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Current Opinion in Genetics & Development

Decoding the architecture and origins of mechanisms for developmental polyphenism

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Developmental polyphenism affords a single genotype multiple solutions to match an organism to its environment. Because polyphenism is the extreme example of how development deviates from a linear genetic blueprint, it demands a genetic explanation for how environmental cues shunt development to hypothetically alternative modules. We highlight several recent advances that have begun to illuminate genetic mechanisms for polyphenism and how this recurring developmental novelty may arise. An emerging genetic knowledge of polyphenism is providing precise targets for testing hypotheses of how switch mechanisms are built — out of olfactory, nutrient-sensing, hormone-reception, and developmental and genetic buffering systems — to accommodate plasticity. Moreover, classic and new model systems are testing the genetic basis of polyphenism's proposed causal roles in evolutionary change.

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Current Opinion in Genetics & Development 2017, **47**:1–8

This review comes from a themed issue on **Evolutionary genetics**

Edited by **Eric S Haag** and **David L Stern**

http://dx.doi.org/10.1016/j.gde.2017.07.015

Introduction

Developmental plasticity, or the ability of a single genotype to produce multiple phenotypes according to environmental cues, is a ubiquitous feature of multicellular organisms. The idea that developmental plasticity itself may be under selection is an old one [1], and the proposition that plasticity can be under genetic control [2] has implied that the selected units of plasticity regulation can be identified. Because developmental plasticity is the complex interplay of genes and environment [3,4], it is possible that genes underlying plastic traits may either lead or follow in the evolution of plasticity [5]. Whatever their history, identified regulatory and target genes that mediate plasticity offer the 'hard entities' that can be analyzed comparatively to reconstruct the mechanistic evolution of plastic responses. A particularly ideal opportunity to identify such genetic components is afforded by developmental polyphenism. Polyphenism, or discontinuous plasticity, can result in such dramatically different phenotypes that they resemble or exceed interspecific differences [4,6]. Although not nearly as common as continuous plasticity, polyphenism makes a compelling research model by virtue of its categorical outputs, which simplify both the phenotypic readout and the ability to isolate discrete genetic modules turned on or off in response to environment. Furthermore, polyphenism is often associated with evolutionary novelty [4,6–9], raising the possibility that a causal relationship between the two can be found. In this review, we highlight recent advances toward uncovering the genetic mechanisms for polyphenism and how they arise during evolution.

Given the recognition that development in general is largely organized as a series of genetic switches [10], it has been logical to predict that polyphenisms, specifically the modular genetic networks corresponding to alternative forms, should be regulated in an analogous way. Developmental observations of polyphenism have led to theoretical models for the genetic architecture of polyphenism switches: for example, they might take the form of compound switches that converge on an ultimate 'switch point' or of a cascade of multiple switches responsive to different cues [4]. In any case, the observation that threshold responses can decide between two discrete phenotypes, such as in response to hormone titers in insects [9], implies that polyphenism 'switch genes' can mediate programmed variability. Numerous studies have associated gene-expression differences with polyphenic morphs, further indicating that transcriptional switches in some form control polyphenism [11–20]. As polyphenism switch mechanisms are revealed, it will be possible to test hypotheses about their architecture and genetic origins. Moreover, the independent evolution of plasticity switches among model systems may ultimately reveal what genetic phenomena, if any, feature generally in the evolution of polyphenism.

Environmental-sensing mechanisms of polyphenism switches

The entry point to a polyphenism decision is expected to be reliable detection of inductive cues, particularly those that will adaptively match phenotype to the selective agents to be experienced [21]. The *Caenorhabditis elegans* dauer, a facultative diapause larva, is tuned to cues of environmental challenges such as starvation and

increased concentrations of crowding pheromones (ascarosides) [22,23]. Environmental-sensing mechanisms illuminated in this model system, given its well-understood genetics [24], can thus provide a detailed reference for polyphenisms of traits such as morphologies or ecotypes. In one recent study connecting olfaction to development [25•], inductive cues were found to repress the calcium/calmodulin-dependent kinase CMK-1/CaMKI in sensory neurons. This resulted in the inhibition of DAF-7/TGF-β and insulin-like protein DAF-28/ILP, which are otherwise required to inhibit dauer entry [26,27]. CaMKI was specifically found to trigger these two pathways in two pairs of neurons — the amphid ASI and 'wing cell' AWC neurons, respectively — and that in the latter pair it interprets the balance of both inhibitory and inductive cues. In another study, endogenous RNAi pathways involving general chromatin modifiers (nuclear Argonaute CSR-1 and the nematode-specific Mutator MUT-16) were also found to regulate dauer-induction pathways in different sets of chemosensory neurons depending on the inductive cue [28•]. By linking the well-understood neuroanatomical and developmental bases of dauer diapause, these findings provide genetic mechanisms for how neuronal and nutrient-sensing pathways interact before they feed into the switch point for a developmental decision.

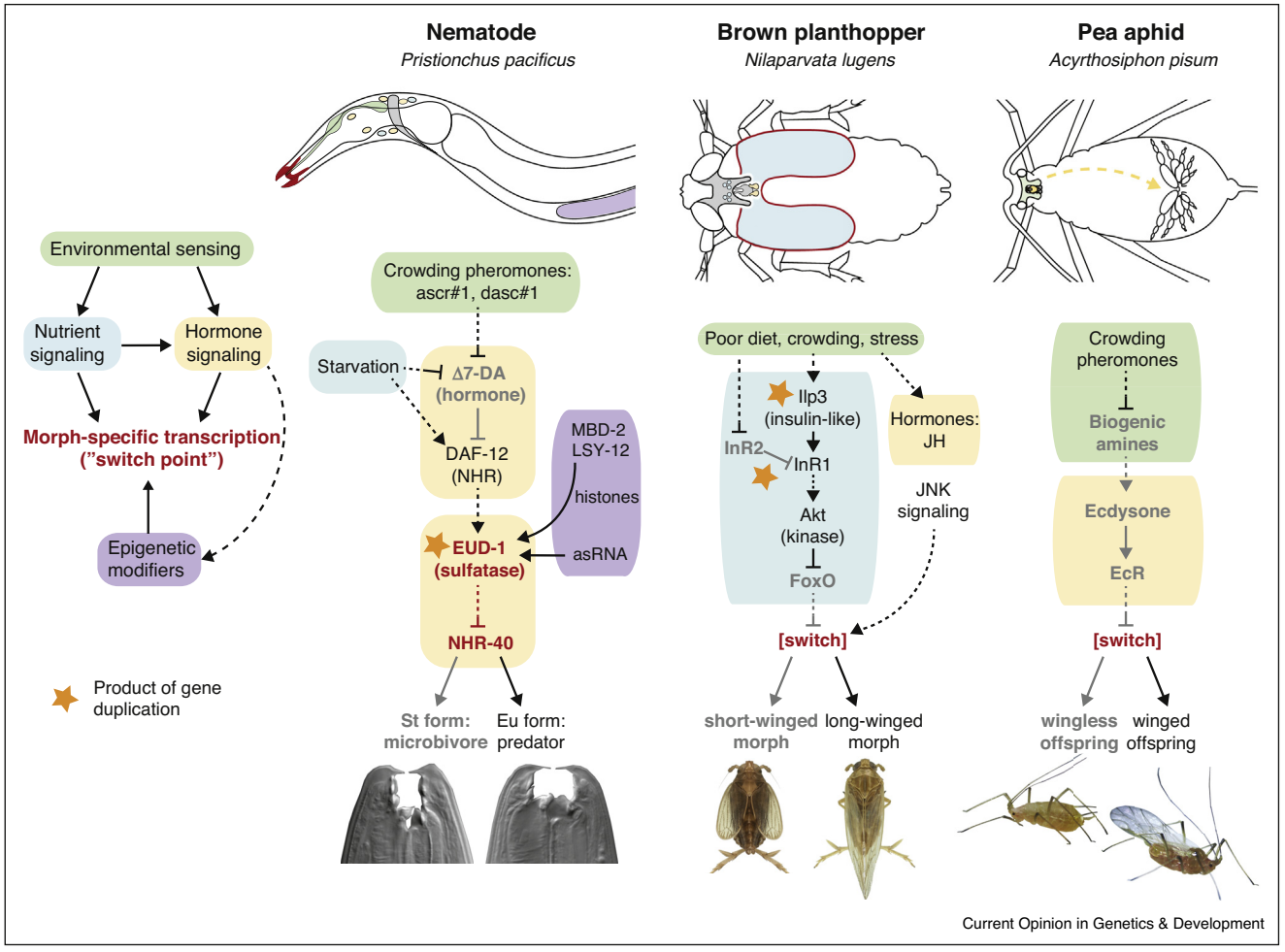
Studies in non-traditional, but ecologically well-described, laboratory models have likewise assayed the effects of genes suspected to mediate polyphenism sensing. In locusts, the neuromodulators serotonin and catecholamine regulate density-dependent phase polyphenism [29,30]. RNAi knockdowns have added two biogenic amine (trace amine neurotransmitter) receptors to the known sensing repertoire for *Locusta migratoria*: specifically the octopamine receptor α (OARα) and tyramine receptor (TAR), which reversed the expected behavioral phase in gregarious and solitary nymphs, respectively [31]. Complementing research on phase polyphenism are functional experiments on the associated body-color polyphenism of locusts. In both *L. migratoria* and *Schistocerca gregaria*, the gene encoding the neuropeptide [His^7]-corazonin was found to induce and maintain the distinct black coloring of the gregarious phase [32,33]. In an alternative insect model, pea aphids (*Acyrthosiphon pisum*), biogenic amines including octopamine have likewise been implicated to act under different polyphenism-induction cues [34]. Although the convergent use of biogenic amines for polyphenism sensing is not surprising, given their neurophysiological importance, the identification of specific receptors allows a more cohesive understanding of polyphenism switch architectures.

Endogenous signaling in polyphenism mechanisms

In addition to the capture of appropriate cues by environmental sensors, nutrient-signaling pathways can also transmit inductive information to the regulatory cascades that decide between developmental programs (Figure 1). In particular, insulin/insulin-like growth factor signaling (IIS) may be a widespread mediator of trait differences, for example their exaggeration through allometric growth [35]. The insulin receptor (InR) has now been functionally linked to a morphological polyphenism in the brown planthopper, *Nilaparvata lugens* [36••]. Although insect wing polyphenisms had been previously described in terms of developmental patterning [37,38], the present study was the first to demonstrate how such a polyphenism is molecularly decided. In the planthoppers, commitment to developing long wings or short wings is promoted by duplicate insulin receptors, InR1 and InR2, respectively. In a logic similar to canonical InR signaling [39,40], InR1 promotes the long-winged, dispersal morph by initiating a phosphorylation cascade that deactivates the transcription factor FoxO. In a surprising modification of the InR pathway, one of the two duplicate receptors (InR2) was found to heterodimerize with and inhibit the other (InR1), but only in developing wing buds. Thus, the functional specialization of the duplicate receptors resulted in polyphenic outcomes by modifying a single, otherwise conserved pathway. Furthermore, the use of a highly pleiotropic nutrient sensor to regulate the planthopper's polyphenism was apparently allowed by restricting the modification to a specific tissue, in which other signaling mechanisms such as stress-activated JNK [41] or hormone cascades [42] are also likely to converge. Taken together, these findings suggest that anatomical compartmentalization of divergent regulatory logic may provide the flexibility for repurposing conserved genetic networks to tissue-specific polyphenisms (*sensu* [43]).

Resource polyphenism is another case of how predictions of nutritional status can influence development, particularly to result in different ecological functions matched to the local ecosystem. An example of resource polyphenism is in the nematode *Pristionchus pacificus*, which develops into one of two feeding-structure morphs: either strict microbivores or omnivores capable of predation, as influenced by population density and microbial food abundance [44–46]. A forward screen for polyphenism-defective mutants first uncovered *eud-1*, which encodes an arylsulfatase and regulates polyphenism downstream of starvation cues, pheromone signaling, and hormone signaling [47]. A screen for suppressors of the phenotype of *eud-1* has since revealed the nuclear receptor NHR-40, which is presumably the transcriptional switch-point between morph-specific genetic modules [48••]. The discovery that *nhr-40* and *eud-1* are expressed in non-overlapping neural tissue suggests the two factors mediate intercellular communication. For example, homologs of EUD-1 in humans cleave steroid hormones to regulate cellular functions such as the modulation of signaling pathways [49]. It is thus possible that EUD-1 deactivates

Figure 1



Generalized logic and empirical examples of polyphenism regulation across three animal models. Above each circuit diagram is an idealized body diagram of the hypothesized sites of polyphenism regulation. Sites of polyphenism (nematode, planthopper) are outlined in red; brains (all taxa) are colored gray where not otherwise highlighted. For the larval nematode, sites for ascaroside pheromone sensing (amphid, green), insulin signaling (head neurons, blue), and dafachronic acid signaling (head neurons, yellow) are predicted based on homology with *C. elegans*; expression of *eud-1* and *nhr-40* has been localized to head and pharyngeal neurons (yellow); chromatin remodeling and antisense RNAs offer a mechanism for carrying signals to the next generation (germline, purple). In the planthopper nymph, sites of insulin signaling (wing buds, neurons in blue) and JH release (corpora allata, yellow) have been demonstrated. Spatial regulation of polyphenism in the aphid is informed by homology with other insect systems: biogenic amine signaling (brain, green) likely occurs upstream of ecdysone signaling (prothoracic glands, yellow) which ultimately regulates polyphenism in the individual's offspring (ovaries, black outline). Planthopper photos by Natasha Wright (Cook's Pest Control); aphid photos by Mary Grantham.

a signal or signaling cascade that otherwise activates NHR-40-mediated transcription. Because NHR-40 promotes the microbivorous morph, the receptor may thereby, under conditions of abundant bacterial food, prevent a 'default' state of becoming a predator. Although this model is still hypothetical, identification of the ligand and immediate targets of this receptor can test this and alternative models of how endogenous signals promote morph-specific transcription.

Factors acting upstream of *eud-1* and *nhr-40* have provided detail on the architecture of the *P. pacificus* resource polyphenism. A screen of existing mutants for potential pleiotropic effects revealed that two conserved histone-modifiers, the histone acetyltransferase LSY-12 and the methyl-binding protein MBD-2, promote the omnivorous morph and specifically regulate the expression of *eud-1* [50••]. Additionally, long non-coding RNAs antisense to *eud-1* were, unexpectedly, found to upregulate *eud-1* expression. The activity of both chromatin remodeling and non-coding RNAs therefore suggests that, and provides details on how, environmental cues may be recorded transgenerationally. In light of this additional complexity, an analysis of other known polyphenism mutants is likely to provide a cohesive picture of the genetic architecture of the switch.

With the identification of factors that control polyphenism in *P. pacificus*, how polyphenism machinery arises in macroevolution can be ultimately reconstructed. For example, *eud-1* is one of three paralogs specific to a lineage including nematodes with polyphenism. Using single-gene and multiple-gene knockouts of *P. pacificus eud-1* paralogs, *eud-1* was shown to be unique among duplicates in being a polyphenism regulator [51]. This finding indicates that the evolution of the polyphenism switch involved gene duplication and functional specialization of this gene. Given the amenability of diplogastrid nematodes to DNA editing, candidate knockouts in other species should increasingly resolve the evolutionary steps taken to build a polyphenism switch.

Recent advances in aphids, an ecological model for transgenerational polyphenism, have likewise uncovered neuroendocrine mechanisms underlying polyphenism decisions. In the pea aphid (*A. pisum*), wingless females are induced by high population density and poor nutrition to produce winged progeny that can disperse to more favorable feeding sites [52]. Using a transcriptional-profiling experiment, induced pea-aphid mothers showed downregulation of genes involved in ecdysone receptor-mediated signaling [34]. To test whether crowding negatively regulates ecdysone signaling, which may in turn be activated by biogenic-amine signaling following olfaction, a follow-up study both inhibited and activated the ecdysone receptor (EcR), which resulted in a higher or lower proportion of winged offspring, respectively [53••]. Thus, in pea aphids, the regulation of a taxonomically restricted trait evolved by repurposing an ancient hormonal signaling pathway.

Ecdysone-mediated polyphenism has evolved convergently with Lepidoptera [54] and is analogous to the co-option of *Pristionchus* mouth polyphenism determination from the taxonomically widespread dauer hormone (Δ7-dafarchronic acid) signaling [45], implying a more general versatility of ancient hormone-reception modules to accommodate novel developmental traits. Surprisingly, the arylsulfatase-encoding homolog of *P. pacificus eud-1* was also noted to be differentially expressed between pea-aphid morphs [34]. Little is known about the developmental roles of arylsulfatases: although a homologous sulfatase is differentially expressed between two sea-urchin (*Heliocidaris*) species with distinct larval forms [55], work in other species suggests a mechanical rather than signaling role for this enzyme [56]. Functional analogy of the aphid arylsulfatase remains to be tested, but an emerging set of molecular tools offers promise for expanding empirical tests to this and other candidate polyphenism regulators in this classic insect model.

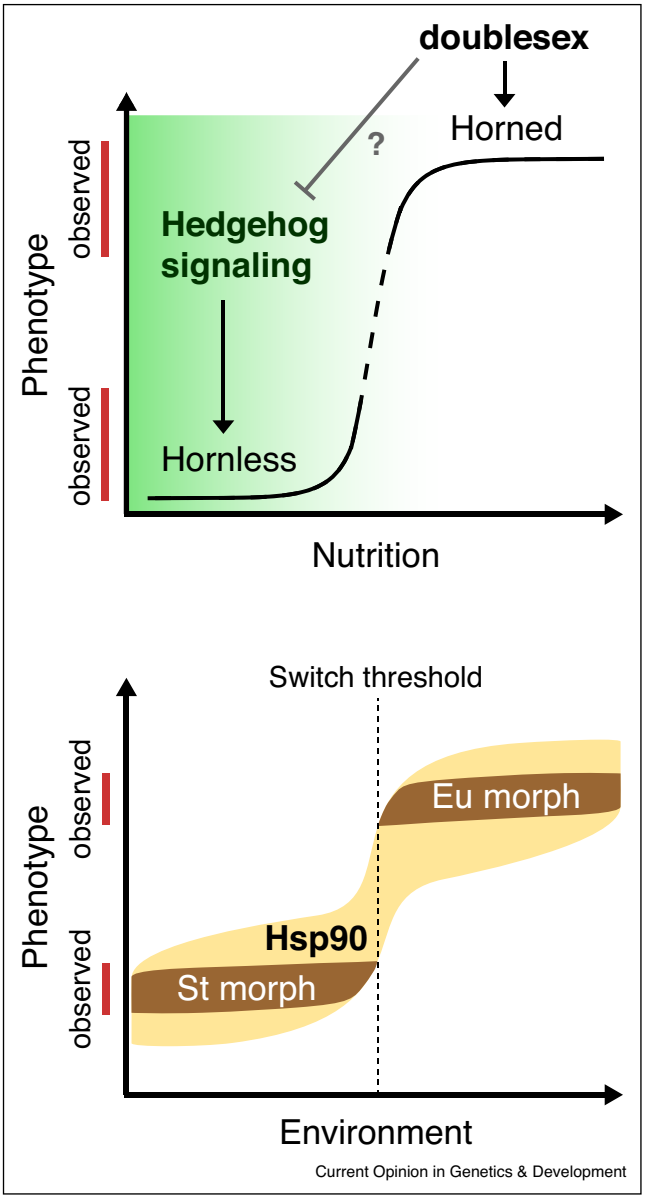
Evolution, refinement, and repurposing of threshold plastic responses

Several evolutionary routes to polyphenism have been proposed, including the recurrence of 'dormant' threshold responses [8] or the novel canalization of continuous plasticity [57]. Identification of the genetic factors that control polyphenism — that is, components required for a step response that do not vary between individuals, but which can be found through genetic perturbations — can distinguish between these alternatives. Following an empirical genetic approach, an increasing number of case studies may ultimately reveal which is the dominant mode of polyphenism evolution. A study of *Onthophagus taurus* dung beetles, in which males show a nutritionally cued polyphenism in the size of their head horns, revealed that Hedgehog (*Hh*) signaling has been co-opted and integrated with nutrition sensing to regulate the decision between two discontinuous morphs [58••]. Although *Hh* is typically a regulator of segment polarity, with additional roles in eye and wing development, functional (RNAi) knockdowns showed that *Hh* also regulates the development of horns, a more recent evolutionary novelty. Hedgehog signaling, which is upregulated in low-nutrition males, specifically flattens the threshold between the two typical (major and minor) morphs to a shallow cline of horn sizes (Figure 2). Because Hedgehog signaling promotes the minor morph, it acts in opposition to *doublesex* (*dsx*), which promotes horn growth in males [59]. It is possible that the two pathways directly interact, as suggested by downregulation of *Hh*, which has *dsx* binding sites, in *dsx* knockdowns in males [60]. Together, these findings suggest that a nutrition-dependent polyphenism is enforced on continuous plasticity by opposing developmental pathways. If so, future research might identify the precise molecular changes that have occurred that allow the co-option of these conserved pathways for the production of a threshold response.

In a case of convergent evolution with *Onthophagus* beetles, *dsx* was found to likewise regulate the caste polyphenism in the ant *Cardiocondyla obscurior* [61•]. In this species, male and female isoforms were regulated in polyphenic tissue (i.e. wings) within each sex to produce polyphenic male and females. This suggests that the pre-existing switch-like behavior of a core regulator may have itself provided the complex regulatory changes needed to effect alternative morphs. In this case, it would be possible that conserved genetic switches either lead or follow the production of discontinuous plasticity.

A recent study of *P. pacificus* also examined the relationship of continuous to discontinuous plasticity, specifically how developmental robustness enforces polyphenism over potentially continuous variation [62••]. The heat-shock protein DAF-21/Hsp90 was found to canalize the mouth polyphenism, as a knockout of the locus released hidden variation outside the normal ranges for the two morphs. Although *daf-21* mutations left the polyphenism intact, rigorously quantified morphologies revealed increased disparity within morphs and closer intermediates between them (Figure 2). This finding indicates that

Figure 2



Developmental buffering mechanisms for polyphenism. In *Onthophagus* beetles (above), Hedgehog (*Hh*) signaling (green) and *doublesex* (*dsx*) work in opposition to one another to repress and promote horn growth, respectively, depending on nutritional input. In well-fed males, *dsx* is necessary for activating horn production. In contrast, low-nutrition males show an increase in *Hh* signaling. Therefore, *Hh* signaling and *dsx* may together impose a threshold (polyphenic) response, possibly in part by direct regulation of *Hh* genes by *dsx*. In *Pristionchus pacificus* nematodes (below), Hsp90 suppresses phenotypic variation (beige) around the microbivorous (St) and omnivorous (Eu) morphs, producing the more discrete phenotypes (brown) observed under wild-type conditions. For both the beetles and nematodes, red bars represent the range of phenotypes naturally observed (i.e. when not subjected to genetic perturbations).

in a species generally observed to have a 'hard' polyphenism, canalizing mechanisms are still required to ensure its exact binary output. Buffering mechanisms allow the build-up of (cryptic) genetic variation that would otherwise be removed by selection in a typical environment, but they may be beneficial when expressed in novel environmental or developmental contexts [63]. The release of cryptic genetic variation has long been predicted to promote morphological evolution [64–66], and the potential role of such variation in the evolution of polyphenism has been shown empirically [67,68]. In *P. pacificus*, such variation was found hidden around the poles of an existing polyphenism, including the genetic variation that could allow divergence both between morphs within species and among morphologies across species.

Evolution of polyphenism downstream of switch mechanisms

Once a polyphenism and switch mechanism are in place, how do the alternative morphs they regulate evolve in response to alternative selection pressures? Theoretical models have predicted that relaxed selection can occur in conditionally expressed genes [69], which include those biased for morphs of a polyphenism. In such a case, polyphenism would not only be a product but a vehicle of molecular evolution. Several analyses of morph-specific constellations of genes have indicated molecular evolution of polyphenism genes downstream of a putative switch. In pea aphids (*A. pisum*), sexual females are present only briefly in the population, unlike the more common asexual females. As predicted, sexual females showed faster rates of evolution in morph-biased genes, a phenomenon attributed to genetic variability due to conditional expression [70]. Similarly, in several species of *Onthophagus* dung beetles, differentially expressed genes in alternative head-horn morphs often showed high divergence rates [71]. In an ant (*C. obscurior*) with four castes, morph-biased genes showed a correlation between the levels of their expression bias and rates of sequence evolution [72•]. All three of these cases suggest that polyphenism influences the rate of morph-biased gene evolution, which could potentially result in the divergence between morphs. Examining this potential effect at a macroevolutionary level, a study of some 90 species of diplogastrid nematodes (including the model *P. pacificus*) showed that the presence of polyphenism statistically correlated with accelerated evolutionary rates [73••]. Moreover, in one clade of *Pristionchus* nematodes, the mouth polyphenism apparently evolved to include as many as five highly disparate and rapidly diverging morphs [74•], implying that the mechanism has undergone a multiplication of switches. Adapting genetic knowledge from *P. pacificus* to this clade may allow an understanding of how polyphenism begets further morphological evolution, even the evolution of additional switches.

Perspective

As genetic details of polyphenism mechanisms are revealed, hypotheses of polyphenism evolution can be tested at the level of specific molecular sequence patterns and genetic regulatory change. Analyses across populations, specifically for polyphenism factors and their direct regulatory targets, will be the most direct route to capturing changes in sensor or switch mechanisms that reflect local adaptation in plastic responses. Additionally, functional research in model species must be grounded in more inclusive comparative contexts if they are to reconstruct how polyphenism switches arise and change. For example, are newly repurposed genes (e.g. *InR2*, *eud-1*) a prerequisite to the new regulatory logic required for polyphenism switches? Or are they later genetic accommodations of plasticity, conditionally expressed from conserved regulatory networks? What were the individual steps required for two ancient regulatory modules, such as nutrient signaling and developmental patterning, to meet in the novel way necessary to regulate polyphenism? Phylogenetic intermediates, beyond isolated models or two-species comparisons, are clearly needed to reconstruct these genetic histories. Fortunately, whole-genomic sequencing and functional tests across multiple species are increasingly feasible, and gene editing by CRISPR/Cas9 has already been applied to polyphenism genetics in non-traditional models [51,62••]. Understanding the evolution of polyphenism, and possibly through polyphenism [75], will surely follow from comparative analyses of its emerging genetic vocabulary.

Conflict of interest statement

Nothing declared.

Acknowledgement

This work was supported by a grant from the National Science Foundation (IOS-1557873).

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- • of special interest
- •• of outstanding interest

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