Multiple Plasticity Regulators Reveal Targets Specifying an Induced Predatory Form in Nematodes

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Abstract

The ability to translate a single genome into multiple phenotypes, or developmental plasticity, defines how phenotype derives from more than just genes. However, to study the evolutionary targets of plasticity and their evolutionary fates, we need to understand how genetic regulators of plasticity control downstream gene expression. Here, we have identified a transcriptional response specific to polyphenism (i.e., discrete plasticity) in the nematode *Pristionchus pacificus*. This species produces alternative resource-use morphs—microbivorous and predatory forms, differing in the form of their teeth, a morphological novelty—as influenced by resource availability. Transcriptional profiles common to multiple polyphenism-controlling genes in *P. pacificus* reveal a suite of environmentally sensitive loci, or ultimate target genes, that make up an induced developmental response. Additionally, in vitro assays show that one polyphenism regulator, the nuclear receptor NHR-40, physically binds to promoters with putative HNF4α (the nuclear receptor class including NHR-40) binding sites, suggesting this receptor may directly regulate genes that describe alternative morphs. Among differentially expressed genes were morph-limited genes, highlighting factors with putative "on–off" function in plasticity regulation. Further, predatory morph-biased genes included candidates—namely, all four *P. pacificus* homologs of *Hsp70*, which have HNF4α motifs—whose natural variation in expression matches phenotypic differences among *P. pacificus* wild isolates. In summary, our study links polyphenism regulatory loci to the transcription producing alternative forms of a morphological novelty. Consequently, our findings establish a platform for determining how specific regulators of morph-biased genes may influence selection on plastic phenotypes.

Key words: **developmental plasticity, differential expression, alternative splicing, switch mechanism, phenotypic variation, *Pristionchus pacificus*.**

Introduction

Developmental plasticity, or the ability to translate a single set of alleles into multiple phenotypes, exemplifies the nonlinear path from genotype to phenotype (West-Eberhard 2003; Pigliucci et al. 2006). This ability is perhaps most extreme in the case of polyphenism, or "developmental conversion," whereby plasticity is multimodal (Smith-Gill 1983; Pfennig et al. 2010; Yang and Pospisilik 2019). However, even if phenotypes are environmentally directed, plasticity itself is often heritable (Bradshaw 1965; Scheiner 1993; Pigliucci 2005). Because polyphenism in particular requires differences in structure or scaling, plastic phenotypes should follow a switch-like response to environmental cues (Nijhout and Wheeler 1982), predicting that polyphenism's genetic basis is the activation of alternative developmental networks (Abouheif and Wray 2002). Moreover, if the resulting phenotypes are each adaptive, these networks must be reliably deployed in response to the correct environmental cues (Moran 1992). Such a coordinated response should require genetic machinery that can receive these cues, integrate them into a switch, and produce the phenotype matching the inductive environment. Indeed, specific genes have been identified to control binary switches between alternative morphologies (Ragsdale et al. 2013; Xu et al. 2015), as have genes expressed under morph-specific induction cues (Brisson et al. 2010; Leichty et al. 2012; Daniels et al. 2014; Berens et al. 2015; Schrader et al. 2015). However, the question of how a molecular switch influences environmentally sensitive loci (sensu Via et al. 1995), specifically to match form to environment as a plastic response, is still largely unanswered.

One challenge to addressing this question is to disentangle genes regulated specifically through polyphenism from those otherwise expressed in tissue- or organism-wide physiological responses, given the wide-ranging effects that morph-inducing environments are expected to have on an individual (Forsman 2015; Corona et al. 2016). Furthermore, standing genetic variation among assayed individuals potentially obscures stereotypic responses expected from a single genotype (Nuzhdin et al. 2004; Whitehead and Crawford 2006). A solution to these challenges would be to study plastic development against standardized environmental and genetic backgrounds, as would be possible by manipulating a defined, genetic switch mechanism. Fortunately, genetic perturbations in laboratory models have identified specific transcription factors, particularly nuclear receptors (NRs), that act at the switch point of plastic developmental responses. For example,

in the nematode *Caenorhabditis elegans*, the DAF-12/Vitamin D receptor controls the shunt to a diapause (dauer) larva under nutritional stress (Antebi et al. 2000). Likewise, the receptor NHR-40 has been identified as a switch-like regulator of morphological polyphenism in the nematode *Pristionchus pacificus* (Kieninger et al. 2016). NRs, which possess both a ligand-binding domain (LBD) and a DNA-binding domain (DBD), directly interpret small-molecule signaling to genetic switches, making them obvious candidates for regulators of environmentally sensitive traits. Indeed, NRs are well known to control developmental polyphenism: for instance, the ecdysone (EcR)-ultraspiracle (USP/RXR) complex regulates seasonal polyphenisms in at least two orders of insects (Rountree and Nijhout 1995; Brakefield et al. 1998; Monteiro et al. 2015; Vellichirammal et al. 2017). Therefore, it is logical to predict that NRs play a general, perhaps essential, role in producing animal polyphenisms. Consequently, the functional study of an NR known to control morphological plasticity may make it possible to determine how a switch engages polyphenism-specific gene repertoires.

The nematode *P. pacificus* offers a system for studying tissue-specific developmental plasticity, specifically in mouthparts that allow the animals to use alternative food resources (Ragsdale 2015; Sommer et al. 2017). Resource polyphenisms are a taxonomically widespread phenomenon that in some cases—such as *P. pacificus*—include alternative forms of complex morphological traits (Smith and Skúlason 1996; West-Eberhard 2003). The polyphenism of *P. pacificus* and other nematodes in its family (Diplogastridae) usually involves a localized morphological phenotype (Fürst von Lieven and Sudhaus 2000), the individual cells of which have been defined (Baldwin et al. 1997; Bumbarger et al. 2013). In this species, animals develop into either of two adult morphs depending on their environmental experiences as larvae (fig. 1): an omnivorous and predatory ("eurystomatous," Eu) morph that has opposing, moveable teeth is more likely to develop under conditions of starvation than if offered abundant diet of microbes (Bento et al. 2010). Additionally, the polyphenism is influenced by local population density, analogous to other systems with inducible carnivores (Collins and Cheek 1983; Hoffman and Pfennig 1999): crowding results in higher concentrations of small-molecule pheromones, specifically ascarosides and derivatives, that induce development of the Eu morph (Bose et al. 2012; Werner et al. 2018). *Pristionchus* nematodes thus respond to competition for bacterial food, becoming able to feed on other nematode species likely to co-occur with them in the wild (Weller et al. 2010; Félix et al. 2018). In contrast, the alternative ("stenostomatous," St) morph, which has a narrow mouth with a single tooth, is a microbe-feeding specialist that is less fit than the Eu morph when starved but develops faster than it when abundant bacteria are available (Serobyan et al. 2014). These two developmental outcomes represent a fitness trade-off, particularly in structures that in large part define the animals' ecological function (Yeates et al. 1993; Wilecki et al. 2015). Moreover, predatory teeth are a novelty of Diplogastridae relative to close outgroups, which are strictly microbivorous

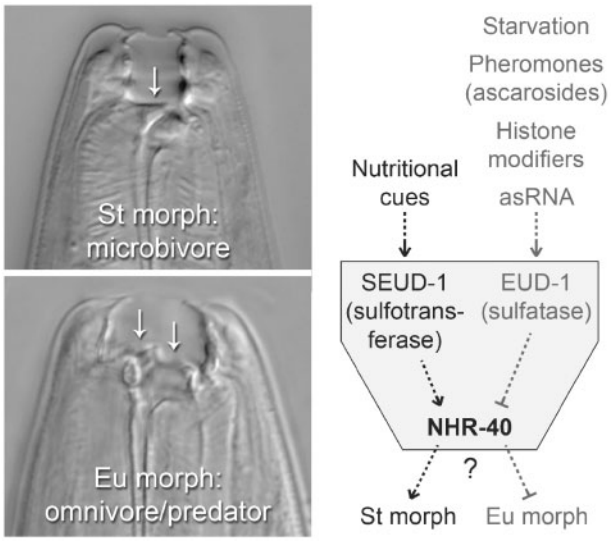

Fig. 1. Resource polyphenism and its regulation in *Pristionchus pacificus*. Alternative morphs develop as influenced by environmental cues: whereas the stenostomatous (St) is promoted by an abundance of food, the eurystomatous (Eu) morph, which can feed on both bacteria and other nematodes, is induced by cues signaling limited availability of bacterial food. Polyphenism in *P. pacificus* is defined by alternative morphological structures: most notably, the St morph has a single, narrow tooth (single arrow), whereas the Eu morph has two opposing teeth (two arrows) that can capture and puncture prey nematodes. The environmental, genetic, and epigenetic factors influencing the polyphenism act through a switch (gray box), whose known components converge on the nuclear receptor NHR-40.

(Kiontke and Fitch 2010; Susoy et al. 2015), implying that insights gained into their alternative development will also ultimately inform how and which genes build novel traits (Moczek et al. 2011).

Two features of *P. pacificus* as a laboratory model offer an entry point to linking a switch mechanism to defined target-gene repertoires of a morphological polyphenism. First, several components of a switch have been genetically characterized for the *P. pacificus* polyphenism (fig. 1). The switch comprises a pair of enzymes with dosage-dependent activity, the sulfatase EUD-1 and sulfotransferase SEUD-1, which alternatively promote the Eu and St phenotypes, respectively (Bui et al. 2018; Namdeo et al. 2018). These factors can completely toggle the switch, acting upstream of NHR-40, the most downstream known regulator of the polyphenism (Bui et al. 2018). Because the switch channels all known environmental sensing and epigenetic modifications that influence the polyphenism decision (Ragsdale et al. 2013; Serobyan et al. 2016; Sieriebriennikov et al. 2018), morph-biased transcription can in principle be distinguished from off-target environmental effects of morph-inducing cues. Second, the ability to genetically manipulate the switch allows comparisons that distinguish between polyphenism-specific transcription and the pleiotropy of individual switch components.

In this study, we have defined precise, polyphenism-specific genetic repertoires, specifically through the manipulation of multiple, completely penetrant plasticity regulators. Moreover, we have used in vitro assays to show that NHR-40

can directly bind to putative target genes, specifically those with predicted NR binding sites, in these repertoires. Among genes specific to a plastic response, we have identified factors offering new regulatory insight into its control, including some that differ correspondingly to naturally occurring phenotypic variation in the polyphenism. Together, our results link a developmental switch to expression differences producing morphological polyphenism, thereby illuminating a genetic mechanism for how plasticity is specified in ecologically dimorphic species.

Results and Discussion

Manipulation of a Switch Gene Reveals Stereotypical Morph-Constitutive Transcription

To identify the ultimate targets of the mouth-polyphenism switch of *P. pacificus*, we compared strains fully penetrant for either the St (microbivorous) or Eu (predatory) morphs, even in the absence of potentially confounding induction cues. Specifically, we sequenced the transcriptomes of mixed-stage nematodes—that is, including all juvenile stages prior the expression of the adult dimorphism—that 1) were fully defective for the switch gene *eud-1* or 2) constitutively overexpressed wild-type (PS312, "California") copies of *eud-1*. For the former strain, we used the null mutant *eud-1(tu445)*, which has a St-constitutive phenotype (Ragsdale et al. 2013). To generate an Eu-constitutive strain whose genetic background (i.e., to the *eud-1* gene) was similar to that of the St strain, *eud-1(tu445)*, we introgressed a fully integrated transgene (*iubIs16*), which contains multiple copies of *eud-1*^{PS312}, into the *eud-1(tu445)* strain above, creating the strain *eud-1(tu445) iubIs16*. This setup allowed us to compare strains differing in a gene with no observed mutant phenotypes other than that of polyphenism control (Ragsdale and Ivers 2016). Importantly, our approach also controlled for environmental influence by sequencing animals of predetermined developmental outcome under common laboratory conditions. The resulting transcriptomes showed highly stereotypical patterns of morph-specific expression across replicates (>97% within-morph correlation; $P < 10^{-10}$, Pearson; supplementary fig. S1, Supplementary Material online).

Multiple Plasticity Regulators Distinguish Polyphenism Targets from Pleiotropic Effects

Having identified morph-biased genes in a genetically and environmentally standardized background, we next sought to identify genes making up a polyphenism-specific response. To distinguish targets of the mouth-polyphenism switch, thereby accounting for unobserved pleiotropy of *eud-1*, we filtered the above morph-biased genes by transcripts enriched in other morph-constitutive lines. Specifically, we sequenced the transcriptomes of two other mutants, *seud-1(iub7)* and *nhr-40(tu505)*, which also have Eu-constitutive phenotypes (supplementary data 2 and 3, Supplementary Material online). Because *eud-1*, *seud-1*, and *nhr-40* show only partial overlap in the timing and location of their expression, gene regulation common to all three Eu-constitutive lines relative to the *eud-1* mutant line should

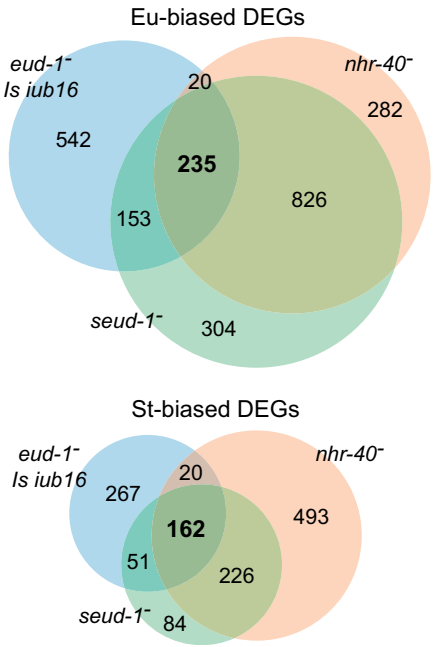

Fig. 2. Morph-biased genes differentially influenced by multiple polyphenism regulators in *Pristionchus pacificus*. Comparisons of three eurystomatous (Eu)-constitutive lines (*eud-1*$^{-}$ *Is iub16*, *nhr-40*$^{-}$, and *seud-1*$^{-}$) to a stenostomatous (St)-constitutive line (*eud-1*$^{-}$), reveal a restricted set of environmentally sensitive loci that are polyphenism-specific (boldface font), as distinct from the pleiotropic effects of one or two polyphenism switch genes. Counts are of genes with transcripts enriched 2-fold or more between morphs.

represent polyphenism-specific expression, as opposed to physiologically related or other off-target effects. Consequently, our comparison showed that less than one-third of genes with 2-fold enrichment between *eud-1*-manipulated lines were differentially expressed in all Eu-constitutive lines (fig. 2). Our approach thus recovered precise, limited suites of environmentally sensitive loci specifying alternative morphologies.

As predicted, the above suites of differentially expressed genes (DEGs) for alternative morphs included obvious candidates explaining biological differences related to resource polyphenism. For example, several DEGs encode products likely to function in lipid metabolism (supplementary tables S1 and S2, Supplementary Material online). Because we sequenced predetermined morphs taking a common diet, particularly in the absence of starvation or carnivory (Lightfoot et al. 2019), the regulation of such genes—where relevant to dietary metabolism—may reflect needs anticipated where alternative morphs naturally appear. Genes regulating longevity were also consistently morph-biased across comparisons (supplementary fig. S2, Supplementary Material online), reminiscent of the transcriptional effects of dauer induction in *C. elegans* (Jones et al. 2001; Golden and Melov 2007). This effect may be due to conservation of some genetic responses underlying dauer and polyphenism regulation in *P. pacificus* (Bento et al. 2010), although many DEGs acting downstream of dietary restriction signals were, surprisingly, St-biased rather than Eu-biased. Together, morph-biased expression

of fat-metabolism and longevity genes suggests that physiological differences are not just part of a global response to induction cues but are also controlled by the polyphenism switch itself. Additionally, morph-biased gene sets revealed several putative transcription factors in each morph-biased transcriptome, including several NRs (supplementary tables S1 and S2 and supplementary data 1–3, Supplementary Material online). Eu-biased genes included those with highest sequence similarity to *C. elegans nhr-34*, *nhr-38*, and *nhr-237*, which to our knowledge have only predicted functions. Among St-biased genes were those similar to *nhr-70*, *nhr-127*, and *nhr-246*, the former of which affects fat storage in *C. elegans* (Arda et al. 2010), and the latter of which has a dauer-enriched function in that species (Shih et al. 2019). Although the functions of NRs enriched in *P. pacificus* have yet to be determined, their morph bias suggests that the polyphenism switch directs a hierarchical network of environmentally sensitive loci, including downstream receptors. In summary, polyphenism-specific DEGs provide glimpses into the genetic networks alternatively activated by known plasticity regulators.

Morph-Limited Transcripts Reveal a Morphogenesis Regulator and Isoforms of a *doublesex* Homolog

Because we recovered transcriptomes from completely morph-constitutive lines, we could specifically distinguish morph-limited transcripts, or those whose expression is unique to a single morph and thus likely to have more trait-specific (i.e., less pleiotropic) function than genes that show only relative differences in expression. Few DEGs showed zero or near zero expression in all comparisons, although those found suggest an "on–off" function specialized for morph-specific developmental networks (supplementary table S3, Supplementary Material online). Although most of the genes thus defined were novel genes, one of the two St-limited genes we recovered (UMM-S139-8.52) was a homolog of *C. elegans phat-1*, which regulates pharyngeal gland morphogenesis in that species (Smit et al. 2008). The pharyngeal gland is an organ more prominent in diplogastrid nematodes than in strictly microbivorous outgroups (Zhang and Baldwin 1999; Riebesell and Sommer 2017), and its disproportionately higher neural connectivity in *P. pacificus* suggests its importance for predatory feeding (Bumbarger et al. 2013). Furthermore, the primary (dorsal) pharyngeal gland empties through the dorsal tooth and its supporting cells, the structures that in large part define the diplogastrid mouth polyphenism (Fürst von Lieven and Sudhaus 2000). Thus, morph-limited transcripts have revealed a candidate we hypothesize to effect morph-specific differences in polyphenic feeding morphology.

We also investigated whether morph-limited transcripts also included alternative splice forms. Extensive alternative splicing is known for vertebrates and insects in response to environmental stresses (Long et al. 2013; Jakšić and Schlötterer 2016), as well as between polyphenic morphs in insect systems (Grantham and Brisson 2018; Price et al. 2018). Between the two *eud-1*-manipulated lines, we identified 257 differentially spliced genes (DSGs), which showed a strong correlation among replicates per morph (supplementary fig. S3 and supplementary data 4, Supplementary Material online). Among these DSGs, the single gene detected to have morph-limited isoforms was the *P. pacificus* ortholog of the doublesex/MAB-3 domain gene *dmd-6* (UMA-S212-3.56), whose *C. elegans* homolog has no previously reported function. Specifically, the two isoforms differed in the presence of an exon (Exon 4 of the long isoform) immediately downstream of the region encoding the DNA binding-domain (fig. 3*A*). We validated this pattern by PCR, confirming expression of the short isoform in *eud-1* mutants and the long isoform in all Eu-constitutive lines (fig. 3*B*). Further, we found that the skipped exon of the short isoform causes a frameshift relative to the open reading frame of the larger exon, such that only the DBD is kept in both isoforms (supplementary fig. S4, Supplementary Material online). If thus translated, the short form might reverse otherwise normal transcriptional regulation by occupying target sites without interacting with its coregulators. This finding suggests that polyphenism in *P. pacificus* may, in addition to known switch machinery, involve the isoform-specific logic of *doublesex* in insect (*Drosophila*) sex differentiation (Burtis and Baker 1989). If indeed a part of polyphenism regulation, this feature would comprise a remarkable convergence with polyphenism regulation in some insects, as has evolved independently in beetles (Kijimoto et al. 2012) and ants (Klein et al. 2016). As highlighted by this example, our results provide a proof of principle that candidate genes or isoforms with putative on–off polyphenism function can be identified, offering potentially new, functionally testable logic for plasticity regulation.

NHR-40 Binds Predicted Target Promoters

NHR-40 is a transcription factor at the known end of the polyphenism switch, showing epistasis over other identified plasticity regulators (Kieninger et al. 2016; Bui et al. 2018). Therefore, morph-biased transcriptomes are likely to include direct targets of the receptor itself. As expected, DEGs included genes with predicted motifs for HNF4α (xGxxCAAAGTYCA; fig. 4*A* and supplementary data 5, Supplementary Material online), the class of NRs including NHR-40 and for which binding has been validated for human (Fang et al. 2012). To determine whether *P. pacificus* (*Ppa*) NHR-40 would be capable of binding motifs predicted from human genes, we confirmed that *Ppa*-NHR-40 has a sequence homologous with, although not identical to, the DNA-binding P-box of human HNF4α (fig. 4*B*). In humans, this feature interacts with the receptor's target motifs (Fang et al. 2012), suggesting similar DNA-binding activity by *Ppa*-NHR-40. To test the hypothesis that NHR-40 binds differentially regulated genes possessing HNF4α motifs, we then performed in vitro screening for its interaction with putative promoter sequences of an Eu-biased gene. The gene we selected was *eud-1*, which was previously found to be down-regulated in *eud-1* mutant alleles (i.e., autoregulated) when compared with the wild-type (Ragsdale et al. 2013) and contains the predicted NHR-40 binding motif in its promoter (1,458 bp upstream of the transcription start site). Using a yeast one-hybrid reporting system, we found that the entire

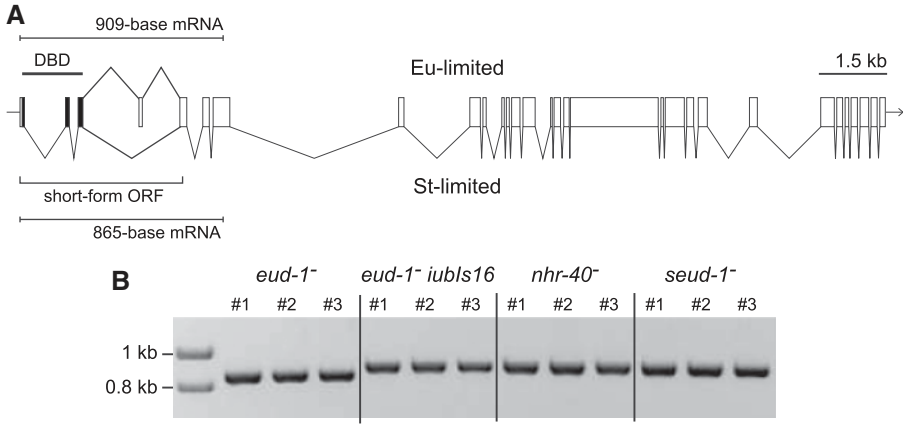

FIG. 3. Morph-limited isoforms of the transcription factor gene *dmd-6* in *Pristionchus pacificus*. (*A*) In *Ppa-dmd-6*, a *doublesex* homolog, an isoform limited to the St morph skips an exon flanking the DNA-binding domain (DBD) and which causes a shift in the short isoform's open reading frame (ORF) downstream of that domain. mRNA lengths shown for cDNA sequences amplified by PCR using a single primer-pair. (*B*) Gel electrophoresis of *Ppa-dmd-6* isoforms in St-constitutive (*eud-1*$^{-}$) and Eu-constitutive (*eud-1*$^{-}$ *iuIs16*, *nhr-40*$^{-}$, *seud-1*$^{-}$) strains.

Ppa-NHR-40 protein, which is encoded by the gene's long isoform (*Ppa-nhr-40a*; Kieninger et al. 2016), bound to the *eud-1* promoter, in contrast to a protein only including the LBD, which reported no interaction (fig. 4C and supplementary fig. S5, Supplementary Material online; table 1 and supplementary tables S5 and S6, Supplementary Material online). Likewise, a fragment expressing only the DBD, encoded by a naturally occurring short isoform (*Ppa-nhr-40b*), bound to the *eud-1* promoter. In short, these results report a positive interaction between NHR-40 and a putative target. Our results also show that the short *Ppa-nhr-40* isoform, which inhibits the Eu-promoting function of the full-length *Ppa-nhr-40* isoform (Kieninger et al. 2016), binds the same targets as the latter isoform. The NR's DBD (i.e., *Ppa*-NHR-40b) thus interacts with targets even in the absence of a LBD, suggesting that NHR-40's regulatory activity is gated not through DNA binding itself but protein–protein interactions, as otherwise common for NRs (Ludewig et al. 2004; Asahina et al. 2006; Fuxman Bass et al. 2016). Because the short protein contains no domain that could receive a ligand or interact with potential coregulators, the two isoforms may potentially compete for direct targets to steer the polyphenism decision.

Because *Ppa*-NHR-40 showed no binding to a random DNA sequence (empty yeast vector), we also wanted to know whether the promoter of another gene possessing an HNF4α motif might also confer binding ability. To test this idea, we cloned a 2-kb promoter for a gene likewise containing a predicted HNF4α motif but from another species, *C. elegans*. The gene we selected was *C. elegans* (*Cel*) *sul-2*, the *C. elegans* ortholog of *eud-1*. In our assay, we found that *Ppa*-NHR-40 (both NHR-40a and NHR-40b) indeed binds the promoter of *Cel-sul-2* (table 1). Although several binding sites are likely to be present in *Ppa-eud-1* and in *Cel-sul-2*, the interaction of NHR-40 with both promoters suggests that the predicted HNF4α motif likely confers this binding ability. Additionally, because our assays showed binding of *eud-1* in particular, our results suggest that *eud-1* could be part of an autoregulatory feedback loop in polyphenism regulation, a possibility to be investigated through in vivo assays. In summary, results from our in vitro tests show that NHR-40 physically interacts with predicted NR target sequences, which in principle include those regulated by NHR-40 itself.

NHR-40 DNA-Binding Activity In Vitro Is Conserved with a Nonpolyphenic Outgroup Species

Having screened potential targets using *P. pacificus* NHR-40, we next asked whether the receptor's activity, as assayed in vitro, is conserved with nematodes that never had mouth polyphenism in their phylogenetic history. The NHR-40 P-box domain is identical in *C. elegans* and *P. pacificus* (fig. 3B), whose most recent common ancestor predated the origin of the diplogastrid mouth polyphenism (Susoy et al. 2015). Therefore, we hypothesized that NHR-40 in the two species can bind the same sequences and that its cooption into polyphenism regulation occurred through other changes, such as to the receptor's other domains, regulatory elements, targets, or ligand. When we screened for protein–DNA interactions, we found that *Cel*-NHR-40 binds to the *eud-1* promoter sequence (table 1). Given this interaction, we also tested whether *Cel*-NHR-40 could bind an endogenous promoter, specifically the same clone of the *Cel-sul-2* promoter we used in our *Ppa*-NHR-40 assays. Our test reported an interaction, showing that NHR-40 binding is similar between *C. elegans* and *P. pacificus* when assayed in vitro. Although it is possible that in vivo tests using the genes' endogenous environments may show activity differences between the two species, our results suggest that other aspects of NHR-40 are more likely to distinguish polyphenism-specific function of NHR-40 in *P. pacificus*.

Natural Variation in Morph-Biased Gene Expression Reflects Phenotypic Variation

Finally, having identified ultimate targets of the *P. pacificus* mouth polyphenism, we asked whether particular candidates could improve our understanding of how polyphenism is executed and possibly evolves in this system. We chose to focus on a notable example: we examined all four *P. pacificus* homologs of *C. elegans* heat-shock gene *Hsp70*/*hsp-70*, which

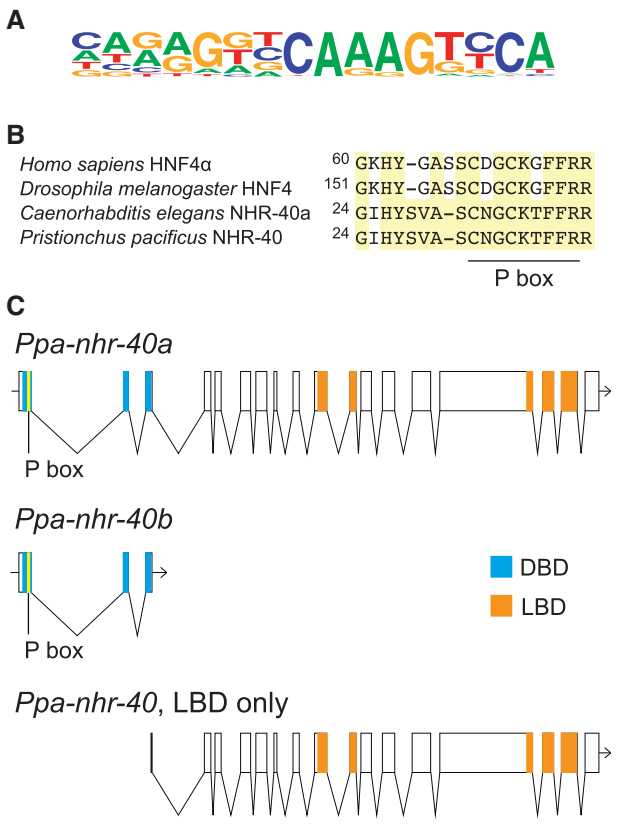

FIG. 4. Predicted binding sites and protein structure of *Pristionchus pacificus* NHR-40. (*A*) Consensus sequence for genome-wide, predicted HNF4α binding motifs in *P. pacificus*. (*B*) Comparison of the conserved, putative P-box of nematode NHR-40 and other metazoan nuclear-receptor homologs. Blue boxes, residues conserved across all taxa. (*C*) Protein forms used for in vitro assays of *P. pacificus* (*Ppa*) NHR-40 binding function. Constructs encoding the long isoform (*Ppa-nhr-40a*) and only the DNA-binding domain (DBD) of the protein (*Ppa-nhr-40b*) include sequences of naturally occurring splice variants. A third, artificial construct included both fragments of the split ligand-binding domain but not the DBD. Yellow, P-box coding sequence.

Table 1. Results of Yeast One-Hybrid Assays for Binding of *Pristionchus pacificus* (*Ppa*) and *Caenorhabditis elegans* (*Cel*) NHR-40 Proteins to Putative Promoter Elements with Predicted Target Motifs.

	Ppa-eud-1	*Cel-sul-2*	Empty Vector
***Ppa*-NHR-40a (full length)**	+	+	−
***Ppa*-NHR-40b (DBD only)**	+	+	−
***Ppa*-NHR-40, LBD only**	−	−	−
Empty vector	−	−	−
***Cel*-NHR-40a**	+	+	−

NOTE.—Target sequences were cloned from *P. pacificus* and *C. elegans* (*Cel*). DBD, DNA-binding domain; LBD, ligand-binding domain (DBD); +, interaction reported; −, no interaction reported.

were consistently highly enriched in all Eu-constitutive lines (table 2 and supplementary fig. S6, Supplementary Material online). The differential expression of these genes suggests that the polyphenism may trigger a stress-like response from a genetic cascade, similar to the enrichment of other heat-shock proteins in FoxO-signaling mutants in *C. elegans* (Murphy et al. 2003; Golden and Melov 2004). All four of these genes possessed HNF4α binding motifs (table 2), suggesting that they may be directly regulated by an HNF4α-class NR such as NHR-40. In contrast, two other *Hsp70*-family (i.e., heat-shock protein family A) genes, which lack HNF4α binding motifs either 2-kb upstream or downstream of their transcription start sites, were significantly or nearly significantly enriched among St-biased genes in all comparisons (table 2). The differential expression of *Hsp70* homologs is illuminating, given that another heat-shock protein, Hsp90/DAF-21, is known to canalize morphological differences in the *P. pacificus* mouth polyphenism: when the *Hsp90* gene is disrupted, less faithful morphological outputs result, including apparent intermediates between morphs (Sieriebriennikov et al. 2017). Because Hsp70 directly interacts with Hsp90 for the latter's chaperone function (Kravats et al. 2018), it is possible that differential expression of Hsp70 ensures, through its activity with Hsp90 (not Eu-enriched), identities of alternative morphs as the result of a switch. Together, differences in *Hsp70* expression suggest a potential mechanism for how the polyphenism decision is delivered to phenotypic outputs, making it an exemplar for studying how polyphenism-controlled expression varies among strains.

Given the morph bias of *Hsp70* homologs, we hypothesized that strain-specific variation in the switch should be followed by differential expression in polyphenism target genes. To test this idea, we asked whether wild haplotypes of *P. pacificus* that differ in their polyphenism ratios under standardized environmental conditions have incurred differences in Hsp70 expression. Specifically, we compared the expression of Eu-biased *Hsp70* genes in the Eu-biased "California" (PS312) strain, which was used for the genetic manipulations herein, to that in two strains (RS5200B, RSB020) known to be highly St-biased in laboratory culture (Ragsdale et al. 2013) and representing distinct populations of *P. pacificus* (Morgan et al. 2012). As predicted, expression was lower in the St-biased strains than in the Eu-biased strain (fig. 5). Differential expression of candidate genes, specifically *Hsp70* homologs, therefore, mirrors differences in the polyphenism ratio across strains, lending further support for their direct involvement in executing polyphenism.

Targets of a Plasticity Switch Reveal a Genetic Model for Resource Polyphenism

By linking a known plasticity regulator to downstream transcription, our study offers a mechanism for how polyphenism proceeds from a genetic switch to the production of alternative morphologies. This mechanism provides a first genetic model for how a definitive "switch point," predicted from numerous examples of polyphenism in nature (West-Eberhard 2003), produces differences between alternative, complex morphological traits. Furthermore, we have illuminated a mechanism for polyphenism between resource-use morphs in particular. Alternative resource use increases the dimensionality of a species' niche, allowing it to exploit ecological opportunity, and consequently resource polyphenisms

Table 2. Morph-Biased Expression, Gene Locations, and HNF4α Binding Motif Locations of *Pristionchus pacificus Hsp70* and Hsp70-Family Homologs.

Homolog	Gene ID	Chr	HNF4α Motif Positions	Log_2FC, *Is iub17*	Log_2FC, *nhr-40*$^-$	Log_2FC, *seud-1*$^-$
Hsp70	UMM-S7-10.26	I	−1,624, −1,505, −1,498, +1,027	6.24	6.51	5.58
	UMM-S233-4.36	IV	−1,926, −1,919, −679, +1,038	5.65	5.94	5.02
	UMM-S233-10.49	IV	−1,912	5.54	5.90	5.00
	UMS-S233-10.85	IV	+1,051	5.65	5.89	5.00
Hsp70-family	UMM-S196-15.33	I	N/A	−1.67	−1.09	−0.88
	UMM-S196-15.41	I	N/A	−1.64	−0.89	−0.55*

NOTE.—Gene identities (ID) from "El Paco" genome annotation (Rödelsperger et al. 2017). Differential expression shown as log fold-change (log_2FC) in the Eu morph in three constitutive lines (*Is iub17*, *nhr-40*$^-$, and *seud-1*$^-$) relative to the St morph (*eud-1*$^-$). Motif positions are nucleotides upstream (−) or downstream (+) of transcription start site. Chr, chromosome of locus.
*$P_{adj} > 0.01$.

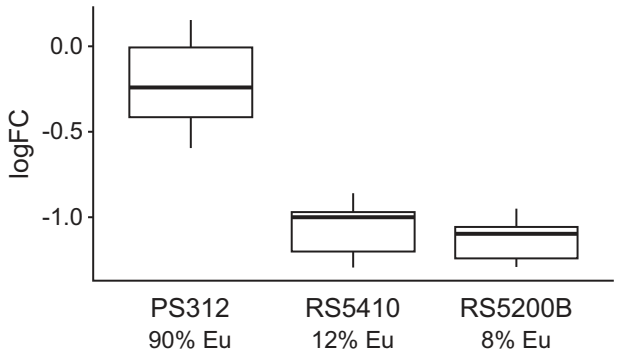

FIG. 5. Comparison of *Hsp70* expression in natural polyphenism variants of *Pristionchus pacificus*. Expression was quantified for wild isolates with different morph-ratios under a common induction environment. Two strains (RS5200B, RS5210), representing different *P. pacificus* populations (Morgan et al. 2012), show a bias toward the stenostomatous (St) morph in laboratory culture (Ragsdale et al. 2013). Collective expression of morph-biased *Hsp70* genes in St-biased strains was significantly lower ($\chi^2 = 23.6$, df = 1, $P < 0.001$) than in the reference strain (PS312), which is biased toward the eurystomatous (Eu) morph under the same laboratory conditions. Mean expression levels of *Hsp70* were calculated as log fold-change (LogFC) normalized to two reference genes (*tbb-4* and *y45F10d*). Center line, median; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range. Mouth-ratio phenotypes (% Eu) of strains shown below strain names.

have been attributed general importance in species diversification (Maynard Smith 1966; West-Eberhard 1989; Smith and Skúlason 1996; Pfennig et al. 2010). Given that resource-use plasticity can lead to genetic differences distinguishing populations in local inductive environments (Parsons et al. 2016), the genetic machinery of its control is an obvious target for its selection, whether to change its sensitivity to induction cues (i.e., plasticity) or to assimilate a single, more locally adaptive form (Ehrenreich and Pfennig 2016). Here, we have revealed how a polyphenism control mechanism creates morph-specific transcriptional profiles, ultimately resulting in alternative forms with different ecological roles. Moreover, we demonstrate that variation in these target genes reflects phenotypic variation in how resource polyphenism responds to a given set of environmental cues.

Our results support a regulatory model whereby the Eu (predatory) morph is inhibited through the activity of NHR-40, whose DBD constitutively binds putative target genes (fig. 6). Although it is not yet known whether *Ppa*-NHR-40 primarily activates or represses its direct targets, functional and phenotypic assays of its natural, short isoform (*Ppa*-NHR-40b) indicate that its activity is gated through its interaction with a third factor. Specifically, all *Ppa-nhr-40* mutants generated thus far have nonsynonymous mutations in the sequence encoding the LBD, and these mutants show a phenotype similar to lines overexpressing *Ppa-nhr-40b* (Kieninger et al. 2016), which we show here to bind putative targets. By the model we propose, the St morph would be continuously promoted, or the Eu morph continuously suppressed, in the absence of induction cues such as starvation and high population density. When these induction cues are strong enough, NHR-40 would then be deactivated, converting between genetic networks to produce the Eu morph. We speculate that suppression of the Eu morph may be achieved by the constitutive release of a hypothetical small-molecule signal—for example, a metabolic product signaling satiety (Chawla et al. 2001)—under well-fed conditions but then possibly reduced under an inductive environment. Although the conditions under which the short isoform *Ppa-nhr-40b* is promoted are still unknown, its repression of *Ppa-nhr-40a* makes it a potential, additional component of the switch mechanism in nature. Finally, the sensitivity of the switch mechanism involves the upregulation of EUD-1 (sulfatase) and downregulation of SEUD-1 (sulfotransferase) under different environments (Werner et al. 2017; Bui et al. 2018). Because these two enzymes could alternatively modify the availability or transport of some signal molecule, they may together indirectly regulate NHR-40 activity. Given that cues of population density and nutritional status are both communicated through sensory neurons (Albert et al. 1981; Meisel et al. 2014; Berendzen et al. 2016), it is possible that the integration of multiple, upstream inductive cues occurs among the cells encompassing the *P. pacificus* polyphenism switch (Bui et al. 2018). Indeed, other factors promoting the St morph upstream of EUD-1 (which promotes the Eu morph), the α-N-acetylglucosaminidases NAG-1 and NAG-2, are expressed in nonoverlapping head and pharyngeal neurons, possibly modifying the same molecules as EUD-1 as part of a signaling mechanism to the switch (Sieriebriennikov et al. 2018). While offering several new predictions to be tested, our results have established an explanatory framework for

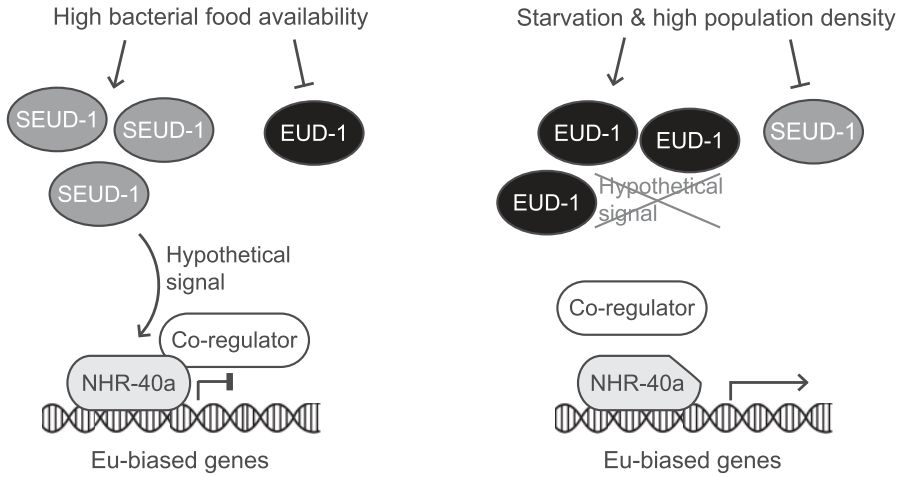

Fig. 6. A genetic mechanism for specifying alternative, resource-use morphologies in *Pristionchus pacificus*. Environmental cues inducing morphs regulate enzymes (EUD-1 and SEUD-1) with opposing function and whose relative expression determines the outcome of the mouth-polyphenism switch in *P. pacificus*. Direct regulation NHR-40a is possible in principle through either repression of eurystomatous (Eu)-biased genes (shown) or activation of stenostomatous (St)-biased genes (not shown). Following assays of NHR-40 activity and downstream transcription, this model provides a putative genetic explanation for how environmental cues are translated into the molecular differences characterizing alternative forms of a morphological trait.

how a switch between alternative resource-morphs is achieved.

Our findings also suggest that multiple NRs may coordinate the alternative, tissue-specific developmental networks specifying these morphs. In nematodes, NRs have undergone a massive radiation, and many of those described control metabolic networks or developmental processes (Gissendanner et al. 2004; Antebi 2015). Moreover, networks involving NRs can be complex, and it is known that metabolic gene regulation in *C. elegans* involves some modules that are replete with NRs (Arda et al. 2010). Here, morph-biased transcriptomes indicate that morphological polyphenism may also rely on signaling through several NRs, both within and downstream of a fully penetrant switch mechanism. Ideally, in vivo experiments could pull out organism-wide targets for NHR-40 and putative downstream receptors, as have been achieved for NRs in *C. elegans* (Shostak et al. 2004; MacNeil et al. 2015), thereby testing the functional importance of *P. pacificus* NRs to individual candidate genes (e.g., *eud-1*, *Hsp70* homologs). By pointing to specific genetic interactions, our study makes it possible to interrogate their roles in building different forms of a novel, complex trait.

Developmental plasticity has been argued to be a precursor to evolutionary innovation, and a wealth of examples attests the strong coincidence between plasticity and novel forms (West-Eberhard 2003; Moczek et al. 2011; Rajakumar et al. 2012; Susoy et al. 2016). If alternative forms first appear when induced under a new environment, that they can be assimilated implies that polyphenism can be selected from standing variation in its incipient regulatory genes or targets from the outset (Waddington 1953; Suzuki and Nijhout 2006). The molecular machinery for alternative morph induction may then hold a genetic explanation for how variation in plasticity impacts the accumulation of variation in plastic traits themselves (Beldade et al. 2011; Projecto-Garcia et al. 2017). For instance, genes or isoforms that are morph-limited may in particular be subject to selection whose coefficient depends on the frequency of a morph's appearance—that is, as controlled by a switch—in a population. Namely, the frequency of exposure of such genes (or isoforms) is predicted to determine the rate at which genetic variation in those genes is culled or amassed, thereby influencing the speed of evolution in their corresponding traits (Lande 2009; Van Dyken and Wade 2010). Indeed, molecular signatures of selection in environmentally induced transcriptomes indicate the potential of morph-biased genes to experience rapid evolution (Purandare et al. 2014; Pespeni et al. 2017; Schrader et al. 2017). How a molecular switch controls the environmental activation of alternative gene networks, particularly those building alternative morphologies, is necessarily fundamental to the fate of variation of polyphenic traits. In principle, signatures of molecular change in both the switch and its targets should reflect divergence in the plastic phenotypes themselves.

Materials and Methods

Nematode Strains and Crosses

Four *P. pacificus* strains were compared in our analyses. Two of these strains manipulated alleles of *eud-1*: 1) the St-constitutive line *eud-1(tu445)* and 2) the Eu-constitutive line *eud-1(tu445) iuIs16*. The former line was used to force the expression of St genes with a null mutant allele, which confers a fully penetrant, St-constitutive phenotype, and this strain had been previously backcrossed to the reference strain (PS312) four times (Ragsdale et al. 2013). The latter line has an integrated transgene, which was inserted onto a chromosome randomly in laboratory culture, that is, without invoking an insertion induction method (Fire 1986). Prior to its integration, the transgene was expressed as a complex extrachromosomal array, Ex[*eud-1*^{PS312}], that carried many copies of wild-type (PS312) *eud-1* and which conferred a fully penetrant, Eu-

constitutive phenotype (Ragsdale et al. 2013). To generate the *eud-1(tu445) iubIs16* line, the inserted transgene (*iubIs16*) was introgressed into the *eud-1(tu445)* line above from a strain with a *eud-1(tu442)* mutant background. For the introgression, outcrossed lines were selected for the presence of a reporter gene, *egl-20*^{promoter}*:: TurboRFP*, included in the insertion and which is expressed in the nematodes' tails throughout their lifetime. The introgression was performed through five rounds of outcrossing to *eud-1(tu445)*. Replacement of the background *eud-1(tu442)* allele with the *tu445* allele was confirmed by PCR with mutation-detection primers as previously described (Bui et al. 2018). Crosses were performed using five males and two hermaphrodite nematodes placed on nematode growth media plates seeded with bacterial lawns grown from 50 µl *Escherichia coli* OP50. The Eu-constitutive phenotype of the resulting line was confirmed according to protocols described previously (Serobyan et al. 2013). The transcriptomes of two other Eu-constitutive lines were also sequenced: 3) an *nhr-40* mutant, *nhr-40(tu505)*, which was isolated from a screen for suppressors of *eud-1*, outcrossed for two generations to *eud-1(tu445)*, and then additionally outcrossed to PS312 to be wild-type at the *eud-1* locus (Kieninger et al. 2016); (iv) a *seud-1* null mutant, *seud-1(iub7); eud-1(tu445)*, which was isolated from a similar suppressor screen and backcrossed to the *eud-1(tu445)* line four times (Bui et al. 2018). All strains were maintained on nematode growth media plates with lawns grown from 300 µl OP50 and kept at ~23 °C.

RNA Extraction, Sequencing, and Read Processing

RNA was extracted from pooled, mixed-staged samples for all nematode lines, as described in detail in the supplementary methods, Supplementary Material online. RNA sequencing, and the subsequent trimming and alignment of sequenced reads, was performed as described in the supplementary methods, Supplementary Material online.

Differential Expression Analysis

Read counts for aligned sequences were determined using the *FeatureCounts* function from the R Bioconductor package Rsubread v. 1.24.2 (Liao et al. 2013). Differential expression analysis was performed in edgeR (Robinson et al. 2010), with *P* values adjusted according to the Benjamini–Hochberg procedure to restrict false discovery rate (FDR). Expressed genes from the FeatureCounts and edgeR output were filtered with an adjusted *P* value (P_{adj}) of 0.01 to limit false positives. For downstream analyses, morph-biased gene sets were conservatively defined to include genes whose transcripts were enriched at least 2-fold between the *eud-1(tu445)* (St-constitutive) and *eud-1(tu445) iubIs16* (Eu-constitutive) samples. This filter on the transcriptome yielded 501 St-enriched and 950 Eu-enriched genes. This gene set was further reduced to represent polyphenism-specific transcription, specifically by excluding expression influenced by *eud-1* but not by other known polyphenism regulators. For this filter, we compared expression of *nhr-40* and *seud-1* mutants each to that of the *eud-1* mutant, defining only those genes relatively up- or downregulated 2-fold in all three Eu lines relative to the St line as polyphenism-specific. To maintain consistency between differential expression and differential splicing analyses, the same BAM files produced after STAR mapping by the rMATS pipeline (see below) were used for differential expression analysis. To define morph-limited genes, we used absolute read counts, as opposed to normalized read counts (e.g., reads per kilobase million sequenced), so as not to exclude shorter genes from discovery. An arbitrary cutoff for inclusion was a mean < 5 reads per replicate for one morph. Orphan genes among morph-limited gene sets were defined as having a BLAST *e*-value of ≤ 0.001 when compared with *C. elegans* genes.

Genetic Pathway and Gene Ontology Analysis

To hone our search for genes and gene categories that were differentially enriched between morphs, we performed Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analysis and Gene Ontology (GO) analysis. KEGG pathway analysis was performed using the generally applicable gene-set enrichment (GAGE) method, which tests for coordinated differential expression of genes in a common pathway, as implemented in R Bioconductor (Luo et al. 2009). KEGG terms were retrieved by performing BLASTp of *P. pacificus* protein sequences against the BlastKOALA (https://www.kegg.jp/blastkoala/) "family_eukaryotes" database. Enriched-pathway analyses were then performed using GAGE default parameters. Significantly enriched pathways ($P_{adj} < 0.01$), as established after a unified FDR correction, were plotted using *plot_pathway* function. Analyses were performed for each of our three transcriptomic comparisons, with features common to all three analyses annotated on a single pathway map (supplementary fig. S2, Supplementary Material online). For GO analysis, the *P. pacificus* reference strain (PS312) GO file (version 270651, updated on February 13, 2019) was retrieved from the European Bioinformatics Institute (https://www.ebi.ac.uk/GOA/downloads). Due to differences in gene annotation system between the more recent "El Paco" genome assembly (Rödelsperger et al. 2017) used herein and the deposited GO file, we converted gene annotations by performing BLASTp between a FASTA including all "El Paco" protein predictions and a UniProt protein FASTA file (updated on October 26, 2018), with an *e*-value cutoff of $\leq 10^{-3}$. GO-term enrichment analysis was performed on genes with 2-fold expression differences in all three Eu-to-St comparisons described earlier. The topGO package (Alexa and Rahnenfuhrer 2013) was used to perform Fisher's exact test calculations to identify overrepresented GO terms within morph-biased gene sets. Of 21,931 *P. pacificus* genes, 12,754 were annotated with GO terms. GO terms with $P < 0.05$ were considered significantly enriched.

Alternative Splicing Analysis

We used the aligned sequences for *eud-1(tu445)* and *eud-1(tu445) Is iub16* to search for alternative splicing events by the "Paired" model in rMATS, using the command-line parameters: *-t single -len 78 -c 0.0001 -analysis -P -novelSS 1*. rMATS was used to calculate the proportion of long and short isoforms at each splice site, providing a differential

percent spliced-in (PSI) statistic as output. The change in PSI between conditions (ΔPSI) was used to determine alternative splicing events specific for each morph, and we used a cutoff value of ΔPSI = 10% to define alternative splicing between morphs (Wang et al. 2008). Statistically significant splicing events were filtered from raw results using a FDR-adjusted *P* value (*q* value) of 0.05 and average read counts per junction of 10 using the *maser* package in R (Veiga 2018).

Motif Discovery

The HOMER (Hypergeometric Optimization of Motif EnRichment) software package (Heinz et al. 2010) was used for motif discovery among potential regulatory sequences. BEDTools (Quinlan and Hall 2010) was used to extract sequences 2-kb upstream and downstream of transcription start sites according to the *P. pacificus* "El Paco" genome assembly. Genome-wide discovery of putative HNF4α motifs was performed using the HOMER script findmotifs.pl under default parameters, allowing a maximum of two mismatches in motifs. To recover the motif locations for HNF4α, the HOMER script annotatePeaks.pl was run under the "tss" mode for a window of 2-kb upstream and downstream of the start of the first exon, using the HNF4α motif file output from the findmotifs.pl script.

Yeast One-Hybrid Screening

Yeast one-hybrid (Y1H) screening was performed using the yeast strain YM4271 according to an established protocol (Reece-Hoyes and Walhout 2012). The cloning vectors pMW#2 (Addgene #13349) and pMW#3 (Addgene #13350) were used as bait vectors, and pPC86 (a gift from the Walhout lab) was used as the prey vector. For assays, *nhr-40* sequences were cloned from a full-length sequence of *Ppa-nhr-40* as previously confirmed by 5′ and 3′ RACE-PCR (Kieninger et al. 2016). Clones included: 1) the entire *Ppa-nhr-40a* sequence, which includes regions coding both the DBD and LBD; 2) the sequence of the natural isoform *Ppa-nhr-40b*, which encodes only the DBD; and 3) a *Ppa-nhr-40* fragment encoding only the LBD, including a loop sequence therein (supplementary table S4, Supplementary Material online). The DBD was predicted based on homology to *C. elegans* NHR-40 and other *C. elegans* HNF4α-class NRs (Brožová et al. 2006). LBD boundaries were estimated as searched against the NCBI Conserved Domain Database (Marchler-Bauer et al. 2017). Target promoter sequences of an Eu-biased gene, *Ppa-eud-1*, and *Cel-sul-2* were cloned using primers listed in supplementary table S5, Supplementary Material online. Cloning of each promoter sequence into its bait vector and of each *nhr-40* fragment into its prey vector was performed by restriction enzyme digestion. Specifically, restriction-enzyme sites were added to both ends of each promoter sequence by PCR, and restriction enzymes (SpeI and SacII for pMW#2; SpeI and XhoI for pMW#3; MluI and NotI for pPC86) were used to generate compatible ends for both PCR products and vectors. The ligation reaction was done using T4 DNA ligase (New England Biolabs) at 16 °C overnight in a thermocycler, and the ligation product was transformed into *E. coli* competent cells. Yeast transformation was performed with both bait vectors simultaneously, and an autoregulation test was used to select the transformants with the least basal expression levels of reporter genes (*URA*, *HIS*, *LACZ*), with 3-aminotriazole (3-AT, Sigma) being used to control the basal level expression of the *HIS* gene. After confirming the presence of promoter sequences by PCR and Sanger sequencing, pPC86 vectors containing *nhr-40* coding sequences were each introduced into the bait clones. We screened for interactions between prey (NHR-40) and bait (promoter sequences) by detecting growth of double-transformed yeast on selective media (-Ura -His -Trp +3AT) as well as by a colony lift β-galactosidase assay. Yeast colony PCR was performed on yeast lysates as previously described (Reece-Hoyes and Walhout 2012).

Validation of Morph-Limited Expression of *dmd-6*

To confirm alternative splicing of *dmd-6* as identified by rMATS, we used reverse transcription PCR (RT-PCR) to detect the presence of alternative isoforms of *dmd-6*, which were predicted to be morph-limited. Total RNA from the St-constitutive line *eud-1(tu445)* and the Eu-constitutive lines *eud-1(tu445) iubIs16*, *nhr-40(tu505)*, and *seud-1(iub7); eud-1(tu445)* was extracted and sequenced as described earlier. First-strand cDNA was synthesized from 1 µg of total RNA using the SuperScript III Reverse Transcriptase kit (Invitrogen) with hexamer random primers, then diluted 5-fold with nuclease-free water. RT-PCR was performed using 1 µl of a 1:5 dilution of cDNA, with the forward primer ATGGTTGAACTAGTAAAGAGT and reverse primer AGGCATGGTTTGAAGAATGG, and using Phusion Green Hot Start II high-fidelity DNA polymerase (Thermo Fisher Scientific). PCR was performed using the following thermocycling conditions: 98 °C for 10 s, 54 °C for 30 s, 72 °C for 1 min, for 32 cycles. PCR products were visualized on a 2% agarose gel using the BioRad Gel Doc System. PCR products from three replicates were excised from the gel and pooled together in a single microtube per sample for DNA extraction using a Zymo Gel Extraction kit (Zymo Research). Purified PCR products were submitted for Sanger sequencing (Eurofins Genomics) to verify the sequences of the two isoforms.

Phylogenetic Analysis

Homology of *P. pacificus Hsp70* and other Hsp70-family homologs was determined using phylogenetic analysis of putative homologs from *P. pacificus* and *C. elegans*, as described in the supplementary methods, Supplementary Material online.

Quantification of Morph-Specific Transcripts across Phenotypic Variants

For qRT-PCR, total RNA was extracted as described earlier and 1 µg of total RNA was used for first-strand cDNA synthesis with random hexamers in a 20 µl reaction using the SuperScript III Reverse Transcriptase kit (Invitrogen). cDNA was diluted 1:5, and 1.5 µl of diluted cDNA was used in a 10-µl PCR on a Roche LightCycler 96 system, using SYBR GREEN reaction mix and the manufacturer's (Roche) software with

GGATCTAATGGAAATCAACAG as forward primer and GTATAACTAATCTATTGCACA as reverse primer. The PCR cycle was: 10 min at 95 °C, followed by 45 cycles of 10 s at 95 °C, 20 s at 52 °C, and 15 s at 72 °C, with a single fluorescent read at the end of each extension. Three independent biological replicates by three technical replicates were run for each condition. Melting-curve analysis was performed to ensure the absence of nonspecific products or primer dimerization, and PCR efficiency was identified with a 5-log titration of pooled cDNA. Relative expression levels were determined using the ΔΔCt method, with PS312 designated as the control group and with the beta-tubulin gene *Ppa-tbb-4* and *Ppa-y45f10d* used as reference genes (Bui et al. 2018). After log fold-change values were calculated, they were used as the response variable in linear mixed models using the R package lme4 (Bates et al. 2013). For this test, nematode strain was specified as a fixed factor, and technical replicate was specified as a random variable to account for the shared variance among the three measures for each biological sample. Significance was assessed by comparing nested models using likelihood ratio tests. The R package lsmeans (Lenth 2016), which can accommodate mixed models, was used to implement a Tukey's HSD test to determine significant differences in gene expression among strains.

Supplementary Material

Supplementary data are available at *Molecular Biology and Evolution* online.

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Author Contributions

L.T.B. and E.J.R. designed the study, conducted the experiments, analyzed the data, and wrote the article.

References

Abouheif E, Wray GA. 2002. Evolution of the gene network underlying wing polyphenism in ants. *Science* 297(5579):249–252.

Albert PS, Brown SJ, Riddle DL. 1981. Sensory control of dauer larva formation in *Caenorhabditis elegans*. *J Comp Neurol*. 198(3):435–451.

Alexa A, Rahnenfuhrer J. 2013. topGO: Enrichment analysis for gene ontology. R package version 2.20.0.

Antebi A. 2015. Nuclear receptor signal transduction in *C. elegans*. *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.64.2.

Antebi A, Yeh WH, Tait D, Hedgecock EM, Riddle DL. 2000. *daf-12* encodes a nuclear receptor that regulates the dauer diapause and developmental age in *C. elegans*. *Genes Dev*. 14(12):1512–1527.

Arda HE, Taubert S, MacNeil LT, Conine CC, Tsuda B, Van Gilst M, Sequerra R, Doucette-Stamm L, Yamamoto KR, Walhout A. 2010. Functional modularity of nuclear hormone receptors in a *Caenorhabditis elegans* metabolic gene regulatory network. *Mol Syst Biol*. 6:367.

Asahina M, Valenta T, Silhankova M, Korinek V, Jindra M. 2006. Crosstalk between a nuclear receptor and β-catenin signaling decides cell fates in the *C. elegans* somatic gonad. *Dev Cell*. 11(2):203–211.

Baldwin JG, Giblin-Davis RM, Eddleman CD, Williams DS, Vida JT, Thomas WK. 1997. The buccal capsule of *Aduncospiculum halicti* (Nemata: Diplogasterina): an ultrastructural and molecular phylogenetic study. *Can J Zool*. 75(3):407–423.

Bates D, Maechler M, Bolker B. 2013. lme4: linear mixed-effects models using S4 classes. R Package version 1.1-7.

Beldade P, Mateus ARA, Keller RA. 2011. Evolution and molecular mechanisms of adaptive developmental plasticity. *Mol Ecol*. 20(7):1347–1363.

Bento G, Ogawa A, Sommer RJ. 2010. Co-option of the hormone-signalling module dafachronic acid-DAF-12 in nematode evolution. *Nature* 466(7305):494–497.

Berendzen KM, Durieux J, Shao LW, Tian Y, eui KH, Wolff S, Liu Y, Dillin A. 2016. Neuroendocrine coordination of mitochondrial stress signaling and proteostasis. *Cell* 166(6):1553–1567.

Berens AJ, Hunt JH, Toth AL. 2015. Comparative transcriptomics of convergent evolution: different genes but conserved pathways underlie caste phenotypes across lineages of eusocial insects. *Mol Biol Evol*. 32(3):690–703.

Bose N, Ogawa A, Von Reuss SH, Yim JJ, Ragsdale EJ, Sommer RJ, Schroeder FC. 2012. Complex small-molecule architectures regulate phenotypic plasticity in a nematode. *Angew Chem Int Ed*. 51(50):12438–12443.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv Genet*. 13(C):115–155.

Brakefield PM, Kesbeke F, Koch PB. 1998. The regulation of phenotypic plasticity of eyespots in the butterfly *Bicyclus anynana*. *Am Nat*. 152(6):853–860.

Brisson JA, Ishikawa A, Miura T. 2010. Wing development genes of the pea aphid and differential gene expression between winged and unwinged morphs. *Insect Mol Biol*. 19(Suppl. 2):63–73.

Brožová E, Šimecková K, Kostrouch Z, Rall JE, Kostrouchová M. 2006. NHR-40, a *Caenorhabditis elegans* supplementary nuclear receptor, regulates embryonic and early larval development. *Mech Dev*. 123(9):689–701.

Bui LT, Ivers NA, Ragsdale EJ. 2018. A sulfotransferase dosage-dependently regulates mouthpart dimorphism in the nematode *Pristionchus pacificus*. *Nat Commun*. 9(1):4119.

Bumbarger DJ, Riebesell M, Rodelsperger C, Sommer RJ. 2013. System-wide rewiring underlies behavioral differences in predatory and bacterial-feeding nematodes. *Cell* 152(1–2):109–119.

Burtis KC, Baker BS. 1989. Drosophila *doublesex* gene controls somatic sexual differentiation by producing alternatively spliced mRNAs encoding related sex-specific polypeptides. *Cell* 56(6):997–1010.

Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. 2001. Nuclear receptors and lipid physiology: opening the X-files. *Science* 294(5548):1866–1870.

Collins JP, Cheek JE. 1983. Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. *Am Zool*. 23(1):77–84.

Corona M, Libbrecht R, Wheeler DE. 2016. Molecular mechanisms of phenotypic plasticity in social insects. *Curr Opin Insect Sci*. 13:55–60.

Daniels EV, Murad R, Mortazavi A, Reed RD. 2014. Extensive transcriptional response associated with seasonal plasticity of butterfly wing patterns. *Mol Ecol*. 23(24):6123–6134.

Ehrenreich IM, Pfennig DW. 2016. Genetic assimilation: a review of its potential proximate causes and evolutionary consequences. *Ann Bot*. 117(5):769–779.

Fang B, Mane-Padros D, Bolotin E, Jiang T, Sladek FM. 2012. Identification of a binding motif specific to HNF4 by comparative analysis of multiple nuclear receptors. *Nucleic Acids Res*. 40(12):5343–5356.

Félix MA, Ailion M, Hsu JC, Richaud A, Wang J. 2018. *Pristionchus* nematodes occur frequently in diverse rotting vegetal substrates

and are not exclusively necromenic, while *Panagrellus redivivoides* is found specifically in rotting fruits. *PLoS One* 13(8):e0200851.
Fire A. 1986. Integrative transformation of *Caenorhabditis elegans*. *EMBO J*. 5(10):2673–2680.
Forsman A. 2015. Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity* 115(4):276–284.
Fürst von Lieven A, Sudhaus W. 2000. Comparative and functional morphology of the buccal cavity of Diplogastrina (Nematoda) and a first outline of the phylogeny of this taxon. *J Zool Syst* 38(1):37–63.
Fuxman Bass JI, Pons C, Kozlowski L, Reece-Hoyes JS, Shrestha S, Holdorf AD, Mori A, Myers CL, Walhout A. 2016. A gene-centered *C. elegans* protein–DNA interaction network provides a framework for functional predictions. *Mol Syst Biol*. 12(10):884.
Gissendanner CR, Crossgrove K, Kraus KA, Maina CV, Sluder AE. 2004. Expression and function of conserved nuclear receptor genes in *Caenorhabditis elegans*. *Dev Biol*. 269(2):399–416.
Golden TR, Melov S. 2004. Microarray analysis of gene expression with age in individual nematodes. *Aging Cell*. 3(3):111–124.
Golden TR, Melov S. 2007. Gene expression changes associated with aging in C. elegans (February 12, 2007), WormBook, ed. *The C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.127.2.
Grantham ME, Brisson JA. 2018. Extensive differential splicing underlies phenotypically plastic aphid morphs. *Mol Biol Evol*. 35(8):1934–1946.
Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C, Singh H, Glass CK. 2010. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell*. 38(4):576–589.
Hoffman EA, Pfennig DW. 1999. Proximate causes of cannibalistic polyphenism in larval tiger salamanders. *Ecology* 80(3):1076–1080.
Jakšić AM, Schlötterer C. 2016. The interplay of temperature and genotype on patterns of alternative splicing in *Drosophila melanogaster*. *Genetics* 204(1):315–325.
Jones SJM, Riddle DL, Pouzyrev AT, Velculescu VE, Hillier LD, Eddy SR, Stricklin SL, Baillie DL, Waterston R, Marra MA. 2001. Changes in gene expression associated with development arrest and longevity in *Caenorhabditis elegans*. *Genome Res*. 11(8):1346–1352.
Kieninger MR, Ivers NA, Rödelsperger C, Markov GV, Sommer RJ, Ragsdale EJ. 2016. The nuclear hormone receptor NHR-40 acts downstream of the sulfatase EUD-1 as part of a developmental plasticity switch in *Pristionchus*. *Curr Biol*. 26(16):2174–2179.
Kijimoto T, Moczek AP, Andrews J. 2012. Diversification of *doublesex* function underlies morph-, sex-, and species-specific development of beetle horns. *Proc Natl Acad Sci U S A*. 109(50):20526–20531.
Kiontke K, Fitch DH. 2010. Phenotypic plasticity: different teeth for different feasts. *Curr Biol*. 20(17):R710–R712.
Klein A, Schultner E, Lowak H, Schrader L, Heinze J, Holman L, Oettler J. 2016. Evolution of social insect polyphenism facilitated by the sex differentiation cascade. *PLoS Genet*. 12(3):e1005952.
Kravats AN, Hoskins JR, Reidy M, Johnson JL, Doyle SM, Genest O, Masison DC, Wickner S. 2018. Functional and physical interaction between yeast Hsp90 and Hsp70. *Proc Natl Acad Sci U S A*. 115(10):E2210–E2219.
Lande R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J Evol Biol*. 22(7):1435–1446.
Leichty AR, Pfennig DW, Jones CD, Pfennig KS. 2012. Relaxed genetic constraint is ancestral to the evolution of phenotypic plasticity. *Integr Comp Biol*. 52(1):16–30.
Lenth RV. 2016. Least-squares means: the R package lsmeans. *J Stat Softw*. 69(1):1–33.
Liao Y, Smyth GK, Shi W. 2013. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Res*. 41:108.
Lightfoot JW, Wilecki M, Rödelsperger C, Moreno E, Susoy V, Witte H, Sommer RJ. 2019. Small peptide-mediated self-recognition prevents cannibalism in predatory nematodes. *Science* 364(6435):86–89.
Long Y, Song G, Yan J, He X, Li Q, Cui Z. 2013. Transcriptomic characterization of cold acclimation in larval zebrafish. *BMC Genomics* 14:612.
Ludewig AH, Kober-Eisermann C, Weitzel C, Bethke A, Neubert K, Gerisch B, Hutter H, Antebi A. 2004. A novel nuclear receptor/coregulator complex controls *C. elegans* lipid metabolism, larval development, and aging. *Genes Dev*. 18(17):2120–2133.
Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ. 2009. GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics* 10:161.
MacNeil LT, Pons C, Arda HE, Giese GE, Myers CL, Walhout A. 2015. Transcription factor activity mapping of a tissue-specific in vivo gene regulatory network. *Cell Syst*. 26(2):152–162.
Marchler-Bauer A, Bo Y, Han LY, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, et al. 2017. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Res*. 45(D1):D200–D203.
Maynard Smith J. 1966. Sympatric speciation. *Am Nat*. 100(916):637–650.
Meisel JD, Panda O, Mahanti P, Schroeder FC, Kim DH. 2014. Chemosensation of bacterial secondary metabolites modulates neuroendocrine signaling and behavior of *C. elegans*. *Cell* 159(2):267–280.
Moczek AP, Sultan S, Foster S, Ledón-Rettig C, Dworkin I, Nijhout HF, Abouheif E, Pfennig DW. 2011. The role of developmental plasticity in evolutionary innovation. *Proc R Soc Lond B*. 278(1719):2705–2713.
Monteiro A, Tong X, Bear A, Liew SF, Bhardwaj S, Wasik BR, Dinwiddie A, Bastianelli C, Cheong WF, Wenk MR, et al. 2015. Differential expression of ecdysone receptor leads to variation in phenotypic plasticity across serial homologs. *PLoS Genet*. 11(9):e1005529.
Moran NA. 1992. The evolutionary maintenance of alternative phenotypes. *Am Nat*. 139(5):971–989.
Morgan K, McGaughran A, Villate L, Herrmann M, Witte H, Bartelmes G, Rochat J, Sommer RJ. 2012. Multi locus analysis of *Pristionchus pacificus* on la Réunion Island reveals an evolutionary history shaped by multiple introductions, constrained dispersal events and rare outcrossing. *Mol Ecol*. 21(2):250–266.
Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C. 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 424(6946):277–284.
Namdeo S, Moreno E, Rödelsperger C, Baskaran P, Witte H, Sommer RJ. 2018. Two independent sulfation processes regulate mouth-form plasticity in the nematode *Pristionchus pacificus*. *Development* 145(13):dev166272.
Nijhout HF, Wheeler DE. 1982. Juvenile hormone and the physiological basis of insect polymorphisms. *Q Rev Biol*. 57(2):109–133.
Nuzhdin SV, Wayne ML, Harmon KL, McIntyre LM. 2004. Common pattern of evolution of gene expression level and protein sequence in *Drosophila*. *Mol Biol Evol*. 21(7):1308–1317.
Parsons KJ, Concannon M, Navon D, Wang J, Ea I, Groveas K, Campbell C, Albertson RC. 2016. Foraging environment determines the genetic architecture and evolutionary potential of trophic morphology in cichlid fishes. *Mol Ecol*. 25(24):6012–6023.
Pespeni MH, Ladner JT, Moczek AP. 2017. Signals of selection in conditionally expressed genes in the diversification of three horned beetle species. *J Evol Biol*. 30(9):1644–1657.
Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol Evol*. 25(8):459–467.
Pigliucci M. 2005. Evolution of phenotypic plasticity: where are we going now? *Trends Ecol Evol*. 20(9):481–486.
Pigliucci M, Murren CJ, Schlichting CD. 2006. Phenotypic plasticity and evolution by genetic assimilation. *J Exp Biol*. 209(12):2362–2367.
Price J, Harrison MC, Hammond RL, Adams S, Gutierrez-Marcos JF, Mallon EB. 2018. Alternative splicing associated with phenotypic plasticity in the bumble bee *Bombus terrestris*. *Mol Ecol*. 27(4):1036–1043.

Projecto-Garcia J, Biddle JF, Ragsdale EJ. 2017. Decoding the architecture and origins of mechanisms for developmental polyphenism. *Curr Opin Genet Dev*. 47:1–8.
Purandare SR, Bickel RD, Jaquiery J, Rispe C, Brisson JA. 2014. Accelerated evolution of morph-biased genes in pea aphids. *Mol Biol Evol*. 31(8):2073–2083.
Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26(6):841–842.
Ragsdale EJ. 2015. Mouth dimorphism and the evolution of novelty and diversity. In: Sommer RJ, editor. Pristionchus pacificus: a nematode model for comparative and evolutionary biology. Leiden: Brill. p. 301–329.
Ragsdale EJ, Ivers NA. 2016. Specialization of a polyphenism switch gene following serial duplications in *Pristionchus* nematodes. *Evolution* 70(9):2155–2166.
Ragsdale EJ, Müller MR, Rödelsperger C, Sommer RJ. 2013. A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell* 155(4):922–933.
Rajakumar R, San Mauro D, Dijkstra MB, Huang MH, Wheeler DE, Hiou-Tim F, Khila A, Cournoyea M, Abouheif E. 2012. Ancestral developmental potential facilitates parallel evolution in ants. *Science* 335(6064):79–82.
Reece-Hoyes JS, Walhout AJ. 2012. Gene-centered yeast one-hybrid assays. *Methods Mol Biol*. 812:189–208.
Riebesell M, Sommer RJ. 2017. Three-dimensional reconstruction of the pharyngeal gland cells in the predatory nematode *Pristionchus pacificus*. *J Morphol*. 278(12):1656–1666.
Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1):139–140.
Rödelsperger C, Meyer JM, Prabh N, Lanz C, Bemm F, Sommer RJ. 2017. Single-molecule sequencing reveals the chromosome-scale genomic architecture of the nematode model organism *Pristionchus pacificus*. *Cell Rep*. 21(3):834–844.
Rountree DB, Nijhout HF. 1995. Hormonal control of a seasonal polyphenism in *Precis coenia* (Lepidoptera: Nymphalidae). *J Insect Physiol*. 41(11):987–992.
Scheiner SM. 1993. Genetics and evolution of phenotypic plasticity. *Annu Rev Ecol Syst*. 24(1):35–68.
Schrader L, Helantera H, Oettler J. 2017. Accelerated evolution of developmentally biased genes in the tetraphenic ant *Cardiocondyla obscurior*. *Mol Biol Evol*. 34(3):535–544.
Schrader L, Simola DF, Heinze J, Oettler J. 2015. Sphingolipids, transcription factors, and conserved toolkit genes: developmental plasticity in the ant *Cardiocondyla obscurior*. *Mol Biol Evol*. 32(6):1474–1486.
Serobyan V, Ragsdale EJ, Müller MR, Sommer RJ. 2013. Feeding plasticity in the nematode *Pristionchus pacificus* is influenced by sex and social context and is linked to developmental speed. *Evol Dev*. 15(3):161–170.
Serobyan V, Ragsdale EJ, Sommer RJ. 2014. Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proc Biol Sci*. 281(1791):20141334.
Serobyan V, Xiao H, Namdeo S, Rödelsperger C, Sieriebriennikov B, Witte H, Röseler W, Sommer RJ. 2016. Chromatin remodelling and antisense-mediated up-regulation of the developmental switch gene eud-1 control predatory feeding plasticity. *Nat Commun*. 7:12337.
Shih PY, Lee JS, Sternberg PW. 2019. Genetic markers enable the verification and manipulation of the dauer entry decision. *Dev Biol*. doi: 10.1016/j.ydbio.2019.06.009.
Shostak Y, Van Gilst MR, Antebi A, Yamamoto KR. 2004. Identification of *C. elegans* DAF-12-binding sites, response elements, and target genes. *Genes Dev*. 18(20):2529–2544.
Sieriebriennikov B, Markov GV, Witte H, Sommer RJ. 2017. The role of DAF-21/Hsp90 in mouth-form plasticity in *Pristionchus pacificus*. *Mol Biol Evol*. 34(7):1644–1653.
Sieriebriennikov B, Prabh N, Dardiry M, Witte H, Röseler W, Kieninger MR, Rödelsperger C, Sommer RJ. 2018. A developmental switch generating phenotypic plasticity is part of a conserved multi-gene locus. *Cell Rep*. 23(10):2835–2843.
Smit RB, Schnabel R, Gaudet J. 2008. The HLH-6 transcription factor regulates *C. elegans* pharyngeal gland development and function. *PLoS Genet*. 4(10):e1000222.
Smith-Gill SJ. 1983. Developmental plasticity: developmental conversion versus phenotypic modulation. *Am Zool*. 23:47–55.
Smith TB, Skúlason S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu Rev Ecol Syst*. 27(1):111–133.
Sommer RJ, Dardiry M, Lenuzzi M, Namdeo S, Renahan T, Sieriebriennikov B, Werner MS. 2017. The genetics of phenotypic plasticity in nematode feeding structures. *Open Biol*. 7(3):160332.
Susoy V, Herrmann M, Kanzaki N, Kruger M, Nguyen CN, Rödelsperger C, Röseler W, Weiler C, Giblin-Davis RM, Ragsdale EJ, et al. 2016. Large-scale diversification without genetic isolation in nematode symbionts of figs. *Sci Adv*. 2(1):e1501031.
Susoy V, Ragsdale EJ, Kanzaki N, Sommer RJ. 2015. Rapid diversification associated with a macroevolutionary pulse of developmental plasticity. *Elife* 2015(4):1–39.
Suzuki Y, Nijhout HF. 2006. Evolution of a polyphenism by genetic accommodation. *Science* 311(5761):650–652.
Van Dyken JD, Wade MJ. 2010. The genetic signature of conditional expression. *Genetics* 184(2):557–570.
Veiga D. 2018. maser: Mapping Alternative Splicing Events to pRoteins. R package version 1.
Vellichirammal NN, Gupta P, Hall TA, Brisson JA. 2017. Ecdysone signaling underlies the pea aphid transgenerational wing polyphenism. *Proc Natl Acad Sci U S A*. 114(6):1419–1423.
Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH. 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol Evol*. 10(5):212–217.
Waddington CH. 1953. Genetic assimilation of an acquired character. *Evolution* 7(2):118–126.
Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. 2008. Alternative isoform regulation in human tissue transcriptomes. *Nature* 465(7221):470–476.
Weller AM, Mayer WE, Rae R, Sommer RJ. 2010. Quantitative assessment of the nematode fauna present on *Geotrupes* dung beetles reveals species-rich communities with a heterogeneous distribution. *J Parasitol*. 96(3):525–531.
Werner MS, Claaßen MH, Renahan T, Dardiry M, Sommer RJ. 2018. Adult influence on juvenile phenotypes by stage-specific pheromone production. *iScience* 10:123–134.
Werner MS, Sieriebriennikov B, Loschko T, Namdeo S, Lenuzzi M, Dardiry M, Renahan T, Sharma DR, Sommer RJ. 2017. Environmental influence on *Pristionchus pacificus* mouth form through different culture methods. *Sci Rep*. 7(1):7207.
West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Syst*. 20(1):249–278.
West-Eberhard MJ. 2003. Developmental plasticity and evolution. Oxford: Oxford University Press.
Whitehead A, Crawford DL. 2006. Neutral and adaptive variation in gene expression. *Proc Natl Acad Sci U S A*. 103(14):5425–5430.
Wilecki M, Lightfoot JW, Susoy V, Sommer RJ. 2015. Predatory feeding behaviour in *Pristionchus* nematodes is dependent on phenotypic plasticity and induced by serotonin. *J Exp Biol*. 218(9):1306–1313.
Xu HJ, Xue J, Lu B, Zhang XC, Zhuo JC, He SF, Ma XF, Jiang YQ, Fan HW, Xu JY, et al. 2015. Two insulin receptors determine alternative wing morphs in planthoppers. *Nature* 519(7544):464–467.
Yang CH, Pospisilik AJ. 2019. Polyphenism – a window into gene-environment interactions and phenotypic plasticity. *Front Genet*. 10:132.
Yeates GW, Bongers T, de Goede RGM, Freckman DW, Georgieva SS. 1993. Feeding habits in soil nematode families and genera – an outline for soil ecologists. *Nematology* 25(3):315–331.
Zhang YC, Baldwin JG. 1999. Ultrastructure of the esophagus of *Diplenteron* sp. (Diplogasterida) to test hypotheses of homology with Rhabditida and Tylenchida. *J Nematol*. 31(1):1–19.