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## The right tools for the job: Regulating polyphenic morph development in insects

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### Abstract

Polyphenism is a form of developmental plasticity in which organisms respond to environmental cues by producing adaptive, discrete, alternative phenotypes known as morphs. The phenomenon is common and important as both a form of adaptation and a source of variation for natural selection. Understanding the evolution of polyphenism will require understanding the proximate factors that regulate alternative morph production. Renewed interest and technological advances have fueled multiple approaches to the latter, including hormone manipulation studies, targeted transcriptomic studies, and epigenetic profiling. We review these studies and suggest that integration of multilayered approaches will be necessary to understand the complex mechanisms involved in regulating alternative morphologies.

### Introduction

Many organisms have evolved the ability to respond to changing environmental conditions by altering development to produce adaptive phenotypes. Such developmental plasticity can be a decisive boon, allowing individuals within populations to adapt to changing environmental circumstances on short, non-evolutionary time scales. If, in addition to being adaptive, the developmental response is discrete and stable, then the phenomenon is referred to as a *polyphenism*, with alternative phenotypes, referred to as *morphs*, typically exhibiting correlated suites of traits [1–3]. The production of alternative phenotypes by the same genetic material is inherently a regulatory process: external cues are perceived by the organism and then converted into a molecular signal or signals that specify development of the appropriate morph (Figure 1). Once morph identity is specified, alternative morphs develop by proceeding down different ontogenies.

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Evolutionary studies of polyphenism typically aim to understand how polyphenic responses evolve and how such plasticity can, in turn, affect the course of evolution. Answers to both of these questions will undoubtedly be informed by a deeper understanding of the proximate mechanisms of polyphenism, in particular its underlying genetics, epigenetics, and development. Fortunately, renewed interest in phenotypic plasticity, combined with technological advances, has provided insights into this area. Here we review recent contributions to the regulation of polyphenic morph development in insects with an eye toward identifying common themes in how these alternative phenotypes are regulated.

### The classic approach: hormone manipulation studies

Although technological advances promise continued progress in understanding the proximate basis of polyphenism, important insights can still be gained through the classic technique of hormone manipulation. As polyphenisms often involve manifold changes throughout the organism, it is not surprising that hormones have long been known to coordinate the development of alternative morphs. In insects, two classes of hormones, ecdysteroids and juvenile hormones (JH), underlie the developmental decisions inherent in many polyphenisms [4,5].

Two studies highlight the power of this technique and the insights they can provide. First, at least two species of the ant genus *Pheidole* possess a “supersoldier” subcaste that is affected by nutritional state and mediated by JH. In several *Pheidole* species that do not normally produce supersoldiers, application of a JH analog can induce supersoldiers. This result suggests that there is an ancestral developmental potential for this plastic response and that activating this latent response likely facilitated parallel evolution of the supersoldier subcaste in multiple *Pheidole* species [6]. Second, a hormonal approach has linked seasonal changes to color and life history adaptation in the butterfly *Bicyclus anynana*. In this species, wet season or dry season morphs are induced depending on the temperature that larvae and pupae experience during development [7]. A recent study has functionally confirmed what had long been suspected about this polyphenism based on titer correlations: ecdysteroids act as part of the switch that controls progression down developmental pathways leading to alternative life histories [8]. This latter study is also one of the first to examine carefully whether each member of the suite of ecologically relevant traits that characterize the alternative morphs is affected by ecdysteroids.

In general, hormonal manipulation studies are most informative when combined with attempts to correlate morph development with direct measurements of hormone titer, with differential expression of hormone regulators, and with correlated changes of morph-specific downstream genes [reviewed in 9]. These two examples and a myriad of hormonal investigations in other polyphenic insects [reviewed in 5] reveal a common theme: hormonal signaling is a critical aspect of regulating polyphenisms. That said, polyphenic ontogenies involve much more than hormones and recent technological advances are revealing some of these additional regulators.

## Transcriptomic approaches

The establishment of model systems for studying plasticity and advances in our ability to describe transcriptomes have fueled rapid progress toward understanding the details of alternative developmental trajectories and the nature of environmentally controlled developmental switches [sensu 10]. In a number of polyphenisms, for example, transcriptomic approaches have identified a large number of gene expression differences between morphs [e.g., 11,12–14], indicating that different genes from a shared genome contribute to each phenotype. Although most of these studies have focused on the differentiation of adult phenotypes, long after morph identity has been specified, a few have used transcriptomics to identify differences in gene expression that regulate alternative morph specification by precise temporal profiling of the transcriptome in response to inducing cues [15–18]. Even with these more targeted studies, however, it can be challenging to identify the changes in gene expression that are indeed responsible for morph specification because often there are numerous correlated changes in gene expression.

The emergence of new model insect systems has allowed us to go beyond correlation and test the potential roles played by particular factors in generating plasticity. The dung beetle, *Onthophagus taurus*, provides an excellent example. These beetles exhibit a male-specific horn polyphenism controlled by nutrition [20]. Small males develop virtually no horns, while large males develop large horns; females have no horns. Kijimoto et al. [21] profiled transcript levels from developing horn tissues and used the data to identify a number of candidate genes for polyphenic horn growth, including the sex determination gene *doublesex* (*dsx*). Subsequent work [18] revealed that, as in *Drosophila*, *dsx* transcripts are sex-specific, and play a role in the expression of primary and secondary sexual traits. Interestingly, male *dsx* is also found at higher levels in the horn primordia of large males than of small males and RNAi against male *dsx* results in the loss of horns in large males while leaving small males unaffected. These experiments suggest that *dsx* is a key regulator of both sexual dimorphism and male horn polyphenism. Presumably *dsx* somehow interacts with JH, which is known to mediate the nutritional control of the horn polyphenism [22,23]. But how does this interaction occur? Studies of male-specific exaggerated trait growth (either horns or mandibles) in other beetle species suggest that *dsx* might act to sensitize a tissue to respond or not respond to a circulating hormone such as JH or insulin-like peptides [24–28]. Whether *dsx* acts in this way in the horn polyphenism is a question for future research.

Transcriptional profiling of the transition between density-induced solitary and gregarious morphs in the migratory locust provides another example. Genome-wide expression assays led to the identification of the dopamine pathway as an exciting candidate for controlling the behavioral changes that accompany phase transitions [29]. RNAi knockdown of genes in the pathway confirmed their causative role, and pharmacological manipulation showed that dopamine promotes gregariousness [29]. In addition to behavioral differences, solitary and gregarious locusts differ in morphological, physiological, and neurochemical features [30,31]. As pointed out in recent reviews of the locust dispersal polyphenism [32,33], despite the established importance of the dopamine pathway in regulating behavioral changes, no one factor controls all of the differences between the morphs. Years of study and the application of wide variety of approaches have revealed that

there must be changes in neuroendocrine factors, peptides, metabolites, transcripts, and epigenetic factors.

These examples provide two lessons, one methodological and one biological. First, a transcriptomic approach can be quite useful in identifying candidate genes and pathways when none are known, if the profiling is done at developmentally appropriate stages and within relevant tissues. Second, while the “switch” metaphor is apt for some polyphenisms (*e.g.*, male beetle horns), it fails to capture the reality of others (*e.g.*, solitary and gregarious locust morphs). In the case of male beetle horns, the environmental cues that trigger the development of one morph versus the other are received during a relatively short window of development and the effects are irreversible. In contrast, the transition between locust morphs is arguably more complicated; it can be intraindividual or transgenerational, partial or complete, and reversible [30]. A greater understanding of the regulation of alternative morphs in a variety of species should open our eyes to the full spectrum of ways that such developmental decisions get made and how such mechanisms can evolve.

## Epigenetic approaches

One of the more exciting and recent approaches to understanding the mechanistic basis of polyphenism has been to investigate whether epigenetic modifications (*e.g.*, acetylation or methylation of histones and methylation of CpGs in DNA) might play a role in either specification or subsequent differentiation of morphs. Such modifications are likely to correlate with any differential transcription involved in the initial morph specification after receiving the inducing environmental cue, since epigenetic changes accompany transcriptional changes [34]. The mitotic inheritance of chromatin states may also then be needed to perpetuate morph identity to the various tissues of a developing organism and allow for the differential transcription required to differentiate alternative phenotypes. A question often posed is whether epigenetic modifications play a role in polyphenism. The answer is undoubtedly “yes”, insofar as such modifications are now taken to be part and parcel of differential transcription [35], which almost certainly plays a role in morph differentiation if not specification. A more specific and perhaps more fruitful question is whether epigenetic modifications stand in the causal chain leading from environmental cue to morph specification and, if so, what types?

Of the various insect models, social insects have thus far contributed most to our knowledge of the role of epigenetic modifications in polyphenism. Inspired by the transcriptional regulatory role that DNA (mostly CpG) methylation plays in vertebrates, most work has focused on DNA methylation. Kucharski et al. [36] were the first to demonstrate a functional role for DNA methylation in morph specification, specifically diet-induced caste specification in honeybees. RNA interference of the DNA methyltransferase *Dnmt3*, responsible for *de novo* methylation of CpG cytosines, causes queens to develop from presumptive worker larvae, suggesting that *de novo* CpG methylation mediates between diet and caste specification. A more recent study has shown that DNA methylation may contribute to caste differentiation *post*-specification [37]. In the ant species *Camponotus floridanus*, environmental conditions cause workers to vary quantitatively in size. Alvarado et al. [37] discovered that this size variation was regulated, at least in part, by global levels

of DNA methylation. Further, they implicated DNA methylation levels at the size-regulating gene *Egfr* and its associated expression level as a significant mediator of this process. Size variation is an important component of caste differences in ants, so this epigenetic effect could be a critical factor underlying morph differentiation.

Although in insects the functional significance of DNA methylation for gene expression is still not entirely clear, in part because methylation tends to occur within the gene body rather than at promoters [reviewed in 39], it may act by preventing or promoting the binding of regulatory proteins and by changing how tightly DNA binds to nucleosomes [46,47]. Another possible role for DNA methylation in insects has been suggested by data from social insects; namely, that it regulates alternative splicing. Several studies in honeybees and ants have noted a correlation between DNA methylation and alternative splicing at the genome-wide level [42,48,49], while a study in the jewel wasp (*Nasonia vitripennis*) has not [50]. Further, interfering with *de novo* DNA methylation alters patterns of exon skipping and intron retention in the honeybee [51]. Could DNA methylation foster plasticity by regulating the production of morph-specific transcripts? The idea is tantalizing, yet so far unsupported. It is notable, for example, that so far no study in insects has functionally tested whether methylation status has an effect on splicing for a particular transcript.

Surprisingly little research has investigated the role that histone modification and noncoding RNAs might play in polyphenism, making it an obvious focus of future studies. Two reports are notable exceptions. First, Simola et al. [52] found a possible role for H3K27 acetylation in caste differentiation in the carpenter ant (*Camponotus floridanus*) by profiling different histone modifications at the genome-wide level in different castes. Second, an excellent, thorough study by Yang et al. [53] found that a microRNA controls dopamine synthesis in the migratory locust (*Locusta migratoria*) by regulating two genes in the dopamine synthesis pathway (*henna* and *pale*). As noted above, dopamine levels have been shown to control the production of solitary versus gregarious forms in this species [29]. Similar approaches promise further insights in the coming years.

## Concluding remarks

Not surprisingly, the regulation of alternative developmental pathways is a complicated, multilayered process, involving both initial specification and subsequent differentiation. Changes occur in hormones, biogenic amines, transcripts, and epigenetic marks. Post-transcriptional changes may also play important roles. Because of this, approaches to dissecting the mechanism of regulation must be multilayered. To date, interactions between changes in gene expression and hormones are well established. For example, studies reviewed above suggest that morph-specific gene expression can sensitize relevant tissues to hormonal action in a morph-specific manner. Further support for this idea is provided by a recent study in the brown planthopper, which showed that two insulin receptors regulate how the wing buds respond to an insulin-like peptide secreted by the brain, producing long-winged or short-winged forms [54]. Although the role of epigenetic modifications in regulating polyphenism is still being investigated, integration is clearly on the horizon.

Proximal studies of insect polyphenism ultimately address the mechanisms that underlie a fundamental feature of life on earth: organisms can sense changes their environment and, in response, alter development to produce adaptive changes in phenotype. An understanding of these mechanisms ought to provide a sound basis for understanding how such plasticity evolves and how it may constrain or facilitate future evolution.

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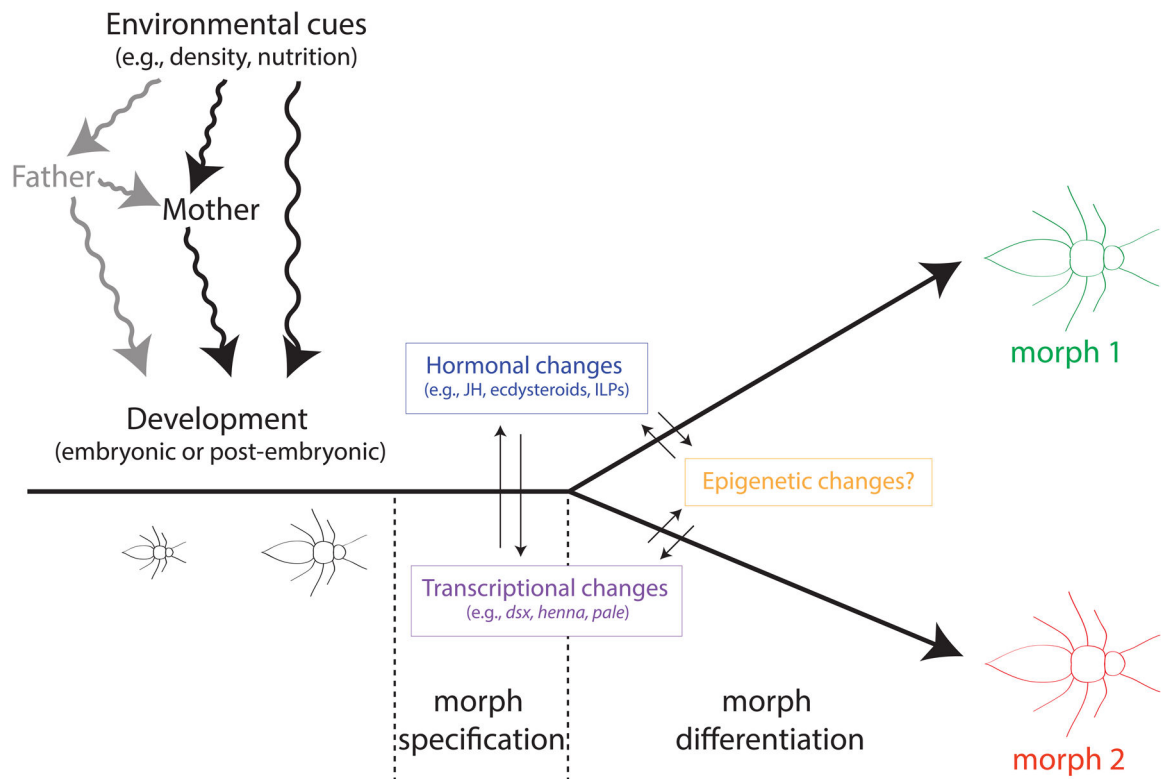
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### Highlights

- Many insects can produce adaptive, polyphenic morphs in response to environmental cues
- The regulatory mechanisms underlying polyphenism are slowly being revealed
- Hormonal and transcriptomic approaches have revealed specific mechanisms
- Epigenetic approaches are promising, yet further study is needed



**Figure 1.**

The process of morph specification and differentiation during development involves a branching ontogeny influenced by hormonal, transcriptional and epigenetic changes, all of which may influence each other. Although images of post-embryonic development are shown, specification and differentiation can just as easily take place during embryonic development. These can influence development directly, or be mediated by a mother, who may receive the cue and transmit this information on to her progeny as a maternal effect. Although less supported and less investigated, paternal mediation (shown in grey) is also possible [55], either in combination with or independently of the mother.