Eco-Evo-Devo: The Time Has Come

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Abstract

The major goal of ecological evolutionary developmental biology, also known as "eco-evo-devo," is to uncover the rules that underlie the interactions between an organism's environment, genes, and development and to incorporate these rules into evolutionary theory. In this chapter, we discuss some key and emerging concepts within eco-evo-devo. These concepts show that the environment is a source and inducer of genotypic and phenotypic variation at multiple levels of biological organization, while development acts as a regulator that can mask, release, or create new combinations of variation. Natural selection can subsequently fix this variation, giving rise to novel phenotypes. Combining the approaches of eco-evo-devo and ecological genomics will mutually enrich these fields in a way that will not only enhance our understanding of evolution, but also of the genetic mechanisms underlying the responses of organisms to their natural environments.

Keywords

Evodevo • Evolution • Ecology • Stochastic variation • Robustness • Environmental stress • Developmental recombination • Genetic accommodation • Genetic assimilation • Ancestral developmental potential • Social interactions • Epigenetics • Developmental plasticity • Polyphenism • Ecoevodevo

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6.1 Introduction

> The time seems to have come when we need to take into account two further aspects of the evolutionary mechanism. In the first place, natural selective pressures impinge not on the hereditary factors themselves, but on the organisms as they develop from fertilized eggs to reproductive adults. We need to bring into the picture not only the genetic system by which hereditary information is passed on from one generation to the next, but also

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> the "epigenetic system" by which the information contained in the fertilized egg is expanded into the functioning structure of the reproducing individual. Each organism during its lifetime will respond in some manner to the environmental stresses to which it is submitted and in a population there is almost certain to be some genetic variation in the intensity and character in these responses. Natural selection will favor those individuals in which the responses are of most adaptive value. Conrad H. Waddington (1959).

Why should an ecological genomicist be interested in Waddington's (1959) prescient words written almost 60 years ago? The short answer is that development – the processes through which the fertilized egg becomes a reproducing adult – matters. Development mediates interactions between genes and the environment, so if the goal of ecological genomics is to "uncover the genetic mechanisms underlying responses of organisms to their natural environments" (Ungerer et al. 2008), then this goal is unattainable without taking development into account. The long answer is that development mediates these interactions in multiple and complex ways affecting the genetic and phenotypic variation available for natural selection to act upon. Waddington (1959) clearly saw the importance of integrating these interactions into evolutionary theory back in 1959, but the time for this integration seems to have come only now with the recent emergence of the field of ecological evolutionary developmental biology or more simply "eco-evo-devo." This field acknowledges the fact that organisms are continually subject to a changing environment, whether it be changes in nutrition, temperature, predators, competitors or all of these simultaneously. It aims to uncover the rules that underlie the interactions between an organism's environment, genes, and development, and by doing so, aims to expand our current view of how evolution works. As the name of this field implies, eco-evo-devo unites the field of evolutionary developmental biology (evo-devo) with ecology, but also includes the subdisciplines of developmental plasticity, epigenetics, and social evolution, where many recent advances are being made. For example, recent books in development plasticity, such as West-Eberhard's (2003) *Developmental Plasticity and Evolution*, and Gilbert and Epel's (2009) *Ecological Developmental Biology*, have breathed new life in our understanding of how developmental plasticity can promote, rather than retard, evolutionary change. In this explosive cocktail of fields and subdisciplines is a conceptual revolution on the horizon – a potentially new way of thinking about evolution, development, and ecology.

The goal of our chapter is to describe, in a series of sections, some key and emerging concepts from eco-evo-devo. Although the sections cover a wide range of different topics, taken together, they contribute to painting a larger picture of the evolutionary process. First, in Endocrine Signaling (Sect. 6.2), we set the tone by highlighting the genes controlling sensitivity to or titers of hormones during development as the potential locus of gene and environment interactions. Second, through our sections on Ancestral Developmental Potential (Sect. 6.3), Stochastic Variation (Sect. 6.5), Social Interactions (Sect. 6.6), and Ecological Interactions (Sect. 6.7), we discuss the different ways in which the environment can play a dual role – the more familiar role as selective agent and the less familiar role as a source or inducer of phenotypic variation. Third, in Developmental Recombination (Sect. 6.4) and Robustness (Sect. 6.8), we discuss how development can then regulate the interactions between genes and their environment in a number of complex ways such that development can mask, release, or create new combinations of genotypic and phenotypic variation produced by this interaction. Finally, in Genetic Assimilation and Accommodation (Sect. 6.9) and Integrating Levels of Biological Organization (Sect. 6.10), we discuss how variation produced by the environment at all levels of biological organization can be fixed by natural selection to create novel phenotypes. We conclude (Sect. 6.11) with a diagram inspired by Waddington (1959) to summarize the interrelations between all of the following sections.

6.2 Endocrine Signaling: A Locus of Gene and Environment Interactions

Phenotypic traits can be under complete genetic control (e.g. mendelian traits), but most traits are plastic and result from a complex interaction between genetic and environmental inputs (West-Eberhard 2003; Gilbert and Epel 2009). One form of plasticity, polyphenism, is the ability of the same genome to produce two or more alternative phenotypes in a single population in response to an environmental cue, such as temperature or nutrition (Nijhout 1999). Polyphenic traits represent powerful models to understand the effects of environmental inputs on development and provide the opportunity to uncover the physiological and genetic mechanisms underlying the susceptibility to undertake or not particular developmental decisions (Jenner and Wills 2007).

Developmental decisions are often mediated by endocrine signaling (Flatt and Heyland 2011). The decision about which developmental trajectory to undertake depends on several key components of endocrine signaling (Nijhout 1999; Zera et al. 2007): (1) the systemic titer of the hormone (circulating concentration of the hormone); (2) the hormonal receptors in target cells, which can show variation in the degree and timing of sensitivity; and (3) the downstream pathways that are activated upon hormone binding. In insects, the role of hormones in developmental decisions has been extensively studied (Nijhout 1998); juvenile hormone (JH), together with ecdysteroids, orchestrate growth, molts and timing of metamorphosis. For example, in the cricket *Gryllus rubens*, wing-length polyphenism is the result of population density during development (Zera and Tiebel 1988). At low densities, during a critical sensitivity period that spans from mid to penultimate larval instars, JH titer drops below a threshold, whereas ecdysteroid titer exceeds a threshold, which result in the development of macropterous individuals. In contrast, at high densities, JH titer remains above the threshold, whereas ecdysteroid titer is below a threshold during the critical period of sensitivity, which leads to the interruption of wing development and micropterous individuals are formed.

The evolution of environmentally sensitive traits may occur through evolutionary changes in any one of the three key components of endocrine signaling highlighted above (Nijhout 1999; Zera et al. 2007). Mutations in genes controlling hormonal titers can bring individuals to cross a hormonal threshold more readily and therefore express an alternate phenotype. Artificial selection experiments in the tobacco hornworm (*Manduca sexta*) have shown that polyphenic traits can evolve through the modification of hormonal titers. Suzuki and Nijhout (2006) selected for a polyphenic line of *M. sexta*, where individuals express a black pigmentation when raised at a low temperature but develop as green when raised at a high temperature (see Sect. 6.9 for details on the mechanisms). In order to determine if a hormonal titer is involved in controlling the expression of the alternative color phenotypes, they ligated the thorax of the larvae to prevent endogenous JH circulation. They subsequently topically applied a JH analogue, methoprene, from low to high concentrations. They found that regardless of the temperature that they were raised at, individuals develop as black at low methoprene concentrations, while they develop into green individuals at higher methoprene concentrations. These results indicate that this polyphenic switch is mediated largely through differences in hormonal titers rather than changes in hormonal sensitivities or downstream effector molecules.

Alternatively, mutations in the threshold itself (for example, a hormone-binding receptor), may allow for a hormonal titer to cross that threshold more readily, and therefore also express an alternative phenotype (Nijhout 1999; Zera et al. 2007). In dung beetles, *Onthophagus taurus*, males can develop, or not, a pair of horns on their head depending on larval nutrition: large males above a critical threshold body size will develop a pair of horns on their heads while smaller males below the critical body size threshold will not. Introduced populations of *O. taurus* in North Carolina and in Australia have rapidly evolved divergence in the critical body

size threshold that separates alternative morphs. Using hormonal manipulations, Moczek and Nijhout (2002) showed that this divergence has evolved through the modification of the response threshold to JH. North Carolina male beetles develop horns when exposed to a lower methoprene concentration, and during an earlier critical period than male beetles from Australia. These results indicate that divergence of polyphenic traits can occur through changes in the degree and timing of sensitivity to endocrine signaling.

Furthermore, the production of effector molecules downstream of a hormonal switch can also be the target for evolution (Nijhout 1999; Zera et al. 2007). Wing polyphenism in ants is universal and evolved only once: across all ant species, reproductive individuals are winged and worker castes are wingless (Abouheif and Wray 2002). The regulation of expression of the wing patterning gene network is under the control of a JH switch that determines the fate of an egg (queen or worker). This gene network is largely conserved between winged castes and other holometabolous insects (Carroll et al. 2005; Tomoyasu et al. 2009; Shbailat et al. 2010), but is evolutionarily labile across wingless castes of different ant species (Abouheif and Wray 2002; Shbailat and Abouheif 2012). For example, in the ant species *Lasius niger* and *Crematogaster lineolata*, the hormonal switch between queens and workers occurs relatively early during development, and therefore, both species have vestigial imaginal discs of similar size. However, the expression of genes in the wing patterning network in vestigial discs of workers differs between the two species. These results indicate that downstream targets of endocrine signaling can also evolve to generate expression differences in gene regulatory networks.

The underlying genetic basis of polyphenic traits, either in terms of changes in hormone titers or sensitivity, remains to be discovered in most cases, although it is most often assumed to be polygenic (Roff and Fairbairn 1991; Roff 1996; Braendle et al. 2005). Using next-generation sequencing tools may help answer questions such as: (1) is the variation in the response to environmental stimuli between taxa a consequence of changes in a few key genes, or small changes in multiples genes? (2) Which kind of genes and gene networks underlie hormonal thresholds and sensitivities? These examples illustrate how environmental responses can be incorporated into developmental decisions through the action of endocrine signaling. Furthermore, the evolution of any key component of the endocrine signaling pathway may lead to the evolution of new variation and phenotypes.

6.3 Ancestral Developmental Potential

The term atavism refers to the sporadic and spontaneous appearance of ancestral phenotypes in individuals of modern wild populations (Darwin 1868; West-Eberhard 2003). Examples of this include individual whales with hindlimbs, snakes with additional skeletal elements and humans with tails (Dubrow et al. 1988; Hall 2003; Tomić and Meyer-Rochow 2011). Generally, this type of variation is often considered to contribute little, if at all, to the evolutionary process (Levinton 1986; Stiassny 2003). There are also several cases of atavistic traits being induced in the lab (Waddington 1957; Weatherbee et al. 1998; Harris et al. 2006; Chan et al. 2010). Examples of these "experimental atavisms" include chickens with teeth, freshwater stickleback fish with pelvic structures, and flies with hindwings. As is the case with spontaneous atavisms, experimental atavisms are given little weight in understanding the evolutionary process (Levinton 1986) and are more commonly known as "hopeless monsters".

Dollo's Law, which posits that once a complex trait is lost it is unlikely to re-evolve, has been an influential concept in phylogenetic systematics (Goldberg and Igić 2008; Wake et al. 2011). However, so many counter-examples have appeared that Dollo's Law can no longer be considered the rule, but rather the exception (Collin and Miglietta 2008). Several instances have been demonstrated where ancestral traits, that have been lost for millions of years, have subsequently re-evolved in derived lineages such as wings in stick insects (Whiting et al. 2003), teeth in

amphibians (Wiens 2011), digit number in lizards (Kohlsdorf and Wagner 2006; Kohlsdorf et al. 2010) shell coiling and mode of development in marine snails (Collin and Cipriani 2003; Collin 2004; Collin et al. 2007) and herbivore defense in plants (Armbruster et al. 2009). This phylogenetic pattern of reversal, which has been called 'taxic atavism' (Stiassny 2003), may be much more common than originally thought (West-Eberhard 2003; Abouheif 2008; Rajakumar et al. 2012). Although these three forms of atavisms (spontaneous, experimental and taxic) have been described in detail in the literature, little effort has gone into determining whether considering them together would in any way be informative to the further understanding of their occurrence or more generally the evolutionary process.

Recently, Rajakumar et al. (2012) united all three types of atavism in a single study of supersoldier development and evolution in the ant genus *Pheidole*. They demonstrated that the supersoldier caste, known for its complex defensive skills and giant heads (Huang 2010), is actually an ancestral feature that was subsequently lost in most species of the group. The supersoldier subcaste then re-evolved in at least eight species, including one species called *Pheidole obtusospinosa* (taxic atavism). Furthermore, they found in nature several anomalous supersoldier-like individuals in one *Pheidole* species that does not have a supersoldier caste (spontaneous atavism). How did this occur? Using hormonal manipulations, they were able to produce supersoldiers in several species (including the species of which they found the spontaneous atavism) that do not have a supersoldier caste (experimental atavism). This result demonstrates that the potential to produce supersoldiers is ancestral and that there exists an ancestral developmental potential for supersoldiers that can be environmentally induced across the genus *Pheidole*.

How do ancestral developmental potentials persist throughout 35–60 millions years of evolution, such that they can be environmentally induced in extant species? In Rajakumar et al.'s (2012) case, pleiotropy or more specifically hormonal pleiotropy, is proposed to be the mechanism that facilitates the retention of dormant ancestral traits. Specifically, the same hormonal process is involved in the production of both soldier and supersoldier ants. If the underlying process of supersoldier development is compromised, soldier development would be affected as well. This would be disadvantageous as the soldier caste performs functions that are critical to the survival of the entire colony. Therefore, although not phenotypically expressed, the ability to produce a supersoldier is preserved in the genome of all *Pheidole* species indirectly through continued selection for soldier production.

Rajakumar et al. (2012) propose that, if recurrently induced by environmental factors in the wild, what begins as a spontaneous atavism can later evolve into a taxic atavism. Both the underlying developmental process and the eventual evolutionary pattern can be elucidated further with the help of phylogenetics and the induction of experimental atavisms in the lab. Initially, anomalous phenotypes that occur in the wild may not appear to be evolutionarily advantageous. However, the anomaly may be a spontaneous atavism that reflects a historically advantageous trait, which has been evolutionarily preserved by pleiotropy. If it is reactivated and similar selective pressures are present (to that of the ancestor) there is a possibility that this atavism may eventually become fixed in the population. Taken together, anomalies that spontaneously appear in the wild are a source of variation for natural selection to act upon.

6.4 Developmental Recombination: A Source of New Combinations

The modular nature of development has been one of the most important discoveries in developmental and evolutionary biology (Schlosser and Wagner 2004; Gilbert and Epel 2009). The organization of development into modules is largely the emergent consequence of genes being organized into interacting networks capable of responding to discrete morphogen and hormonal thresholds (Schlosser and Wagner 2004; Flatt

et al. 2005). Developmental modules are critical for distinguishing and giving identity to populations of cells within and between tissues (Carroll et al. 2005: Schlosser and Wagner 2004; Davidson 2006). The key implication of the modular nature of development for eco-evo-devo is that developmental modules are quasi-independent, meaning that when these modules are subject to genetic perturbation or environmental stresses they will respond in an almost independent manner from one another. On the one hand, this quasi-independence of developmental modules helps confer robustness during development (see Sect. 6.8) because genetic or environmental perturbations can be confined to specific modules. However, when the genetic perturbation or environmental stress is too great and robustness is compromised, "cryptic variation" is released (Gibson and Dworkin 2004). Examples of the accumulation and the release of cryptic genetic variation include ribozymes, which exhibit higher adaptation rate with accumulated cryptic genetic variation (Hayden et al. 2011) and T cell adaptive immunity where cryptic alleles drive rapid adaptation of activation responses when the cellular population is presented with a novel environment during infection (Whitacre et al. 2012). Several other examples are vulval development in *C. elegans* (Duveau and Félix 2012), feeding strategies in toads (Ledón-Rettig et al. 2010), and wing development in *Drosophila* (Dworkin 2005). While the release of cryptic variation is thought to be instrumental in trait evolution, much less attention has been given to the fact that the variation released may not be completely random, but rather, may reflect variation within and between independent developmental modules. The dissociation or formation of different combinations of modules is called "developmental recombination" which selection can subsequently act upon (West-Eberhard 2003, 2005). For example, stripe patterns in zebras and related equine species appear to be modular in their appearance in nature as described by Darwin (1868). It is possible that particular stripes are controlled independently from one another by different developmental modules and these can be recombined such that they can occur in different combinations in closely-related species.

Rajakumar et al. (2012) provide another example of developmental recombination with the evolution of supersoldiers in the ant genus *Pheidole*. It has previously been shown that the soldier caste in *Pheidole* develops as the result of a pulse of JH that crosses a discrete threshold during a critical time period (Wheeler and Nijhout 1981, 1983; Abouheif and Wray 2002). An additional JH threshold at a second critical period is present in the species *Pheidole obtusospinosa* that regulates the development of an additional 'supersoldier' caste (Rajakumar et al. 2012). When JH is applied during this second critical period, the supersoldier caste is consistently produced. Surprisingly, supersoldiers can also be induced in species that do not have a supersoldier caste due to the activation of an ancestral, but cryptic, JH threshold (Rajakumar et al. 2012). This demonstrates that the second threshold in *Pheidole obtusospinosa* emerged from the re-evolution of a cryptic JH threshold. This has occurred either through the evolution of the threshold itself or the regulation of JH production. The key indicator of supersoldier development is wing imaginal discs, each of which develop as independent modules. Induced supersoldiers exhibited quantitatively and qualitatively more variability in wing imaginal discs as compared to species, like *Pheidole obtusospinosa*, that naturally evolved supersoldiers. They found there was more variation in wing imaginal disc number, size, asymmetry and gene expression (Rajakumar et al. 2012). This novel variation of developmental modules, i.e., wing imaginal discs, generated by developmental recombination can undergo selection. It is likely that this is the type of variation, following the induction of a cryptic hormonal threshold, which was under selection during the course of supersoldier evolution in *Pheidole obtusospinosa*. Through the reorganization of existing developmental modules, the process of developmental recombination may more generally provide a source of variation for selection.

Many of the studies highlighted above were only possible due to recent advancements of molecular and genomic techniques. We are now beginning to appreciate the importance of the

reorganization of developmental modules in generating novel phenotypes, the next step will be to apply this framework to a life-history context in order to make better predictions of the ecological role and adaptive function of this type of variation. Furthermore with the use of comparative genomics and transcriptomics, we can more precisely identify the molecular makeup of different developmental recombinants that arise in nature.

6.5 Stochastic Variation: Molecules and Beyond

Biological systems are far more dynamic and noisy than originally assumed (McAdams and Arkin 1999). Noise at the level of molecular interactions can permeate to higher biological levels leading to stochastic variation in gene expression, and in turn, can contribute to differences in phenotype (Kilfoil et al. 2009). In most cases, developmental systems are robust and will buffer this stochastic variation in gene expression (see Sect. 6.8). However, recent studies have demonstrated that stochastic variation in gene expression has been co-opted during evolution to play an important role in influencing developmental decisions, where an initially stochastic expression of genes is stabilized to determine cell fates (Losick and Desplan 2008). An example of this occurs in the early *Drosophila* embryo, where all cells in proneural clusters initially have the capacity to differentiate into neuroblasts (Heitzler and Simpson 1991, reviewed in Losick and Desplan 2008). One cell in the cluster stochastically expresses more Delta protein than other cells, and as a consequence, it differentiates into a neuroblast while all others become epidermal cells. Cell fate decisions based on the stabilization of stochastic gene expression occur in other multicellular organisms, in different tissue types, and permeates to higher levels of biological organization (Kilfoil et al. 2009). Kilfoil et al. (2009) suggest that stochastic decisions in gene expression may permeate up to the level of individual organisms, such that particular social and behavioral decisions emerge stochastically. For example, reproduction in colonies of the ant species *Harpagnathos saltator* appear to be regulated in a stochastic manner, where an initially stochastic decision is stabilized and made permanent (Hölldobler and Wilson 2008, reviewed in Kilfoil et al. 2009). Queens and workers in *H. saltator* are both capable of reproduction; however, worker reproduction is inhibited by the presence of the queen (Peeters et al. 2000). The removal of the queen results in several antagonistic interactions between workers leading to the emergence of a small group of workers that become reproductively active (Hölldobler and Wilson 2008). These reproductive workers, who are at the top of the colony's dominance hierarchy, emerge because they are the first to acquire a distinct cuticular hydrocarbon profile that signals their fertility to the rest of the low-ranking workers in the colony (Hölldobler and Wilson 2008). Kilfoil et al. (2009) proposed the following model: removal of the queen triggers stochastic variation in the activity levels of enzymes involved in the synthesis of cuticular hydrocarbons. The stochastic expression of these enzymes in workers biases particular individuals towards becoming reproductive workers. Later, positive and antagonistic behavioral interactions between individuals further amplify the differences, resulting in the establishment of a small group of high-ranking reproductive individuals, while the rest of the workers in the colony remain reproductively quiescent. This example suggests that stochastic variation in gene expression may contribute in important ways to phenotypic evolution and behavioral decisions at higher levels of organization. Furthermore, Kilfoil et al. (2009) raise the possibility that phenotypic variation due to stochastic variation in gene expression should be acknowledged formally as a category of phenotypic variation in quantitative genetics (called V_s). Ecological genomics will be critical for defining and quantifying this category of variation, as well as in overcoming the challenges of distinguishing this type of variation from those derived from deterministic processes. Understanding how stochastic variation that is abundant at the molecular level permeates to higher levels of organization is an important arena where ecological genomics and eco-evo-devo meet.

6.6 Social Interactions: Generators of Behavioral and Phenotypic Variation

Social interactions can be thought of as a network and can be not only a source of information that individuals respond to, but also feed information back onto themselves. The way individuals tune their behavior to their social context and how accurate they are at tuning into the social signals affects not only their survival but their overall fitness. Therefore, while traditionally we have considered the importance of interactions between species and at the ecosystem level, we should not disregard the effect of group composition within species. The examples we present below show that social interactions can generate adaptive and novel phenotypic variation upon which natural selection can act upon. Social interactions, for the purpose of this chapter are not limited to social species (Tinbergen 1971), and encompass both an individual's reaction to the presence of at least one other individual of the same species and how their interactions can influence each other.

Social interactions can initiate top-down influences on an individual's phenotype; that is, changes in social interactions can lead to changes in individual behavior that in turn can change gene expression. For example, in the guppy *Poecilia reticulata*, social interactions in the form of mating preferences can affect an individual's behavior. Males choose the social context that will make them more attractive to female guppies based on the appearance of other males (Gasparini et al. 2013). In the African cichlid fish *Astatotilapia burtoni*, social interactions not only affect male behavior but also affect neural gene expression (Renn et al. 2008). Depending on social status, males can show two possible phenotypes: dominant or subordinate. When dominance status changes as a consequence of social interactions, changes in pigmentation and behavior take place within minutes, which is followed by gene expression changes in the brain. This is followed within a couple of weeks by changes in reproductive physiology and the dominant male phenotype is manifested (Renn et al. 2008; Fernald and Maruska 2012).

Social interactions can also influence development. In reptiles, for example, temperature plays a major role in influencing not only sex determination but also developmental timing. McGlashan et al. (2012) have shown in the freshwater turtle *Emydura macquarii* that synchronicity in hatching times are socially driven. Synchronicity in hatching times appears to be influenced by embryo-embryo communication. In this case, the authors suggest that temperature independent developmental timing could be achieved through changes in thyroid hormone production cued to CO_2 concentration in the nest or detection of sibling's heart rate. Interspecific interactions may have ultimately driven the evolution of this socially generated synchronicity because hatching time affects survival in the face of predation. Therefore, social interactions in this case can result in developmental timing changes which permeate to higher levels of organization and result in species success in complex interspecific interactions.

In the previous two examples we discuss how social interactions can generate adaptive phenotypic responses in developing and adult individuals. However, social interactions can also induce novel phenotypic variation during development that is relevant for evolution (see Sects. 6.3, 6.4, and 6.9). Some of the best documented examples come from social insects, where social interactions regulate caste determination (Hölldobler and Wilson 1990, 2008). A good example of social interactions influencing caste determination during development comes from the work by Wheeler and Nijhout (1981, 1983) on the determination of different types of worker subcastes in colonies of the ant genus *Pheidole*. The worker caste in this genus is composed of small 'minor workers' and large, big-headed, 'soldiers,' where minor workers make up 95 % of the colony and perform tasks related to foraging and brood rearing and soldiers make up the other 5 % and specialize mainly in tasks related to defence. Remarkably, colonies can maintain and even slightly adjust this ratio according to changes in their ecological environment (Yang et al. 2004). Passera (1977), Passera et al. 1996 and Wheeler and Nijhout (1984) showed that in

circumstances where the percentage of soldiers in the colony is too high, adult soldiers can inhibit the development of future soldiers using a contact pheromone. This contact pheromone exploits the mechanism normally used by *Pheidole* colonies to determine minor workers and soldiers during development. This developmental mechanism is based on a switch (threshold) that is regulated by the levels of JH, where larvae that produce high levels of JH during a critical period develop into soldiers, and those that produce low levels of JH develop into minor workers. The contact pheromone, which adult soldiers use to inhibit the future development of soldiers when there are too many soldiers in the colony, is thought to reduce the sensitivity of the larva to JH and larvae that would normally develop into soldiers develop instead into minor workers. For example, when Wheeler and Nijhout (1984) treated larva with relatively moderate levels of JH in a colony that has no soldiers, these larvae developed into soldiers, but when they treated larvae in a colony that has 100 % soldiers, they developed into minor workers. The following experiment, however, shows how social interactions can induce phenotypic variation relevant for evolution – when Wheeler and Nijhout (1984) treated larvae with relatively high levels of JH in a colony with 100 % soldiers, the JH treatment was too high for adult soldiers in the colony to completely inhibit the development of these larvae into soldiers. While some larvae still developed into soldiers, some larvae developed into exceptionally large small-headed minor workers that were as large in size as the big-headed soldiers! This shows that social interactions can influence development to produce phenotypic variations not normally observed in the colony. Indeed, the induction of phenotypic variation through social interactions may have played a role in the evolution of the minors and soldiers in this genus. Pie and Traniello (2007) showed that body size is the most variable trait across *Pheidole* species. Ecological genomics can play a critical role in helping to uncover the genes expressed during development of castes in ant species as they socially regulate their colonies to respond to the ecological pressures that surround them. In general, there is great opportunity in these and other systems for ecological genomics to enrich our understanding of how social interactions can generate adaptive and novel phenotypic variation upon which natural selection can act on.

6.7 Ecological Interactions

The role of the environment is twofold: through the action of natural selection, certain phenotypes will be selected in certain environments, but simultaneously, the environment can induce phenotypic variation through plasticity, thereby influencing the ecology of the organism. It is becoming clear that the dual role of the environment may often create a feedback loop that simultaneously influences the evolution of a trait. The fact that selection can act at various stages during the ontogeny of plastic traits can facilitate a rapid reaction and evolution of populations to a changing environment. Plastic phenotypes can subsequently be fixed through genetic assimilation and accommodation (Waddington 1957; West-Eberhard 2003, see Sect. 6.9), therefore facilitating the evolution of adaptive phenotypes.

An example of the environment acting as both a selective force while simultaneously acting as an inducing force in giving rise to different phenotypes can be found in North American Spadefoot toads (Pfennig and Murphy 2000; Ledón-Rettig and Pfennig 2011). These amphibians inhabit xeric habitats and among them all species of *Spea* genus have the ability to produce alternative larval phenotypes: omnivorous larvae that are small and feed on detritus, and carnivorous larvae that are large and feed exclusively on small insects or other anuran larvae. This resource polyphenism is dependant on multiple environmental cues, including nutrition and density in the ephemeral ponds where larvae develop (Pfennig and Murphy 2000; Ledón-Rettig and Pfennig 2011). Spadefoot toads may reach high densities in wetlands, are key for nutrient cycling within ponds, and it has been shown that their larvae can influence the entire trophic structure of these ecosystems (Ghioca-Robrecht and Smith 2010). In allopatry, *S. multiplicata* and *S. bombifrons* both exhibit this resource polyphenism. Interestingly, when they occur in sympatry, the

competitive interaction between larvae of both species have promoted differences in expression of this resource polyphenism: *S. multiplicata* has a tendency to produce omnivorous phenotypes, while *S. bombifrons* has a tendency to produce carnivorous phenotypes in sympatry (Pfennig and Murphy 2000). Therefore, their phenotypic plasticity has permitted the persistence of the two species in sympatry by reducing competitive interactions between them. This example illustrates how the environment acts bi-directionally; both as a selective force in favoring certain phenotypes over others, and simultaneously as an inducing force giving rise to different phenotypes. This eco-evolutionary feedback modifies the ecological interactions among species within communities, and will ultimately affect the evolutionary trajectories of each *Spea* species in these sympatric populations.

Another well-described example where the environment plays a selective role, but may also be playing an inducing role, is the parallel evolution of stickleback phenotypes. Parallel evolution of recurrent phenotypes in similar environments is nearly universal in the natural world and is generally considered an indication that the traits evolved by natural selection (Futuyma 1998; West-Eberhard 2003). These parallel selection pressures can explain in part this outcome, but the additional explanation of the existence of shared ancestral developmental potentials across replicates that respond in a comparable manner when placed into the same environmental conditions has been examined recently (see Sect. 6.3). The parallel evolution of three-spined sticklebacks in freshwater habitats from a marine ancestor is probably one of the most extensively studied systems to uncover the ecological drivers and genetic bases of parallel evolution in wild populations (Schluter 2000). Many freshwater populations show repeated evolution of the same limnetic and benthic ecotypes that differ in several morphological traits and diet. Notably, limnetic ecotypes feed primarily on zooplankton, and have a long and slender mouth whereas benthic ecotypes feed on larger invertebrates and have a short and wide mouth (Bell and Foster 1994). These ecotypes occupy different trophic niches and their respective evolution has therefore affected the ecological interactions within these freshwater communities (Bell and Foster 1994). The role of natural selection in the evolution of these freshwater phenotypes is well-established at both the phenotypic and the genetic level (Schluter 2000; Colosimo et al. 2005), but in comparison, the role of a common developmental potential in their parallel evolution has not received as much attention (West-Eberhard 2005). Wund et al. (2008, 2012) tested the hypothesis that the recurrent evolution of freshwater ecotypes is the result of a plastic developmental potential present in the marine ancestor. They found that when marine sticklebacks were reared on either a "limnetic diet" or a "benthic diet", the phenotypic plasticity of the head and mouth parallels the phenotypic divergence observed among freshwater ecotypes, supporting the role of a developmental potential in the marine ancestor in the recurrent evolution of the limnetic ecotypes, as well as repeated genetic assimilation in this system (see Sects. 6.3 and 6.9).

In the last few years, many organisms have had their genomes published, and exploiting the full potential of these data may reveal insights into the genetic bases of ecological adaptation and recurrent environmental induction of phenotypes. The genome of the three-spined stickleback has recently been published, and quite remarkably, accompanied with 20 additional genome-wide comparisons across populations to detect genomic regions that are repeatedly and consistently associated with the marine-freshwater divergence (Jones et al. 2012). The highly replicated nature of the system, the presence of the ancestral population together with the genomic resources available provides the unique opportunity to identify the genetic bases of this developmental potential and discover the mechanisms underlying its evolution. The presence of fragile sites (specific loci that preferentially exhibit gaps and break on metaphase chromosomes (Durkin and Glover 2007) in the stickleback genome have already been previously identified as targets for repeated evolution of ecologically relevant traits (Chan et al. 2010) and further exciting discoveries are without doubt awaiting to be realized. With genomes becoming available for more and more species, and even for several populations

of the same species, similar approaches for understanding the dualistic role of the environment in selecting and inducing phenotypes may be undertaken in the coming years.

6.8 Robustness: A Regulator of Variation

With the advent of population genetics in the twentieth century followed by molecular population genetics it became evident that natural populations have abundant genetic variation (Lewontin 1974). At the gene expression level, this variation gets further compounded by stochasticity of cellular processes (see Sect. 6.5) (Landry 2009). In addition to these 'internal' sources of variation, organisms must also face variations in their biotic and abiotic environments during development, such as geographical location, seasons, abrupt changes in weather, predatory relations, social interactions, and nutrition (see Sects. 6.6 and 6.7). Some of these variations are predictable but others are often rapid and unpredictable. Given that organisms face variation in both genotype and the environment during development, it is surprising that organisms mostly develop a robust phenotype. Robustness – also known as 'canalization of development' (Waddington 1942) – is the persistence of an organismal trait (organism or organ, gene expression pattern or activity, a cellular process) under different stochastic, environmental and genetic conditions or perturbations (Félix and Wagner 2008). Waddington and Schmalhausen independently characterized robustness in the mid 1900s, although they used different terminology (Waddington 1942; Schmalhausen 1949). When detailed studies of developmental systems at the genetic and molecular levels became available in the last few decades, the problem of robustness was revisited (Gilbert 1991; Eshel and Matessi 1998; Siegal and Bergman 2002; West-Eberhard 2003).

Robustness mostly acts to conceal the underlying variation in the genotype and the responsiveness of the organism to varying environments. In special cases, like that that of polyphenism, robustness allows only a few or specific phenotypic outcomes in response to specific environments. By acting against expression of variation at the phenotypic level, robustness results in accumulation of cryptic variation in the population both in the genotype and in the responsiveness of the organism to the environment. It is only under certain variations or conditions in the genotype or the environment that robustness becomes compromised thus exposing these phenotypes to natural selection.

Genetic and simulation data reveal that regulation of robustness could happen at the gene interaction level in a network involving feedback mechanisms (Crickmore et al. 2009; Holloway et al. 2011). In the same context the degree of robustness of a gene in a network would depend on its additive, dominance, or epistatic relationships with other components of the network (Proulx and Phillips 2005). It has also been shown that gene network hubs contribute to robustness (Levy and Siegal 2008). The genetic or epigenetic regulators that produce robust organisms during development in presence of variation in the genotype and the environment are in the early phases of their exploration (Masel and Siegal 2009). The developmental mechanisms that these regulators employ to achieve robustness still remain as fragmented case examples (Braendle and Félix 2009; Félix 2012; Gursky et al. 2012). One type of regulators of robustness are heat shock proteins that act as capacitors (a term borrowed from electronics), which implies that they accumulate a large amount of variation in an input and transmit it in a controlled manner (Rutherford and Lindquist 1998). This mechanism often involves miRNA (Pal-Bhadra et al. 2004) or piwi RNA pathways (Gangaraju et al. 2011). Redundancy (Wagner 2005) and modularity (von Dassow et al. 2000; Ma et al. 2006) are some other mechanisms that have been proposed. Identifying the molecular genetic mechanisms that are involved in the buffering mechanism of robustness, and their compromise that brings about release of cryptic variation in the genome, are necessary for a complete understanding of how shape, form, and proportion are generated during development and how robustness contributes raw material for

natural selection to act upon. Emerging tools and concepts highlighted in this book allow us to identify genome-wide contributors of robustness and to integrate ecological concepts into specific mechanisms of robustness in the near future.

6.9 Genetic Assimilation and Accommodation: Fixation of Environmentally – Induced Variation

In classical evolutionary genetic models, genetic variation is thought to represent an important source of raw material for evolution (Futuyma 1998; Rockman and Wray 2002; Wray et al. 2003). However, in recent years, phenotypic variation arising from developmental plasticity has been proposed as an equal, if not more, significant source of raw material for evolutionary change (West-Eberhard 2003, 2005). According to this model, developmental systems generally produce robust phenotypes until they become compromised due to the presence of an environmental or genetic perturbation. This results in a systemic response, exposing phenotypic variants to natural selection. Thereafter, through a process called genetic accommodation, natural selection increases the environmental sensitivity of the developmental program such that an environmentally induced trait is always induced when it encounters a recurrent environmental cue (West-Eberhard 2003, 2005). Therefore, genetic accommodation increases phenotypic plasticity often leading to multiple phenotypic outcomes, such as in polyphenism. Alternatively, in a process called genetic assimilation, natural selection decreases environmental sensitivity of the developmental program such that an environmentally induced trait is constitutively expressed in the absence of the recurrent environmental cue (Waddington 1942; Schmalhausen 1949; Waddington 1956). Therefore, genetic assimilation decreases phenotypic plasticity resulting in the evolution of a single phenotypic outcome. Both of these processes alter environmental sensitivity by acting on the genes that control the frequency and form of a trait (genetic accommodation increases while genetic assimilation decreases environmental sensitivity).

In the 1950s, Conrad H Waddington was able to demonstrate the process of genetic assimilation by repeatedly selecting for four-winged flies after an environmental perturbation (Waddington 1957). Gloor (1947) had discovered that environmental perturbation of fruit fly embryos with ether can result in transformation of the third thoracic segment into a duplicate of the second thoracic segment such that these flies develop four wings. A few years later Waddington showed that these four-winged flies could become fixed in the population using artificial selection (Waddington 1957). Waddington repeatedly selected flies with this phenotype after ether shock. This resulted first in an increase in the frequency of the phenotype until after some generations homozygous females consistently produce four-winged individuals without the ether treatment (Waddington 1957). Following up on Waddington's experiments, Gibson and Hogness (1996) demonstrated that this phenotypic response to ether correlated with a loss of expression of *Ultrabithorax* (*UBX*) gene in the imaginal discs of the third thoracic segment.

Elegant work by Suzuki and Nijhout (2006) with the tobacco hornworm moth *M. sexta* provided compelling evidence of the process of genetic accommodation. Wild-type *M. sexta* larvae are green. In some cases, mutant larvae arise that develop a black pigmentation. When these mutants are heat-shocked (42 °C), a spectrum of pigment phenotypes (between green and black) is generated. They established a genetic line for larvae that more readily developed green by selecting for the green variants each generation. After only 13 generations, most larvae would develop green following the heat-shock treatment. Most importantly, when exposed to low temperatures, the larvae developed black but, when exposed to temperatures above 28.5 °C, the larvae would develop green. The response-curve to this temperature continuum was sigmoidal indicating the trait was now polyphenic. It turns out that the black mutant larvae produce very low amounts of JH, whereas the heat-shock induced green larvae

produce significantly more JH (see Sect. 6.2). Therefore, beginning with a single phenotype, selection was able to generate individuals that have an increased plastic response that is mediated by a hormonal threshold (resulting in two phenotypes). Interestingly, genetic assimilation is also possible with this *Manduca* model. In parallel to selecting for a genetic line that could respond to temperature with increased plasticity (genetic accommodation), they also selected for a "monophenic" line, that after seven generations consistently produced black larvae regardless of temperature level due to decreased plasticity (genetic assimilation). Finally, it is known that a sister species of *M. sexta* (*Manduca quinquemaculata*; Hudson 1966) is naturally polyphenic: at low temperatures the larvae develop black and at high temperatures the larvae develop green. Therefore, due to shared developmental modules arising from common ancestry, it is possible that cryptic genetic variation in the genome of *Manduca sexta* includes polyphenic combinations. This evolutionary contribution to cryptic genetic variation is likely due to the presence of an ancestral developmental potential in this group (see Sect. 6.3).

It is not exactly known how genomic loci contribute to fixing of the phenotype via genetic assimilation or genetic accommodation. For example, in Waddington's (1957) artificial selection experiment, when the chromosome providing the four-winged phenotype was brought into the context of a wildtype genome or individual wildtype chromosomes, the phenotype could not be reproduced. This experiment shows that during artificial selection, chromosomal loci scattered throughout the genome contributed to the genetic assimilation of the four-winged phenotype. In this case, and more generally, these contributing loci could come from standing genetic variation or *denovo* mutations in the form of: allelic variants, cis- and trans-regulators, downstream target genes, promoters or coding regions. Advances in ecological genomics make it possible to uncover these loci precisely to understand the mechanistic basis of genetic assimilation and genetic accommodation.

6.10 Integrating Levels of Biological Organization

A major challenge in eco-evo-devo is to uncover the relationships within and among different levels of biological organization; from the level of molecules, to cells, tissues, organs, and organ systems, all of which combine to make up the individual organism. Levels of organization external to the individual organism extend to higher levels, such as groups, populations, and communities. Each level includes multiple members of the same level, which interact to form higher levels of organization in the form of nested hierarchies (MacMahon et al. 1978; Zylstra 1992; Valentine 2003; Jagers op Akkerhuis 2008; Findlay and Thagard 2012). Beyond discussions on the units or levels of selection (Lewontin 1970; Dawkins 1978; see discussion in Pigliucci and Kaplan 2006), many have recognized that organismal complexity is produced from networks of both top-down and bottom-up interactions (Valentine 2003; Longo et al. 2012).

Research in eco-evo-devo over a number of years has shown that evolutionary and developmental changes at some levels of biological organization can either be associated or dissociated with other levels (Abouheif 1997; Wray 1999). For example, across species, the same or homologous phenotypes do not necessarily use the same or homologous genes or gene networks (Wray and Abouheif 1998). To understand how such associations or dissociations evolve, we have to explicitly consider the organization and interaction among hierarchical levels. Gene networks, which can be considered as a distinct level of biological organization from its constituent genes, provides an example (Abouheif 1999). Modularity is a general and well-characterized feature of gene networks that allows for the maintenance of obligate linkages, while at the same time allows some flexibility and redundancy of other linkages in the network (Von Dassow et al. 2000; Ma et al. 2006). Furthermore, network modules as a whole can be co-opted during evolution to function in different processes. A good example of this is

the signal transduction Receptor Tyrosine Kinase (Ras-RTK) pathway, which has been co-opted during evolution to transmit signals between the cell surface and nuclear genes during the development of skin in mammals, eyes in fruit flies, and the female genitalia in nematodes (Gilbert 2010). Therefore, the modularity and co-option of network modules during development and evolution is one of the many ways in which dissociations between genes, gene networks, and phenotypes can evolve (see Sect. 6.4 for further discussion).

Associations and dissociations can also occur between the organ to embryo level. For example, Nijhout and Emlen (1998) show that removal of the hindwing imaginal discs in the butterfly *Precis coenia*, leads to a proportional increase in the final size of the forewings. Similarly, selection for increased or decreased size of horns in male *Onthophagus acuminatus* beetles produces a compensatory (opposite) change in eye size (Nijhout and Emlen 1998). In this case, associations and dissociations can arise because while production of the organ itself (the disc in the butterfly and the horn in the beetle) may be autonomous, it is their interactions that influence the final form of the organism. Furthermore, Abouheif and Wray (2002) showed that although the wingless phenotype in worker castes is evolutionarily conserved across all 15,000 species of ants, the underlying wing organs (wing imaginal discs), as well as the gene network that is responsible for the growth and patterning of these organs, are disrupted in different ways in different species. Because wing development in winged and wingless castes in ants is environmentally determined through the action of hormones, it raises the possibility that the environment may have played a significant role in facilitating the evolution of these associations and dissociations in ants (see Sect. 6.9).

Together, these examples show that associations and dissociations can occur among multiple levels at the same time. Development integrates all of the levels of biological organization outlined above, producing an individual organism that incorporates the interactions, outcomes and variation at multiple levels of organization during its own lifetime and transferring these from one generation into the next (Hall 2013). The tools and concepts of ecological genomics will facilitate the explicit consideration of multiple levels of hierarchical organization into eco-evo-devo studies.

6.11 Conclusion

Indeed "the time seems to have come ..." as Waddington (1959) so eloquently stated, to integrate the complex interactions between environment, genes, and development into our understanding of the evolutionary process. Although each of our individual sections document a particular aspect of this complex interaction, together, our sections tell the larger story of eco-evo-devo and its wider implications on evolutionary theory. We attempt to summarize this larger story in Fig. 6.1, which was actually inspired by the figure in Waddington's (1959) article that attempts to "take into account two further aspects of the evolutionary mechanism."

In Fig. 6.1, the development of an individual in a single generation is represented from top to bottom, from a fertilized egg to a reproducing adult, where arrows represent interactions between environment, genes, and development. Development must buffer the effect of *de novo* and standing genetic variation, as well as stochastic variation, to produce a robust phenotype (Interactions represented by grey arrows). While this type of variation is most often masked or buffered leading to the accumulation of cryptic genetic variation, natural selection can occasionally act upon this variation to produce alternative phenotypes that are stochastically or genetically determined. As a consequence of environmental change (organisms dispersing, constructing, modifying, or suddenly experiencing new environments) developing individuals are subject to environmental stress either directly or indirectly through new ecological and social interactions (represented by black arrows). This environmental stress can either perturb or be buffered by robustness (indicated by a double-headed arrow). If the environmental stress perturbs robustness, it can result in the release of cryptic genetic and phenotypic variation as well as in the induction of

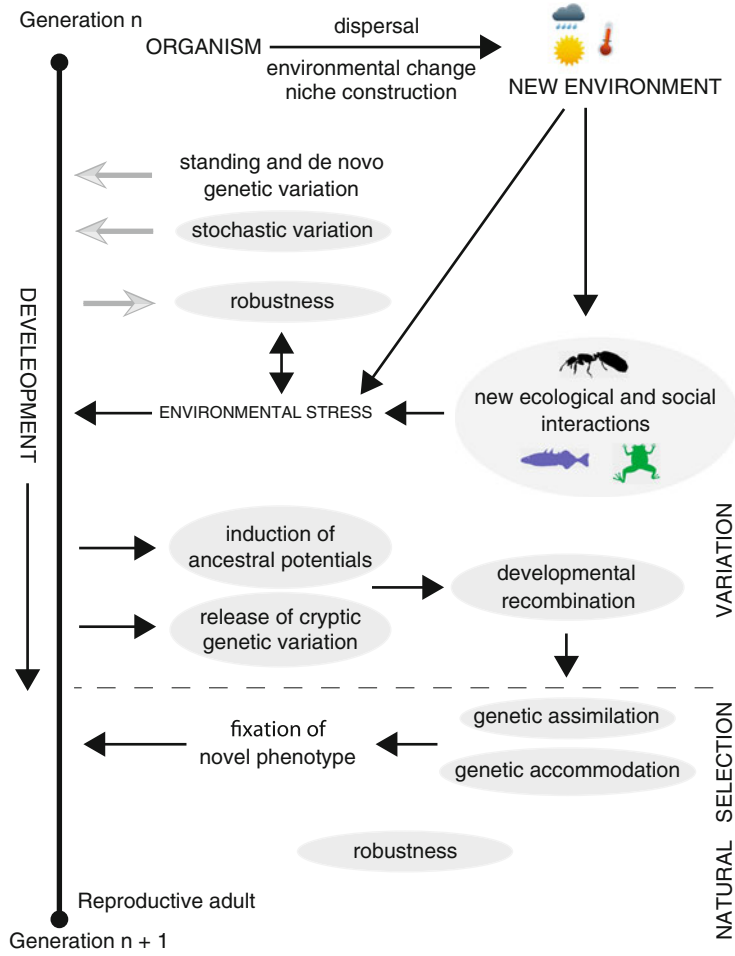

Fig. 6.1 The development of an individual during a single generation (from n to n + 1) is represented by a *thick black line* to the *left* from *top* to *bottom*, beginning with fertilization and ending with a reproducing adult. Shaded *grey ovals* correspond to Sects. 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, and 6.9 in the main text (some sections are combined into a *single oval*). *Grey arrows* show the interaction of endogenous sources of variation (standing genetic variation, de novo variation and stochastic variation) with the robustness of the developmental system of the organism. *Black arrows* highlight the developmental and evolutionary consequences following dispersal, niche construction and modification, and sudden changes in the environment of an organism. *Double-headed arrow* highlights the direct interaction between robustness and the environment. The area above the dotted line represents sources of variation, whereas the area below the dotted line represents evolutionary processes where natural selection is acting. Figure 6.1 is inspired by that found in Waddington (1959)

ancestral developmental potentials. The modular nature of gene networks will subsequently cause the variation released by environmental stress to appear in potentially new combinations.

If any of this variation provides an advantage to the reproducing adult, then this variation will be fixed by natural selection through either the process of genetic accommodation or genetic assimilation. The mechanism through which the fixation of environmentally induced phenotypes occurs is most likely to involve standing genetic variation, although de novo mutations and epigenetic mechanisms also contribute. Finally, these fixed phenotypes become robust to further environmental and genetic variation.

This eco-evo-devo view of the evolutionary process takes into account both genetic and epigenetic systems. We will end this chapter by

raising an important quandary for the ecological genomicist – if we view the evolutionary process as one where both genetic and epigenetic systems are important, then where should the focus of the ecological genomicist be: the genes responsible for the adaptive trait itself or the genes underlying the environmental responsiveness of the adaptive trait? This distinction is fundamental and important for understanding the genetic basis of adaptation, yet this has received little attention from biologists. Clearly, there is still much to learn about the rules underlying interactions between genes and the environment during development, the near future is likely to yield great insight in this direction.

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