed toad, *Xenopus laevis*, also modifies its body color so that it matches various environmental backgrounds. The hormone determining this adaptation is α -MSH (α -melanophore-stimulating hormone) synthesized and secreted by the pituitary under central neural control. Various neurotransmitters are involved in the release of this hormone by the pituitary. Using axonal tract tracing, R. Tuinhof et al. (1994) have identified details of the neural circuit (from retina to the hypothalamus), whose signals to the pituitary stimulate the release of the hormone and make the adaptive coloration possible (Tuinhof et al., 1994). Neurons of the suprachiasmatic nucleus project to the pituitary α -MSH (melanophore-stimulating hormone)-producing cells, where they release neurotransmitters regulating α -MSH synthesis (Tuinhof et al., 1994). Another serotonin center in raphe nucleus innervating the pituitary is also involved in the regulation of the secretion of the hormone (Ubink et al., 1999) (see also section *Generation of Information for Adaptive Camouflage in Xenopus* in chapter 2).

Larvae of salamanders of the family *Ambystomatidae*, when kept in total darkness, blanch after about one hour. When illuminated they darken in a dark background and brighten when the background is white. Predictably, removal of eyes prevents these camouflage responses in salamanders.

In arthropods neurohormones regulate color changes and movement of pigments during light-and-dark adaptation of their eyes (Pearse et al., 1987). It seems that this neural circuit might be less complex than some known behavioral circuits. It is important, in this regard, to remember that melanocytes (pigment cells) of the human skin derive from neural crest cells which, on the way of their migration to the ectoderm first have made a stop in the developing brain.

It is not surprising that other color-adaptable animals when finding themselves in a uniformly colored or monochrome environment such as snow-covered polar areas, over time (within a sufficient number of generations) switch from this adjustable coloration to a fixed white body color matching the white color of the environment.

Mimicry is a special form of camouflage where a species (the mimic) develops morphologies and behaviors resembling those of other species (the model). During the Batesian mimicry [after the British naturalist and explorer, Henry Walter Bates (1825-1892)], the mimic resembles a model that is not attractive or even is abominable to the predator, whereas during Müllerian mimicry [after the German zoologist Johann F. T. Müller (1821–1897)] two species, both abominable to their predators evolve similar morphology to deter the predator. The adaptation of the mimic to the model may be profitable to the mimic in the case of Batesian mimicry, or may be profitable for both mimicry partners in cases of Müllerian mimicry. A hypothesis presented by C. Darwin (1874) posits that Batesian mimics began

to evolve at a remote past, when the model and mimic were much more similar. Even if right, the hypothesis would only account for a limited number of known cases of mimicry (Turner, 1977).

Juveniles of the predator coral fish, fang blenny (*Plagiotremus rhinorhynchos*), develop a striking resemblance to their prey, cleaner wrasse (*Labroides dimidiatus*) but only when in the proximity of about 1 meter from the prey; when removed from the prey/model, they lose the mimetic coloration and restore the normal color (Moland and Jones, 2004).

Environmental stress (heat shock or cold shock, e.g.) can modify the wing patterns in butterflies in such a way that they resemble certain wing pattern mutants (A. Kühn and K. Henke, 1936), implying that morphological changes attributed to mutations are not always or necessarily related to genes, as it has been for a long time taken for granted. A temperature-induced pattern modification that makes butterflies resemble other species has also been described (Nijhout, 1985).

There are many known cases of coloration and mimicry that do not offer any advantage to the carriers, what casts doubt on the role of the natural selection in the evolution of the mimicry. By considering the gradual evolution to be impossible and “saltational” origin unacceptable, Turner developed a two-step hypothesis, according to which, a mimicry starts with a major mutation achieving a sudden rough resemblance to the model, and is followed by the establishment of further (modifier) genes at other loci, which will improve the pattern (Turner, 1977). This hypothesis succeeded in overcoming the difficulty arising by the obvious impossibility of accumulation of small, nonadaptive mutations, but it contradicts Fisher’s assertion that carriers of major mutations always suffer severe, deleterious side effects. Besides, as Orr and Coyne argue, while differences in characters determined by a single gene are easily identifiable, it is very difficult to discriminate between many genes of small effect and the presence of major genes amid many modifiers. Hence, experimentation has “little power to detect the presence of major genes” (Orr and Coyne, 1992) and no reliable evidence exists to demonstrate the existence of such major genes.

Suddenly occurring changes in the body coloration and patterning are beyond the explanatory power of neoDarwinian paradigm. Camouflage and mimicries usually involve activation and expression of such a great number of genes that it is impossible even to imagine how numerous individual changes in numerous genes could have been accumulated often without offering any advantage to the carriers. As T.H. Morgan would put it more than a century ago:

There is no need to question that in some cases animals may be protected by their resemblance to other animals, but it does not follow, despite the vigorous assertions of some modern Darwinians, that this imitation has been the result of selection. (Morgan, 1903)

With neural mechanisms proven to regulate camouflage (cryptic coloration), there is no visible reason for excluding the possibility of the involvement of the central nervous system in the evolution of mimicries. Any case of camouflage and mimicry implies, as a *sine qua non*, perception of the body color and neural representation of the model in the brain of the camouflant or mimic. While the neural processing of visual stimuli leading to the cascade of events that change the color and pattern to produce cryptic coloration is experimentally determined in numerous cases we are still ignorant on the mechanisms used by the mimics for transmitting the mimicry to their offspring.

It could be argued that the insect, the fish and clawed frog are aware of the fact that the mimicry and adaptive coloration contributes to their survival no more than an amoeba knows that its debris-engulfing routine is necessary for its survival. This argument is hardly relevant since the control of the CNS on adaptive changes of color in both fish and *Xenopus* takes place on an unconscious level, and for the color adaptation to take place is not necessary for these animals to “know” what they are doing. The unconscious instinctive “knowledge” is all they need to adapt their color or pattern to the background.

The conscious-unconscious dualism is an anthropocentric view rather than an evolutionary principle. No matter at what level of the CNS activity the adaptive coloration might be controlled and regulated,

it is an *adaptive*, non-random phenomenon, based on the innate instinct that contributes to animal's camouflage and survival.

Commenting on the insect mimicry, more than a century ago, Alfred Taylor wrote:

Here, in this common British butterfly, we have the whole problem set before us - vivid colour, the result of intense and long continued effort; grand display, the object of that colour; dusky, indefinite colour, for concealment; and the "instinctive" pose, to make that protective colour profitable. The insect knows all this in some way (Taylor, 1886)

Summarizing the evidence on the mechanisms of adaptive coloration presented in this section it may be said that no changes in genes are involved and no hypothetical mechanism has been ever presented to show how changes in genes may induce adaptive coloration. Vast majority of the known cases of adaptive coloration could reasonably be explained based on the experimental evidence on the neural mechanisms of coloration determined for a number of species.

Polyphenisms Polyphenisms in Invertebrates

Populations of a marine bryozoan, *Membranipora membranacea*, are polymorphic for inducible spine type and consist of a constitutively spined type (6.2%), which produces spines in the absence of the predator stimulus, an unspined type (13.4%), and an inducible type (80%), which produces spine when detects the presence of its predator in the environment (Harvell, 1998). This is a complex case that comprises both polyphenism and predator-induced plasticity (in the case of the inducible type) triggered by the perception of the predator or its kairomones in the bryozoan's brain.

Butterflies of the families *Nymphalidae*, *Pieris* and *Papilionidae*, produce pupae that display different body colors depending on the color of the pupation site. The peacock butterfly *Inachis io* (*Nymphalidae*), the white butterfly, *Pieris brassicae* (*Pieridae*), and *Papilio polyxenes* (*Papilionidae*), as pupae, display one of two alternative cryptic colors, green-yellow or brown-black, depending on the prevailing color at the pupation site. In order for the pupae to be able to adopt the body color that better matches the color of its surroundings, they first have to perceive the prevailing color of the pupation site. This perception takes place in the insect's brain and in the insect's brain is also synthesized the first signal of the signal cascade leading to production of the cryptic body color (green or brown). That signal is a neuropeptide, PMRF (pupal melanization-reducing factor), which is released from the brain into the hemolymph. PMRF is located throughout the entire central nervous system, but its release during the pre-pupal stage is controlled by neural stimulation from the brain.

When pupation of butterflies of the species *Inachis io* and *Pieris brassicae* takes place in a light-green background, the pupa synthesizes PMRF, which starts a signal cascade that leads to accumulation of lutein into cuticle and appearance of the green-yellow body color. When pupation takes place on a dark site, PMRF is not produced, lutein is not incorporated into the cuticle and the pupa appears black. Butterflies of the *Papilionidae* family, although using the same neurohormone for determining their cryptic body color, seem to rely on a different mechanism:

If the plasticity in pupal color in *I. io* and *P. polyxenes* is controlled by PMRF, then the neural control of the hormone's release would have to be different in the two species. In *I. io* (and *P. brassicae*) the hormone is released when pupation is on or in green vegetation, while in *P. polyxenes* it is released when pupation is on a brown surface. (Starnecker and Hazel, 1999)

Experiments on *Graphium sarpedon nipponum* (Frühstorfer, 1903) have shown that this butterfly determines its protective body color during the pupal stage, according to the color of the pupation site: green - on white background and reddish-brown - on black background.

Pupae of *Papilio xuthus*, however, use not the background color but the smoothness of the surface of leaves and twigs, on which they molt, as cues for determining their body color. This indirect cue also leads to adaptive coloration. On leaves and new twigs, which are smooth and green, they become green, while on dead branches, which have rough surface and brownish color, they become grayish-brown. For explaining the mechanism of the body colors in these cases Hiraga has presented the “tactile signals accumulation model”, according to which body coloration is related to accumulation of tactile signals and perception in the pupal brain of the smoothness/roughness of the surface of the pupation site (Hiraga, 2005; figure 11.3).

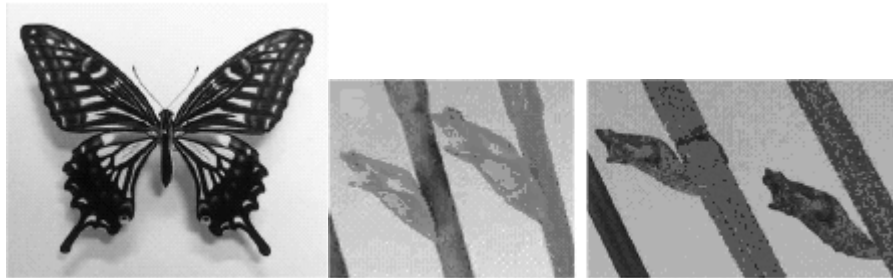


Figure 11.3. Adult butterfly *Papilio xuthus* and its pupae developing lighter on smooth twigs and darker on rough twigs (From Hiraga, 2005).

In some cases, social and olfactory stimuli also stimulate adaptive responses in the form of changes in phenotype and life history. So, e.g. exposure of female cockroaches of the species *Nauphoeta cinerea* to conspecific males or even to male pheromones alone, affects their time of reproduction, increases the number of offspring they produce, and increases the biases of producing parthenogenetic offspring in this facultative parthenogenetic species (Moore and Moore, 2003).

A proportion of eggs of *Drosophila mercatorum* develop parthenogenetically into adult flies. The proportion of unfertilized eggs, which develop parthenogenetically into adult flies increases with the decrease of the population density (Kramer and Templeton, 2001), suggesting that social or olfactory factors play a role of in determining the parthenogenetic development.

Whether a female individual in a bee colony will become a worker or queen is determined by the kind of food they are provided with. At a biochemical level it is also known that the fate of those individuals depends on the level of circulating juvenile hormone in the fourth larval stage, which in turn is determined by brain signals that stimulate (allatotropins) or inhibit (allatostatins) the synthesis of JH (juvenile hormone). The “caste-determining” effect of the food, thus, is determined in the CNS.

In North American field crickets *Gryllus firmus* and *Gryllus rubens*, formation of wings is determined by lack of juvenile hormone, as it may be concluded from the fact that in nonpolyphenic winged female crickets, *Gryllus assimilis*, formation of wings is prevented by stimulating JH secretion (Zera, A.J. et al., 1998), which is under strict control of brain neurohormones (allatotropins and allatostatins). A.J. Zera and coll. (1998) have produced analogous flightless female offspring with enlarged ovaries in the nonpolyphenic cricket, *G. assimilis*, by simply applying a juvenile hormone analog on adult females. *In vivo* experiments have demonstrated that flight-capable morphs of *Gryllus firmus*, synthesize greater amounts of total lipids and triglycerides necessary for flight, whereas flightless morphs synthesize greater amounts of phospholipids deposited in the eggs and ovary. However, the

local administration of juvenile hormone in flight-capable morphs increases secretion of phospholipids similarly to the flightless morphs (Zhao and Zera, 2002). Moreover, experimental increase of the level of JH in flight-capable crickets not only increases the ovarian size, similarly to flightless morphs, but it also leads to the loss of flight muscles by histolysis (Zera and Cisper, 2001).

Populations of the common pond skater (a long-legged insect gliding over water), *Gerris lacustris* (L.) (Heteroptera: Gerridae), in Bavaria, Germany, show remarkable environmentally-induced discrete phenotypic differences in life history and morphology. Populations living in the field ponds are bivoltine (produce two generations annually) with predominantly long-winged individuals, while forest pond populations are univoltine with an increased proportion of short-winged flies (Pfennig and Poethke, 2006).

Seasonal Polyphenism in Insects

Each year the African butterfly *B. anynana* produces two different seasonal morphs: a wet-season generation of individuals with large marginal eyespots of several colors and a transverse band on the ventral side of the wings, and individuals with small eyespots of fewer colors and no band on their wings in the dry-season (figure 11.4).

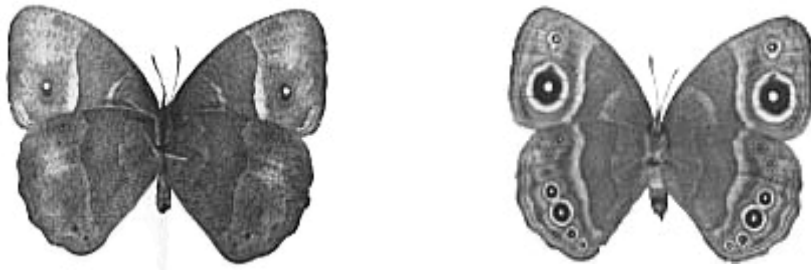


Figure 11.4. Dry-season morph (left) and wet-season morph (right) of *Bicyclus anynana*.

Both patterns are adaptive under respective conditions in environment: the patterning of the dry season morph is cryptic, resembling the brown leaves on the forest floor, whereas the conspicuous patterning of the mobile wet-season morph might serve as a warning to predatory birds and lizards.

It is observed that earlier secretion and higher level of ecdysteroids in the young pupae is responsible for the wet-season morph with large eyespots and a transverse band on their wings (Koch et al., 1996; Brakefield et al., 1996) and ecdysteroid treatment, depending on temperature, may enlarge eyespots by locally regulating the synthesis of pigments. Recall that production of ecdysteroids is under the control of the insect CNS: it is stimulated by the prothoracicotropic hormone (PPTH), a brain neurohormone, and inhibited, at least in some insects, by another hormone and by direct neural control (Chapman, 1998d).

The tropical butterfly, *Bicyclus anynana*, also responds to the predictable seasonal changes in temperature by adaptively changing the egg size and this change is predominantly nongenetically, maternally, determined (Steigenga et al., 2005). In order to perform the eyespot-inducing function, ecdysteroids have to bind their nuclear receptor, EcR (ecdysteroid receptor), which forms a heterodimer with USP (ultraspiracle protein). The final color of the scale cells in *B. anynana* wings coincides with expression of nuclear ecdysteroid receptors (EcRs) and the patterns of EcR expression in the wet-season and dry-season butterflies are different (Koch et al., 2002).

The complex patterning of the wing eyespots results from a complex spatial pattern of the activity of the hormone but ecdysteroid hormones are released in the haemolymph and are uniformly distributed all over the butterfly wing and body. Hence, it is plausible that the patterning and the color of the wings may be determined by the patterns of expression of the EcR (ecdysteroid receptor) in the butterfly wings.

The close correlation between expression of EcRs (ecdysteroid receptors) and colored scale cells in *B. anynana* wings arises the critical question: what determines the expression patterns of EcRs, and ensuing patterning and colors, in *B. anynana*'s wings?

It is known that ecdysteroids themselves upregulate EcR expression, but given the fact that they circulate with haemolymph, they cannot determine the spatial pattern of expression of EcR in the eyespot foci. A specific inducer of EcR expression in insects is local innervation, as it has been demonstrated in experiments with other insects where denervation prevents upregulation of EcRs by ecdysteroids. This is also corroborated by the fact that denervation (axotomy of the motoneuron) of the DEO1 (dorsal external oblique1) muscle in *Manduca sexta* prevents almost totally expression of the EcR-B1 (figure 11.5) and the growth of the muscle. The inducer of EcR-B1 expression released from the motoneuron is probably a diffusible factor because the effects on the EcR-B1 expression are observed beyond the contact of the muscle with the arbor (Hegstrom et al., 1998).

Based on their experiments, investigators concluded:

Innervation regulates the choice of EcR isoforms expressed in growing muscle. (Hegstrom et al., 1998).

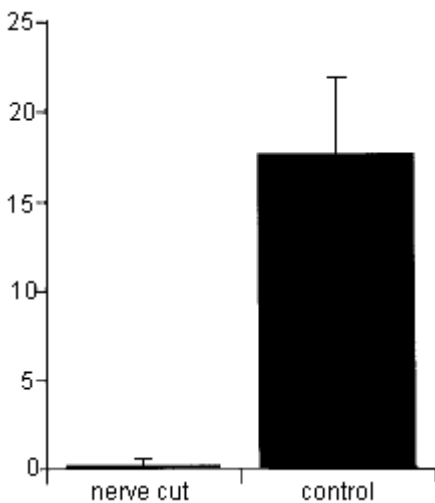


Figure 11.5. Effect of unilateral axotomy on the number of myonuclei expressing EcR-B1 within a 10,000 mm² square of the central portion of the intact and denervated fiber. Axotomy was performed on diapausing pupae; 72 hr later the pupae were injected with 20E to initiate adult development (From Hegstrom et al., 1998).

The main external cue activating the neurohormonal mechanism for seasonal diphenism in *B. anynana* is the temperature during larval-pupal stages (temperatures >24°C induce wet-season eyespot morph and temperatures under 20°C - dry-season, eyespotless morph).

By manipulating temperatures and selecting (for absence of eyespots under low temperature) for up to

20 generations investigators succeeded in obtaining monophenic forms of *B. anynana*, which produce only one of the phenotypes (with or without eyespots) even when reared under alternative environmental temperature. This is an experimentally induced evolutionary change involving no change in genes.

Seasonal polyphenism is also observed in many other insects. The black swallowtail butterfly, *Papilio polyxenes*, e.g., produces larvae of darker color in fall and larvae of lighter color in summer. The polyphenism seems to be adaptive since darker larvae, which are produced when the photoperiod is shorter and the environmental temperature lower, have higher growth rate (Hazel, 2002).

In response to experimental winter-approaching cues and predator cues, the damselfly *Lestes sponsa*, shifts to the so-called cohort splitting, which is diapausing and overwintering by minimizing the developmental rate (Johansson et al., 2001) until spring. Remember: cues such as photoperiod and

temperature are analyzed in the central nervous system and the CNS is the site where the external stimuli are related to the changes in color of the larvae. The overwintering morph of the comma butterfly, *Polygonia c-album*, has the underside of the wings of gray-brown coloration, while the summer morph has much lighter coloration. The summer morph is believed to have evolved “not as anti-predator adaptation but more likely as a result of the benefit conferred upon directly developing butterflies that can reallocate resources from soma (gray-brown coloration, e.g.) to reproduction and in so doing deinvest in soma and cryptic coloration”. By avoiding diapause, i.e. by developing directly, the summer morph can allocate more resources to reproduction. Hence, it is believed that the summer morph evolved from the overwintering morph (Wiklund and Tullberg, 2004).

The European map butterfly, *Araschnia levana* L., exhibits strong seasonal polyphenism. It has two seasonal forms: the short-day spring generation developing from diapausing pupae into red butterflies with black spots, and the long-day summer generation of black color with a vertical white stripe that develops from non-diapausing pupae but also develops larger body and wings and shows greater mobility. The seasonal diphenism of *A. levana* L. is also regulated by timing of ecdysteroid secretion (Koch, 1987), which is under neural photoperiodic control (Fric et al., 2004). Switching from one morph to the other is determined by the length of the photoperiod (short photoperiods induce formation of the spring morph and long photoperiods - the summer morph) (Koch and Bückmann, 1987).

Larvae of a bivoltine race of the silkworm, *B. mori*, can change from the summer morph into autumn morph, and the reverse, under action of other neurohormones, such as the Br-SG (brain-subesophageal) complexes, various neuropeptides, such as SMPH (summer morph-producing hormone), DH (diapause hormone), etc.

NeoDarwinian Explanation of the Seasonal Polyphenisms in Insects

The neoDarwinian explanation of the seasonal polyphenism of *B. anynana* presented here is based on the interpretation of the phenomenon in the case of some *Drosophila* spp. of California by Theodosius Dobzhansky, one of the founders of the neoDarwinian evolutionary synthesis. These *Drosophila* species in spring produce generations adapted to warm weather whereas the autumn offspring is adapted to cold weather (Dobzhansky, 1971d):

The readaptation to warmth need not be any more difficult than the adaptation to cold was; both will depend on the availability of genetic raw materials on which natural selection can act. But the readaptation may occur in two ways. (Dobzhansky, 1971c)

Dobzhansky tried to explain the phenomenon with the presence of genetic variability, i.e. the presence of the necessary adaptive genes in the population's gene pool:

If old genes, adaptive to warmth were not completely eliminated from the population during the cold phase, they may now be selected and the gene pool may revert to its old state... a microevolutionary change occurs every year and is undone as the season changes. (Dobzhansky, 1971c)

Decades after this hypothesis was presented there is no hint on the existence of “genes adaptive to warmth” in these species. But the strange statement that “a microevolutionary change occurs every year and is undone as the season changes” may be responsible for adaptation of myriad of *Drosophila* individual contradicts population genetic knowledge.

My imagination is too weak to envisage how any “microevolutionary change” could be related to the seasonal polyphenism of *B. anynana*, but for the sake of argument, let's take it for granted that such an event would be possible. This, however, would cost the population an exceptionally high death rate

every six months. This does not occur because if it did this high death rate could not have escaped the observation.

Epigenetic Explanation of Seasonal Polyphenisms in Insects

In all examples of seasonal polyphenism presented in this subsection a neural component is involved in determining the color and patterning of insect wings. External stimuli related to the appearance of seasonal polyphenism are perceived in the CNS of the insect. The changes in wing patterning between the two seasonal morphs of *B. anynana*, e.g., are systematic and affect all the individuals in populations, a fact that excludes any involvement of gene mutations in expression of the seasonal polyphenism. The basic difference (presence-absence of eyespots in the wings) in the East African butterfly is determined, on the one hand, by the timing of expression of the ecdysteroid hormone, which is secreted under strict neural control, via the brain hormone, PTTH (prothoracicotrophic hormone) and, on the other, by the expression of the EcR (ecdysteroid receptor) exclusively in the wing eyespots, which is regulated by local innervation, at least in the case of expression of this receptor in insect muscles (Hegstrom et al., 1998).

Wing Polyphenism in Insects

It is thought that wing polyphenism in ants evolved only once, 125 million years ago (Abouheif and Wray, 2002).

The flesh fly, *Sarcophaga argyrostoma* (Robineau-Desvoidy, 1830), like most flesh flies, is ovoviviparous. In autumn, under short day conditions, it gives birth to offspring that diapause as pupae, whereas during summer-time, it produces long-day nondiapausing and direct-developing generations. Using techniques of artificial uterus, investigators found that this photoperiod-related developmental plasticity, although determined during the intrauterine period is not induced maternally, but is determined by the embryonic central nervous system, after it becomes operative during intrauterine life. Investigators conclude that photosensitive period in embryos

May begin when the embryonic central nervous system is sufficiently developed and continues through larval development and the period of post-feeding “wandering” to come to an end before or at puparium formation. (Kenny et al., 1992).

Clearly, no changes in genes, DNA or selection are involved in the evolution of the dramatic change of this life history character.

The alternation of sexual and asexual generations is characteristic for many aphid species. In the aphid *Megoura viciae* Buckton (Homoptera, Aphididae), the photoperiod and temperature determine whether the female will reproduce sexually or parthenogenetically. In response to long days (16-hour days) and temperatures higher than 15⁰ C, the aphid loses its wings, and switches from the normal ovipara production of the adult gynopara to production of vivipara. The same response follows administration of the JH (juvenile hormone) (Hardie, 1981). Both temperature and day length are perceived in the CNS. Hence, it is logical to infer that external stimuli (temperature and density) received by sensory organs and processed in the brain enable the latter to appropriately respond by secreting neurohormones that induce (allatotropins) or inhibit (allatostatins) secretion of JH in corpora allata, leading to formation of wings or inhibition of wing formation respectively.

Not only is the function of corpora allata and JH secretion by these glands under the control of the CNS but it is experimentally determined that the photoperiodic mechanism of alternation of sexual and asexual generations in the aphid *M. viciae* Buckton, is neurally determined and its effector is a neurosecretion of the insect protocerebrum (Steel and Lees, 1977). Thus, the seasonal switch to two

alternative reproductive modes in this insect is epigenetically regulated by neural mechanisms involving no changes in genes or genetic mechanisms in general.

Experimental Polyphenisms in Insects

Daphnia magna females produce only female offspring, but their oocytes, when exposed to aqueous solutions of the crustacean hormone methyl farnesoate (a terpenoid synthesized, under neural control, by the mandibular organ), during the late ovarian development, develop exclusively into males (Olmstead and LeBlanc, 2002; Olmstead and LeBlanc, 2003). The reprogramming of the oocytes to produce males, by the same genotype that produces females shows that sex in *Daphnia* is determined non-genetically, epigenetically.

Cases of male polyphenisms (production of winged and wingless males) reported in some ant species of the genus *Cardiocondyla*, such as *Cardiocondyla obscurior*, e.g., are very interesting not only because they are observed among of the individuals of the same sex (males) of the same species but also because of the different nature of the external stimuli that induce their appearance. Experimental data show that this polyphenism is related to environmental stress rather than any genetic polymorphism (Cremer and Heinze, 2003; Schrempf and Heinze, 2006). The main inducing factor of the caste polyphenism in these ants is a sudden drop of at least 5°C in temperature but other stressors, such as reduction of the colony size by experimental splitting of the colony and food shortage also lead to increased production of winged males. Depending on the environmental conditions, ant colonies are able to flexibly allocate resources between two alternate (winged or wingless) male morphs. They invest more in producing exclusively wingless worker males, when conditions are favorable, or, to the contrary, under unfavorable conditions, they invest in producing the expensive dispersal form of winged males. Investigators have proven that this male diphenism is not genetic and is not transmitted via the egg cell, but is determined during the larval development in an adaptive response to the environmental conditions (Cremer and Heinze, 2003). Experimental evidence led them to the conclusion that it is not the male larva itself that determines expression of the winged phenotype but it is a change in the workers' social behavior toward larvae, the antennation (touching the larvae with antennae) that "instructs" these larvae to develop into winged male morphs (Schrempf and Heinze, 2006). Obviously, the tactile information that is provided to larvae is transmitted to the sensory organs, and processed in the brain, before larvae determine the developmental pathway they have to switch for developing into winged or wingless males.

The caste structure of the damp-wood termite, *Zootermopsis nevadensis* (Isoptera: Termopsidae), has been experimentally changed, by the treatment with a juvenile hormone analog, which induces formation of a new intermediate caste, featuring both soldier and winged morphology. The phenotype of the intercaste individuals depends on the time when nymphs are treated with the analog (Miura et al., 2003). Let's remember that juvenile hormone synthesis and secretion is induced by signals of cerebral origin.

Recent studies on the migratory locust, *Locusta migratoria*, and the silkworm, *Bombyx mori*, have shed additional light on the mechanisms controlling polyphenisms in insects. A larval brain neuropeptide, [His⁷]-corazonin (as well as another neuropeptide, [Arg⁷]-corazonin isolated from the larval brain of the silkworm) seem to be responsible for body color polymorphisms of the migratory locust. Injection of this brain peptide alone in the young adults of the grasshopper *Oedipoda miniata* (Brakefield et al., 1996) and alone or in combination with JH (juvenile hormone) in migratory locust instars (Tanaka, 1995; Tanaka, 2000a; Tanaka, 2000b; Yerushalmi and Pener, 2002) produces a variety of body-color patterns depending on the time and the dose of injection.

Experimental stimulation of JH secretion, which is induced by brain allatotropins, prevents formation of wings in normally nonpolyphenic winged female crickets (Zera et al., 1998).

A proportion of eggs of the nonparthenogenetic species *Drosophila mercatorum* are able to

parthenogenetically develop into adult flies. The fact that proportion of unfertilized eggs that are able to develop into adult flies increases with the decrease of the population density (Kramer and Templeton, 2001), suggests a role of social factors in determining the parthenogenetic development. Spontaneous transition of whole populations of *Drosophila mercatorum* to parthenogenetic reproduction has been observed in laboratory (Takenaka-Dacanay and Carson, 1991). Recall, social stimuli are perceived and processed in the central nervous system.

A bivoltine race (Daizo) of the silkworm, *Bombyx mori*, produces two seasonal moths, autumn and summer morphs, in response to long- and short-day photoperiods, respectively. Transplantation or injection of the extracts of the Br-SG (brain-suboesophageal ganglion) complexes as well as injection of the neurohormone DH (diapause hormone) from long day pupae (which normally would develop into autumn morphs) into short-day pupae transforms the latter into autumn morphs. Decerebration of early pupae, like transplantation of BrSG complexes, also makes short-day males shifting toward developing into autumn morphs. Another neurohormone, SMPH (summer morph-producing hormone) induces transformation of long-day larvae into summer morphs (Yamanaka et al., 2000).

Polyphenisms in Vertebrates

Cichlid fish are widely known for their exceptionally rapid morphological evolution and speciation in East African lakes but, in the Western hemisphere, they exhibit surprising polyphenisms.

Mexican cichlid fish are developmentally extravagant, displaying several phenotypes of teeth and digestive apparatuses in individuals of the same brood, even when raised on soft food in the laboratory (Sage and Selander, 1975). This enables the offspring to take advantage of a variety of edibles - snails, algae, fish, and arthropods. Thus, the parents increase chances that some individuals of their offspring will survive even under unpredictable hostile environmental conditions.

Although a great number of genes is involved in the development of the above divergent features, no environmental agent is responsible for the phenomenon and no “mutant genotype” has been shown to exist. While any imaginable neoDarwinian explanation fails to account for the phenomenon, an alternative epigenetic explanation would be that generation of different morphs in a single brood can be induced by differential distribution of maternal factors in each individual of the brood, similarly to the epigenetic process that is experimentally demonstrated to occur in other cases to be described later (*Urosaurus ornatus*, e.g.).

Rearing tadpole larvae of the salamander *Hynobius retardatus* together with heterospecific larvae of the frog *Rana pirica* increases proportion of the broad-headed, cannibal morph, in comparison with the normal salamander morph (Michimae and Wakahara, 2002). Larvae of this salamander develop broad carnivorous head not only in response to visual stimuli but also in response to experimentally induced mechanical vibrations resembling those of flapping tails of tadpoles, in the water, which helps tadpoles to capture and handle the prey (Michimae et al., 2005). Remember, vibrational stimuli are neurally perceived and processed in the CNS before the developmental pathway for developing carnivorous head is activated.

Some salamanders of the genus *Ambystoma* (Ambystomatidae family), depending on the environment temperature, may undergo indirect metamorphic development, with a larval aquatic stage and a terrestrial reproductively mature stage or remain paedomorphic, i.e. they may reach reproductive maturity while still at the larval stage in water (retaining external gills and tail with fin margin) and avoid the terrestrial stage of life cycle. The developmental flexibility of the life histories of *Ambystoma mexicanum* (axolotl) and *A. tigrinum*, known as facultative paedomorphosis, has been for a long time subject of intensive investigations for determining the mechanisms enabling them to perform this adaptive developmental switch (Denoel et al., 2005).

Other times, salamanders of the above species are polymorphic, i.e. in the same population individuals

of both types (paedomorphic and metamorphic) coexist, even though there seems to be no advantage from the maintenance of this life history polyphenism (Denoel et al., 2005).

It is interesting to point out that, during the last

3000 years, the ratio of paedomorphic to metamorphic specimens in the population of *A. tigrinum* from Lamar Cave (Yellowstone National Park, WY, USA) has remained unchanged (Bruzgul et al., 2005). Most salamanders develop by metamorphosis, which enables them to use both aquatic and terrestrial habitats. However, species of salamanders are also known that avoid metamorphosis and remain paedomorphic aquatic species during the whole life cycle (figure 11.6).

Administration of thyroid hormones under laboratory conditions, induces metamorphosis in neotenic axolotls, a fact that together with the evidence that nuclear receptors for the thyroid hormone are expressed and are functional in this species, and no relationship is found to exist between the allelic variation of thyroid hormone receptor genes and paedomorphosis [“there is no relationship between TR allelic variation and life

cycle modes among other Mexican ambystomatids” (Voss et al., 2000)], suggests that low levels of thyroid hormones in blood may be the cause of paedomorphosis in these species (Safi et al., 2004).

Some salamander species express an alternate developmental mode in which they forego metamorphosis and remain in the aquatic habitat throughout their lifetimes. Tadpoles of the pinewoods tree frog, *Hyla femoralis*, respond with morphological changes to the presence in environment of non-contact cues, such as metabolites (their nature is still unknown) of digestion of conspecific preys released by the predator and alarm pheromones released by the threatened conspecific prey. The morphological changes include deeper and shorter tails, changes in tail fin coloration and reduced body size (LaFiandra and Babbitt, 2004). Recently, it has been shown that tadpoles of this anuran respond to cues of the larval migratory dragonfly predator *Anax junius* by morphological changes that are graded proportionally to the assessed risk of predation (Richardson, 2006).

Females of the polyandrous lizard, *Uta stansburiana*, show a strange ability for selectively using sperm from large body sires to produce within the same clutch male offspring,

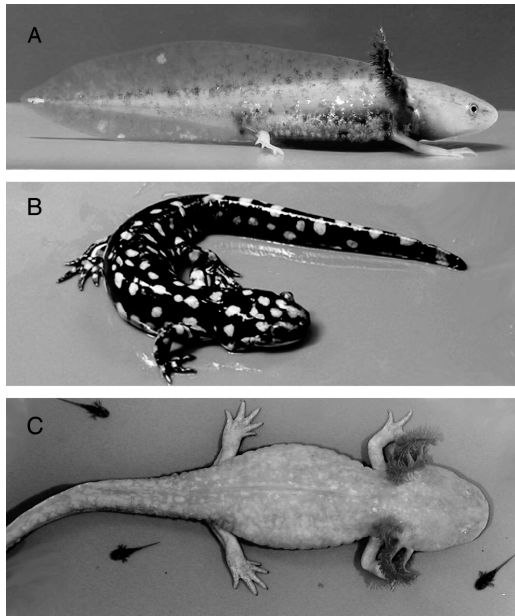


Figure 11.6. Larval and adult stages of *Ambystoma* (A) Larval *A. mexicanum*. (B) Adult *A. tigrinum* (metamorphic). (C) Adult *A. mexicanum* (paedomorphic) (From Voss and Smith, 2005).

and sperm from small body sires to produce female individuals. It is not known how females make this selection, but obviously females receive visually the information about the body size of sires and that perception takes place in the CNS (Calsbeek and Sinervo, 2004).

A similar pattern of adapting sex ratio to the mate quality has been observed in experiments with the blue tit in Sweden; when mated with males of brighter ultra-violet coloration, blue tit females increase proportion of the male offspring (Griffith et al., 2003).

The tree lizard, *Urosaurus ornatus*, gives birth to offspring, which develop as orange- or blue-throated morphs. Both morphs belong to the same genotype. It is demonstrated that it is an epigenetic

mechanism, the amount of maternal testosterone in their hatchling that determines the proportion of the above phenotypes (Ketterson and Nolan, 1999).

It is known that birds also deposit in eggs sex steroids, which contribute to the early development of the offspring. Recent evidence shows that the influence of maternal steroids on the offspring may extend beyond the embryonic development. So, e.g. maternal androgens of the eggs of the black-headed gull contribute to the development of the nuptial plumage almost one year after hatching (Eising et al., 2006).

Experimental Polyphenisms in Vertebrates

Male offspring of mosquito fish (*Gambusia affinis*) that prey on mosquitoes modify body shape and show better locomotory performance (figure 11.7). The differences in morphology and swimming performance observed between populations under predation and predation-free populations persist for many years under predation-free laboratory conditions (Langerhans et al., 2004; Langerhans and DeWitt, 2004). Langerhans et al. (2004) succeeded in validating their ecomorphological prediction that fish coexisting with their predator fish “evolve a larger caudal region and a shallower anterior body/head region in order to increase burst-swimming speed region”, which is necessary for their antipredator behavior.

Pregnant placentotrophic viviparous scincoid lizard, *Pseudemoia pagenstecheri*, adaptively manipulates the morphology and life history of its offspring according to a number of environmental stimuli it experiences during the pregnancy. In response to the olfactory perception of lizard-eating snakes during pregnancy, it gives birth to offspring of bigger body mass and very long tail, thus reducing the offspring’s vulnerability to the predator snake (Shine and Downes, 1999).

The spade-foot toad, *Scaphiopus multiplicatus*, produces offspring of two distinct forms: fast-growing carnivorous and slow-growing omnivorous individuals, at particular ratios. Both morphs have significantly different digestive tract morphology and dietetic requirements. Experimental treatment with thyroxine induces production of a third, quite different morph (Storz, 2004).

Experiments conducted by R.J. Denver (1997) shed some light on the signal cascades triggered by environmental stimuli in desert amphibians. As tadpoles, these amphibians live in temporary ponds that contain water for unpredictable periods of time. In the years of low precipitations, the ponds dry up earlier. This causes a habitat stress to which the tadpoles of that and some other species respond by changing their behavior and by speeding up the metamorphosis to transform into adult amphibians, able to live on dry land. Denver attributes this polyphenism to the earlier activation of a cerebrally activated neuroendocrine stress pathway:

The lowering of the level of the water in the environment makes the hypothalamus to produce more CRH (corticotrophin-releasing hormone – N.C.), which stimulates pituitary to produce hormones that stimulate thyroid and adrenal glands whose products help organism to cope with stress, in this case by losing their tail and beginning the growth of their limbs. (Denver, 1997)

Let’s remember that the hormone that stimulates the thyroid gland to secrete TH (thyroid hormone) is the pituitary TSH (thyroid-stimulating hormone), which, in turn, is upregulated by hypothalamic neurohormone TRH (thyrotropin-releasing hormone). In turn, the hypothalamic TRH neurons

Receive a dense input from neurons originating in other brain areas, which release catecholamines at their synaptic contacts with the cell bodies and dendrites of TRH-containing neurons. (Strand, 1998e)

In the Australian lizard, *Bassiana dupperreyi* (Gray, 1938), Scincidae, sex is determined both genetically (the species has heteromorphic sex chromosomes) and by the incubation temperature, but

the incubation temperature over-rides chromosomal sex determination (Shine et al., 2002). Administration of corticosterone in pregnant lizards, *Lacerta vivipara*, leads to reduction of juvenile body size and increases survival rates of juvenile male offspring (Meylan and Clobert, 2005).

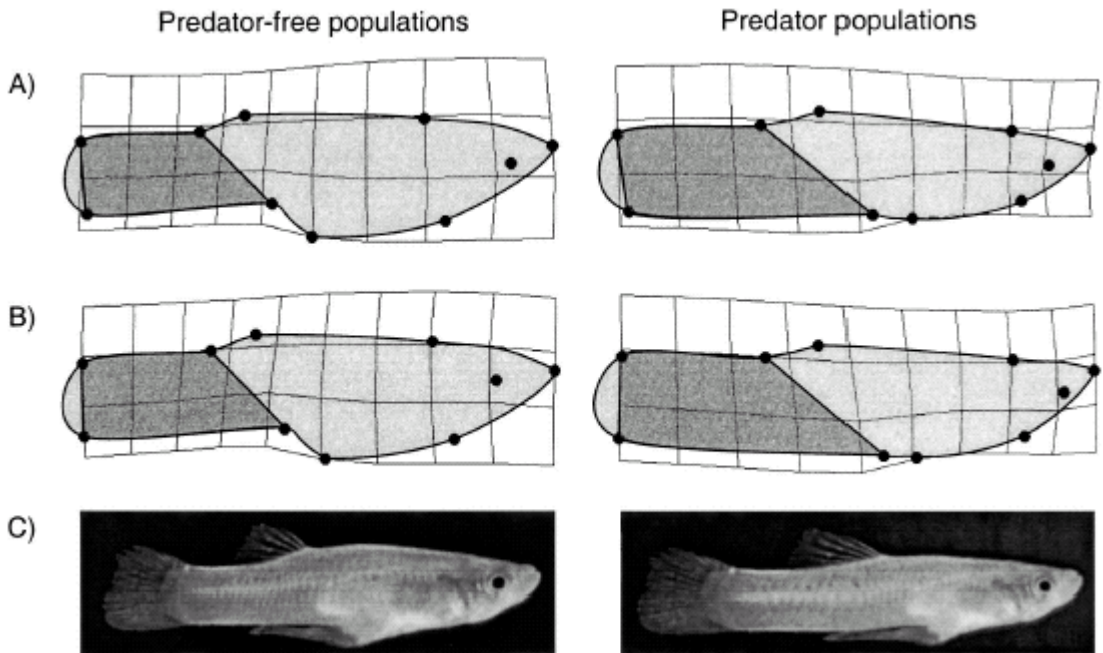


Figure 11.7. Visualization of morphological divergence between predator regimes for *G. affinis* males. Thin-plate spline transformations depict morphological differences in (A) 2001, (B) 2003, and (C) both years combined as described by canonical axes derived from the predator regime effect. Photographs in (C) represent deviations in landmark configurations between predatory environments applied to a single photograph of an individual with an intermediate canonical score (From Langerhans et al., 2004).

It has been believed that, in distinction from reptiles and fish, the sex in birds is genotypically determined. Recent experiments with the Australian brush turkey, *Alectura lathami*, a megapode with heteromorphic Z and W sex chromosomes, have shown that in birds too, environmental temperature may considerably change the sex ratio from 1:1 when egg incubation from the stage 1 takes place at 34^o C, to 3:1 for males at 31^o C, and 3:1 for females at 36^o C (Goeth and Booth, 2005). Again, the epigenetic determination of the sex in the offspring overrides the genetic sex determination.

Based on the fact that birds deposit glucocorticoids in their eggs, experiments have been conducted to demonstrate the possible roles of glucocorticoids in the offspring phenotype. Administration of corticosterone in the eggs of the yellow-legged gull (*Larus michahellis*) was shown to delay hatching, reduce begging display, and decrease the body weight (Rubolini, et al., 2005).

Experiments with four small shrew species (tiny mouse-like mammals) of the *Sorex* genus, have shown that when reared under extreme environmental stress, these mammals exhibit increased changes in mandibles. The functionally integrated groups of traits were those that changed more both within and between the species under experimental conditions (Badyaev and Foresman, 2000; Badyaev and Foresman, 2004).

Predator-induced Defenses

Predator-induced defenses are discrete changes in morphology, physiology, behavior, or life history that invertebrates develop in response to detection in the environment of the natural predators or their chemical cues. It is believed that the developmental plasticity induced by chemical cues in aquatic animals led to evolution of special neural structures for detecting these cues (Wisenden, 2000).

Upon detecting kairomones of its predator, *Daphnia magna* increases production of yolk proteins, shortens the time until the first reproduction, and reduces the size of the brood. These changes lead to production of more yolk proteins than are needed, a phenomenon that is known as parental optimism (Stibor, 2002).

Planktonic organisms delay hatching to avoid the offspring being eaten by the predator, but *Daphnia curvirostris*, when its predator *Gambusia holbrooki* Girard is present in wetlands for long periods of time, from spring to late fall, cannot afford delaying hatching (Angeler, 2005).

In response to filtrates of 10 different species of organisms belonging to not only rotifers but also to remotely related species of other phyla, a rotifer, *Keratella testudo*, produces posterior spines, which make it less vulnerable to its predators (Stemberger and Gilbert, 1987).

The marine colonial bryozoan, *Membranipora membranacea*, produces spines within two days of exposure to its waterborne predator extracts and the type of spines produced varies in accordance with the concentration of the cue (Harvell, 1998).

J.J. Gilbert (1980) observed that *Asplanchna brightwelli* produces different morphs depending on the quantity of prey it perceives in the environment. That perception of the prey arises in the CNS according to visual or other input that the animal receives through the sense organs. Since no changes in genes are involved in this defensive plasticity, the correlation between the amount of the prey and the morphology of the offspring implies a (causal?) relation between the *perception* of the prey quantity and the changes in the gamete that activate the developmental program for producing the adaptive morphological change in the offspring.

Snails *Litorina obtusata* in the northern Gulf of Maine have thinner shells than their southern conspecifics. And this for a good reason: they do not have to bother carrying a heavier shell in that northern habitat, where their predator, *Carcinus maenus*, is very rare or even absent. But, when the northern snails are transplanted to the southern, predator-rich habitat their shell becomes thicker and their body mass larger, and inversely, the southern snails transplanted to the North develop thinner and lighter shells and larger bodies.

The blue mussel *Mytilus edulis*, when outplanted to the sites of high predation by the crab *Carcinus maenus* also produces a thicker shell (Leonard et al., 1999). Other marine snails exposed to predatory crabs increase shell thickness at a degree comparable to “historical transitions in thickness previously attributed to selection by invading predator” (Trussell and Smith, 2000; Trussell, 2001).

The freshwater snail, *Helisoma trivolis*, shows a remarkable flexibility in the phenotypic changes it displays in response to different predators. It responds to the presence of water bugs with a particular suite of changes in behavior, morphology, and life history (the extent and type of the habitat, body mass at reproduction, shell width and height, number of eggs laid, etc.), which is distinct from the suite of changes it generates in the presence of crayfish (Hoverman et al., 2005).

On detecting, in nature or under laboratory conditions, the presence of their predator, the brook trout *Salvelinus fontinalis*, the larvae of the mayfly, *Drunella coloradensis*, develop longer caudal filaments, which enhance their survival rate under predation (Dahl and Peckarsky, 2002).

The largemouth bass, *Micropterus salmoides* (Centrarchidae, Perciformes) responds with a behavioral plasticity when detects the Ostariophysan alarm pheromone released by the finescale dace, *Phoxinus neogaeus* (Cyprinidae); it avoids visiting the space where it smells the pheromone (Brown et al., 2001).

D.N. Reznick et al. (1990) introduced guppies from a high predation site into a low predation site, above a waterfall barrier in two streams in Trinidad. In another experiment they mimicked the above natural processes in laboratory conditions. To their surprise, an exceptionally high rate of evolution (“seven orders of magnitude greater than rates inferred from the paleontological record” (Reznick et al., 1990) was

observed. Precious as it is, the study, unfortunately, was applauded for what it did not prove:

Although lab studies have shown similarly fast rates of natural selection, this is one of the few examples from a natural environment. (Reznick et al., 1990)

The investigators take it for granted that guppies' rapid evolution or difference observed between the experimental and control populations was a "response to natural selection". But it might not have been, since:

They present no evidence of any selective predation or spontaneous mutations or presence of relevant alleles of the type the natural selection acts upon. On the contrary, what they present, seems to suggest the opposite, i.e., that the population of guppies has evolved in an extraordinary fast, nonmutational, nongradual, and yet adaptive manner: the population of guppies is affected by the morphological change as a whole (what excludes the possibility of favorable, i.e. adaptive mutations, which are extremely rare events), and *their population within several generations has changed adaptively as a whole* excluding the possibility of the involvement of natural selection in evolution of the morphological adaptation.

The above reasoning is supported by the careful analysis of C. Broenmark and J.G. Miner (1991), who demonstrated in similar laboratory experiments that crucian carp (*Carassius carassius*) raised in pond sections with piscivorous pike (*Esox lucius*) increased its body depth, which represents a morphological defense against pike. Taking nothing for granted, Broenmark and Miner, considered two possible causes of the appearance of the adaptive morphology: (1) selective predation and (2) predator-induced phenotypic modification of body shape. Based on results of their experiments, they concluded against selection as a factor in the experimental evolution of guppies:

The small variance in the depth and the absence of overlap between treatments suggested no polymorphism with regard to this trait in the original population; thus, selective pressure on genetically determined morphs does not account for the increase in body depth. (Broenmark and Miner, 1992)

The authors, thus, exclude a possible role of natural selection in the rapid evolution of the defensive morphology in the crucian carp. Obviously, a crucial initial step in the evolution of the fish population is perception of the predator threat in the brain of the crucian carp. Although we do not know the developmental pathways and signal cascades linking the perception of the predator to the adaptive change in morphology in this particular case, some relevant experimental evidence will be presented later in the next chapter, *Transgenerational Developmental Plasticity*.

Red-eyed treefrogs, *Agalychnis callidryas*, attach their eggs to vegetation overhanging water. When attacked from egg-eating snakes, *the whole clutch* of eggs of the treefrog hatch immediately and fall into the water below, thus reducing mortality rate from snake predation, although in the water also they face aquatic predators. This response is not a general response but a specific response to the mechanical disturbance caused by the snake, as is proven in experiments where touching and pulling of the eggs does not induce hatching (Warkentin, 1995).

In Panama, the social wasps, *Polybia rejecta*, also prey on the eggs of the treefrogs, killing about one quarter of them. Most undisturbed eggs hatch relatively late in order to survive aquatic predator attacks, but wasp-attacked eggs hatch immediately and most of hatching embryos escape aquatic predators. In distinction from the case of snake attack, when the whole clutch hatches immediately, in the case of wasp attacks, eggs hatch individually, proportionally to the consuming capacity of the predator (Warkentin, 2000).

Tadpoles of the frog, *Rana dalmatina* respond to the presence of the fish predator threespine stickleback, *Gasterosteus aculeatus*, by developing a longer tail and more massive tail muscle (for a higher acceleration speed and higher promptness to escape), which helps tadpoles not for swimming (sticklebacks are much faster) but to quickly reach a refuge such as mud or plant debris. In response to the presence of dragonfly larvae they develop deeper tail fin, which helps them to swim faster (Teplitski et al., 2005a; Teplitskiy et al., 2005b).

On detecting predators and with no refuge available, two salamander species, *Ambystoma barbouri* and *Ambystoma texanum*, change their body color so that it better matches the background, whereas *A. texanum*, additionally, shows a tendency to move toward places that better match their body pattern and color (Garcia and Sih, 2003).

No changes in genes are involved in the development of predator-induced defenses described so far: these

defenses develop in all the individuals of a population and whole populations perfectly return to the “defenseless” state after disappearance or removal of the predator. From this view, the neoDarwinian idea that “predators are the selecting agent” (Slogget and Weisser, 2002) is not accurate. Natural selection, as it is generally understood, acts by gradually accumulating, over long periods of time, gene mutations that very rarely are “useful”, but there is no available evidence that predator-induced defenses evolved as a result of changes in genes.

Inborn Developmental Plasticity

Developmental Polymorphisms are not Genetic Polymorphisms

Presence of different morphs (individuals of distinct discrete morphologies) in the same brood, has been considered to represent genetic polymorphism. Obviously this is a misnomer for the fact that morphs occur within the same brood, i.e. belong to the same genotype, excludes the possibility that genetic factors may be involved in the phenomenon. Hence, and to emphasize the developmental (not genetic) origin of such morphs the term developmental polymorphism will be used here instead of genetic polymorphism.

The flightless bug, *Pyrhocoris apterus* (L), develops nonfunctional wings and produces diphenic morphs for wing length and flight muscles. The macropterous (long-winged) morph develops flight muscles, while the brachypterous (short-winged) morph only develops rudimentary flight muscles because of the arrested growth during development. The flight muscles in macropterous morphs grow until the adulthood and then they are histolyzed, in a process that is accompanied by a series of neuroendocrine changes. Histolysis of the flight muscles in this insect is prematurely induced by administration of a juvenile hormone analog (Socha and Šula, 2006). The fact that JH synthesis and secretion is under control of brain neurohormones (allatostatins and allatotropins) suggests that development of morphs with or without wings and flight muscles is under ultimate neural control.

In the adult cricket, *Gryllus bimaculatus* the histolysis of flight muscles is neurally regulated via local innervation. Motor neurons innervating the flight muscle M112a send signals for timing the histolysis of muscle fibers. Decapitation of insects prevents the muscle histolysis:

JH causes breakdown of flight muscles and motor neurons are involved in the onset of degeneration in the presence of JH. Both endocrine and neural factors are important for flight muscle degeneration (Shiga et al., 2002).

Administration of ecdysone retards the degeneration of flight muscles in adult crickets *Acheta domestica*, but has no effect on denervated flight muscles indicating that a neural factor is necessary for the action of the hormone in muscle degeneration (Srihari et al., 1975).

Females of several damselfly species offer examples of color polyphenisms, in which some females display male dark color (andromorph color) and others the typical female green color (gynemorph color) (Joop et al., 2005).

Once a year some aphids produce a sexual generation. Production of males and females by parthenogenetic XX females necessitates elimination of an X chromosome from the eggs that will produce males; this elimination is again mediated by a brain neuropeptide acting directly on oocytes (Chapman, 1998c).

A cotton aphid, *Aphis gossypii*, Glover (Hemiptera: Aphididae), normally produces offspring of four phenotypes with different colors (light green, dark green, and yellow), different body size, with or without wings, and with distinct life histories.

An interesting case in the crayfish, *Procambarus clarkii*, may shed additional light on the neural mechanisms underlying the production of more than one morph in polyphenic invertebrates. Male individuals of this small crustacean belong to one of two distinct morphotypes, the reproductive form Is and the non-reproductive form IIs. The morphological differences between these two forms consist of Is having larger chelae (claws) on the ischiopodites of walking legs and also having spines (lacking in IIs) on the ischiopodites. Experimental evidence shows that whether an individual will grow as an Is adult morph or as a non-reproductive IIs morph, depends not on any genetic differences but on the level of the hormone methyl farnesoate (with functions similar to the insect juvenile hormone) secreted by the mandibular organ, which is under strict cerebral control (see next chapter on the mechanism of transgenerational phenotypic

plasticity).

Dispersal polyphenism (production of various morphs in order to increase the dispersal capability) in the cricket, *Gryllus firmus*, is represented by three morphs: one flight-capable morph with long wings and fully developed flight muscles, a flightless morph with reduced wings and flight muscles, and the third with fully developed wings but incapable of flight because of histolysis of flight muscles.

A proportion of unfertilized eggs of the mayfly, *Stenonema femora*, develop parthenogenetically and even some female flies produce mixed broods of sexually and parthenogenetically developing offspring despite the fact that all of them are of the same genotype (Ball, 2001). Cases of spontaneous transformation of whole populations into parthenogenetic populations of *Drosophila mercatorum* have been observed in laboratory (Takenaka-Dacanay and Carson, 1997).

Developmental Polymorphism of Wings in Insects

A number of environmental cues such as feeding, photoperiod, crowding, temperature, pheromones, host plant conditions, etc. have been found to influence or determine wing developmental polymorphism in insects. This invertebrate group displays a high degree of lability for wing development, which, among other things, manifests itself in the fact that not rarely the same female insect gives birth to two or more offspring types with different wing morphology or even with or without wings. This developmental lability might have facilitated both the evolutionary acquisition and loss of wings in insects.

The proximate cause of the wing developmental polymorphism seems to be JH (juvenile hormone). As mentioned earlier, wing discs in *Precis coenia* cease growing in the presence of JH and their growth can experimentally be inhibited by JH (Miner et al., 2000). A simple hormonal manipulation, topical administration of JH, leads to formation of wingless females of crickets *Gryllus firmus* and *Gryllus rubens*, which are naturally winged (alates).

How easy the developmental switching from activation to inactivation of the gene regulatory networks responsible for the development of wings in insects is, may be illustrated by the fact that, under natural conditions, *Gryllus firmus* produces offspring consisting of three different morphs (with long wings, with reduced wings, and with wings but lacking flight muscles) (Zera et al., 1998).

Alate females of another insect, the fire ant, *Solenopsis invicta*, are induced to cast their wings when JH is locally applied and local application of the JH antagonist, precocene, prevents the dealating action of JH (Burns et al., 1999). Precocene also induces development of long wings in 30% of short wing-programmed larvae of the ectoparasit *Melittobia digitata* (Consoli and Bradleigh Vinson, 2004).

Occurring among individuals of the same genotype, the wing developmental polymorphism, precludes involvement of changes in genes and any neoDarwinian explanation of the phenomenon of wing developmental polymorphism in insects.

Penetrance: A Bet-hedging Strategy?

Penetrance is a widely recognized hereditary phenomenon that is considered self-explanatory, even though no serious attempt has been made to elucidate its causal basis. There is no universally accepted definition of penetrance, but usually it is defined as proportion of individuals of a population that express a certain genotype or allele. Let's consider here only two contemporary definitions of penetrance:

Percentage of individuals that show at least some degree of expression of a mutant genotype. (Klug and Cummings, 1997),

and

The frequency in the population with which a dominant or homozygous recessive allele manifests itself in the phenotype of an individual. (Russell, 2006)

Both the above definitions restate that which they are intended to explain. As circular definitions they do no more than prove our ignorance on the nature and causes of the phenomenon.

According to the first definition, penetrant phenotypes are expression of a mutant genotype. Is this true? Cases are known of penetrance even in wild type individuals and individuals of the same genotype may

express or not the penetrant phenotype. Remember, penetrance is also observed in full siblings, i.e. in the offspring of the same genotype.

The second definition implies that

1. Dominant alleles may not be phenotypically expressed. If so, by definition, they are not dominant alleles.

2. Homozygous recessive alleles may not be phenotypically expressed. This again contradicts the widely recognized definition of recessivity: recessive genes in homozygous state will be phenotypically expressed. Hence, in the form presented above, the second definition of penetrance is invalid and we are going to consider the penetrance in the meaning of the first definition.

The most obvious fact resulting from studies on penetrance is that the phenomenon does not obey Mendelian inheritance. What we really know in the cases of penetrance is that a certain phenotype is expressed at a certain proportion of individuals in a population, but we do not know the gene involved in expression of the character or even whether any gene is responsible for the character. Hence, the concepts of high penetrant genes and low penetrant genes do nothing more than confirm our ignorance on the causes of variability in expression of phenotypic characters.

As far as the causal basis of the phenomenon is concerned, conventionally, penetrance is loosely considered to result from an interaction of genes with the environment, but in no case it has been possible to elaborate beyond this general theoretical statement. Sometimes, we are told that an allele may not be penetrant because of the presence of its dominant allele (*dominant suppression*) or because of another locus (*epistasis*) that prevents or modifies its expression.

The fact that in some cases the mutant allele, even in homozygous state, is not expressed has been attributed to hypothetical presence of another gene that prevents expression of the mutant genotype. Such counteracting alleles are named *suppressors* some of which, we are told, behave as dominant and some as recessives. Validation of the above statements requires identification of the dominant allele or epistatic and/or suppressor genes. No empirical evidence has ever been presented to substantiate these purely theoretical speculations.

Attempts to speculatively relate penetrance to dominant or recessive epistasis have not been elaborated and no epistatic interactions have been identified or demonstrated in any case of incomplete penetrance. Even if for the sake of argument one would admit that epistatic (dominant or recessive) actions may be involved in penetrance, the appearance of penetrance in homogenous groups of individuals of the same genotype rejects the genetic explanation of penetrance.

The fact that geneticists have not been able to demonstrate genetic causes of penetrance (the role of dominant suppression, dominant epistasis, and recessive epistasis, in penetrance remains to be demonstrated) compels D.J. Fairbanks and W.R. Andersen to refer to a very vague, unspecified causal basis of the “genetic background” of penetrances:

Frequently they are related to the genetic background, which refers to all genes of the individual except the one under study. (Fairbanks and Andersen, 1999)

a statement that clearly does not do much to reduce the theoretical confusion. Most described cases of penetrance do not offer any known evolutionary advantage, and hence no role in their evolution has been attributed to natural selection. This is the reason why some geneticists have admitted the possibility of a “nongenetic” cause for the incomplete penetrance of the human polydactyly, which generally is considered to be a dominant condition (Fairbanks and Andersen, 1999).

It is demonstrated that a number of environmental agents such as temperature, nutrition, drugs and, in some cases, the maternal age and the age of the individual can affect the penetrance of a character. Given the fact that genes/genotype are the same during the lifetime of an organism, these facts suggest that non-genetic factors are responsible for the age-related determination of the phenomenon.

In the fruit fly alone, temperature changes are known to be able to change the penetrance of many alleles from 0% to 100%. (Rothwell, 1988)

indicating that no “penetrant” alleles are responsible for the penetrance. Such facts suggest that penetrance or non-penetrance of a character may result from epigenetic regulatory mechanisms, “designed” to adapt the organism to its environment.

Let’s consider a few examples of the appearance of discrete phenotypes in individuals of the same

genotype, under the same environmental conditions, in order to understand how difficult is to distinguish between the actual concepts of polyphenism and penetrance

Example 1. Although no yolk occurs “in typical mammalian eggs, embryonic mammals develop a yolk sack, a reminder of their genetic relationship with egg-laying reptiles. In man a vestige of the yolk sack (Meckel’s diverticulum) remains in about 2% of the adult population. Its average position is on the ileum, one meter before the ileocolic valve, and its average length is about 5 cm” (Kent, 1973d). A geneticist would say that the character is 2% penetrant in human population, although no genetic factor has been identified or alleged to be related to the penetrance of “yolk” character in humans. An evolutionary biologist would consider this case an atavistic vestigial appearance of an ancestral character but I would find it difficult argue against someone that would consider it a case of inborn developmental plasticity, i.e. a developmental polymorphism.

Example 2. In mayfly, *Stenonema femora*, a proportion of unfertilized eggs develop parthenogenetically and some female flies even produce mixed broods of sexually and parthenogenetically developing offspring (Ball, 2001). Expression of the parthenogenetic trait in a proportion of the brood is generally considered to be a case of developmental plasticity but from a genetic point of view it also fits well to the definition of penetrance as a percentage of a population expressing “a dominant or a homozygous recessive allele” or “a mutant genotype”. However, no mutant genotype or suppressor gene is involved in this parthenogenetic penetrance.

Example 3. The cryptic moth, *Phigalia titea*, according to T. Sargent, prefers light backgrounds and all the time this moth settles on white oaks (Sargent, 1969). This background is very masking for the pale majority of its population, but not for the melanic form. Being more visible to moth predators, the melanic form is selected against but it is still present in constant proportions because 20% of the offspring of both melanic and pale forms are melanic. By definition this is a case of penetrance, in which no mutation (it is beyond any stretch of imagination that, a specific adaptive mutational event would occur systematically in 20% of the individuals of the moth population and still be considered a mutation), no suppressors and no epistatic interactions are involved. Again, depending on the perspective, the case may be considered both an example of penetrance and an instance of developmental polymorphism.

Example 4. Studies in humans show that polydactyly, an inherited dominant anomaly, displays a partial (different from 100%) penetrance; often individuals whose pedigrees show that they have the dominant allele for polydactyly do not express it phenotypically. Fairbanks and Andersen (1999) believe that this is a case of nonpenetrance for which the cause is unknown and may be genetic, non-genetic or both. The fact that the phenomenon of penetrance is characterized by expression in a certain proportion of a population of individuals of the same genotype of alternative phenotypes, both under the influence of environmental factors or in absence of such influences, calls into question the very status of penetrance as a separate biological phenomenon different from polyphenisms or phenotypic plasticity. How can one differentiate it from cases of developmental polymorphism where only a proportion of the offspring of the same genotype express a particular trait?