Testing the Adaptive Decoupling Hypothesis in a Hypermetamorphic and Sexually Dimorphic Insect

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Abstract

Complex life cycles—especially those with distinct larval and adult stages separated by a complete metamorphic event—are common in animals. The adaptive decoupling hypothesis (ADH) proposes that metamorphosis is an adaptation for optimizing expression of traits across life stages that experience opposing selection pressures. Similarly, sex-biased expression of traits is thought to evolve in response to sexually antagonistic selection. Both hypotheses predict that traits will be decoupled (i.e., genetically uncorrelated) among developmental stages and sexes, but direct comparisons between stage-specific and sex-specific decoupling are rare. Additionally, tests of the ADH have been hampered by a lack of suitable traits for among-stage comparisons and by uncertainties regarding how much decoupling is to be expected. To fill these voids, we characterize transcriptome-wide patterns of gene-expression decoupling in the hypermetamorphic and sexually dimorphic insect, *Neodiprion lecontei*. This species has three ecologically and morphologically distinct larval stages separated by molts, as well as a complete metamorphic transition that produces dimorphic adult males and females. Consistent with the ADH, we observe that: (1) the decoupling of gene expression becomes more pronounced as the ecological demands of developmental stages become more dissimilar and (2) gene-expression traits that mediate changing ecological interactions show stronger and more variable decoupling than expression traits that are likely to experience more uniform selection. We also find that gene-expression decoupling is more pronounced among major life stages than between the sexes. Overall, our results demonstrate that patterns of gene-expression decoupling can be predicted based on gene function and organismal ecology.

Significance Statement

8 out of 10 animals undergo a metamorphic transition at some point in their life. The adaptive decoupling hypothesis proposes that metamorphosis is widespread because it enables natural selection to act independently on traits expressed at different stages. Using four ecologically distinct life stages of the redheaded pine sawfly, we show that the dissimilarity of gene expression profiles can be predicted based on the ecological dissimilarity of the life stages and on whether these genes impact ecological interactions. We also find that gene expression is more dissimilar between drastic life stages than between different sexes. Together, these results provide strong support for a core assumption of the ADH: that metamorphosis enables organisms to optimize genetic correlations across development.

Introduction

An estimated 80% of animal species have complex life cycles (CLCs) wherein metamorphosis separates two or more discrete, post-embryonic life stages (1). Although disagreements persist over diagnostic criteria, the general consensus is that metamorphosis involves an irreversible transformation in morphology that is typically accompanied by a pronounced change in ecology (2). This change results in specialized stages optimized for distinct ecological niches (2-6). Furthermore, metamorphosis may facilitate independent adaptations for optimal growth at some stages and optimal reproductive success in others (3, 7, 8). One explanation for the prevalence of CLCs stressed that independent adaptations at the different phases allow for optimal growth at some stages and optimal reproductive success at other stages (3, 7). Central to this explanation is the idea that pleiotropy creates genetic correlations across ontogeny that constrain evolution when traits necessary for one stage are detrimental to another (9).

The adaptive decoupling hypothesis (ADH) proposes that metamorphosis evolved as a mechanism for reducing genetic correlations between life stages, thereby enabling the independent evolution of traits when opposing selection pressures are experienced during different life stages (1, 3, 4, 6, 9, 10). Thus, a key prediction of the ADH is that there will be a lack of genetic correlation between traits expressed at different life stages (3). To date, tests of this prediction have been mixed, with some studies supporting, others refuting, and two studies with mixed results (please see (11-15) and references therein). Importantly, however, the ADH does not require that all traits are decoupled across metamorphosis. Rather, if metamorphosis is an adaptation that enables organisms to optimize genetic correlations across life stages, decoupling may evolve only when genetic correlations constrain adaptation (i.e., when correlated traits are selected in opposing directions). Thus, a more accurate prediction of the ADH is that the degree of genetic decoupling for a particular trait will increase with the strength of opposing selection across life stages. Testing this nuanced prediction will require examining many different traits across life-stage transitions that vary in the extent to which they experience antagonistic selection.

Whole-transcriptome gene-expression data obtained from multiple life stages provide an ideal collection of traits for evaluating the extent to which patterns of genetic decoupling fit predictions of the ADH (12). First, because all stages of the life cycle must be encoded by a single genome, dramatic phenotypic changes that accompany metamorphosis must be mediated by changes in gene expression. Second, transcriptomes provide a large number of quantitative traits, all measured in comparable units of gene expression, that can be readily compared across life stages. Third, the genes included in a transcriptome cover a wide range of biological functions that should vary somewhat predictably in the extent to which they are exposed to opposing selection across the life cycle. Following the logic of the ADH, this variation in selection should generate predictable variation in genetic decoupling. For example, the ADH predicts that genes involved in basic cellular functions (i.e., housekeeping genes) will be more genetically coupled than genes that mediate ecological interactions that change across the life cycle.

The ADH also predicts that transcriptome-wide patterns of decoupling will vary with the magnitude of the ecological change accompanying metamorphosis. Arguably, the most extreme metamorphic transformations occur in holometabolous insects, whose defining characteristic is a distinct pupal stage that separates two completely different body plans (16-18). Importantly, this profound transformation enables one stage to be optimized for feeding and growth (the larval stage) and a second for dispersal and reproduction (the adult stage). The genetic independence of larval and adult traits proposed by the ADH may explain, in part, why holometabolous insects are one of the most evolutionarily successful and diverse lineages on the planet accounting for an estimated 80% of insect species (1, 3, 4, 6, 8-10, 19). In some holometabolous lineages, pronounced ecological and morphological transformations also occur between successive larval instars. This phenomenon, which has been dubbed hypermetamorphosis (20), provides a valuable opportunity to test the prediction that transcriptome-wide levels of genetic decoupling between life stages will increase with the dissimilarity of the fitness landscapes to which they are adapting.

Looking beyond metamorphosis, the rationale underlying the ADH applies more generally to any scenario in which a single genome expresses multiple distinct phenotypes that are subject to antagonistic selection (12). Arguably the best-studied scenario of genetic correlations evolving in response to antagonistic pleiotropy involves alternative phenotypes of a single life stage: adult males and adult females. Just as metamorphosis is thought to reduce genetic correlations across life stages of organisms with CLCs, sex-biased gene expression can enable the independent evolution of male and female traits, leading to the evolution of sexual dimorphism (21-25). An important distinction between CLCs and sexual dimorphism is that in only in CLCs must all alternative phenotypes (i.e., life stages) have non-zero fitness for a novel pleiotropic allele to spread in a population (12). Therefore, all else being equal, greater evolutionary constraint may result in more pronounced trait decoupling between ecologically distinct life stages compared to that observed between the sexes.

To date, only a handful of studies have evaluated the prediction that gene-expression traits will be genetically decoupled across metamorphic boundaries as it pertains to the adaptive decoupling hypothesis (13, 15, 26). Furthermore, no study has evaluated whether overall levels of genetic decoupling vary predictably with type of life-cycle transition or gene function, and only a single study has directly compared patterns of sex-specific and stage-specific gene expression (25). To these ends, we take advantage of a hypermetamorphic and sexually dimorphic species of insect with a well-characterized life history and annotated genome, the redheaded pine sawfly *Neodiprion lecontei* (Figure 1). Based on the ADH and related hypotheses, we predict that: (1) genetic decoupling will increase as the ecological demands of different life stages become more dissimilar, (2) compared to gene-expression traits that are likely to experience uniform selection across the life stages, gene-expression traits that mediate changing ecological interactions will exhibit stronger and more variable patterns of decoupling, and (3) all else equal, genetic decoupling should be more pronounced for life-stage transitions than for separate sexes.

Results and Discussion

Life history of *Neodiprion lecontei* and data collection

Making *a priori* predictions about how genetic decoupling will change over the course of development requires detailed knowledge of an organism's ecology. Decades of natural history research on the redheaded pine sawfly (*Neodiprion lecontei*, order: Hymenoptera; family: Diprionidae) suggest that in addition to the profound change that occurs during the pupal stage, two metamorphic transitions occur within the larval stage (please see (27-29) and references therein) (Figure 1A). First is a shift from a "cryptic" to an "aposematic" feeding larval morph. The

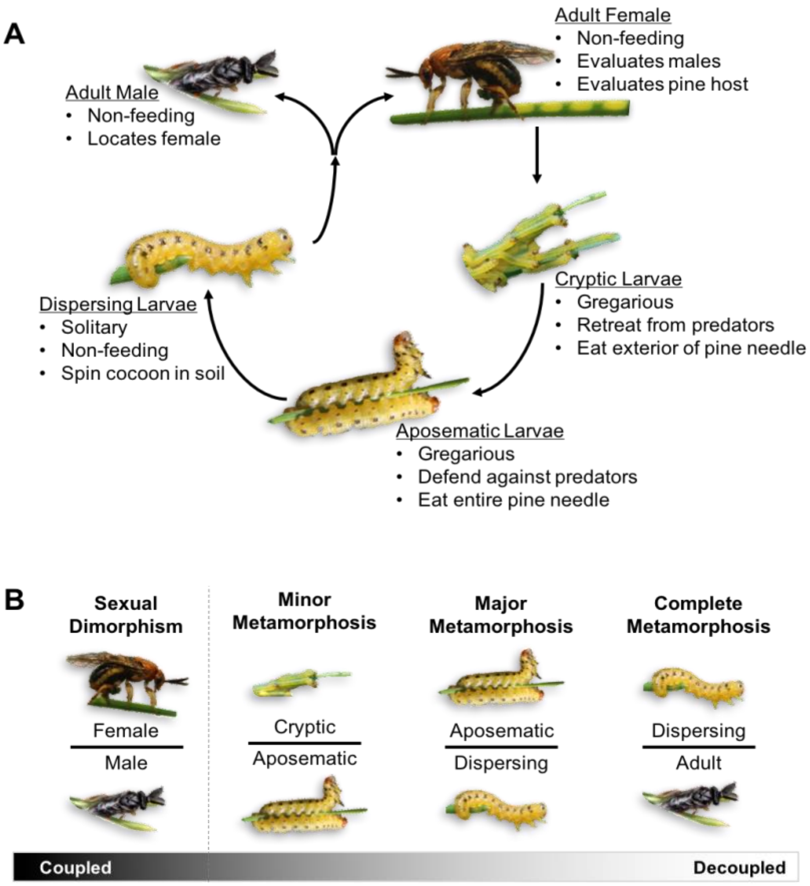

Figure 1: Ecological changes during N. lecontei development generate predictions regarding gene-expression decoupling between the sexes and across different metamorphic transitions. (A) The life cycle of the red-headed pine sawfly includes two distinct feeding larval stages, a non-feeding larval stage, and sexually dimorphic adults. **(B)** Based on this life cycle, metamorphic transitions include: minor metamorphisis (between the two feeding larval stages), major metamorphisis (between the feeding and non-feeding larval stages), and complete metamorphosis (between larval stage and adult). The gradient represents our prediction that gene expression will be most coupled between the feeding larval stages and the adult sexes while it is most decoupled for stages separated by complete metamorphosis.

cryptic morph lacks high-contrast pigmentation, ingests only the exterior of pine needle while avoiding the resinous core, and retreats to the base of the needle when predators are near. By contrast, the aposematic morph is heavily pigmented, consumes entire needles, and sequesters host resins for use in dramatic anti-predator defensive displays. Second, a more pronounced change occurs between the final two larval instars when the aposematic morph molts into a morphologically distinct "dispersing" final instar. While both cryptic and aposematic morphs are gregarious and needle-feeding, the dispersing morph is solitary, non-feeding, and migrates to the litter or soil, where it spins a cocoon. Finally, the most dramatic metamorphic transition occurs within the cocoon, where individuals pupate and transform into sexually dimorphic adults. The adult stage is very short (~4.5 days (30)) and dedicated entirely to reproduction as adults have no digestive system. Adult males and females are highly specialized for sex-specific tasks. Males are excellent fliers and use bipectinate antennae to detect female pheromones from considerable distances. In contrast, females remain near the cocoon eclosion site and use serrate antennae to search for suitable oviposition sites in *Pinus* needles (31). Like most hymenopterans, *N. lecontei* females lay a combination of fertilized and unfertilized eggs that will develop into diploid females and haploid males, respectively.

If metamorphosis is an adaptation that optimizes trait decoupling between ecologically distinct life stages, the extent of decoupling should increase with the magnitude of antagonistic selection between life-stages. On the basis of ecological changes that occur over the life cycle of pine sawflies (Figure 1A), we predict the greatest decoupling will be observed between expression traits separated by the pupal stage (larvae and reproductive adults--"complete metamorphosis"), followed by dispersing and feeding larvae ("major metamorphosis") and the least between cryptic and aposematic feeding larvae ("minor metamorphosis") (Figure 1B). Additionally, because traits expressed in different individuals may be less evolutionarily constrained than traits expressed in multiple life stages of a single individual, we predict trait decoupling between the sexes will be less extreme than that observed between life stages (Figure 1B). As chemosensory genes have been implicated in mediating insect-host and male-female interactions in numerous systems (32-34), we predict that these genes will exhibit highly variable levels of decoupling, with at least some genes exhibiting strong decoupling. To test these predictions, we generated expression data for 9,304 genes via whole-transcriptome sequencing of males of each *N. lecontei* life stage and adults of both sexes (See Supplemental Methods for more details).

Decoupling of gene-expression profiles increases with ecological dissimilarity of *N. lecontei* life stages

To determine whether genetic decoupling changes predictably across different types of metamorphic transitions, we compared whole-transcriptome profiles of different life stages and sexes in two ways. First, to visualize overall similarity or dissimilarity of gene expression across all tissues and life stages, we conducted a principle component analysis (PCA). Consistent with our prediction that differences in gene-expression traits would be most pronounced between ecologically dissimilar life stages, stages separated by intermediate and complete metamorphosis were clearly distinct as shown in the first principle component (PC1) of our PCA (Figure 1B and Figure 2A). By contrast, there are no clear distinctions between cryptic and aposematic larvae or between males and females along either of the first two gene-expression PCs. PC2 primarily separated tissues within each life stage (Figure 2A). Along this axis, larval and adult heads are

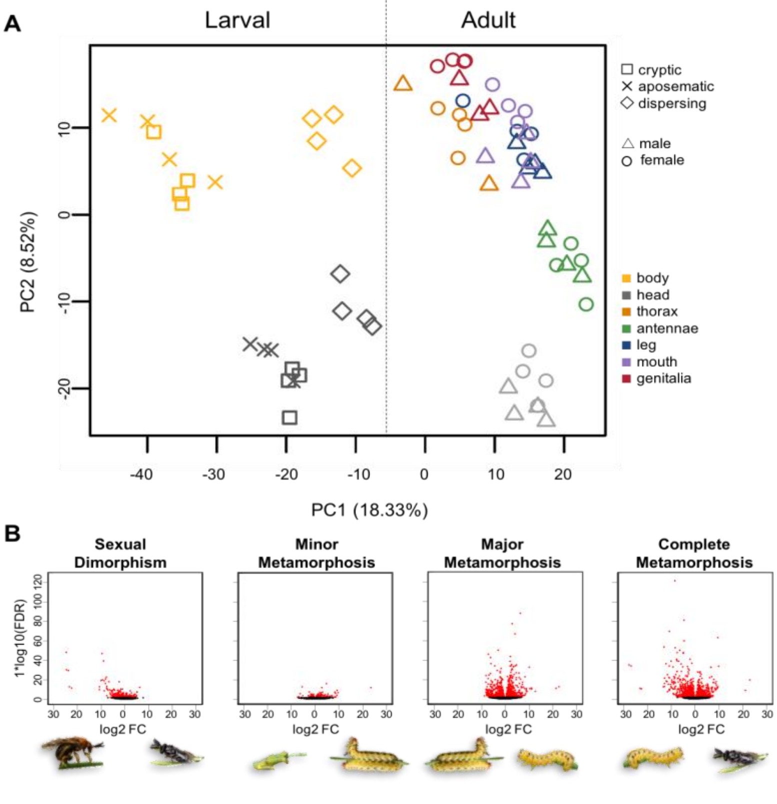

Figure 2: Transcriptome-wide patterns of gene-expression decoupling are consistent with predictions of the ADH. (A) Principle component analyses of 9,304 putatively annotated genes for the head (gray) and body (yellow) tissues of cryptic (☐), aposematic (✕), and dispersing (◇) larvae as well as the head (grey), antenna (green), mouthpart (purple), leg (navy), genitalia (red), and thoracic (orange), tissues of adult males (Δ) and females (○). **(B)** Volcano plots with the log$_2$-fold change of gene expression and log$_{10}$ FDR-adjusted P-values between the sexually dimorphic adults and between each of the metamorphic transitions, arranged according to predictions as in Figure 1B. Each point represents a single gene; red points are significantly differentially expressed between the stages/sexes.

clearly separate from other tissues as are the adult antennal tissues. Based on these results, we analyzed gene-expression decoupling among stages/sexes in two ways: all tissues combined and heads only.

To quantify how decoupling changes across different metamorphic boundaries and between the sexes, we determined the number of differentially expressed genes (DEGs) in each of the four metamorphic/sexually dimorphic comparisons (Figure 1B). We first looked at decoupling across metamorphic transitions. Regardless of whether we looked at all tissues or heads only, both the magnitude of differential expression (log fold-change) and significance (Benjamini-Hochberg adjusted p-value) increased in accordance with ADH predictions (Figure 2B for all tissues). Specifically, the percentage of DEGs increased with ecological dissimilarity of the life stages compared: 2.2% of genes were differentially expressed between cryptic and aposematic larvae, 24.7% between aposematic and dispersing larvae, and 25.1% between dispersing larvae and adult males (heads only: 3.0%, 17.6%, and 31.1%, respectively). All differences in the proportion of genes that were DEGs among life stages were highly significant (Fisher's exact tests; $P < 1 \times 10^{-20}$) except for the all-tissue dispersing larvae vs. male comparison ($P = 0.59$).

Despite males and females being more morphologically dissimilar than the feeding and dispersing larval stages, the percentage of DEGs between sexually dimorphic adults (all tissues: 7.0%; head only: 6.5%) was far less than that observed across both the major and complete metamorphic transitions (Fisher's exact tests; $P < 1 \times 10^{-208}$)(Figure 2B). In contrast, we observed a higher percentage of DEGs between the sexes than between the two feeding larval stages (Fisher's exact tests; $P < 1.0 \times 10^{-20}$). Taken together with the PCA, these results indicate that genetic decoupling between the sexes is more or less on par with the minor metamorphic transition between cryptic and aposematic larvae and far less pronounced than the decoupling observed between more extreme life-stage transitions. Overall, these transcriptome-wide analyses support the ADH prediction that genetic decoupling increases as the ecological demands of the different life stages become more dissimilar, as well as the prediction that genetic decoupling tends to be more pronounced for major metamorphic transitions than for sexual dimorphism.

Chemosensory genes vary in decoupling across life stages

If natural selection has optimized levels of decoupling, as predicted by the ADH, then decoupling will be most pronounced for gene-expression traits that mediate stage- or sex-specific ecological interactions. Furthermore, if selection optimizes patterns of gene-expression decoupling, the extent of gene-expression correlations across life stages/sexes should vary even among genes belonging to the same narrow functional category depending on precise ecological roles (e.g., (13)). In some cases, antagonistic selection may favor decoupling of chemosensory gene expression traits--for example, males and females may express different genes to optimize mate- and host-finding, respectively. However, in other cases, selection may favor positive genetic correlations across life stages. For example, each stage interacts with the host plant and these interactions may rely on the same host chemical cues (Figure 1A). Furthermore, a positive genetic correlation between female oviposition and larval-feeding preferences are expected to facilitate host adaptation (32, 35). Thus, while correlated chemosensory expression may impede adaptation in the presence of antagonistic selection, such correlations could speed adaptation when there is concordant selection across the life stages (12, 36, 37).

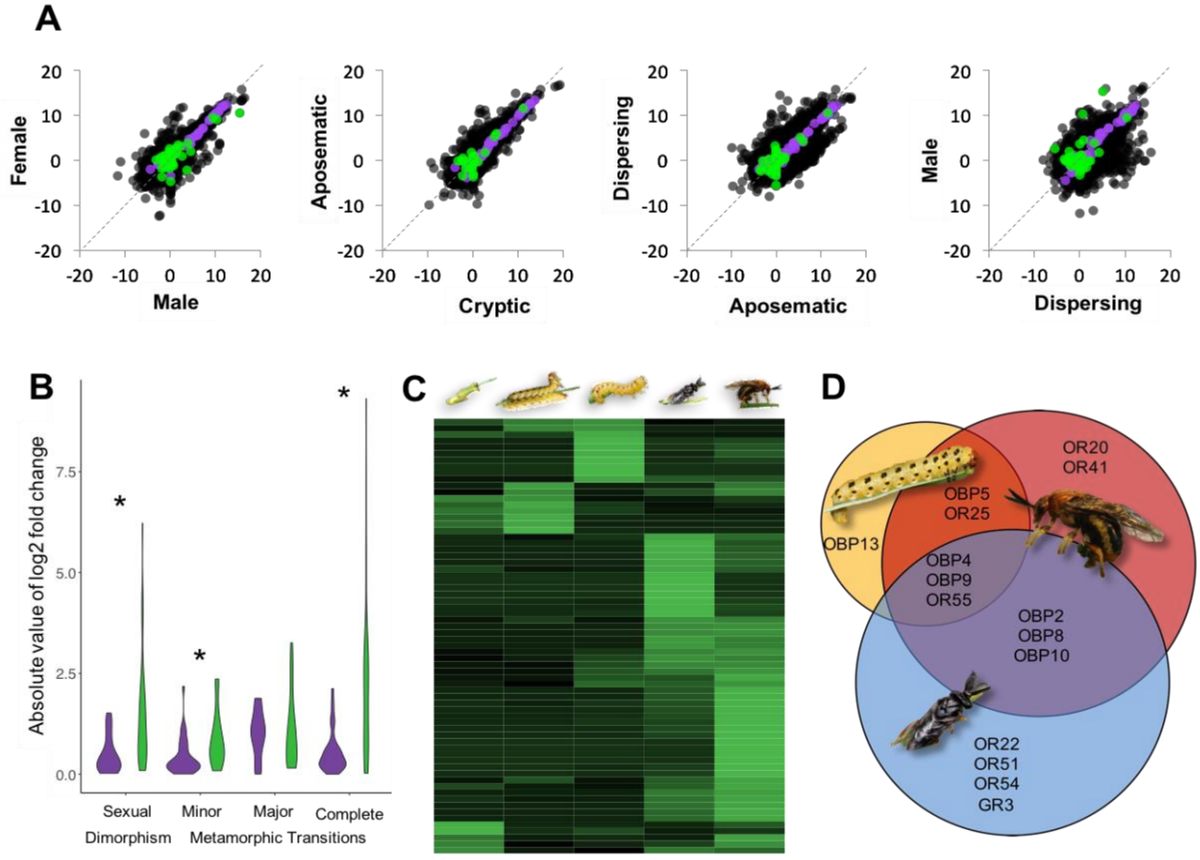

Figure 3: Compared to a group of house-keeping genes, chemosensory genes have higher and more variable levels of expression decoupling across development and between the sexes. (A) Correlation plots of the sexually dimorphic adults and each metamorphic stages for all genes in the transcriptome (black points), Ribosomal protein L genes (purple points), and chemosensory genes (green points). Each point represents $\log_2$ of the average expression level across all tissues/replicates for each life stage/sex. The dotted line represents equal expression in the stages/sexes being compared; deviation from this line represents gene-expression decoupling. **(B)** Violin plots showing the distributions of the absolute value of the $\log_2$-fold change between each comparison (calculated as in Figure 2B) for Ribosomal protein L genes (purple) and chemosensory genes (green). Comparisons are: adult male vs. adult female (MvF), cryptic vs. aposematic larvae (CvA), aposematic vs. dispersing larvae (AvD), and dispersing larva vs. adult male (DvM). Asterisks indicate comparisons for which decoupling is significantly higher for chemosensory genes than for RPL genes. **(C)** Heatmap of expression for each chemosensory gene in 4 male life stages and in adult females (from left to right: cryptic larvae, aposematic larvae, dispersing larvae, adult male, and adult female). Expression is adjusted such that each row has the same total amount of expression to reveal expression patterns for genes expressed at relatively low levels. Whereas some genes have high expression in only one life stage, others are more widely expressed. **(D)** Venn diagram of chemosensory genes with expression levels in the top 10% of all genes expressed in each tissue for feeding larvae, adult males, and adult females. Genes include all olfactory receptors (ORs), gustatory receptors (GRs), and odorant binding proteins (OBPs). Whereas some chemosensory genes exhibit highly sex- and stage-specific expression, others are less decoupled and expressed in multiple stages/sexes.

To evaluate the prediction that patterns of decoupling will reflect opportunities for antagonistic selection, we compared gene-expression traits between two comparably sized sets of genes with very different functions. The first group consists of housekeeping genes (the ribosomal protein L genes, hereafter RPLs), which are expected to show conserved expression across sexes and life stages. The second is set of manually curated chemosensory genes (including odorant receptors, gustatory receptors, odorant-binding proteins), which are expected to experience more antagonistic selection and, therefore, exhibit more variable decoupling. As a first step to examining variation in decoupling among genes belonging to different functional categories, we calculated average expression level for each gene in each stage/sex. We then overlaid RPL and chemosensory gene-expression correlation plots on transcriptome-wide plots for each metamorphic event and between the sexually dimorphic adults (Figure 3A). Whereas all RPL genes have nearly identical expression levels in comparisons between the sexes or stages (e.g., those that fall on the dotted line), among-stage/sex correlations are much more variable for chemosensory genes. While several chemosensory genes fall along the dotted line (equal levels of expression), others are among the most decoupled genes in the transcriptome (e.g., those falling at the edges of the transcriptome-wide cloud of points). Furthermore, chemosensory genes have higher variability (e.g., longer tails) and significantly higher levels of gene-expression decoupling than RPLs in 3 out of 4 comparisons (Figure 3B; Mann-Whitney U test $P < 1.0 \times 10^{-3}$) with the exception of the aposematic vs. dispersing larvae comparison ($P = 0.90$). Overall, these results are consistent with a scenario in which selection has favored coupling for some chemosensory genes and stage-specific decoupling for others.

To gain additional insight into how chemosensory gene expression changes across the life cycle, we created a heat map of stage-specific expression for each gene (Figure 3C). Whereas some chemosensory genes are expressed only during a single life stage, others are expressed at moderate to high levels in multiple life stages. To identify potential candidate genes involved in the detection of either stage-specific (decoupled) or shared (coupled) environmental cues, we examined chemosensory genes that were highly expressed (top 10th percentile of normalized expression) in at least one of the three following stages: feeding larvae, adult females, or adult males (Table S3). This analysis revealed several strongly decoupled chemosensory genes expressed in a single stage/sex in addition to genes that were highly expressed by two or more stages and may be targets of concordant selection (Figure 3D). For example, chemosensory genes that are highly expressed by both larvae and females could be involved in detecting host-plant cues. Similarly, genes highly expressed in both sexes of reproductive adults may play roles in conspecific mate recognition. Genes expressed by all three stages may be important for detecting conspecific or host cues that remain critically important throughout the life span. Notably, although all larvae were males, we did not identify any genes that were highly expressed in larval and adult males to the exclusion of adult females, suggesting once again that ecology is a better predictor of gene-expression patterns than sex in this species. Taken together, these analyses reveal that even functionally similar genes can vary widely in the extent to which their expression is coupled or decoupled across distinct developmental stages. Moreover, this variation in gene-expression decoupling can generate predictions regarding potential facilitators and constraints on adaptation to novel ecological conditions.

Implications for the Adaptive Decoupling Hypothesis

According to the ADH, complex life cycles are pervasive in nature because they facilitate the independent evolution of traits expressed at different life stages, thereby removing constraints that may otherwise slow or prevent adaptation to novel environments (3). Although intuitively appealing, evidence for a key prediction of the ADH—that traits are decoupled across metamorphosis—has been mixed (please see (11-15) and references therein). Here we argue that if metamorphosis is an adaptation for optimizing genetic correlations across life stages, genetic decoupling may evolve only for traits that experience antagonistic selection across ontogeny. In other words, the ADH does not necessarily predict that all traits will be decoupled, but rather that trait decoupling should be somewhat predictable given sufficient knowledge of stage-specific selection pressures. To evaluate the predictability of trait decoupling, we took advantage of extensive natural history data for the redheaded pine sawfly to generate *a priori* predictions regarding the decoupling of gene-expression traits. Consistent with the ADH, we found that ecological differences between different life stages (Figure 1) enabled us to accurately predict how the decoupling of gene-expression traits varies across development in the transcriptome (Figure 2) and in a curated set of ecologically important chemosensory genes (Figure 3). We also found that decoupling of gene expression tended to be more pronounced across major and complete metamorphic transitions than between sexes (Figures 2 and 3).

Although our results are consistent with the ADH, additional data are needed to confirm that genes with decoupled expression contribute to stage-specific adaptations. Moving forward, four complementary approaches can reveal whether decoupled gene-expression traits are adaptive, as predicted by the ADH. First, if decoupled genes contribute disproportionately to adaptation, genes exhibiting the most stage-biased expression patterns should also reveal a history of positive selection (e.g., elevated dN/dS relative to the rest of the genome and higher expression level divergence). Although this prediction has been confirmed by several studies for sex-biased genes (23, 24, 38-40), it has rarely been tested in the context of stage-biased expression across metamorphic boundaries (but see (25)). Second, the ADH predicts that genes with decoupled expression will be associated with phenotypes that experience differential selection over ontogeny. This prediction can be evaluated using genetic mapping approaches (e.g., (15, 41)). Third, experimental genomics can connect genetic variants directly to fitness at different life stages (e.g., (42-45)). Following exposure to a selection regime that favors different traits at different ontogenetic stages, the ADH predicts that genes with the most decoupled expression will exhibit the most pronounced allele frequency shifts. Fourth, because each of the first three approaches reveals correlation rather than causation, functional approaches are needed for definitive evidence that putatively adaptive alleles lack antagonistic pleiotropic effects across metamorphic boundaries.

Our finding that genes with decoupled expression contribute to stage-specific adaptation supports the prediction that metamorphosis is an adaptation for enabling trait decoupling. However, to fully evaluate the ADH, comparative data are needed to address two additional predictions: (1) metamorphosis is favored under ecological conditions that result in pervasive antagonistic pleiotropy across the life cycle and (2) metamorphosis facilitates trait decoupling. To disentangle the ecological and genetic correlates of metamorphosis from shared phylogenetic history, comparative tests of the ADH should focus on lineages that contain multiple independent origins of particular metamorphic phenotypes. For example, within holometabolous insects, hypermetamorphosis has evolved multiple times (20). Likewise, gains and losses of complex life cycles have been demonstrated in numerous taxa and is particularly well documented in insects and amphibians (3, 46-50).

Beyond metamorphosis, the logic of the ADH applies to any situation in which pervasive antagonistic selection favors the evolution of reduced genetic correlations. Although theories explaining sexual dimorphism are conceptually similar to the ADH, the evolutionary trajectories of sexually antagonistic selection may differ predictably from those under ontogenetically antagonistic selection. However, few studies have directly compared patterns of sex-biased and stage-biased gene expression. In one notable exception, Perry et al. (2014) evaluated how patterns of sex-biased expression in gonadal tissue change between three developmental stages of *Drosophila melanogaster*. In contrast to our finding that sex-biased expression was modest compared to stage-biased expression (Figure 2), they reported greater decoupling between the sexes than between larvae and prepupae. This apparent discrepancy could be explained by minimal antagonistic selection between wandering *Drosophila* larvae and prepupae, the lack of a distinct molt between these observed stages, or tissue specificity. Given that the intensity of sexually antagonistic selection is likely to be highly variable across taxa and traits (51-53), studies in diverse taxa will be needed to evaluate the prediction that, all else equal, decoupling of stage-biased expression consistently exceeds sex-biased expression. A final layer of complexity is that if the genetic targets and/or intensity of sexually antagonistic selection vary across an organism's life cycle, patterns of sex-biased gene expression may also be decoupled over ontogeny. In support of this prediction, Perry et al. (2014) reported that 18-30% of sex-biased genes exhibited stage-specific sex-biased expression with 4.5% of genes having opposing sex bias at different stages. Importantly, sex- and stage-specificity impacted evolutionary rates with male-biased genes evolving most rapidly when expressed throughout development while female-biased genes evolved most rapidly when expressed in larvae only. Overall, these results are consistent with the view that sexually antagonistic and stage-specific selection can lead to predictable differences in patterns of gene expression and molecular evolution.

Overall, our transcriptomic analysis of a hypermetamorphic and sexually dimorphic sawfly demonstrates that patterns of gene-expression decoupling can be surprisingly predictable. These findings shed light on seemingly contradictory results reported in previous tests of the ADH and set the stage for follow-up studies on the genetic basis of stage-specific adaptation. However, rigorously testing the ADH and better understanding its relation to sexual dimorphism will ultimately require analyses of gene-expression decoupling in diverse taxa that vary in metamorphic and sexually dimorphic phenotypes. To gain maximal insight from decoupling analyses in other taxa, *a priori* predictions derived from in-depth knowledge of organismal ecology are essential. Although much work remains, these data are critical to understanding why metamorphosis is one of the most successful developmental strategies on the planet.

Materials and Methods

To characterize genetic decoupling between the life stages and sexes and across different functional categories of genes, we designed a sampling scheme that optimized our ability to detect changes in gene expression across a wide range of gene-expression levels, while balancing the cost of library preparation and high-coverage RNA sequencing. To reduce noise stemming from among-population variation, we obtained tissues from a laboratory colony established from *N. lecontei* individuals that were originally collected in 2016 from a single, isolated *Pinus mugo* bush in Lexington, KY, USA (38.043602°N, 84.496549°W). To minimize both environmental effects and opportunity for adaptation to the laboratory environment, we reared all individuals under uniform laboratory conditions for a minimum of one, but no more than two, generations. To ensure that we did not confound metamorphic changes in expression with changes in sex-biased expression that occur during development (39), we restricted all among-life-stage comparisons to haploid males. Only in the adult stage did we directly compare male and female expression.

To compare whole-body patterns of expression to tissue-specific patterns of expression and to increase our ability to detect genes with tissue-specific expression, we prepared separate libraries for head and body tissues dissected from larvae. Additionally, because previous studies have found that the expression of sex-biased genes is highly tissue specific (22), we prepared libraries from six different tissues dissected from male and female adults: the leg, genital, and thoracic tissues from the body and the mouthpart, antennal, and the remaining head tissue. In total, we prepared 2-4 biological replicates for each unique life-stage/tissue combination, for a total of 77 barcoded libraries (Table S1). We then pooled these libraries and sequenced them on a single run of a PE150 Illumina Hiseq4000. After filtering, we obtained over 766 million high-quality reads, with a minimum of 13 million reads per life stage/tissue combination (Table S1).

We utilized these reads to create a genome-guided *de novo* transcriptome (Table S2). Functional annotation of this transcriptome produced 9,304 genes with high similarity to predicted *N. lecontei* or insect genes, which we retained for all downstream analyses (Table S2). Using programs included in the trinity pipeline in combination with custom scripts, we determined normalized gene expression counts, completed principle component analyses, identified differentially expressed genes, and determined highly expressed genes for different tissues and life stages. Additional information is available in SI Materials and Methods.

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