Received: 29 August 2020 | Revised: 10 June 2021 | Accepted: 11 June 2021

DOI: 10.1111/mec.16041

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Support for the adaptive decoupling hypothesis from whole-transcriptome profiles of a hypermetamorphic and sexually dimorphic insect, *Neodiprion lecontei*

Danielle K. Herrig | Kim L. Vertacnik | Anna R. Kohrs | Catherine R. Linnen

Department of Biology, University of Kentucky, Lexington, Kentucky, USA

Correspondence
Danielle K. Herrig, Department of Biology, University of Kentucky, Lexington, KY, USA.
Email: danielle.herrig@uky.edu

Funding information
Kentucky Science and Education Foundation, Grant/Award Number: KSEF-3492-RDE-019; U.S. Department of Agriculture, Grant/Award Number: 2016-67014-2475

Abstract

Though seemingly bizarre, the dramatic morphological and ecological transformation that occurs when immature life stages metamorphose into reproductive adults is one of the most successful developmental strategies on the planet. The adaptive decoupling hypothesis (ADH) proposes that metamorphosis is an adaptation for breaking developmental links between traits expressed in different life stages, thereby facilitating their independent evolution when exposed to opposing selection pressures. Here, we draw inspiration from the ADH to develop a conceptual framework for understanding changes in gene expression across ontogeny. We hypothesized that patterns of stage-biased and sex-biased gene expression are the product of both decoupling mechanisms and selection history. To test this hypothesis, we characterized transcriptome-wide patterns of gene-expression traits for three ecologically distinct larval stages (all male) and adult males and females of a hypermetamorphic insect (*Neodiprion lecontei*). We found that stage-biased gene expression was most pronounced between larval and adult males, which is consistent with the ADH. However, even in the absence of a metamorphic transition, considerable stage-biased expression was observed among morphologically and behaviourally distinct larval stages. Stage-biased expression was also observed across ecologically relevant Gene Ontology categories and genes, highlighting the role of ecology in shaping patterns of gene expression. We also found that the magnitude and prevalence of stage-biased expression far exceeded adult sex-biased expression. Overall, our results highlight how the ADH can shed light on transcriptome-wide patterns of gene expression in organisms with complex life cycles. For maximal insight, detailed knowledge of organismal ecology is also essential.

KEYWORDS
adaptation, antagonistic pleiotropy, differential gene expression, evolutionary constraint, holometabolous insects, metamorphosis, transcriptomics

1 | INTRODUCTION

An estimated 80% of animal species have complex life cycles wherein metamorphosis separates two or more discrete, post-embryonic life stages (Wilbur, 1980). 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 (Bishop et al., 2006). This

change produces specialized stages optimized for distinct ecological niches (Benesh et al., 2013; Bishop et al., 2006; Ebenman, 1992; Istock, 1967; Moran, 1994). One explanation for the prevalence of complex life cycles is that independent adaptations at the different stages allow each stage to specialize: one optimized for growth and another for reproduction (Bryant, 1969; Moran, 1994; Truman & Riddiford, 2019). Central to this explanation is the idea that pleiotropy creates genetic correlations across ontogeny that constrain evolution when traits beneficial for one stage are detrimental to another (Haldane, 1932). The adaptive decoupling hypothesis (ADH) proposes that metamorphosis evolved as a mechanism for breaking developmental and genetic links, thereby facilitating their independent evolution when opposing selection pressures are experienced by different life stages (Ebenman, 1992; Haldane, 1932; Moran, 1994).

Two key predictions of the ADH are that metamorphosis (i) reduces genetic correlations and (ii) increases evolutionary independence between traits expressed in different life stages. Empirical tests of these predictions using various species have been mixed, with some studies supporting the ADH (Aguirre et al., 2014; Anderson et al., 2016; Goedert & Calsbeek, 2019; Medina et al., 2020; Parichy, 1998) and others refuting (Crean et al., 2011; Fellous & Lazzaro, 2011; Watkins, 2001). To further complicate matters, different studies have used different criteria for evaluating the ADH. For example, whereas some studies have interpreted genetic correlations significantly different from zero ($r \neq 0$) as evidence against the ADH (Fellous & Lazzaro, 2011), others have argued that genetic correlations that are less than one ($|r| < 1$) support the ADH (Aguirre et al., 2014; Phillips, 1998) (also see arguments in Collet & Fellous, 2019; Goedert & Calsbeek, 2019, and references therein). The rationale for the latter interpretation is that the ADH does not require that genetic constraints are absent, only that they are reduced by metamorphosis relative to scenarios lacking metamorphosis (Aguirre et al., 2014; Collet & Fellous, 2019; Goedert & Calsbeek, 2019; Moran, 1994). The ADH also does not require that all traits expressed at different life stages evolve independently, only that those experiencing opposing selection pressures are able to resolve antagonism more readily when separated by metamorphosis (Moran, 1994). We note, however, that traits need not experience antagonistic selection to exhibit genetic and evolutionary independence, as the ADH predicts that the developmental mechanisms underlying metamorphosis will decouple many traits at once (Bossuyt & Milinkovitch, 2000; Moran, 1994).

One potentially powerful approach for testing the ADH is to compare trait correlations within and among life stages. The ADH predicts that traits separated by metamorphosis will have greater genetic and evolutionary independence, on average, than traits expressed at different time points within a single life stage (Moran, 1994; Watkins, 2001). Importantly, different traits are likely to vary greatly in both their underlying developmental mechanisms (how immature phenotypes develop into adult phenotypes) and historical selection pressures (the extent to which immature and adult phenotypes have been selected in opposing directions). Variation in these two factors will determine the observed genetic and evolutionary correlations among traits expressed at different life stages. To account for this variation, testing predictions of the ADH may require data from a large and diverse collection of traits (Collet & Fellous, 2019). However, most ADH tests to date have investigated only a handful of traits, and only rarely have direct comparisons been made between metamorphic and nonmetamorphic life-stage transitions (but see Aguirre et al., 2014; Hilbish et al., 1993; Phillips, 1998; Watkins, 2001).

Whole-transcriptome gene-expression data obtained from multiple life stages provide an ideal collection of traits for evaluating predictions of the ADH (Collet & Fellous, 2019). 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 thousands of quantitative traits, all measured in comparable units of gene expression. Third, expressed genes cover a wide range of biological functions with differing selection pressures. Finally, genes are expressed in diverse tissues that should vary in the extent to which they are developmentally decoupled. For example, whereas some adult tissues develop from pre-existing immature structures (e.g., via repatterning of immature precursors), others develop de novo from undifferentiated embryonic cells (e.g., via developmental compartmentalization) (Alberch, 1982; Moran, 1994; Parichy, 1998; Watkins, 2001). Despite this potential, few studies have evaluated patterns of gene expression in light of the ADH (but see Fellous & Lazzaro, 2011; Jacobs et al., 2006; Saenko et al., 2012; Wollenberg Valero et al., 2017).

One way to gain insight into how metamorphosis impacts the genetic and evolutionary independence of gene-expression traits is to evaluate patterns of differential gene expression among distinct developmental stages. In particular, a pattern of stage-biased gene expression may be an indicator that a gene-expression trait evolved in response to ontogenetically antagonistic selection in the past (see Connallon & Knowles, 2005; Mank, 2017; Mank et al., 2008 for an analogous argument in the context of sexually antagonistic selection). If gene-expression traits separated by metamorphosis are less constrained by genetic correlations than traits that are not separated by metamorphosis, life stages that are separated by metamorphosis should show a corresponding increase in transcriptome-wide levels of differential expression. Notably, within a developmental transition type (i.e., metamorphic or nonmetamorphic), the magnitude of stage-biased expression may vary considerably across gene-expression traits due to variation in: (i) selection history (stronger antagonistic selection will generate greater differential expression) and (ii) tissue specificity (tissues with greater developmental decoupling will evolve differential expression more readily).

Although any organism with a complex life cycle can be used to test these predictions, some of the most dramatic metamorphic transformations occur in holometabolous insects, whose defining characteristic is a distinct pupal stage that separates two completely different body plans (Gilbert et al., 1996; Heming, 2003; Kristensen, 1999). The independence between larval and adult traits

may explain, in part, why holometabolous insects are one of the most evolutionarily successful and diverse lineages on the planet (Rainford et al., 2014; Truman & Riddiford, 2019; Wigglesworth, 1954). In some holometabolous lineages, pronounced ecological and morphological transformations also occur between successive larval instars. This phenomenon, known as hypermetamorphosis (Belles, 2011), provides an opportunity to evaluate how genetic correlations and evolutionary independence across ontogeny vary as a function of different types of developmental transitions.

Looking beyond metamorphosis, mechanisms for trait decoupling may evolve in any scenario in which a single genome expresses multiple distinct phenotypes that are subject to opposing selection pressures (Abbott, 2010; Collet & Fellous, 2019; Lande, 1980; Morris et al., 2013; Pennell et al., 2018). Arguably the best-studied scenario of trait decoupling evolving in response to antagonistic pleiotropy involves sexually dimorphic males and females (Mank, 2017). Just as stage-limited gene expression may arise from opposing selection pressures across ontogeny, sex-biased gene expression is thought to be the product of sexually antagonistic selection (Connallon & Clark, 2013; Connallon & Knowles, 2005; Ellegren & Parsch, 2007). However, the developmental and genetic mechanisms that decouple traits between life stages are likely to be different from the mechanisms that decouple traits between the sexes (Connallon & Knowles, 2005; Mank et al., 2008), as are the consequences of ontogenetically vs. sexually antagonistic selection (Collet & Fellous, 2019). Direct comparisons between sex-biased gene expression and stage-biased gene expression can inform how selection and decoupling mechanisms interact to produce observed patterns of trait evolution. However, few studies have directly compared patterns of sex-biased and stage-biased gene expression (but see Ometto et al., 2011; Perry et al., 2014).

To gain greater insight into the factors that shape variation in gene-expression traits, we take advantage of a hypermetamorphic and sexually dimorphic species of insect with a well-characterized ecology, the redheaded pine sawfly (*Neodiprion lecontei*, order: Hymenoptera; family: Diprionidae) (Figure 1). In addition to complete metamorphosis, there are two pronounced transitions that occur within the larval stage of the redheaded pine sawfly (Figure 1a). The first transition is a relatively minor shift from a "cryptic" to an "aposematic" feeding larval morph (hereafter, "minor transition"). The cryptic morph is lightly pigmented, ingests only the exterior of pine needles and retreats to the base of the needle when predators are near. By contrast, the aposematic morph is heavily pigmented and ingests the entire needle to sequester the toxic pine resins for use in antipredator defensive displays (Lindstedt et al., 2020). A more striking transition occurs when the aposematic morph moults into a "dispersing" final instar (hereafter, "major transition"). The dispersing morph is solitary, nonfeeding, less intensely pigmented, and migrates to the litter or soil to spin a cocoon. Complete metamorphosis occurs within the cocoon. The nonfeeding adult stage is dedicated to reproduction. Sexually dimorphic adults are specialized for sex-specific tasks. Males are adept 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 (Anderbrant, 1993).

The hypermetamorphosis and sexual dimorphism exhibited by the redheaded pine sawfly, combined with ecological changes throughout its ontogeny, provide an excellent opportunity to evaluate gene-expression traits in light of the ADH. We hypothesize that patterns of differential expression among stages will be determined by a combination of ecology and development (Figure 1b). To test this hypothesis, we performed whole-transcriptome sequencing of males at four distinct *N. lecontei* life stages (three larval stages and adult) as well as adult females. With these data, we asked how the presence and magnitude of differential gene expression varies as a function of: (i) the type of developmental transition (changes in ecology and/or metamorphosis), (ii) ontogenetic changes vs. sexual dimorphism, and (iii) the functional roles of individual genes. Consistent with our predictions, our data indicate that both developmental transition type and gene function can have a profound impact on patterns of stage-biased expression.

2 | MATERIALS AND METHODS

2.1 | Tissue dissection in laboratory-reared *N. lecontei*

To obtain a large number of individuals for tissue dissection, we established laboratory colonies of *Neodiprion lecontei* from a single population of larvae collected in 2016 from a *Pinus mugo* bush in Lexington, KY, USA (38.043602°N, 84.496549°W). We reared colonies on *Pinus* foliage using standard rearing protocols (Bendall et al., 2017; Harper et al., 2016) for a minimum of one generation before collecting tissues. A summary of the tissues we dissected for each stage can be found in Figure S1. To ensure that all male tissues were haploid, we collected all larvae and adult males from the progeny of virgin females. Prior to dissections, we prepared frozen dissection dishes by freezing 1× PBS (phosphate-buffered saline) in glass petri dishes at −20°C overnight. To obtain larval tissues, we collected instars of haploid male larvae from rearing boxes containing *Pinus* foliage and kept them in a 22°C growth room with an 18:6-hr (L:D) photoperiod. We immediately placed live larvae on frozen dissection dishes, removed the heads from the bodies, and flash-froze both tissues on dry ice prior to storage at −80°C.

To obtain adult tissues, we collected cocoons from rearing boxes and stored them in individual gelatin capsules in the same growth room as the larvae until emergence. Because adults are nonfeeding and do not live long at room temperature, we transferred adults to a 4°C refrigerator to induce a "chill-coma" within 30 hr of emergence. This prolonged life until tissues could be collected (storage time range: 2–20 days; mean: 14 days). We chose a maximum duration of cold storage of 20 days because this duration is short enough to ensure adults are still fully viable and fertile. Importantly, *N. lecontei* adults recover from the 4°C chill-coma within minutes and immediately resume normal mating and host-seeking behaviours. Although

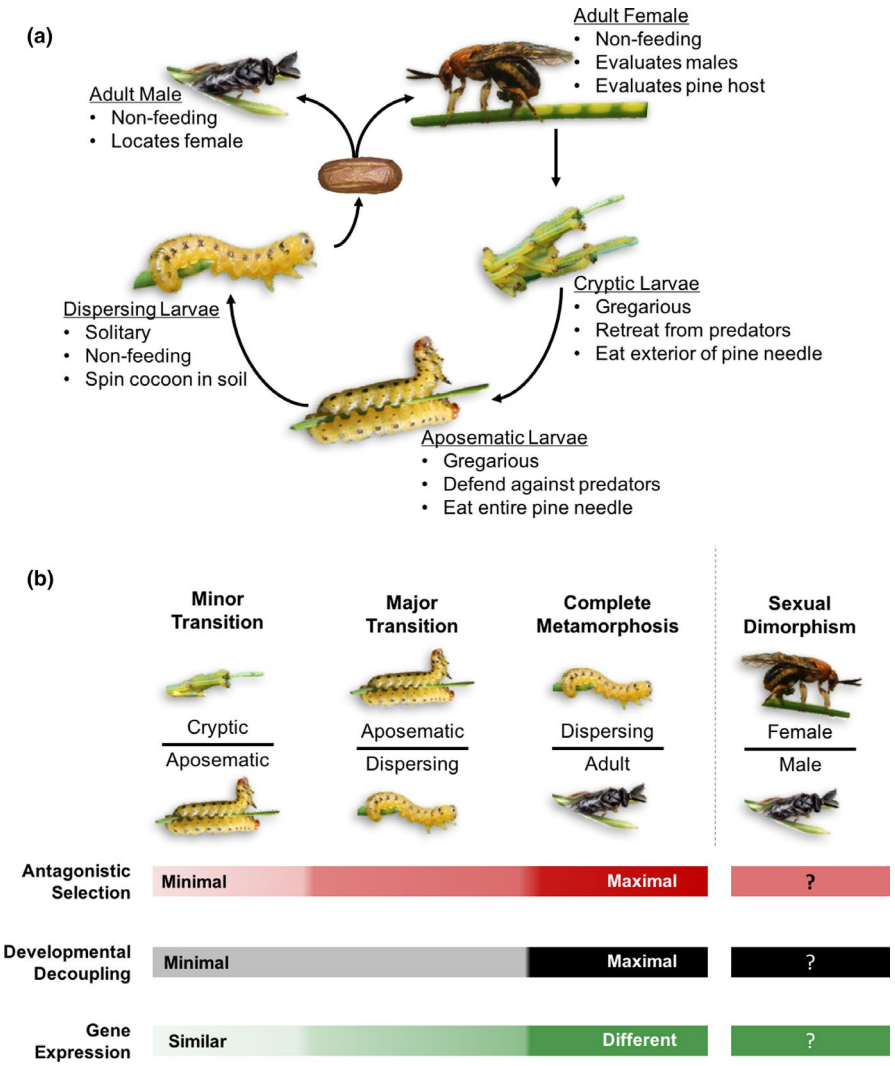

FIGURE 1 Ecological and developmental changes during *N. lecontei* development generate predictions regarding differential gene expression between the sexes and across different metamorphic transitions. (a) The life cycle of the redheaded pine sawfly includes two distinct feeding larval stages, a non-feeding larval stage, and sexually dimorphic adults. Bullet points highlight key traits that change across development. (b) Based on this life cycle, important life cycle transitions include: minor transition (between cryptic and aposematic larval stages), major transition (between the aposematic and dispersing larval stages), and complete metamorphosis (between the larval and adult stages). The gradients represent our predictions regarding the amount of antagonistic selection, developmental decoupling, and differential gene expression that occur between life stages and sexes. Question marks (?) for sexual dimorphism reflect uncertainties regarding how developmental decoupling mechanisms and selection differ between ontogeny and sex determination [Colour figure can be viewed at wileyonlinelibrary.com]

adults are likely to encounter low temperatures in the field, it is nevertheless possible that cold storage could contribute to differences in gene expression between adults and larvae (Freda et al., 2017; Pujol-Lereis et al., 2014; Zhao et al., 2021). To reduce this effect, we warmed adults to room temperature (22°C) and enclosed them in a mesh cage containing *Pinus* seedlings for a minimum of 15 min to ensure that they exhibited normal host- and mate-seeking behaviour. Then, we enclosed each individual in a small plastic cup and sedated it via cold exposure (dry ice) for ~1 min. We placed the sedated adult on frozen dissection dishes to harvest six tissues: antennae, mouthparts, heads (without antennae and mouthparts), legs, thorax and the terminal segment of the abdomen (which includes the ovipositor in females or copulatory organ in males). We flash-froze these tissues and stored them at −80°C until RNA extraction.

2.2 | RNA extraction, library preparation and sequencing

We extracted RNA from all tissues with RNeasy Plus Micro and Mini Kits (Qiagen). To account for family-level variation in gene-expression traits, each RNA extraction contained tissues from a single laboratory-reared family (each represented by 2–18 individuals, depending on the tissue). Prior to starting standard kit protocols, we disrupted tissues using a TissueLyser LT bead mill (Qiagen) and 5-mm stainless steel beads. We disrupted tissues (no buffer) for up to four 30-s periods of 60 oscillations s^{-1}, placing tissues on dry ice between periods to ensure that the RNA did not degrade during the disruption process. We then quantified RNA using Quant-iT RNA Assay Kits (Invitrogen by ThermoScientific) and assessed quality using a 2100 Bioanalyzer and RNA 6000 Nano Kits (Agilent).

To prepare RNA-sequencing (RNA-seq) libraries for Illumina sequencing, we used a TruSeq Stranded mRNA High Throughput Kit (Illumina), following the manufacturer's protocols. For larval tissues, we made each library from RNA extracted from members of a single family. For adult tissues, which required more individuals to obtain sufficient material, we used one to three families per library (with equal RNA contributions from each family). For most tissues, we made libraries for four biological replicates, with different families in each replicate. The exceptions were male copulatory organs (for which only three high-quality biological replicates could be produced) and male thoraxes (for which only two high-quality biological

replicates could be produced), resulting in a total of 77 libraries (Table S1). We quantified the resulting cDNA using Quant-iT DNA Assay Kits (Invitrogen by ThermoScientific) and evaluated quality with a 2100 Bioanalyzer and DNA 1000 Kits (Agilent). We then pooled libraries that passed quality inspection and sequenced this pool with 150-bp paired-end reads on an Illumina HiSeq4000 at the University of Illinois at Urbana-Champaign Roy J. Carver Biotechnology Center.

2.3 | Processing of RNA-seq data to produce a guided *de novo* transcriptome

To demultiplex and quality-trim raw reads, we used TRIMMOMATIC version 0.36 (Bolger et al., 2014) and a phred-based quality cut-off score of 30. We also removed any remaining TruSeq indexed universal adapter present at the beginning of reads using FASTX_CLIPPER version 0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/). After filtering, we used TOPHAT2 (Kim et al., 2013) to map retained reads to the *N. lecontei* version 1.0 assembly (Vertacnik et al. 2016). While an annotated genome was available to us (NCBI *Neodiprion lecontei* annotation release 100), the annotation was based on limited tissue sampling and RNA-seq reads. Therefore, to capture all potential transcripts, we used the mapped reads to produce a genome-guided *de novo* transcriptome assembly using TRINITY version 2.4.0 (Haas et al., 2013). Because TRINITY is known to overestimate the actual number of contigs present in transcriptomes (https://github.com/trinityrnaseq/trinityrnaseq/wiki), we performed additional filtering to retain meaningful contigs. First, we used CD-HIT version 4.6 (W. Li & Godzik, 2006) to identify nonredundant contigs (minimum of 200 bp), BOWTIE2 version 2.3 (Langmead & Salzberg, 2012) to map the reads, and RSEM version 1.2.31 (B. Li & Dewey, 2011) and custom Python (version 3.5.1) scripts to identify contigs with at least one transcript per million in at least two biological replicates.

To functionally annotate contigs that were retained after filtering, we performed two sets of BLASTX version 2.6 (Altschul et al., 1990) searches. First, we used predicted *N. lecontei* nonredundant proteins available on NCBI and a set of manually curated *N. lecontei* odorant-binding proteins (OBPs) , odorant receptors (ORs) and gustatory receptors (GRs) (Vertacnik et al. 2021). For transcripts that did not map with 90% identity to putative *N. lecontei* genes, we performed an additional BLAST search against the insect nonredundant protein database (Pruitt, 2004) (e-value of 0.001). We used these annotations to collapse the TRINITY isoforms into genes for all downstream analyses.

2.4 | Comparing differential-expression patterns among different developmental transitions

To determine whether stage-biased gene expression differs among different types of ontogenetic transitions and to compare stage-biased gene expression to sex-biased gene expression, we first used BOWTIE2 (Langmead & Salzberg, 2012) to align reads to the curated *de novo N. lecontei* transcriptome. We estimated the abundance of each transcript using RSEM via the TRINITY package utility program ALIGN_AND_ESTIMATE_ABUNDANCE.PL (Haas et al., 2013; Li & Dewey, 2011). Using the utility program ABUNDANCE_ESTIMATES_TO_MATRIX.PL, we created a complete count matrix of transcript abundance, normalized gene expression for comparisons of relative abundances (transcripts per million), and normalized expression correcting for highly expressed genes to obtain absolute abundances (TMM, third-quartile normalization) (Haas et al., 2013).

Next, we compared whole-transcriptome profiles of different life stages and sexes in two ways. First, to visualize overall gene expression trends across all tissues and life stages, we conducted a principal component analysis (PCA) using the PtR scripts within the TRINITY package (Haas et al., 2013). Second, to quantify how the prevalence and magnitude of stage-bias varies across different life-stage transitions and between the sexes, we identified differentially expressed genes (DEGs) for each of the four comparisons illustrated in Figure 1b. To identify DEGs, we used the TRINITY utility program RUN_DE_ANALYSIS.PL to implement DESEQ2 version 1.8, which fits a generalized linear model (GLM) to the read count data (Haas et al., 2013; Love et al., 2014), with biological replicates grouped by life stage and sex (cryptic larvae, aposematic larvae, wandering larvae, adult males and adult females). After model fitting, we performed two types of hypothesis tests: (i) Wald tests to identify DEGs between life stage/sex pairs corresponding to our four comparisons of interest (three developmental transitions and adult males vs. adult females), and (ii) likelihood ratio tests to identify DEGs across any of the five groups (for use in "Comparing differential-expression patterns among different functional categories and genes"). Both types of tests used Benjamini–Hochberg correction for multiple testing.

For a gene to be considered differentially expressed among life stages or sexes, we required an adjusted p-value <.05 and a $\log_2$(fold change) >1 or <−1 (corresponding to a doubling or halving of gene expression, respectively). To determine whether the proportion of genes that were differentially expressed varied among the four developmental/sex comparisons, we used global and pairwise Fisher's exact tests and accounted for multiple testing with the "fisher.multcomp" function from the RVAIDEMEMOIRE version 0.9–78 R package (implemented in R 4.0.3). We also computed Clopper–Pearson 95% confidence intervals for each proportion using the "BinomCI" function from the DESCTOOLS version 0.99.40 R package. To determine if the absolute magnitudes of differential expression for DEGs ($|\log_2(FC)|$) differed across the four comparisons, we used a Kruskal–Wallis rank-sum test ("kruskal.test" function) followed by Dunn's post hoc test for multiple pairwise comparisons ("dunnTest" function in the FSA version 0.8.32 R package). To evaluate the potential impact of tissue sampling on our results, we quantified the number of DEGs and magnitude of expression differences among life stages and sexes in two data sets: all tissues and heads only.

We then further explored the relationship between tissue specificity and stage-biased/sex-biased gene expression. To identify genes that were differentially expressed among tissues for a particular comparison, we used DESEQ2 to fit additional GLMs to read

count data grouped by tissue type. We fit three separate GLMs, corresponding to tissues present in comparisons among: larval stages (larval heads and bodies), larval and adult males (all male tissues), and adult males and females (all adult tissues). For each GLM, we used a likelihood ratio test to identify DEGs among tissues in that comparison. Finally, for each pairwise life stage (or sex) comparison (Figure 1b), we identified genes that were differentially expressed: between life stages/sexes only, among tissues sampled for those stages, and both between life stages/sexes and among tissues.

2.5 | Comparing differential-expression patterns among different functional categories and genes

For comparing functional categories of genes, we first conducted a global test of differential expression across all life stages and sexes using the likelihood ratio test implemented in DESEQ2 (see above). We then used these data to evaluate the relationship between gene function and patterns of stage-biased (or sex-biased) expression in two ways. First, we used GOSEQ version 1.36 to identify gene ontology (GO) categories that were over- and underrepresented in the list of DEGs identified in the global test (implemented via the TRINITY utility program RUN_GOSEQ.PL). We then used REVIGO version HTML4 to visualize these gene categories and present the graph of Biological Processes retained after this analysis (Supek et al., 2011). Second, we examined individual genes and asked whether those that were highly differentially expressed among life stages/sexes had known functions related to changing ecological selection pressures across ontogeny (Figure 1a).

2.6 | Case study: Chemosensory genes vs. ribosomal protein L genes

As a complement to the global gene expression analyses, we used a case study approach to compare two groups of genes that we expected, a priori, to experience different selective regimes. For this analysis, we explored patterns of gene expression for 68 manually curated chemosensory genes (including odorant receptors, gustatory receptors, odorant-binding proteins; Vertacnik et al. 2021), which may experience variable selection across development (Figure 1a). We therefore expected these genes to exhibit more extreme and more variable stage/sex bias compared to a group of housekeeping genes (the *ribosomal protein L* genes, hereafter RPLs) of a similar family size (40 genes). To visualize how patterns of stage- and sex-biased expression of our "variable" and "uniform" selection categories compare to the rest of the transcriptome, we first condensed each gene in the transcriptome to a single expression value per stage/sex. For each gene, we calculated the $\log_2$ of the average normalized expression level across all tissues and replicates for each life stage. We then overlaid RPL and chemosensory gene-expression values on transcriptome-wide values for each developmental transition and between the sexually dimorphic adults. To determine whether the distribution of expression differences between the stages/sexes differed between RPLs and chemosensory genes, we first calculated the absolute value of the average $\log_2$-fold change for each comparison with DESEQ2. Then, we used the "wilcox.test" function in R to implement nonparametric Wilcoxon rank-sum tests (Mann–Whitney *U* tests) to test for differences between the two gene families.

Because our analyses revealed highly variable patterns of stage- and sex-biased expression across chemosensory genes (see below), we went a step further to identify chemosensory genes with the highest and lowest levels of expression in each life stage. First, we produced heat maps to visualize variation in patterns of gene expression among stages/sexes across individual chemosensory genes. Because there was substantial variation in the maximum expression of chemosensory genes, we first used the scale function so that the total expression of each gene was equal and the heat map function to visualize the normalized expression levels using R 3.3.2 (R Core Team, 2016). Second, we identified chemosensory genes that had expression levels in the top 10th percentile in the feeding larvae, adult males and adult females to further pinpoint genes with divergent or shared expression across life stages.

3 | RESULTS

3.1 | Sequencing and transcriptome assembly

In total, we obtained over 787 million high-quality paired-end 150-bp reads, with an average of 39.4 million reads per tissue type (Table S1; accession numbers will become available upon publication). After filtering, we retained over 766 million reads (Table S2). An average of 88.4% of these reads mapped to the *Neodiprion lecontei* genome. Our genome-guided *de novo* transcriptome assembly identified 132,243 contigs. After filtering, we retained 58,353 contigs. Across our 77 libraries, average mapping rate to these contigs was 85.5% (range: 74.9%–91.6%). Our putatively annotated transcriptome contained 16,714 contigs representing 9,304 predicted insect genes. Using these 16,714 contigs as the transcriptome, a high mapping rate was maintained across all libraries (mean: 76.7%; range: 60.5%–88.6%).

3.2 | Patterns of stage-biased expression depend on both developmental decoupling and ontogenetic changes in ecology

Consistent with our prediction that differences in gene-expression traits would be most pronounced between ecologically dissimilar and developmentally decoupled life stages (Figure 1b), stages separated by the major transition and by complete metamorphosis were clearly distinct as shown in the first principal component (PC1) of our PCA, which captured 18.33% of the variation (Figure 2a). By contrast, there were no clear distinctions between cryptic and aposematic larvae (minor transition) or between adult males and females along either of the first two gene-expression PCs. PC2 primarily

separated tissues within each life stage and captured 8.52% of the variation (Figure 2a). Along this axis, gene expression in larval and adult heads was clearly distinct from expression in other tissues, as was expression in the adult antennal tissues. Similar trends were evident in PCAs for subsets of the data (head only, nonhead only, larvae only and adults only; Figure S2). Based on these results, we analysed gene-expression differences among stages/sexes in two ways: all tissues combined and heads only (Tables S3,S4 respectively).

Regardless of whether we looked at all tissues or heads only, both the magnitude ($\log_2$ fold-change) and significance (false discovery rate [FDR]-adjusted *p*-value) of stage-biased expression increased in accordance with our predictions (Figure 1b). Specifically, both the horizontal and vertical spread of volcano plots—representing maximum absolute $\log_2$ fold-change and minimum adjusted *p*-value, respectively—increased from minor transition to major transition to complete metamorphosis (Figure 2b,s). Qualitatively, the magnitude and significance of sex-biased expression (adult males vs. adult females) was most similar to patterns of stage-biased expression between cryptic and aposematic larvae (minor transition, Figure 2b,c).

For the combined analysis of all tissues, the proportions of genes that were differentially expressed between life stages or sexes in the four comparisons were: 0.016 for the minor transition; 0.20 for the major transition; 0.20 for complete metamorphosis; and 0.045 for adult sex (Figure 2b, Table 1; Table S3). Proportions of DEGs differed among the four comparisons (Fisher's exact test; $p = 5.0 \times 10^{-4}$) and in all post hoc pairwise tests (FDR-corrected $p < 1 \times 10^{-24}$), except for major transition vs. complete metamorphosis ($p = .48$). The median magnitudes (absolute value of $\log_2$ fold-change) of DEGs for each of the four comparisons were: 2.03 for the minor transition; 2.09 for the major transition; 2.47 for complete metamorphosis; and 2.05 for adult sex. The fold-change of DEGs differed significantly among the four comparisons (Kruskal–Wallis $p = 2.9 \times 10^{-8}$), but the only comparisons that were significantly different in post hoc pairwise tests were major vs. complete and complete vs. sex (FDR-adjusted $p = 1.5 \times 10^{-7}$ and 1.0×10^{-4}, respectively).

For the analysis of head tissue only, the proportion of DEGs were: 0.022 for the minor transition; 0.14 for the major transition; 0.26 for complete metamorphosis; and 0.040 for adult sex (Figure 2c; Table 1; Table S4). The proportion of DEGs differed significantly among the four comparisons (Fisher's exact test; $p = 5.0 \times 10^{-4}$) and in all post hoc pairwise tests (FDR-corrected $p < 1 \times 10^{-8}$), including the major transition vs. complete metamorphosis comparison ($p < 1 \times 10^{-70}$). The median DEG magnitudes for the four comparisons were: 1.93 for the minor transition; 2.09 for the major transition; 2.66 for complete metamorphosis; and 1.81 for adult sex. The magnitude of differential expression differed significantly among the four comparisons (Kruskal–Wallis $p = 9.7 \times 10^{-16}$) and in all post hoc pairwise tests (FDR-adjusted $p < .0024$), except for minor vs. major and minor vs. sex (FDR-adjusted $p = .52$ and.14, respectively).

Overall, compared to the "all-tissue" DEG results, the "head only" results revealed a much stronger signal for a difference between the major transition and complete metamorphosis in terms of the both the proportion and the magnitude of DEGs (Figure 2b vs. 2b; Table 1 and statistical results summarized above). This difference suggests that differences in tissue collection across life stages (Figure S1) contributed to observed patterns of stage-biased gene expression. Consistent with this interpretation, life stages separated by metamorphosis had the greatest number of genes that were differentially expressed both among life stages and among tissues (Figure S3). Nevertheless, the global PCA (Figure 2a) and PCAs for subsets of the data (Figure S2) indicate that life stage (but not sex) explained more variation in gene expression than tissues.

3.3 | Functional categories and genes related to ecological differences among stages exhibit stage-biased expression

Of 13,470 GO categories in our data set, we identified 466 GO categories that were overrepresented (3.5%) and 532 categories that were underrepresented (3.9%) in our DEGs. Genes involved in DNA functions, cellular organization and signalling were underrepresented (Figure 3). Among overrepresented GO categories were those involved in metabolism, translation and moulting. Notably, only the cryptic and aposematic larval stages ingest food while the dispersing larval and adult stages do not, which may correspond to the overrepresentation of metabolism-related GO categories.

Looking more closely at individual genes, the top DEGs (i.e., top ~1%, as judged by the *p*-value in the global life stage/sex comparison) include several genes with functions closely related to changes in ecology across development (Figure 1a; Tables S3 and S4). For example, *esterase FE4-like* (elevated in heads and bodies of aposematic feeding larvae) is a gene thought to be involved in resistance to organophosphate insecticides and may be essential to ingesting and sequestering toxic pine resins (Field et al., 1993; Wei et al., 2019). Additionally, a gene encoding a subunit of the COP9 signalosome complex, which has been implicated in social behaviour and immunity (Tong et al., 2015), is differentially expressed across life stages (Tables S3 and S4). While its expression is detectable in all tissues at fairly high levels (TMM >75), it is elevated in the gregarious feeding larval stages. Putative pigmentation, immunit, and chemosensory genes were also among the top DEGs between life stages, as were several chemosensory genes (Tables S3,S4).

3.4 | Case study: Chemosensory genes have variable levels of stage-bias and sex-bias

We found that the magnitude of stage/sex-biased gene expression for chemosensory genes, some of which may experience variable selection across development, was highly variable (Figure 4a). While several genes had similar levels of expression across life stages (those falling along the dotted lines), others were among the most DEGs in the transcriptome (those falling at the edges of the transcriptome-wide cloud of points, see also Tables S3, S4). This contrasted with a family of housekeeping genes—the RPLs—that should experience

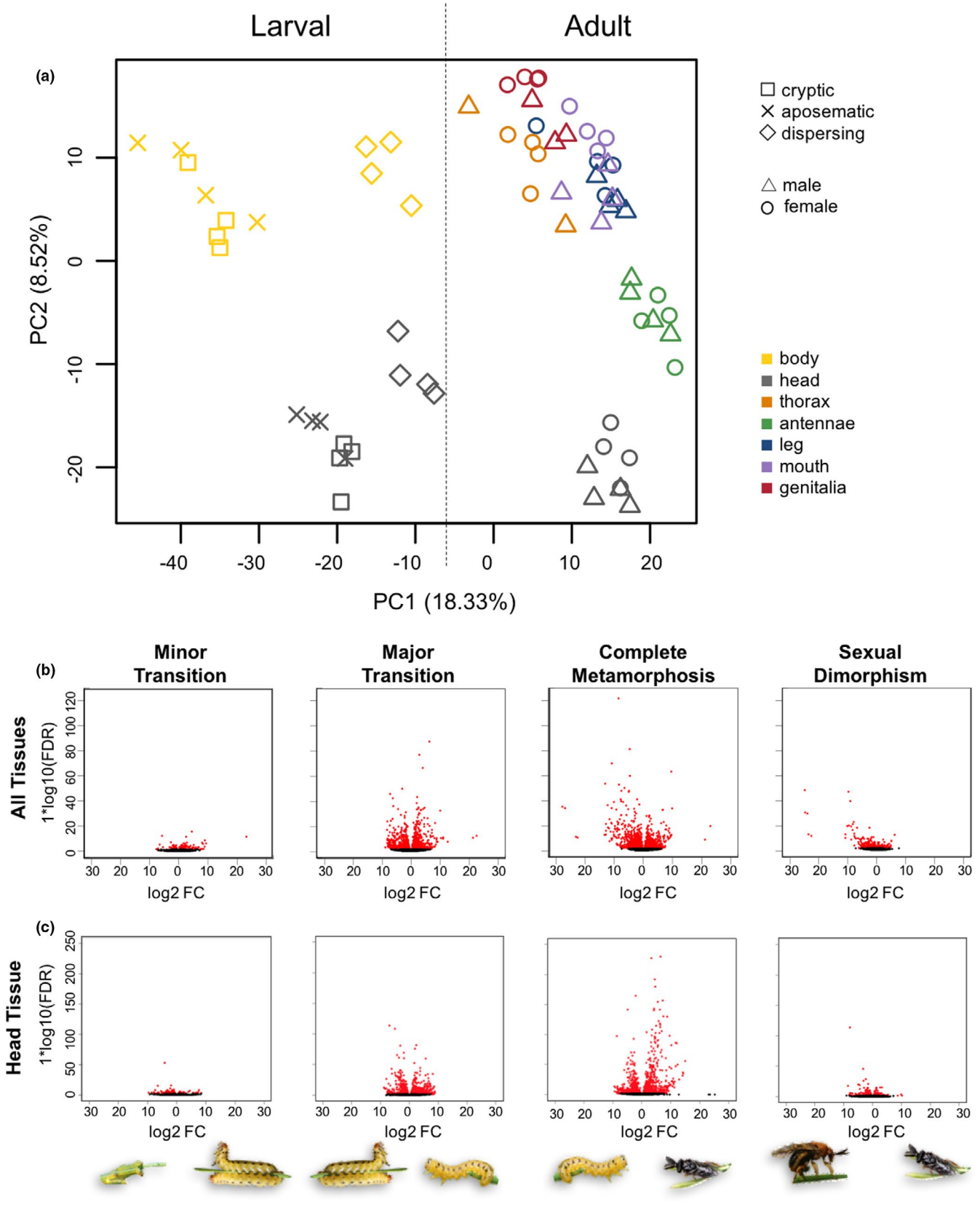

FIGURE 2 Transcriptome-wide patterns of gene-expression differences are consistent with the ADH. (a) Principle component analyses of 9, 304 putatively annotated genes for tissues (indicated by color) and stages (indicated by shape). (b) Volcano plots with the log2 fold change of gene expression and log10 FDRadjusted *p*-values between the sexually dimorphic adults and between each of the developmental transitions for all tissues, arranged according to predictions as in Figure 1b. Each point represents a single gene; red points are significantly differentially expressed between the stages/sexes. (c) Volcano plots as in B using head tissue only [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Prevalence and magnitude of differential expression across developmental transitions and between the sexes

Tissue	Comparison	Expressed genes[a]	Number of DEGs[b]	Proportion of DEGs (CI)[c]	Median magnitudes (IQR)[d]
All tissues	Minor	7,019	114	0.016 (0.013–0.019)	2.03 (1.45–4.44)
	Major	7,262	1,455	0.20 (0.19–0.21)	2.09 (1.46–3.60)
	Complete	7,938	1,553	0.20 (0.19–0.20)	2.47 (1.64–3.90)
	Sex	8,143	369	0.045 (0.041–0.050)	2.05 (1.47–3.15)
Head tissue	Minor	6,408	142	0.022 (0.019–0.026)	1.93 (1.36–3.62)
	Major	6,263	850	0.14 (0.13–0.14)	2.09 (1.41–3.86)
	Complete	6,557	1,710	0.26 (0.25–0.27)	2.66 (1.67–4.29)
	Sex	6,626	264	0.040 (0.035–0.045)	1.81 (1.32–2.95)

[a]Total number of genes expressed in each comparison.

[b]Number of genes that are significantly differentially expressed (p_{adj} < .05) and have an absolute $\log_2$(FC) of ≥1.

[c]Proportion of genes that are differentially expressed (DEGs) with Clopper–Pearson 95% confidence intervals.

[d]Median magnitudes of the absolute $\log_2$(FC) for each comparison and their interquartile ranges.

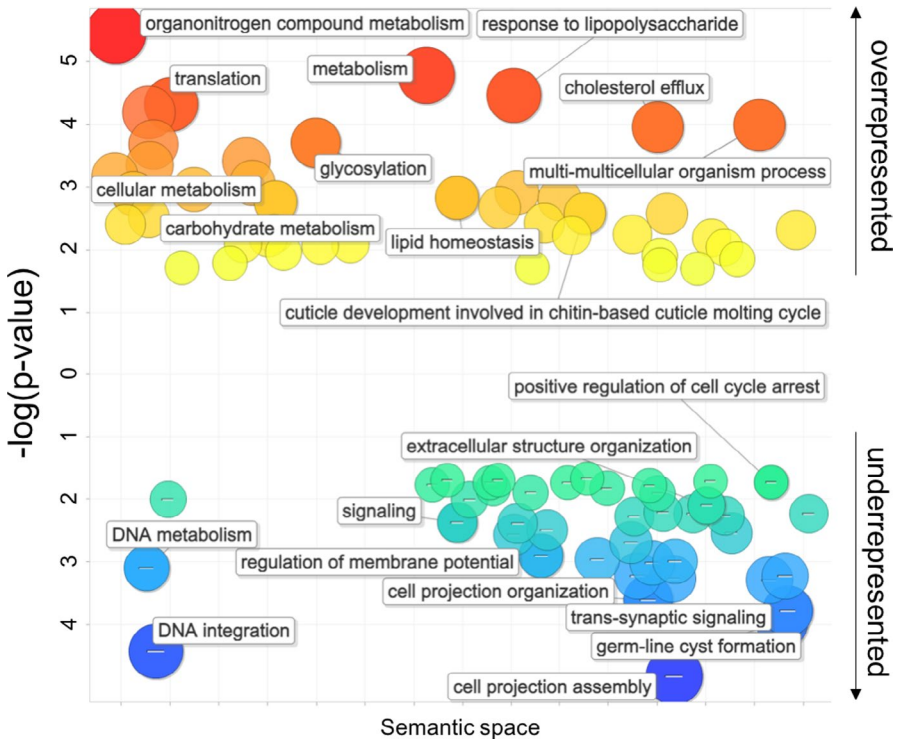

FIGURE 3 GO terms that were overrepresented (top) and underrepresented (bottom) in the global analysis of genes that were differentially expressed among stages and sexes. Semantic space is an indication of GO term similarity as defined by Revigo. The color and size of the dots are representations of the −log(*p*-value) where larger dots are more significantly over/underrepresented. As the dots become red, they are more significantly overrepresented and as they become blue, they are more underrepresented [Colour figure can be viewed at wileyonlinelibrary.com]

fairly uniform selection and had nearly identical expression levels in all comparisons between stages or sexes. Moreover, chemosensory genes had significantly higher fold-changes than RPLs in a global comparison across all four developmental/sex comparisons ($p = 1.7 \times 10^{-10}$) and in three out of four individual comparisons (Wilcoxon rank-sum tests: $p = .90$ for the major transition; $p < .0016$ for all remaining comparisons; see Figure 4b and Table S3).

Looking more closely at individual chemosensory genes, heat maps revealed that while some genes were expressed only during a single life stage, others were expressed at moderate to high levels in multiple stages/sexes (Figure 4c). Similarly, analysis of the highest 10% of expression for each stage/sex identified stage-specific (larval vs. adult), sex-specific (adult male vs. adult female) and universally expressed chemosensory genes that may hold clues to gene function (Figure 4d; Table S6). Particularly intriguing are two genes that were highly expressed in male larvae and adult females (OR25 and OBP5), but not adult males (Figure 4d).

4 | DISCUSSION

The (ADH postulates that complex life cycles are pervasive in nature because they facilitate the independent evolution of traits expressed at different life stages by removing constraints that may otherwise slow or prevent adaptation to novel environments (Moran, 1994). To investigate how selection history and developmental decoupling interact to produce observed trait patterns, we evaluated patterns of stage-biased gene expression in a hypermetamorphic

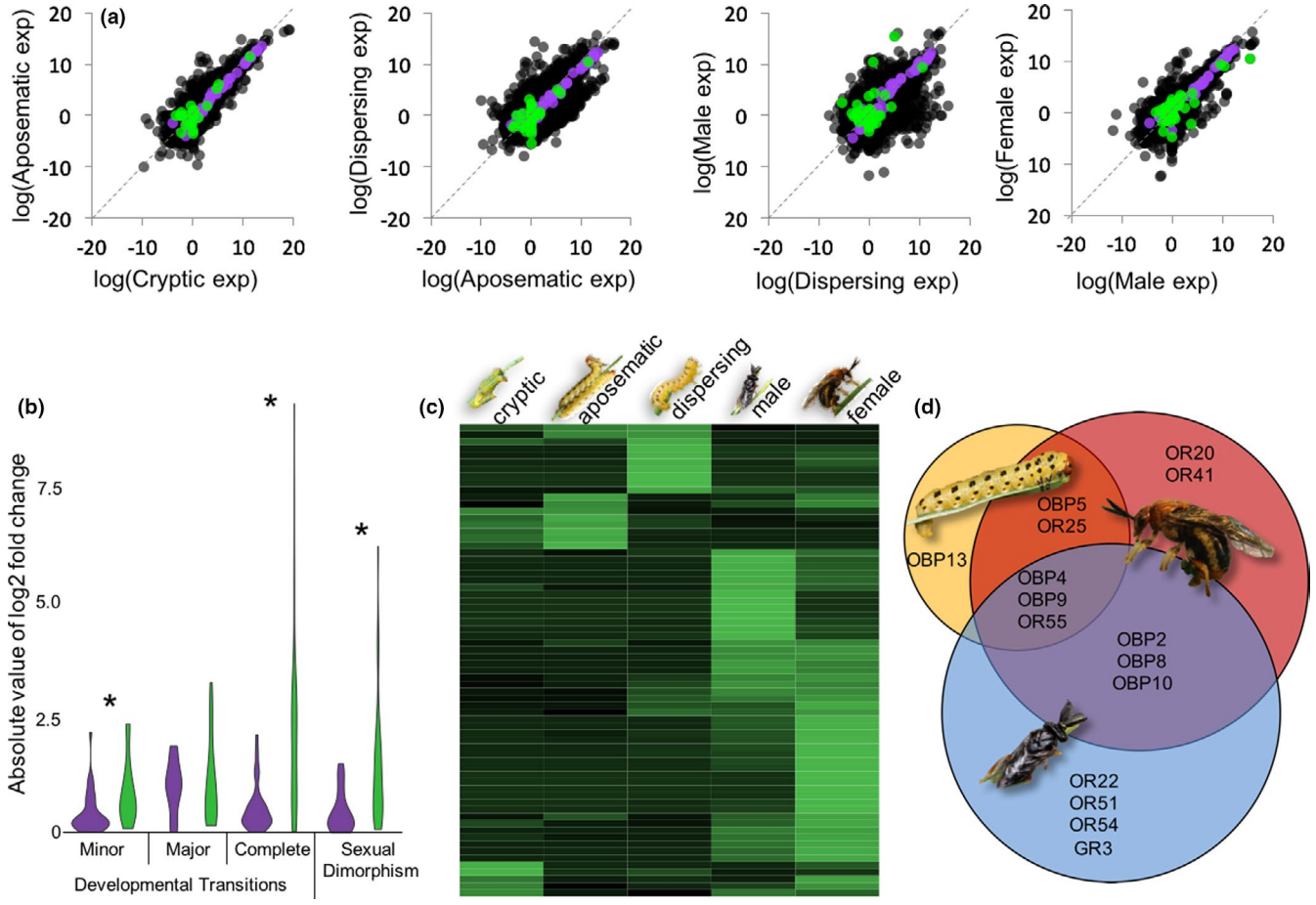

FIGURE 4 Compared to a group of house-keeping genes, chemosensory genes have higher and more variable levels of stage-biased and sex-biased expression. (a) Correlation plots of the sexually dimorphic adults and each metamorphic stage for all genes in the transcriptome (black points), Ribosomal protein L genes (purple points), and chemosensory genes (green points). Each point represents log2 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 biased gene expression. (b) Violin plots showing the distributions of the absolute value of the log2-fold change between each comparison (calculated as in Figure 2b) for Ribosomal protein L genes (purple) and chemosensory genes (green). Comparisons are for adult male vs. adult female (Sexual Dimorphism) and the three developmental transitions: cryptic vs. aposematic larvae (Minor), aposematic vs. dispersing larvae (Major), and dispersing larva vs. adult male (Complete). 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 four 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 expressed in multiple stages/sexes [Colour figure can be viewed at wileyonlinelibrary.com]

insect, the redheaded pine sawfly (Figure 1a). We hypothesized that differential expression would increase with ecological differences that create opportunities for antagonistic selection, but that expression differences would be constrained among life stages not separated by complete metamorphosis (Figure 1b). Consistent with this hypothesis, we found that: (i) differential expression increased with ecological dissimilarity and that the most extreme gene-expression differences were between life stages separated by a complete metamorphic event (Figure 2; Tables S3, S4); (ii) ecologically relevant gene categories exhibited stage-biased expression (Figure 3; Tables S3–S5); and (iii) stage-biased gene expression was more pronounced and variable in a group of genes likely to experience variable selection across development (chemosensory genes) compared to a group of genes likely to experience more uniform selection (housekeeping genes) (Figure 4). Additionally, we found that gene-expression differences tended to be more pronounced between different life stages than between the sexes (Figure 2; Tables S3, S4). Here, we compare patterns of gene expression in *Neodiprion lecontei* to other studies investigating trait differences under ontogenetically and sexually antagonistic selection and, in light of this body of work and limitations of our own study, make suggestions for future research on the ADH.

4.1 | Impact of developmental decoupling mechanisms on patterns of gene expression

During insect metamorphosis, the larval stage disintegrates and reforms into an entirely different adult body plan. According to the ADH, the primary benefit of metamorphosis is that it breaks genetic and developmental constraints, allowing for specialization of the life stages (Moran, 1994; Rolff et al., 2019; ten Brink et al., 2019). While transitions in ecology and minor developmental changes can occur in the absence of metamorphosis, the ADH predicts that the evolution of traits spanning nonmetamorphic transitions are more constrained by genetic correlations than traits spanning a metamorphic transition. However, direct comparisons between metamorphic and nonmetamorphic genetic correlations are rare. Some notable exceptions include investigations of: shell size in hard clams (Hilbish et al., 1993); body-size traits in wood frogs (Phillips, 1998); and body-size and locomotor performance traits in Pacific tree frogs (Watkins, 2001). While two of these studies reported markedly higher genetic correlations for traits measured at different time points within metamorphic phases compared to those measured in different phases (Hilbish et al., 1993; Phillips, 1998), a third study found no difference between within-phase and between-phase genetic correlations (Watkins, 2001). With so few traits investigated in these studies, however, it is difficult to draw firm conclusions regarding the ADH. In addition, because metamorphosis evolved independently in different animal taxa, the developmental mechanisms that decouple traits are likely to vary across taxa (Bishop et al., 2006; Phillips, 1998). Notably, none of these previous studies compared trait correlations within and between phases of holometabolous insects.

Overall, our results for a holometabolous insect support the ADH prediction that stage-biased gene expression (the product of genetic correlations between stages and stage-specific selection) would be most pronounced between stages that are separated by metamorphosis. Comparisons between wandering male larvae and adult males had the largest number of DEGs and the highest magnitude of expression differences (Figure 2b,c; Table 1). However, these results should be interpreted with caution because there are two elements of our experimental design that may have contributed to differences in gene expression between larvae and adults. First, only the adults were exposed to extended cold exposure (2–20 days) prior to tissue collection. Importantly, cold exposure can induce changes in gene expression in insects (see Freda et al., 2017; Zhao et al., 2021 and references therein). We note, however, that the 4°C storage that we used was more moderate than most experiments (typically 0°C) that have investigated the impact of cold stress or chill-comas on gene expression. We also induced the chill-coma within 30 hr of emergence as studies in other species have shown better recovery in younger individuals (Colinet et al., 2012). Under our conditions, recovery from chill-coma was very fast (<1 min) and behaviourally, the adults we dissected were indistinguishable from nonchilled adults. Nevertheless, more work is needed to determine how temperature impacts gene expression in different life stages of *N. lecontei*.

The second factor that could have increased differences in gene expression between larvae and adults is that different tissues were dissected from adult and larval samples (Figure S1). However, our results indicate that variation in tissue sampling probably obscured signals of stage-biased expression rather than enhanced them (Figure S3). For example, when all tissues were analysed together, the proportion of genes that were differentially expressed did not differ significantly between complete metamorphosis and the major transition. By contrast, the difference in stage-biased expression between metamorphic and nonmetamorphic transitions was much more pronounced when we examined expression traits from the heads only (Figure 2b,c; Table 1). Nevertheless, the large number of DEGs observed for the nonmetamorphic major transition was striking. This observation could be explained by a history of strong antagonistic selection between these stages and/or additional genetic or developmental decoupling mechanisms that exist for the moult separating feeding and wandering larvae. Measures of genetic correlations and investigation into developmental mechanisms underlying this transition are needed to disentangle these potential explanations. Analysis of stage-bias across different tissue types—especially those that vary in developmental links between larval and adult stages—would also be informative.

4.2 | Impact of ecology (antagonistic selection) on patterns of gene expression

Ecological shifts are central to proposed hypotheses for the origin, long-term persistence and success of lineages with complex life cycles (Ebenman, 1992; Istock, 1967; Wilbur, 1980). However, because ecological change and developmental decoupling are inextricably linked during metamorphosis, it can be difficult to tease apart their contributions to observed patterns of stage-biased gene expression among life stages. To gain additional insight into the potential contribution of selection to patterns of gene expression, we examined variation in stage-bias not among life stages but across the transcriptome. Consistent with our hypothesis that ecology shapes patterns of stage-biased gene expression (Figure 1b), many of the top DEGs (Tables S3, S4; Figure 4) and GO categories (Figure 3) were relevant to ecological differences between *N. lecontei* life stages (Figure 1a). Here, we compare our findings to previous studies for three important gene categories that probably mediate changing ecological conditions across development in many organisms: immunity, pigmentation and chemosensation.

If immunity is costly and pathogen exposure risk varies across development, selection should favour stage-biased expression for immunity genes (Hanson et al., 2019, and references within). If changes in pathogen exposure risk coincide with metamorphosis, developmental decoupling mechanisms could facilitate the evolution of stage-biased expression of immune genes. Consistent with this prediction, a 2011 study found no evidence of a genetic correlation between larval and adult expression levels of *Drosomycin*, an antimicrobial peptide (AMP) gene, in *Drosophila melanogaster* (Fellous

& Lazzaro, 2011). By contrast, transcription levels for a second AMP gene were significantly correlated between larvae and adults, an observation that led the authors to conclude that the ADH was not supported. However, because the estimated genetic correlation for the second AMP gene was less than one, some degree of independent evolution is still possible (Aguirre et al., 2014; Fry, 1996; Lande, 1980). Thus, an alternative explanation for the contrasting genetic correlations between the two AMP genes is that only one gene experienced strong stage-specific selection. Similarly, because some parasites and pathogens of *N. lecontei* are highly stage-specific (Coppel & Benjamin, 1965; Wilson et al. 1992), some—but not necessarily all—immune genes may exhibit highly stage-biased expression. Consistent with this prediction, while not all *N. lecontei* immune-related genes (Vertacnik et al. 2021) are differentially expressed across life stages, multiple immunity genes are among the most differentially expressed across the transcriptome (Tables S3,S4.

Pigmentation also frequently evolves under conflicting selection pressures across ontogeny. In insects, coloration is subject to diverse selection pressures—including abiotic factors (thermoregulation, UV resistance, desiccation tolerance), predation and sexual selection—that are likely to change over the course of development (Medina et al., 2020; True, 2003). Consistent with the ADH, an analysis of larval and adult coloration in 246 butterfly species found that colour evolution is strongly decoupled across butterfly development (Medina et al., 2020). Notably, whereas selection stemming from predation appears to constrain larval colour evolution, sexual selection on adult males gives rise to extensive interspecific variation in adult colour. A separate genetic analysis in another lepidopteran (*Bicyclus anynana*) demonstrates that melanism in larvae and adults is controlled by separate loci (Saenko et al., 2012). Similarly, pine sawflies have pronounced stage-biased expression for several pigmentation genes (Tables S3,S4). Notably, among our top DEGs were several candidate pigmentation genes (*yellow/major royal jelly* genes, *pale* and *cameo2*) identified in a quantitative trait locus (QTL) mapping analysis of among-population variation in larval colour traits (Linnen et al., 2018).

Because the importance of different gustatory and olfactory cues varies across development, chemosensory traits are also likely to experience ontogenetically variable selection. Consistent with this prediction, studies in diverse insect taxa have revealed highly variable patterns of stage-bias among different chemosensory genes (Colgan et al., 2011; Yang et al., 2018). Similarly, the degree of stage-bias varies across chemosensory genes in *N. lecontei* (Figure 3). Assuming that opportunities for developmental decoupling are fairly similar across genes that are expressed in the same chemosensory tissues, variable patterns of stage-specific expression across chemosensory genes probably reflect different selection histories (Agrawal & Stinchcombe, 2009; Vertacnik & Linnen, 2017). For example, chemosensory genes that are highly expressed by both feeding larvae and egg-laying females could be involved in detecting host-plant cues import to both stages, but not to adult males (Figure 4d). By contrast, chemosensory genes that are highly expressed in a single stage only may be related to highly stage-specific cues. For example, male-specific genes might be related to female pheromone detection. Notably, we did not identify any genes that were highly expressed in male larvae and adult males to the exclusion of adult females, suggesting that ecology is a better predictor of chemosensory gene-expression patterns than sex in this species. Although these expression patterns are suggestive, additional studies are needed to determine the function of different chemosensory genes. Nevertheless, the variability in patterns of stage-bias expression we observed among functionally similar genes highlights the importance of taking ecology and stage-specific selection pressures into account when testing predictions of the ADH.

4.3 | Comparing stage-biased and sex-biased gene expression

Genetic and developmental decoupling mechanisms may evolve in any situation in which pervasive antagonistic selection favours mechanisms that reduce genetic correlations. Although theories explaining sexual dimorphism are conceptually similar to the ADH, the developmental differences between sexes and stages probably impact the mechanisms by which antagonistic selection can be resolved. To date, few studies have directly compared patterns of sex-biased and stage-biased gene expression, with two notable exceptions. First, consistent with our results, a comprehensive analysis of gene expression in different developmental stages, sexes and castes of two ant species revealed that developmental stage had the largest impact on gene-expression profiles (Ometto et al., 2011). Second, in contrast to our finding that sex-biased expression was modest compared to stage-biased expression (Figure 2), a study in *Drosophila melanogaster* using gonadal tissue in three developmental stages (prepupal larvae, pupae and adults) reported greater gene-expression differences between the sexes than among the developmental stages (Perry et al., 2014). This discrepancy could be explained by reduced antagonistic selection among different *D. melanogaster* life stages and/or differences in tissue sampling across studies. 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 change over ontogeny (Perry et al., 2014). Given that the intensity of sexually antagonistic selection is likely to be highly variable across taxa, traits and tissues (Connallon & Clark, 2013; Connallon et al., 2018; Pennell & Morrow, 2013), studies in diverse taxa will be needed to evaluate whether there are consistent differences between stage-biased and sex-biased expression.

4.4 | Limitations and future directions for ADH research

Although the patterns of gene-expression differences we observed in *N. lecontei* are consistent with the ADH, additional data are needed to: (i) evaluate the relationship between stage-biased expression and

genetic independence, (ii) demonstrate that stage-biased genes contribute to stage-specific adaptations, and (iii) establish that metamorphosis is an adaptation for trait decoupling. Here, we discuss strategies for evaluating each of these additional predictions.

First, if stage-biased expression is a reflection of underlying genetic independence, stage-biased genes should have reduced genetic correlations among life stages relative to unbiased genes, and alleles that contribute to expression variation in one life stage should not have pleiotropic effects on another life stage. These predictions can be evaluated by quantitative genetic analysis and QTL mapping, respectively (Freda et al., 2017; Saenko et al., 2012). One study that used both approaches investigated genetic independence of thermal hardiness between larval and adult *D. melanogaster* (Freda et al., 2017). Whereas *D. melanogaster* larvae live in thermally stable rotting fruits and are only present in the warm months, flying adults experience a more variable thermal environment and are exposed to low temperatures during the overwintering generation. Consistent with the ADH and with opposing selection on thermal hardiness across the life stages, thermal hardiness is not genetically correlated across metamorphosis in this species. Additionally, larval and adult traits map to different loci (Freda et al., 2017). Similar studies are needed that investigate a variety of traits in different species.

Second, the prediction that genes with stage-biased expression contribute to stage-specific adaptation could be evaluated using multiple complementary approaches. For example, if stage-biased genes contribute disproportionately to adaptation, genes exhibiting the most stage-biased expression patterns should also reveal a history of positive selection (e.g., evidence of recent selective sweeps or elevated rates of nonsynonymous substitutions relative to the rest of the genome) (Vitti et al., 2013). Although this prediction has been confirmed by several studies for sex-biased genes (Assis et al., 2012; Drosophila 12 Genome Consortium, 2007; Mank et al., 2010; Proschel et al., 2006; L. Yang et al., 2016), it has rarely been tested in the context of stage-biased expression across metamorphic boundaries (but see Perry et al., 2014). An alternative approach would be to use experimental genomics to connect genetic variants directly to fitness at different life stages (Egan et al., 2015; Gloss et al., 2016; Gompert et al., 2019; Ingvarsson et al., 2017). Following exposure to a selection regime that favours different traits at different ontogenetic stages, using the logic of the ADH, we would predict that genes with the most stage-biased expression will exhibit the most pronounced allele frequency shifts.

Third, to more directly test the hypothesis that metamorphosis itself is an adaptation for increasing genetic independence of traits, comparative data are needed to evaluate two additional predictions: (i) metamorphosis is favoured under ecological conditions that result in pervasive antagonistic pleiotropy across the life cycle and (ii) 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 (Belles, 2011). Likewise, gains and losses of complex life cycles have been demonstrated in numerous taxa and are particularly well documented in insects and amphibians (Badets & Verneau, 2009; Bonett & Blair, 2017; Emmanuelle et al. 2010; Moran, 1994; Poulin & Cribb, 2002; Wiens et al., 2007).

5 | CONCLUSIONS

Overall, our transcriptomic analysis of a hypermetamorphic and sexually dimorphic sawfly reveals that patterns of gene expression are in line with predictions of the ADH. 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. Rigorously testing the ADH and better understanding its relationship to sexual dimorphism will ultimately require analyses of patterns of gene expression and genetic correlations in diverse taxa that vary in metamorphic and sexually dimorphic phenotypes. Developmental and genetic studies are also needed to uncover the mechanistic basis of trait decoupling in different tissues, transition types and taxa. To gain maximal insight from decoupling analyses in other taxa, in-depth knowledge of organismal ecology is essential. Although much work remains, these data are critical to understanding why metamorphosis is one of the most successful developmental strategies on the planet.

ACKNOWLEDGEMENTS

For assistance with collecting and maintaining sawfly colonies, we thank current and past members of the Linnen laboratory. For constructive comments on versions of this manuscript, we thank Emily Bendall, Kathryn Everson and three anonymous reviewers. For computing resources, we thank the University of Kentucky Center for Computational Sciences and the Lipscomb High Performance Computing Cluster. Funding for this research was provided by the Kentucky Science and Education Foundation (KSEF-3492-RDE-019; to C.R.L.), the United States Department of Agriculture National Institute of Food and Agriculture (2016-67014-2475; to C.R.L.) and the National Science Foundation (DEB-CAREER-1750946; to C.R.L.).

AUTHOR CONTRIBUTIONS

D.K.H., K.L.V. and C.R.L. participated in the study design. D.K.H., K.L.V. and A.R.K. participated in the data collection. D.K.H. and C.R.L. analysed the data. D.K.H. and C.R.L. drafted the manuscript. All authors read and approved the final manuscript.

DATA ACCESSIBILITY STATEMENT

All raw reads have been deposited at NCBI short read archive (BioProject: PRJNA668791; SRR12814047-SRR12814123). This Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under the accession GJGF00000000. Gene expression count matrix and normalized (TMM) matrix are available on the Linnen Lab GitHub (https://github.com/LinnenLab/Herrig_etal_2021) as well as all scripts used for analyses (perl, R, and python).

ORCID

Danielle K. Herrig https://orcid.org/0000-0002-4843-206X

Catherine R. Linnen https://orcid.org/0000-0001-5715-456X

REFERENCES

Abbott, J. K. (2010). Intra-locus sexual conflict and sexually antagonistic genetic variation in hermaphroditic animals. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1703), 161–169. https://doi.org/10.1098/rspb.2010.1401

Agrawal, A. F., & Stinchcombe, J. R. (2009). How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society B: Biological Sciences*, *276*(1659), 1183–1191. https://doi.org/10.1098/rspb.2008.1671

Aguirre, J. D., Blows, M. W., & Marshall, D. J. (2014). The genetic covariance between life cycle stages separated by metamorphosis. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1788), 20141091. https://doi.org/10.1098/rspb.2014.1091

Alberch, P. (1982). Developmental constraints in evolutionary processes. In J. T. Bonner (Ed.), *Evolution and development*. Dahlem Workshop Reports (Life Sciences Research Report) (Vol. *22*, pp. 313–332). Springer. https://doi.org/10.1007/978-3-642-45532-2_15

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/s0022-2836(05)80360-2

Anderbrant, O. (1993). In M. R. Wagner, & K. F. Raffa (Eds.), *Sawfly life history adaptions to woody plants*. Academic Press.

Anderson, B. B., Scott, A., & Dukas, R. (2016). Social behavior and activity are decoupled in larval and adult fruit flies. *Behavioral Ecology*, *27*(3), 820–828. https://doi.org/10.1093/beheco/arv225

Assis, R., Zhou, Q., & Bachtrog, D. (2012). Sex-biased transcriptome evolution in Drosophila. *Genome Biology and Evolution*, *4*(11), 1189–1200. https://doi.org/10.1093/gbe/evs093

Badets, M., & Verneau, O. (2009). Origin and evolution of alternative developmental strategies in amphibious sarcopterygian parasites (Platyhelminthes, Monogenea, Polystomatidae). *Organisms Diversity & Evolution*, *9*(3), 155–164. https://doi.org/10.1016/j.ode.2009.02.003

Belles, X. (2011). Origin and evolution of insect metamorphosis. *Encyclopedia of Life Sciences (ELS)*. https://doi.org/10.1002/9780470015902.a0022854

Bendall, E. E., Vertacnik, K. L., & Linnen, C. R. (2017). Oviposition traits generate extrinsic postzygotic isolation between two pine sawfly species. *BMC Evolutionary Biology*, *17*(1), 26. https://doi.org/10.1186/s12862-017-0872-8

Benesh, D. P., Chubb, J. C., & Parker, G. A. (2013). Complex life cycles: why refrain from growth before reproduction in the adult niche? *American Naturalist*, *181*(1), 39–51. https://doi.org/10.1086/668592

Bishop, C. D., Erezyilmaz, D. F., Flatt, T., Georgiou, C. D., Hadfield, M. G., Heyland, A., Hodin, J., Jacobs, M. W., Maslakova, S. A., Pires, A., Reitzel, A. M., Santagata, S., Tanaka, K., & Youson, J. H. (2006). What is metamorphosis? *Integrative and Comparative Biology*, *46*(6), 655–661. https://doi.org/10.1093/icb/icl004

Bolger, A. M., Lohse, M., & Usadel, B. (2014). TRIMMOMATIC: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bonett, R. M., & Blair, A. L. (2017). Evidence for complex life cycle constraints on salamander body form diversification. *Proceedings of the National Academy of Sciences*, *114*(37), 9936–9941. https://doi.org/10.1073/pnas.1703877114

Bossuyt, F., & Milinkovitch, M. C. (2000). Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. *Proceedings of the National Academy of Sciences*, *97*(12), 6585–6590. https://doi.org/10.1073/pnas.97.12.6585

Bryant, E. H. (1969). A system favoring the evolution of holometabolous development 1,2. *Annals of the Entomological Society of America*, *62*(5), 1087–1091. https://doi.org/10.1093/aesa/62.5.1087

Colgan, T. J., Carolan, J. C., Bridgett, S. J., Sumner, S., Blaxter, M. L., & Brown, M. J. (2011). Polyphenism in social insects: insights from a transcriptome-wide analysis of gene expression in the life stages of the key pollinator, *Bombus terrestris*. *BMC Genomics*, *12*, 623. https://doi.org/10.1186/1471-2164-12-623

Colinet, H., Sciaussat, D., Bozzolan, F., & Bowler, K. (2012). Rapid decline of cold tolerance at young age is associated with expression of stress genes in *Drosophila melanogaster*. *Journal of Experimental Biology*, *2*, 253–259. https://doi.org/10.1242/jeb.076216

Collet, J., & Fellous, S. (2019). Do traits separated by metamorphosis evolve independently? Concepts and methods. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1900), 20190445. https://doi.org/10.1098/rspb.2019.0445

Connallon, T., & Clark, A. G. (2013). Evolutionary inevitability of sexual antagonism. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1776), 20132123. https://doi.org/10.1098/rspb.2013.2123

Connallon, T., Débarre, F., & Li, X.-Y. (2018). Linking local adaptation with the evolution of sex differences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1757), 20170414. https://doi.org/10.1098/rstb.2017.0414

Connallon, T., & Knowles, L. L. (2005). Intergenomic conflict revealed by patterns of sex-biased gene expression. *Trends in Genetics*, *21*(9), 495–499. https://doi.org/10.1016/j.tig.2005.07.006

Coppel, H. C., & Benjamin, D. M. (1965). Bionomics of Nearctic pine-feeding diprionids. *Annual Review of Entomology*, *10*, 69–96. https://doi.org/10.1146/annurev.en.10.010165.000441

Crean, A. J., Monro, K., & Marshall, D. J. (2011). Fitness consequences of larval traits persist across the metamorphic boundary. *Evolution*, *65*(11), 3079–3089. https://doi.org/10.1111/j.1558-5646.2011.01372.x

Drosophila 12 Genomes Consortium (2007). Evolution of genes and genomes on the Drosophila phylogeny. *Nature*, *450*(7167), 203–218. https://doi.org/10.1038/nature06341

Ebenman, B. (1992). Evolution in organisms that change their niches during the life cycle. *The American Naturalist*, *139*(5), 990–1021. https://doi.org/10.1086/285370

Egan, S. P., Ragland, G. J., Assour, L., Powell, T. H. Q., Hood, G. R., Emrich, S., Nosil, P., & Feder, J. L. (2015). Experimental evidence of genome-wide impact of ecological selection during early stages of speciation-with-gene-flow. *Ecology Letters*, *18*(8), 817–825. https://doi.org/10.1111/ele.12460

Ellegren, H., & Parsch, J. (2007). The evolution of sex-biased genes and sex-biased gene expression. *Nature Reviews Genetics*, *8*(9), 689–698. https://doi.org/10.1038/nrg2167

Emmanuelle, J., Gwenaelle, G., & Armelle, C. (2010). Evolutionary lability of a complex life cycle in the aphid genus Brachycaudus. *BMC Evolutionary Biology*, *10*(1), 295. https://doi.org/10.1186/1471-2148-10-295

Fellous, S., & Lazzaro, B. P. (2011). Potential for evolutionary coupling and decoupling of larval and adult immune gene expression. *Molecular Ecology*, *20*(7), 1558–1567. https://doi.org/10.1111/j.1365-294X.2011.05006.x

Field, L. M., Williamson, M. S., Moores, G. D., & Devonshire, A. L. (1993). Cloning and analysis of the esterase genes conferring insecticide resistance in the peach-potato aphid, *Myzus persicae* (Sulzer). *The Biochemical Journal*, *294*(Pt 2), 569–574. https://doi.org/10.1042/bj2940569

Freda, P. J., Alex, J. T., Morgan, T. J., & Ragland, G. J. (2017). Genetic Decoupling of thermal hardiness across metamorphosis in *Drosophila melanogaster*. *Integrative and Comparative Biology*, *57*(5), 999–1009. https://doi.org/10.1093/icb/icx102

Fry, J. D. (1996). The evolution of host specialization: Are trade-offs overrated? *The American Naturalist*, *148*, S84–S107. https://doi.org/10.1086/285904

Gilbert, L. I., Tata, J. R., & Atkinson, B. G. (1996). *Metamorphosis: Postembryonic reprogramming of gene expression in amphibian and insect cells*. Academic Press.

Gloss, A. D., Groen, S. C., & Whiteman, N. K. (2016). A genomic perspective on the generation and maintenance of genetic diversity in herbivorous insects. *Annual Review of Ecology, Evolution, and Systematics*, *47*(1), 165–187. https://doi.org/10.1146/annurev-ecolsys-121415-032220

Goedert, D., & Calsbeek, R. (2019). Experimental Evidence That Metamorphosis Alleviates Genomic Conflict. *The American Naturalist*, *194*(3), 356–366. https://doi.org/10.1086/704183

Gompert, Z., Brady, M., Chalyavi, F., Saley, T. C., Philbin, C. S., Tucker, M. J., Forister, M. L., & Lucas, L. K. (2019). Genomic evidence of genetic variation with pleiotropic effects on caterpillar fitness and plant traits in a model legume. *Molecular Ecology*, *28*(12), 2967–2985. https://doi.org/10.1111/mec.15113

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., Couger, M. B., Eccles, D., Li, B. O., Lieber, M., MacManes, M. D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C. N., … Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, *8*(8), 1494–1512. https://doi.org/10.1038/nprot.2013.084

Haldane, J. B. S. (1932). The time of action of genes, and its bearing on some evolutionary problems. *The American Naturalist*, *66*(702), 5–24. https://doi.org/10.1086/280406

Hanson, M. A., Lemaitre, B., & Unckless, R. L. (2019). Dynamic evolution of antimicrobial peptides underscores trade-offs between immunity and ecological fitness. *Frontiers in Immunology*, *10*, https://doi.org/10.3389/fimmu.2019.02620

Harper, K. E., Bagley, R. K., Thompson, K. L., & Linnen, C. R. (2016). Complementary sex determination, inbreeding depression and inbreeding avoidance in a gregarious sawfly. *Heredity (Edinb)*, *117*(5), 326–335. https://doi.org/10.1038/hdy.2016.46

Heming, B. S. (2003). *Insect development and evolution*. Comstock Pub. Associates.

Hilbish, T. J., Winn, E. P., & Rawson, P. D. (1993). Genetic variation and covariation during larval and juvenile growth in *Mercenaria mercenaria*. *Marine Biology*, *115*(1), 97–104. https://doi.org/10.1007/bf00349390

Ingvarsson, P. K., Hu, J., Lei, L., & de Meaux, J. (2017). Temporal fitness fluctuations in experimental Arabidopsis thaliana populations. *PLoS One*, *12*(6), e0178990. https://doi.org/10.1371/journal.pone.0178990

Istock, C. A. (1967). The evolution of complex life cycle phenomena: An ecological perspective. *Evolution*, *21*(3), 592–605. https://doi.org/10.1111/j.1558-5646.1967.tb03414.x

Jacobs, M. W., Degnan, S. M., Woods, R., Williams, E., Roper, K. E., Green, K., & Degnan, B. M. (2006). The effect of larval age on morphology and gene expression during ascidian metamorphosis. *Integrative and Comparative Biology*, *46*(6), 760–776. https://doi.org/10.1093/icb/icl015

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., & Salzberg, S. L. (2013). TOPHAT2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology*, *14*(4), R36. https://doi.org/10.1186/gb-2013-14-4-r36

Kristensen, N. P. (1999). Phylogeny of endopterygote insects, the most successful lineage of living organisms. *European Journal of Entomology*, *96*, 237–253.

Lande, R. (1980). Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution*, *34*(2), 292–305. https://doi.org/10.1111/j.1558-5646.1980.tb04817.x

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with BOWTIE 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Li, B., & Dewey, C. N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics*, *12*(1), https://doi.org/10.1186/1471-2105-12-323

Li, W., & Godzik, A. (2006). Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics*, *22*(13), 1658–1659. https://doi.org/10.1093/bioinformatics/btl158

Lindstedt, C., Bagley, R., Calhim, S., Jones, M., & Linnen, C. (2020). The impact of life stage and pigment source on the evolution of novel warning signal traits. *bioRxiv*. https://doi.org/10.1101/2020.10.20.346700

Linnen, C. R., O'Quin, C. T., Shackleford, T., Sears, C. R., & Lindstedt, C. (2018). Genetic basis of body color and spotting pattern in redheaded pine sawfly larvae (Neodiprion lecontei). *Genetics*, *209*(1), 291–305. https://doi.org/10.1534/genetics.118.300793

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Mank, J. E. (2017). The transcriptional architecture of phenotypic dimorphism. *Nature Ecology & Evolution*, *1*(1), https://doi.org/10.1038/s41559-016-0006

Mank, J. E., Hultin-Rosenberg, L., Zwahlen, M., & Ellegren, H. (2008). Pleiotropic constraint hampers the resolution of sexual antagonism in vertebrate gene expression. *The American Naturalist*, *171*(1), 35–43. https://doi.org/10.1086/523954

Mank, J. E., Nam, K., Brunstrom, B., & Ellegren, H. (2010). Ontogenetic complexity of sexual dimorphism and sex-specific selection. *Molecular Biology and Evolution*, *27*(7), 1570–1578. https://doi.org/10.1093/molbev/msq042

Medina, I., Vega-Trejo, R., Wallenius, T., Symonds, M. R. E., & Stuart-Fox, D. (2020). From cryptic to colorful: Evolutionary decoupling of larval and adult color in butterflies. *Evolution Letters*, *4*(1), 34–43. https://doi.org/10.1002/evl3.149

Moran, N. A. (1994). Adaptation and constraint in the complex life cycles of animals. *Annual Review of Ecology and Systematics 25*, 573–600. https://doi.org/10.1146/annurev.es.25.110194.003041

Morris, M. R., Goedert, D., Abbott, J. K., Robinson, D. M., & Rios-Cardenas, O. (2013). Intralocus tactical conflict and the evolution of alternative reproductive tactics. *Advances in the study of Behavior*, *45*, 447–478. https://doi.org/10.1016/b978-0-12-407186-5.00007-0

Ometto, L., Shoemaker, D., Ross, K. G., & Keller, L. (2011). Evolution of gene expression in fire ants: the effects of developmental stage, caste, and species. *Molecular Biology and Evolution*, *28*(4), 1381–1392. https://doi.org/10.1093/molbev/msq322

Parichy, D. M. (1998). Experimental analysis of character coupling across a complex life cycle: Pigment pattern metamorphosis in the tiger salamander, *Ambystoma tigrinum tigrinum*. *Journal of Morphology*, *237*(1), 53–67. https://doi.org/10.1002/(sici)1097-4687(199807)237:1<53:aid-jmor5>3.0.co;2-p

Pennell, T. M., Holman, L., Morrow, E. H., & Field, J. (2018). Building a new research framework for social evolution: intralocus caste antagonism. *Biological Reviews*, *93*(2), 1251–1268. https://doi.org/10.1111/brv.12394

Pennell, T. M., & Morrow, E. H. (2013). Two sexes, one genome: the evolutionary dynamics of intralocus sexual conflict. *Ecology and Evolution*, *3*(6), 1819–1834. https://doi.org/10.1002/ece3.540

Perry, J. C., Harrison, P. W., & Mank, J. E. (2014). The ontogeny and evolution of sex-biased gene expression in *Drosophila melanogaster*. *Molecular Biology and Evolution*, *31*(5), 1206–1219. https://doi.org/10.1093/molbev/msu072

Phillips, P. C. (1998). Genetic constraints at the metamorphic boundary: Morphological development in the wood frog, *Rana sylvatica*. *Journal of Evolutionary Biology*, *11*(4), 453–463. https://doi.org/10.1046/j.1420-9101.1998.11040453.x

Poulin, R., & Cribb, T. H. (2002). Trematode life cycles: short is sweet? *Trends in Parasitology*, *18*(4), 176–183. https://doi.org/10.1016/s1471-4922(02)02262-6

Proschel, M., Zhang, Z., & Parsch, J. (2006). Widespread adaptive evolution of Drosophila genes with sex-biased expression. *Genetics*, *174*(2), 893–900. https://doi.org/10.1534/genetics.106.058008

Pruitt, K. D. (2004). NCBI Reference Sequence (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Research*, *33*(Database issue), D501–D504. https://doi.org/10.1093/nar/gki025

Pujol-Lereis, L. M., Rabossi, A., & Quesada-Allué, L. A. (2014). Analysis of survival, gene expression and behavior following chill-coma in the medfly *Ceratitis capitata*: Effects of population heterogeneity and age. *Journal of Insect Physiology*, *71*, 156–163. https://doi.org/10.1016/j.jinsphys.2014.10.015

Rainford, J. L., Hofreiter, M., Nicholson, D. B., & Mayhew, P. J. (2014). Phylogenetic distribution of extant richness suggests metamorphosis is a key innovation driving diversification in insects. *PLoS One*, *9*(10), e109085. https://doi.org/10.1371/journal.pone.0109085

Rolff, J., Johnston, P. R., & Reynolds, S. (2019). Complete metamorphosis of insects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1783), 20190063. https://doi.org/10.1098/rstb.2019.0063

Saenko, S. V., Jerónimo, M. A., & Beldade, P. (2012). Genetic basis of stage-specific melanism: A putative role for a cysteine sulfinic acid decarboxylase in insect pigmentation. *Heredity*, *108*(6), 594–601. https://doi.org/10.1038/hdy.2011.127

Supek, F., Bošnjak, M., Škunca, N., & Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS One*, *6*(7), e21800. https://doi.org/10.1371/journal.pone.0021800

R Core Team. (2016). *R: A Language and Environment for Statistical Computing*. R Foundation of Statistical Computing.

ten Brink, H., de Roos, A. M., & Dieckmann, U. (2019). The evolutionary ecology of metamorphosis. *The American Naturalist*, E116–E131. https://doi.org/10.1086/701779

Tong, X. W., Chen, B., Huang, L. H., Feng, Q. L., & Kang, L. (2015). Proteomic analysis reveals that COP9 signalosome complex subunit 7A (CSN7A) is essential for the phase transition of migratory locust. *Scientific Reports*, *5*, 12542. https://doi.org/10.1038/srep12542

True, J. R. (2003). Insect melanism: The molecules matter. *Trends in Ecology & Evolution*, *18*(12), 640–647. https://doi.org/10.1016/j.tree.2003.09.006

Truman, J. W., & Riddiford, L. M. (2019). The evolution of insect metamorphosis: a developmental and endocrine view. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *374*(1783), 20190070. https://doi.org/10.1098/rstb.2019.0070

Vertacnik, K. L., Geib, S. M., & Linnen, C. R. (2016). Neodiprion lecontei genome assembly Nlec1.0, NCBI/GenBank. Publication no. 10.15482/USDA.ADC/1235565.

Vertacnik, K. L., Herrig, D. K., Keating Godfrey, R., Hill, T., Geib, S. M., Unckless, R. L., Linnen, C. R. (2021). Ecological correlates of gene family size: the draft genome of the redheaded pine sawfly Neodiprion lecontei (Hymenoptera. Diprionidae). doi:https://doi.org/10.1101/2021.03.14.435331

Vertacnik, K. L., & Linnen, C. R. (2017). Evolutionary genetics of host shifts in herbivorous insects: insights from the age of genomics. *Annals of the New York Academy of Sciences*, *1389*(1), 186–212. https://doi.org/10.1111/nyas.13311

Vitti, J. J., Grossman, S. R., & Sabeti, P. C. (2013). Detecting natural selection in genomic data. *Annual Review of Genetics*, *47*(1), 97–120. https://doi.org/10.1146/annurev-genet-111212-133526

Watkins, T. B. (2001). A quantitative genetic test of adaptive decoupling across metamorphosis for locomotor and life-history traits in the Pacific Tree frog, hyla regilla. *Evolution*, *55*(8), 1668–1677. https://doi.org/10.1111/j.0014-3820.2001.tb00686.x

Wei, L., Gao, S., Xiong, W., Liu, J., Mao, J., Lu, Y., & Li, B. (2019). Latrophilin mediates insecticides susceptibility and fecundity through two carboxylesterases, esterase4 and esterase6, in Tribolium castaneum. *Bulletin of Entomological Research*, *109*(4), 534–543. https://doi.org/10.1017/s0007485318000895

Wiens, J. J., Kuczynski, C. A., Duellman, W. E., & Reeder, T. W. (2007). Loss and re-evolution of complex life cycles in marsupial frogs: Does ancestral trait reconstruction mislead? *Evolution*, *61*(8), 1886–1899. https://doi.org/10.1111/j.1558-5646.2007.00159.x

Wigglesworth, V. B. S. (1954). *The physiology of insect metamorphosis*. University Press.

Wilbur, H. M. (1980). Complex life cycles. *Annual Review of Ecology and Systematics*, *11*, 67–93. https://doi.org/10.1146/annurev.es.11.110180.000435

Wilson, L. F., Wilkinson, R. C., & Averill, R. D., (1992). *Redheaded pine sawfly- its ecology and management*. U.S. Department of Agriculture.

Wollenberg Valero, K. C., Garcia-Porta, J., Rodríguez, A., Arias, M., Shah, A., Randrianiaina, R. D., Brown, J. L., Glaw, F., Amat, F., Künzel, S., Metzler, D., Isokpehi, R. D., & Vences, M. (2017). Transcriptomic and macroevolutionary evidence for phenotypic uncoupling between frog life history phases. *Nature Communications*, *8*(1), https://doi.org/10.1038/ncomms15213

Yang, H., Cai, Y., Zhuo, Z., Yang, W., Yang, C., Zhang, J., Yang, Y., Wang, B., & Guan, F. (2018). Transcriptome analysis in different developmental stages of Batocera horsfieldi (Coleoptera: Cerambycidae) and comparison of candidate olfactory genes. *PLoS One*, *13*(2), e0192730. https://doi.org/10.1371/journal.pone.0192730

Yang, L., Zhang, Z., & He, S. (2016). Both male-biased and female-biased genes evolve faster in fish genomes. *Genome Biology and Evolution*, *8*(11), 3433–3445. https://doi.org/10.1093/gbe/evw239

Zhao, D., Zheng, C., Shi, F., Xu, Y., Zong, S., & Tao, J. (2021). Expression analysis of genes related to cold tolerance in *Dendroctonus valens*. *PeerJ*, *9*, e10864. https://doi.org/10.7717/peerj.10864

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Herrig, D. K., Vertacnik, K. L., Kohrs, A. R., & Linnen, C. R. (2021). Support for the adaptive decoupling hypothesis from whole-transcriptome profiles of a hypermetamorphic and sexually dimorphic insect, *Neodiprion lecontei*. *Molecular Ecology*, 30, 4551–4566. https://doi.org/10.1111/mec.16041