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The relative impact of parental and current environment on plant transcriptomes depends on type of stress and genotype

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Through developmental plasticity, an individual organism integrates influences from its immediate environment with those due to the environment of its parents. While both effects on phenotypes are well documented, their relative impact has been little studied in natural systems, especially at the level of gene expression. We examined this issue in four genotypes of the annual plant *Persicaria maculosa* by varying two key resources—light and soil moisture—in both generations. Transcriptomic analyses showed that the relative effects of parent and offspring environment on gene expression (i.e. the number of differentially expressed transcripts, DETs) varied both for the two types of resource stress and among genotypes. For light, immediate environment induced more DETs than parental environment for all genotypes, although the precise proportion of parental versus immediate DETs varied among genotypes. By contrast, the relative effect of soil moisture varied dramatically among genotypes, from 8-fold more DETs due to parental than immediate conditions to 10-fold fewer. These findings provide evidence at the transcriptomic level that the relative impacts of parental and immediate environment on the developing organism may depend on the environmental factor and vary strongly among genotypes, providing potential for the interplay of these developmental influences to evolve.

1. Introduction

The developing organism's phenotype reflects not only its immediate environment but, in many cases, that of its parents. Both immediate and parental influences have been characterized in a wide range of taxa for functional and life-history traits (reviewed by [1,2]) and at the underlying level of gene expression (e.g. [3–5]). Current and parental environmental effects on development (often termed *immediate* and *transgenerational plasticity*, respectively) are known to vary depending on the factor or stress in question, in many cases providing specifically adaptive adjustments (reviewed by [6–9]; see [10]). Moreover, genotypes may differ in their precise patterns of phenotypic response to a given current or parental factor [11–14]. Although numerous studies have revealed both immediate and inherited environmental influences on the phenotypes of developing individuals, the relative impact of these two types of environmental influence on gene expression, their functional similarity or distinctness, and the generality of these patterns are as yet poorly understood, in four key ways.

First, studies that test for both current and parental effects on offspring (reviewed by [15]) seldom explicitly compare the magnitude of these two aspects of plasticity [16,17]. Instead, parental effects on offspring development are often presumed to be minor relative to the effects of the offspring's immediate environment [18–20]. Although in many published cases transgenerational effects on phenotypes are indeed subtle [16], it is not known whether they are inherently

less pronounced than immediate effects. Indeed, several studies have revealed stronger parent- than current-environment effects on offspring phenotypes: parent environment influences germination behaviour in *Arabidopsis thaliana* more strongly than immediate germination conditions [21,22]; maternal photoperiod in *Daphnia* has been shown in some contexts to have a greater impact on an offspring individual's egg production strategy than its immediate photoperiod [23]; and maternal thermal environment can result in a greater number of differentially expressed genes than the immediate water temperature in the marine fish *Gasterosteus aculeatus* [3]. Theory suggests that, for certain environmental factors, parental conditions may better predict an offspring's selective environment, favoring the evolution of strong transgenerational plasticity relative to immediate response to environmental factors that affect fitness [17,19,20,24,25]. Hence the relative impact of parental and immediate environments may differ depending on the system and the organism's evolved developmental reliance on these alternative sources of information about a given cue.

Second, the relative impact of immediate and transgenerational effects may vary with the type of environmental factor. In plants, for example, light and soil moisture represent critical resources for survival and growth that are patchily distributed in natural habitats, varying from amply available to stressfully limited [26]. As a result, plants have evolved a robust set of transcriptional and phenotypic responses to shade and drought stress that promote access to these resources through changes to relevant traits (reviewed in [2]). Modulation in gene expression patterns is a well-characterized component of response to the shade cast by neighboring plants [27,28]. Such changes frequently involve the expression of genes directly related to shade avoidance and tolerance, including photoreceptor gene networks [29–31] and growth hormone regulation and response genes [32,33]; at the trait level, well-known responses to shade include increased leaf biomass allocation, shoot elongation, and producing structurally thinner leaves with greater surface area (reviewed in [28,34,35]). Recent studies have also demonstrated the potential for plants that experience shade as parents to induce these developmental traits in their offspring [36–38], but it is not known whether this is achieved via the same gene expression changes.

Likewise, plants show pronounced molecular and developmental responses to limited soil moisture. Immediate drought stress is known to affect expression of numerous genes associated with aspects of drought tolerance, such as cell wall thickening, osmotic adjustment and various metabolic processes [39–41]. Typical developmental responses include increased root biomass allocation [42], more extensive root systems [26], and cuticular thickening [43]. Parental drought is also implicated in similar effects on offspring phenotypes [44,45]. Although immediate and parental stress effects are thus well documented for both shade and drought, it is not known whether their relative impact remain consistent across these distinct stresses.

Third, it is also not known whether the relative impacts of immediate and parental plasticity are consistent among different genetic backgrounds in natural systems or if, instead, variation in this relationship may be a little recognized aspect of genetic variation (see [46]). If so, this raises the intriguing possibility that patterns of developmental integration of current and inherited environmental influences (e.g. the prioritization of one signal over the other in specific circumstances) may be subject to adaptive evolution [19,20,24,25,47]. It is well established that genotypes express different patterns of plasticity in response to the immediate environment (i.e. genotype–environment interaction [48]), providing the substrate for adaptive evolution of developmental plasticity [9,11,13,49–51]. Similarly, genotype-based studies of transgenerational plasticity typically reveal genetic variation for response to parental conditions (e.g. [52–57]). However, there has been very little examination of genetic variation for the *relative contributions* of parent- and offspring-environmental effects to offspring phenotype.

Finally, whether parental and current-environment effects on gene expression patterns are similar or distinct in nature is as yet an open question. While an environmental stress experienced during the parental generation can lead to the same trait changes as that stress does when encountered directly by the offspring (e.g. [23,36,52,58,59]), it is not known whether these transgenerational and immediate phenotypic responses result from concordant differential expression of the same genes. Although to our knowledge this has not been directly examined, available data suggest that inherited parental and immediate environmental influences may in fact yield rather different gene ontologies: several studies show that unlike transcription changes due to current environmental factors, conditions experienced by the parent act on genes involved in regulation of transcription and other RNA processes [3,60,61]. Sikkink *et al*. [62] tested effects of heat stress in *Caenorhabditis elegans* and found that gene expression changes due to parent versus offspring stress were only weakly correlated and led to dissimilar phenotypic effects.

To address these issues, we surveyed the transcriptome of our study system using RNA-seq to investigate the relative effects of parental and immediate (offspring) environment for two key resource stresses in plants, light and soil moisture. In order to describe the nature and diversity of the molecular plant response systems that have evolved in natural habitats, we studied field-sourced genotypes of *Persicaria maculosa* Gray (= *Polygonum (sensu latu) persicaria* L.), an annual plant found in natural populations in northeast North America across a range of light and soil moisture conditions [63,64]. Previous studies of *P. maculosa* genotypes have documented adaptive plasticity in response to both immediate and parental levels of light and moisture (e.g. [44,45,58,65–68]). The species is an excellent study system for testing plasticity because of its mixed breeding system [69]. The combination of self- and cross-fertilization provides naturally occurring genotypic diversity due to outcrossing, yet allows for the generation of highly inbred lines with no inbreeding depression [55]. Hence isogenic replicate plants of distinct *P. maculosa* lines can be raised in alternative parental and developmental conditions to characterize genotype-specific transgenerational and immediate plastic responses.

2. Material and methods

(a) Study system

We studied 4 genetic lines of *P. maculosa*, an annual generalist plant of allotetraploid origin [64]. To include diverse naturally evolved genotypes, we collected achenes (single-seeded fruits) from randomly chosen mature plants ≥ 1 m apart in 3 established,

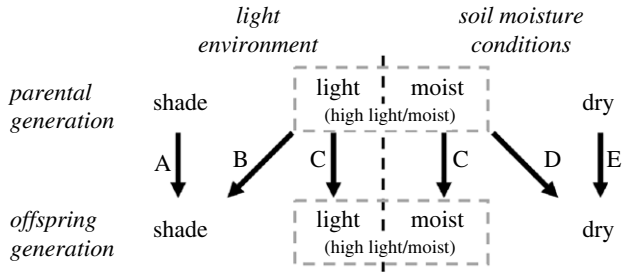

Figure 1. Experimental design for light environment and soil moisture treatments (separated by dotted vertical line indicating separate analyses). Arrows A–E represent experimental combinations of parent and offspring treatments. Plants in the high light/moist treatment (grey dotted boxes) provided a control comparison for both shade and dry plants in that generation. To assess the effect of the parent light treatment on transcription, we compared the shade-grown offspring of isogenic shade (A) versus high light (B) parent plants. The effect of offspring light treatment was examined by comparing offspring of high light parents when grown in a shade (B) versus high light (C) offspring treatment. We assessed the effect of offspring soil moisture treatment by comparing offspring of moist soil parent plants grown in a moist soil (C) versus dry soil (D) offspring treatment, and the effect of parent soil moisture by comparing dry soil-grown offspring of isogenic moist (D) versus dry (E) parent plants. To maintain a feasible experimental scale, we did not test the effects of alternative parental light and moisture level in the corresponding non-stressful offspring treatment (i.e. offspring high light and offspring moist soil).

geographically separate field populations occupying the species' typical range of habitats: MHF, Northfield, Massachusetts (MA): open, moist pasture; NAT, Natick, MA: open, mesic cultivated farmland; TP, Dover, MA: patchy, partly tree-shaded mesic field (site details in [63]; two genotypes were used from the NAT population which previous work has shown to be very genetically diverse). This sample provides insight to the species' genetic diversity for plastic response but was not designed to test adaptive population-level differences, which are beyond the scope of the study. A random subset of field-collected achenes (one per randomly chosen field parent) were propagated via self-fertilization and single-seed descent for four generations under uniform favorable greenhouse conditions (full sun with field-capacity soil moisture) to produce highly inbred experimental lines (hereafter 'genotypes'; see [48]).

(b) Parental generation

Achenes from each of four experimental genotypes (MHF1, NAT1, NAT2, and TP2) were grown to reproductive maturity in one of three randomly assigned greenhouse treatments: full sun with moist soil (high light/moist), full sun with dry soil (dry), or simulated shade with moist soil (shade). Note that the parental high light/moisture treatment provided a stress-free (control) comparison for both the parental dry and the parental shade treatments (figure 1).

(c) Offspring generation

Mature achenes from one (self-fertilized) parent plant for each genotype × parent treatment combination were germinated on petri plates and transplanted into pots (3 replicate seedings per pot). Experimental pots (4 genotypes × 5 [parent treatment × offspring treatment] combinations × 3 replicates = 60 pots) were raised in a randomized complete block design in a Conviron E2 growth chamber (Controlled Environments, Winnipeg, Canada) in one of five parent treatment × offspring treatment combinations—parent high light/moist × offspring high light/moist; parent shade × offspring shade; parent dry × offspring dry; parent high light/moist × offspring shade; parent high light/moist × offspring dry (figure 1; note that this design is partial rather than full factorial as the study does not aim to comprehensively address the question of potential adaptive match versus mismatch between parent and offspring stresses; see [16]). Previous studies have confirmed that the experimental 'dry' and 'shade' stress treatments strongly reduce biomass and reproduction in *P. maculosa* (e.g. [67,70]); field populations of the species can encounter the full range of resource levels tested [63]. 11–12 d post-transplant, leaf tissue from each replicate pot of 3 seedlings was harvested, pooled and flash frozen for RNA extraction (Promega SV Total RNA Isolation System Kit, Promega Corporation, Madison, WI, USA).

(d) De novo transcriptome sequencing, assembly and annotation

We submitted all 60 RNA samples to the National Genomics Infrastructure (NGI) at Uppsala University, Uppsala, Sweden for RNA sequencing. Libraries were prepared for each sample using an Illumina TruSeq Stranded mRNA with Poly-A selection Library Prep kit (Illumina, San Diego, CA, USA), which were subsequently paired-end sequenced (2 × 150) on an Illumina NovaSeq 6000 platform utilizing an S1 flow cell. In addition to the short read sequences, we submitted a pool of 5 samples from genotype TP2 representing all 5 Parent/Offspring treatment combinations for long-read sequencing following PacBio's Iso-Seq protocol (Pacific Biosciences of California Inc., Menlo Park, CA, USA) using a PacBio Sequel sequencing platform at the NGI, Uppsala, Sweden.

Because no reference genome of *P. maculosa* was available, we assembled the Illumina short read and PacBio long read data into a *de novo* transcriptome using Trinity software (v. 2.8.4) [71] following the protocol in Feiner *et al*. [72], with minor changes made to optimize for this data set (full assembly and analysis details in electronic supplementary material). Trinity assembled 48,022 transcripts, representing 33,828 predicted genes. The N50 for the transcriptome was 1,938 nucleotides (nt), with a median contig length of 1,015 nt and a mean contig length of 1,322.12 nt. We annotated the transcriptome using Trinotate (v. 3.2.0) [73], a software suite that makes use of a variety of other annotation tools. In brief, TransDecoder (v. 5.5.0, https://github.com/TransDecoder/TransDecoder) generated putative amino acid sequences, and BLASTx and BLASTp (BLAST + v. 2.9.0) [74] were used to search nucleic and amino acid sequences against the UniProtKB/Swiss-Prot database (retrieved 19 December 2019). A list of gene ontology (GO) terms for each transcript was generated based on the BLAST matches.

(e) Transcript quantification and differential expression analysis

Transcript abundances were quantified with kallisto quant using default settings [75], and transcripts with low expression were discarded from the analysis. We analysed for differentially expressed transcripts in R (v. 3.6.2) [76] using the sleuth package (v. 0.30.0) [77], fitting a generalized linear model for each transcript while accounting for variation in transcript abundances across replicates.

To assess the effects of the two qualitatively different stressors, we separately analysed parent and offspring shade versus high light samples, and parent and offspring dry versus moist soil samples. 21,383 transcripts were included in the light treatment analyses after filtering for low-count transcripts, and 20,007 passed filtering for the soil moisture analyses. For each of the two stress types, we tested for differential expression using the model ~genotype + parent environment + offspring environment + genotype:parent environment + genotype:offspring environment +

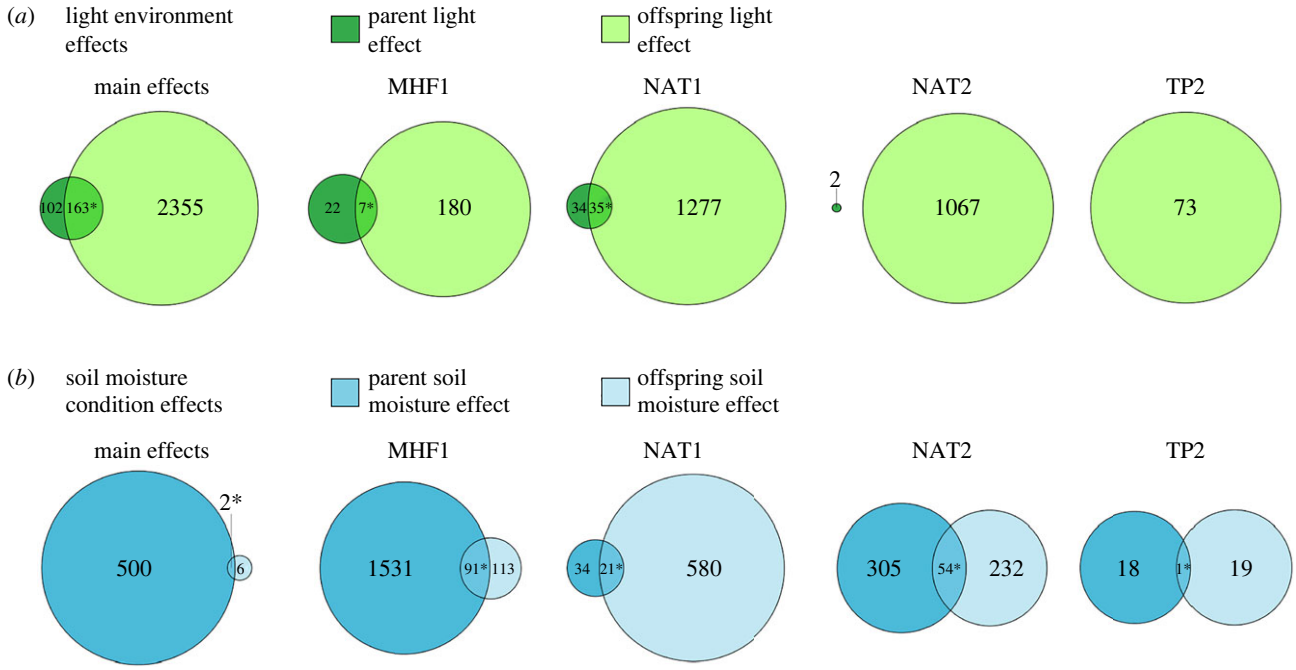

Figure 2. Euler diagrams showing the number of differentially expressed transcripts (DETs, $q \leq 0.1$) due to effect of parental environment, offspring environment and the intersection (transcript changes common to both generations' effects). Main effects (pooled across genotypes) and effects on each genotype are shown for (*a*) light treatment (shade versus high light) and (*b*) soil moisture treatment (dry versus moist soil). For each diagram in (*a*) and (*b*), numbers within the non-overlapping segments represent DETs unique to either the parent or offspring effects, while the number in the intersection represents DETs that were significantly differentially expressed due to both parent and offspring effects. Diagrams are scaled to an equivalent total area; individual circles within each diagram are scaled relative to the total area based on the number of DETs due to each effect. In the case of light environment, more DETs were found due to offspring than parent environment; the opposite was true for soil moisture. Note genotypic variation for the absolute and relative impact of parent and offspring effects of both light and soil moisture treatment (visualized as variation in the relative size of the segments for each genotype-specific diagram). * = intersection greater than upper limit of 95% CI for a random overlap (see Material and methods; no overlaps were found to be below the CI lower limit).

LF1 + LF2 + LF3 [+ LF4], where LF1, LF2, LF3 and LF4 are latent factors constructed using the lfmm_ridge function from the lfmm package (v. 1.0) [78] and LF4 was only used in the soil moisture analyses. Differential expression was calculated via likelihood ratio tests. Tests were corrected for false discovery via the Benjamini–Hochberg method ($q \leq 0.1$) [77]. To examine the relative breadth of response to the parent and offspring growth conditions, we calculated the ratio of the number of parent effect DETs to the number of offspring effect DETs both for the main effect and individual genotypes for both the light and soil moisture comparisons. In addition, we performed a co-expression network analysis using WGCNA [79], of which the details and findings can be found in the electronic supplementary material.

(f) Transcript overlap analysis

Individual DETs that appeared in multiple sets of interest (i.e. the overlap or intersection between any two or more given sets of transcripts) were quantified and visualized using the R package VennDiagram (v. 1.6.20) [80]. We calculated overlaps between the sets of DETs resulting from parent and offspring effects of either high light versus shade (hereafter 'light effect overlap') or dry versus moist soil ('soil moisture effect overlap') for both the main and individual genotype effects. Additionally, the four-way overlap among the main effect DETs was quantified, as well as four-way overlaps among sets of DETs for all genotypes for each of the main effects. Significance for the amount of overlap was calculated using a 95% confidence interval derived from a 10,000 iteration random sampling bootstrap analysis.

(g) Gene ontology enrichment analyses

We completed an exploratory GO term enrichment analysis of the DETs using the topGO package for R (v. 2.38.1) [81] with the *de novo* transcriptome as a background reference. Separate enrichments were carried out for parent and offspring effects for both the light and soil moisture analyses; these analyses were limited to GO terms in the 'biological process' category. We ran topGO using a Fisher's exact test with the default algorithm (a weighted elimination algorithm; see [82]), utilizing transcript counts for each GO term to calculate enrichment while accounting for the GO hierarchical structure, and we set a significance threshold of weighted $p \leq 0.05$.

3. Results

(a) Differential expression due to light environment

Expression of certain transcripts changed as a result of both current and parental shade versus high light, with the number of DETs resulting from the main effect of offspring light environment nearly 10-fold greater than the main effect of the parent treatment (2,518 and 265 DETs, respectively, giving a parent DET : offspring DET ratio [P : O] of 0.102; figure 2*a*, table 1). Within individual genotypes, we observed DETs due to offspring light environment for all genotypes and effects of parent light environment were found in 3 of the 4 (figure 2*a*, table 1). Consistent with the much larger main effect of current (offspring) than parent light environment, we found more DETs due to offspring than parent effects for every experimental genotype (P : O DET ratio < 1; table 1). These patterns were reflected within the co-expression network as well, with far more and larger modules responding significantly to offspring effects than parent effects, as well as considerable genotypic variation

Table 1. Quantity of significant DETs ($q \leq 0.1$) for main and genotype-specific effects of parent and offspring environment light and moisture treatments. A ratio of parent : offspring effect DETs (P : O) > 1 represents a broader impact of parent treatment transcription relative to the impact of offspring treatment within the given effect, while P : O < 1 indicates a broader effect of offspring treatment relative to parent treatment. All comparisons involving the impact of high light versus shade had a P : O < 1. However, comparisons involving moist versus dry soil were more varied, with the main effect and genotypes MHF1 and NAT2 with P : O > 1, genotype NAT 1 with P : O < 1, and TP2 with P : O ≈ 1. DET counts for $q \leq 0.05$ and $q \leq 0.01$ can be found in electronic supplementary material, table S1.

treatment comparison	effect or genotype	parent treatment DETs	offspring treatment DETs	parent DETs/offspring DETs (P : O)
high light versus shade	main effect	265	2518	0.102
	MHF1	29	187	0.155
	NAT1	69	1312	0.053
	NAT2	2	1067	0.002
	TP2	0	73	0.000
moist versus dry soil	main effect	502	8	62.750
	MHF1	1622	204	7.951
	NAT1	55	601	0.092
	NAT2	359	286	1.255
	TP2	19	20	0.950

within the vast majority of the modules (electronic supplementary material, data S2).

(b) Differential expression due to soil moisture conditions

As with light environment, transcripts were differentially expressed as a result of both current and parental soil moisture treatments. In this case, however, the relative impact of parent and offspring effects varied strongly among genotypes (figure 2*b*, table 1), and overall we found many more DETs due to parent than offspring conditions (main effect of parent dry versus moist soil = 502 DETs compared with main effect of offspring dry versus moist soil = 8 DETs for a P : O effect ratio of 62.750; table 1). Although both parent and offspring soil moisture treatments led to DETs in all genotypes, in two genotypes there were more DETs as a result of parent relative to offspring moisture conditions (MHF1 P : O effect ratio = 7.951; NAT2 P : O = 1.255), in one genotype there were more DETs due to offspring than parent moisture conditions (NAT1 P : O = 0.092), and in one genotype parent and offspring conditions resulted in nearly identical numbers of DETs (TP2 P : O = 0.950; figure 2*b*, table 1). Similar patterns emerged within the co-expression network as well: parent and offspring soil moisture conditions influenced a roughly equivalent number of and similarly-sized modules, and genotypic variation was found in the majority of modules (electronic supplementary material, data S2).

(c) Parent and offspring effect overlap analysis

We observed a significant intersection between parent and offspring effect DETs for both light environment and soil moisture conditions. With respect to the main effects of light (see 'Transcript overlap analysis' above), we identified 163 transcripts as being differentially expressed due to both the parent and offspring light environment (figure 2*a*), much higher than expected by chance based on bootstrapped random sampling (CI 97.5 percentile = 40 DET overlap). Greater than expected parent and offspring light effect overlaps also occurred within two genotypes (MHF1: 7 DETs overlap, 97.5 percentile = 2 DET overlap expected by chance; and NAT1: 35 DETs overlap, CI 97.5 percentile = 9 DET overlap expected by chance; figure 2*a*), and we saw no light effect overlaps in the remaining two genotypes. With respect to DET overlap among genotypes, there was no four-way intersection among all sets of genotype-specific DETs for parent light environment (electronic supplementary material, figure S2*a*). In response to offspring light environment, 7 DETs changed in all four genotypes (97.5 percentile = 0 DET overlap; electronic supplementary material, figure S2*b*), including a heat shock protein (HSP70), a methyltransferase (XPL1), a cellulose synthase (CESA2) and a phototropic-response protein (NPY2).

When examining the effects of soil moisture conditions, we found a significant overlap of two transcripts identified as being differentially expressed due to both the parent and offspring main effects (CI 97.5 percentile = 1 DET overlap expected by chance; figure 2*b*). We also detected a significant overlap between parent and offspring soil moisture effect overlap within all genotypes (MHF1 91 DETs overlap, CI 97.5 percentile = 24 DET expected overlap; NAT1: 21 DETs overlap, CI 97.5 percentile = 5 DET expected overlap; NAT2: 54 DETs overlap, CI 97.5 percentile = 10 DET expected overlap; TP2: 1 DETs overlap, CI 97.5 percentile = 0 DET expected overlap; figure 2*b*). We observed no four-way overlap among the genotypes for either the parent or offspring soil moisture effects (electronic supplementary material, figure S2*c*,*d*). Additionally, there were no DETs shared among the four main effects (parent light, offspring light, parent soil moisture and offspring soil moisture effects; figure 3).

(d) Gene ontology enrichment analyses

Among the top enriched GO terms (i.e. significantly enriched GO terms with the smallest *p* values) for gene expression changes associated with parent shade versus high light were terms primarily related to regulation of transcription

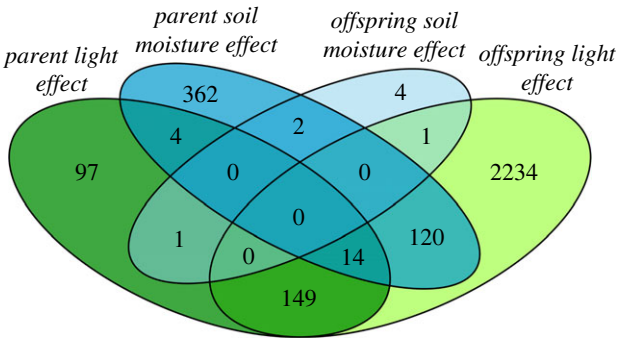

Figure 3. Venn diagram showing overlap in DETs ($q \leq 0.1$) due to parental and immediate environment main effects. Numbers within the overlaps represent DETs found in response to multiple environmental effects, while numbers occurring in only one ellipse represent DETs due to a single environmental factor. No DETs were found in the four-way intersection of these effects, suggesting a lack of shared molecular mechanism among all examined effects. Because the DETs in the Soil Moisture and Light effect sections were identified in separate analyses using different sets of transcripts, statistical tests for significant overlap were not performed.

and of developmental processes, and response to various abiotic stimuli (electronic supplementary material, table S2*a*). The top GO terms for effects of offspring light environment were more varied, with terms relating to response to direct stimuli including light and heat, chloroplast localization, morphogenesis, and other developmental and metabolic processes (electronic supplementary material, table S2*b*). Interestingly, similar to the parent light enrichment, GO terms associated with parental effects of dry versus moist soil consisted largely of terms involved in regulation of transcription, developmental processes and some metabolic processes (electronic supplementary material, table S2*c*). Only eight DETs were associated with the main effect of offspring soil moisture conditions. Of those, three were annotated: AT4G21870, a heat shock protein; RHA1A, a zinc finger protein; and AT3G16370, a lipase/acylhydrolase.

4. Discussion

Despite intense interest in both immediate and inherited environmental influences on phenotypic expression, few data are available regarding the potential effects of parental environments on individual animal or plant transcriptomes. Hence, although several studies have provided qualitative insight by testing the significance of both parent- and offspring-environment effects on phenotypes within the same study organism (reviewed by [16,83]), explicit comparisons of the relative transcriptome impact of these alternative sources of environmental influence are lacking. We have found only one published study that allows a direct quantitative comparison of parental and immediate transcriptome effects. Shama *et al*. [3] tested the effects of controlled immediate, maternal and grandmaternal thermal environments on transcriptome-wide expression changes in a pooled sample of pectoral fin muscle tissue from field-based three-spined stickleback (*G. aculeatus*) families. Interestingly, that study found that maternal temperature environment had the greatest impact on gene expression, while the immediate environment had the least impact.

Our study of naturally occurring *P. maculosa* genotypes raised in contrasting offspring or parental light and moisture levels revealed a more complex, context-dependent interplay of environmental influences across generations: we found no consistent pattern regarding the relative impact of parental and current environmental effects on transcription. Instead, although the developing individual's current environment generally altered expression of a greater number of DETs than did conditions during its parent's generation, depending on the type of environmental stress and the genotype the opposite also occurred—in certain cases, parental environment led to more DETs than immediate conditions. These patterns of transcriptome change are consistent with patterns of adaptive plasticity for developmental phenotypes in the same and closely related *P. maculosa* genotypes including total biomass, mean and specific leaf area, whole-plant leaf area, and root extension (details in electronic supplementary material, box S1) [36,58].

(a) The relative impact of parental and offspring environment on the number of DETs differed for the two environmental stresses tested

Although plant plasticity in response to immediate light and moisture conditions is comparatively well studied, including at the transcriptome level (see [11]), little is known regarding the effects of parental resource stresses on offspring gene expression. Published cases include Zheng *et al*. [84], who identified several transcripts that were differentially expressed over six successive generations of drought in *Oryza sativa*, and Liu *et al*. [85], who documented parental drought-induced changes in miRNA and mRNA expression in *Triticum turgidum*. Our study revealed that the architecture of transcriptional change in response to parental versus current stress differed for two major plant resources. In the case of ample versus limited light, far more transcripts changed expression level in response to developmental conditions than parental treatment. This pattern was consistent for every genotype in the study. In contrast, with respect to moist soil versus drought stress, the main effect of parental environment was far greater than that of the immediate, offspring treatment; however, this relationship was far less consistent across genotypes (as with any analysis of variance, a significant main factor effect can result from either many subtle changes in transcript number across several genotypes, or from a few transcripts that change number dramatically in certain genotypes; see next section). Similar patterns to these were also observed within co-expression networks, with more modules affected of the offspring light than by parent light environment but relatively equal contributions from offspring and parent soil moisture conditions.

Environmental conditions including resource availability can directly enter gene regulatory pathways as external cues are transduced to cellular components via metabolic feedbacks, hormone translocation and protein cascades (references in [1,2,49,86]). Through these mechanisms, immediate levels of both light and moisture strongly influence the phenotypes of developing plants, leading to functionally appropriate adjustments to resource-collecting tissues (e.g. [26,34,35,38,67,87,88]). Broad changes in the transcriptome may also be induced by immediate levels of both light [30,33,89] and soil moisture [41,90], though their phenotypic consequences are often unknown. Parental levels of both factors have also been shown to transgenerationally influence plant phenotypes

[37,91,92], including in *P. maculosa* [36,44,45,58]; in this species, DNA methylation changes plus stable or increased seed provisioning jointly mediate effects of parental light and moisture stress on offspring [36,44,55].

While it is often assumed that the immediate environment will have a larger effect than the parental environment on an individual's phenotype [16,18–20], theory predicts that whether the parental or offspring environment has a stronger impact will reflect how reliably each one predicts the selective environment that individual will encounter: strong transgenerational plasticity is likely to evolve when the parental environment is a better indicator than the current environment and greater within-generation plasticity when the current environment is a better predictor [19,20,24,25]. In the case of ample light versus shade, the consistently greater impact of the developmental environment may indicate a primary predictive role of current light conditions.

In *P. maculosa* habitats, leaves and branches of herbaceous neighbors as well as larger woody plants create a fine-grained mosaic of sunny and shaded microsites into which offspring (as seeds) are passively dispersed (generally 0.5–1 m from the mother plant; R. Waterman, unpublished data). Because shade cast by trees and perennials remains spatially constant over many generations while the herbaceous community changes every season, the parent-to-offspring autocorrelation of sunny and shaded microsites at the individual plant scale is moderate but noisy (e.g. Pearson correlation of 0.57 from 2019 to 2020 [93]). This modest environmental autocorrelation combined with unreliable cues due to within- and across-generation variability may only weakly favour persistent transgenerational effects [94] despite the limited dispersal distance in this system (see [25]). We speculate that by several weeks into development (the stage at which RNA was extracted in the present experiment), a plant's current light environment may more strongly signal its future growth conditions such that relatively greater reliance on immediate cues would be expected to evolve [25,47]; further investigation is required to confirm this speculation, including testing the effects of parental shade stress on development of unstressed offspring. By contrast, moisture conditions in the field reflect site soil composition and topography, resulting in somewhat larger spatial patches likely to remain consistently wet or dry relative to each other from year to year but varying in concert due to seasonal weather. Since within-generation precipitation generates unreliable immediate cues, and because an anticipatory signal of drought stress from a parent *P. maculosa* plant boosts offspring survival in dry soil [45], this system may have evolved to generate such a signal [94] associated with substantial transcriptome changes. We note that to comprehensively address the extent to which responses are adaptive, further research using fully factorial designs may add valuable insights [16]. Additionally, we outline three general caveats which apply to this and other RNA-seq datasets in electronic supplementary material, box S2.

(b) The relative impact and transcriptional effects of immediate and parental resource stress varied among genotypes

The effects of contrasting light and moisture conditions during development on transcription (i.e. number of DETs due to a given offspring treatment difference) varied considerably among the four experimental *P. maculosa* genotypes. Genotypic differences in patterns of developmental response to immediate environmental conditions (genotype by environment interaction variance [48]) are a prevalent feature of natural systems (reviewed by [51]; e.g. [14,95–99]). It is this aspect of genetic variation that provides the potential for patterns of developmental plasticity to evolve under natural selection [9,12,13,50]. While innumerable studies document such $G \times E$ for morphology, life-history, and other plant and animal traits, there is also a growing literature showing genotypic differences for immediate environmental effects at the transcriptome level that (presumably) underlies changes to developmental phenotypes (reviewed in [100]; e.g. [11,101–103]). Our findings add further evidence of genotypic variation for transcriptional response patterns to developmental conditions. Indeed, $G \times E$ was observed in the co-expression networks for both environmental stresses (electronic supplementary material).

To date, the literature documenting genotypic variation for parental environment effects is less extensive. Such genotype × parental environment interaction effects on phenotypes have been reported for traits including vertebrate body size [104] and disease risk [105]; invertebrate parasite resistance [106]; and plant life-history [46,107,108], defense structures [52,60], biomass allocation, and morphology [44,55,66]. Our data revealed pronounced variation among *P. maculosa* genotypes for the number of DETs induced by parental light and moisture stress as well as $G \times$ *parent* E interaction effects in the co-expression networks, confirming the general observation that genotypes differ in the environmental effects transmitted from parents to offspring by revealing such variation at the transcriptome level. These results are consistent with previous studies documenting significant genotype × parental environment interaction variance within populations of this species, in response to both parental light levels [36,66] and parental soil moisture [45].

Our data also provide new insights with respect to genotypic variation for environmental response: genotypes differed in the relative impact of parental and immediate conditions on the transcriptome. In the case of light environment treatments, all genotypes showed a greater offspring than parental effect on transcription, but the ratio of these effects varied considerably (from 0.002 to 0.155). With respect to soil moisture, depending on genotype the relative effect of parental conditions varied from less than to much greater than the effect of the individual's immediate environment (from 0.092 to 7.951). Since our experimental *P. maculosa* genotypes were field-sourced, they represent naturally occurring variation for the degree to which parental versus immediate conditions influence offspring transcriptomes and presumably the resulting phenotypes.

Genotypic variation in natural populations may thus include not just different levels of responsiveness to immediate and parental conditions, but—because parental and offspring effects vary independently of each other among genotypes—variation in the *relationship* between these two aspects of environmental response. As a result, this relationship can potentially evolve under natural selection [20,24]. Several simulation models have examined this issue by varying the relative phenotypic impact of parental and immediate environments among genotypes [19,25,47]. In these models, as noted in the preceding section, variation in the relative reliability of parental and current environmental 'cues' for predicting an organism's selective conditions led to adaptive evolution of their relative impact on individual response.

Empirical tests of these models require data on the spatial distribution and cross-generational autocorrelation of alternative environments in naturally evolving systems. In the case of fine-grained environmental variables such as light and shade, resolving these patterns is particularly challenging. Available data (summarized in the previous section) indicate that cross-generation microsite correlations for both light and soil moisture are highly variable within field populations of this colonizing species, providing a generally noisy target for natural selection. This may allow the persistence of genetic variation which can then contribute to adaptive evolution of the relative impact of transgenerational effects—if a site becomes temporally more consistently moist or dry, for instance, or if a new population is founded in a location with either very strong or very weak cross-generation environmental correlations.

(c) Parental and offspring effects led to broadly different transcriptome changes

We found surprisingly little overlap in the transcriptional effects of parental and immediate levels of a given resource in terms of individual transcripts. Additionally, functional categories for the effects of offspring light and soil moisture conditions were almost completely distinct from each other as well as from their respective parent effects. GO enrichments for offspring light DETs were consistent with functional genomics studies showing effects of a plant's current light conditions on light response gene networks [29,30] and genes linked to regulation of, and response to, plant growth hormones [32,33]. The immediate soil moisture environment transcript annotations also agreed with the literature: ROS stress is known to accompany drought stress due to increased photorespiration [109], and heat shock proteins have been linked to drought response in numerous systems [110–112]. These studies indicate that a plant's transcriptional response to its current conditions may largely reflect expression changes in genes related to a specific environmental stress.

In contrast, GO terms for regulation of RNA processes and transcription were among the top enriched terms in response to both parental light and parental moisture. Note that despite similar ontologies, only a small number of DETs changed in response to both parental environmental factors (figure 3), suggesting that different genes are likely involved in each transgenerational regulatory response. No terms relating to these transcriptional processes were identified among top enriched in either offspring enrichment analysis (electronic supplementary material, table S2). Differential expression of transcription-related genes has been shown to be specifically associated with parental effects in other transgenerational animal and plant studies, including maternal thermal environment effects in stickleback [3], parental CO_2 concentration effects in the reef fish *Acanthochromis polyacanthus* [61], and effects of simulated herbivory on parental *Mimulus* plants [60]. That parental environment effects related to RNA processes and transcriptional regulation have been observed in such diverse cases suggests that the developmental influence of parental environment may be mediated in part via epigenetic regulation of transcripts which then alter downstream gene expression.

In *P. maculosa*, parental and immediate shade influence seedling development in similar ways [36,65]; the same is true for parental and immediate drought stress [45,58,68]. Yet at the level of transcription, the present study found largely dissimilar parental and offspring effect GO enrichments as well as a generally low overlap of DETs that were associated with both parent and immediate levels of either resource stress. This indicates that even when transgenerational and within-generation plasticity yield similar phenotypic outcomes, they may do so through distinct changes in gene expression; instead, parental effects may act on genes that participate in cellular regulatory systems which then influence downstream expression of transcripts that produce said phenotypic response. Although these downstream expression changes may be subtle and hence require greater statistical power to be individually detected, they may collectively contribute to significant trait change. To evaluate the extent to which parental and immediate environments may differently participate in developmental phenotypes in natural systems, fully factorial transcriptomics studies with very high replication may be necessary to provide sufficient power to discover such subtle expression changes.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. Raw RNA-seq reads are available at the NCBI Sequence Read Archive at accession PRJNA886646. The assembled transcriptome and other data files are available at the Dryad repository: https://doi.org/10.5061/dryad.jsxksn0fp [113].

Supplementary material is available online [114].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. T.S.E.: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; N.F.: funding acquisition, investigation, methodology, validation, writing—review and editing; M.F.A.: formal analysis, methodology, writing—review and editing; J.D.C.: investigation, methodology, writing—review and editing; S.E.S.: conceptualization, funding acquisition, investigation, methodology, supervision, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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