Intergenerational Effects of Early Life Starvation on Life-History, Consumption, and Transcriptome of a Holometabolous Insect

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ABSTRACT: Intergenerational effects, also known as parental effects in which the offspring phenotype is influenced by the parental phenotype, can occur in response to parental early life food-limitation and adult reproductive environment. However, little is known about how these parental life stage-specific environments interact with each other and with the offspring environment to influence offspring phenotype, particularly in organisms that realize distinct niches across ontogeny. We examined the effects of parental early life starvation and adult reproductive environment on offspring traits under matching or mismatching offspring early life starvation conditions using the holometabolous, haplo-diploid insect *Athalia rosae* (turnip sawfly). We show that the parental early life starvation treatment had context-dependent intergenerational effects on the life-history and consumption traits of offspring larvae, partly in interaction with offspring conditions and sex, while there was no significant effect of parental adult reproductive environment. In addition, while offspring larval starvation led to numerous gene- and pathway-level expression differences, parental starvation impacted fewer genes and only the ribosomal pathway. Our findings reveal that parental starvation evokes complex intergenerational effects on offspring life-history traits, consumption patterns as well as gene expression, although the effects are less pronounced than those of offspring starvation.

Introduction

Intergenerational effects, more commonly known as parental effects, are defined as causal influences of parental phenotype on offspring phenotype that are likely mediated by non-DNA sequence-based inheritance (Wolf and Wade 2009; Perez and Lehner 2019). Together with transgenerational effects, which occur across several generations, they play a key role in the ecology and evolution of organisms (Badyaev and Uller 2009) and impact the responses of individuals to changing environmental conditions (Sánchez-Tójar et al. 2020). One important environmental factor that can rapidly change during an organism's lifetime and across generations is food availability. Periods of starvation are commonly encountered by insects in the wild (Jiang et al. 2019) and, when experienced early in life, often have long-lasting effects on an individual's phenotype, affecting life-history, consumption, and behavior (Miyatake 2001; Wang et al. 2016; McCue et al. 2017). Intergenerational effects of starvation events on offspring developmental trajectories have been found to differ depending on the exact starvation regime, sex, and traits that are considered (Saastamoinen et al. 2013; McCue et al. 2017; Paul et al. 2019). To fully elucidate the adaptive nature of intergenerational effects, studies should encompass different phases in development of the parental life cycle and how such life stage-specific experience affects offspring phenotype (English and Barreaux 2020).

The early life environment can shape the phenotype of an individual and its offspring via distinct routes (Monaghan 2008; Burton and Metcalfe 2014; Taborsky 2017). Individuals experiencing a benign environment in early life may pass on their superior condition to their offspring (van de Pol et al. 2006), often called silver spoon or carry-over effects (Monaghan 2008), resulting in offspring that are better able to deal with stressful conditions (Franzke and Reinhold 2013). Similarly, negative effects of a poor start in life might also be passed on, resulting in offspring less able to cope with stressful conditions (Naguib et al. 2006). Alternatively, such stressed

individuals may produce offspring that are buffered against stressors, for example, through parental provisioning (Valtonen et al. 2012; Pilakouta et al. 2015; Hibshman et al. 2016). Finally, identical environmental conditions experienced in a certain stage (i.e. a match between parental and offspring environment) may be predicted to be optimal for the offspring (match-mismatch scenario). Evidence for such predictive adaptive effects has been found in several species (Raveh et al. 2016; Le Roy et al. 2017), but is weak in others (Uller et al. 2013). Each of these avenues of intergenerational effects are not mutually exclusive. For example, offspring in mismatched environments may do relatively better if their parents were of high quality, due to silver spoon effects (Engqvist and Reinhold 2016). Fully reciprocal designs are needed to determine the relative importance of the experience in a certain life stage or generation on such developmental plasticity (Groothuis and Taborsky 2015).

In addition, parental investment in offspring can vary depending on the condition of the parent's mate (Ratikainen and Kokko 2010). Both males and females have been shown to enhance investment in response to perceived increases in mate quality or attractiveness (Cunningham and Russell 2000; Cornwallis and O'Connor 2009). Changes in parental investment based on mate cues may potentially counteract or augment the effects of the parental early life environment on offspring phenotype. For example, poor early life conditions can lead to poor quality of mates and therefore affect mating success and reproductive investment (Hebets et al. 2008; Vega-Trejo et al. 2016). As niches often shift across an individual's lifetime, both parental early and later life environments and their interaction must be considered.

With discrete phases during development, insects, particularly holometabolous insects, present ideal organisms in which to investigate how intra- and intergenerational environmental cues, such as food limitation and mate quality, might influence individual phenotypes (English and Barreaux 2020). Periods of low

resource availability during larval development are known to influence developmental trajectories with knock-on effects on adult size, reproductive success and adult starvation resistance (Boggs and Niitepõld 2016; Wang et al. 2016). Individuals may respond to periods of starvation with increased food uptake (Regalado et al. 2017) as well as compensatory growth (i.e. a faster growth rate) or catch-up growth (i.e. attaining a minimum size by prolonging the larval development) (Hector and Nakagawa 2012). Moreover, starvation leads to substantial shifts in gene expression within individuals (Moskalev et al. 2015; Jiang et al. 2019; Etebari et al. 2020; Farahani et al. 2020). However, little is known about how long these effects may persist (McCue et al. 2017) and to what extent parental starvation may affect gene expression of offspring experiencing similar or dissimilar conditions.

In the present study, we investigated the effects of parental early life starvation and parental reproductive environment on offspring (i.e. intergenerational effects), under matching or mismatching larval starvation conditions, using the turnip sawfly, *Athalia rosae* (Hymenoptera: Tenthredinidae). Adult females oviposit on various species of Brassicaceae, including crop species, on which *A. rosae* can become a pest (Riggert 1939; Sawa et al. 1989). The leaf-consuming larvae can readily experience periods of starvation when their hosts are overexploited (Riggert 1939). The adults are nectar-feeding and in addition collect neo-clerodanoid-like compounds (hereafter called ‘clerodanoids’) from non-Brassicaceae plants, which improve their mating probability (Amano et al. 1999) and affect the social interactions between adults of both sexes (preprint Paul et al. 2021; preprint Paul and Müller 2021). We measured various key life-history traits to assess the combined and interactive effects of parental and offspring larval resource availability and parental clerodanoid exposure on the offspring. Moreover, we measured effects of parental and offspring early life starvation regimes on consumption and gene expression of offspring. We predicted that 1) there are stronger intragenerational than intergenerational effects of

larval starvation treatment on all parameters measured; 2) matching conditions between parental and offspring starvation treatment are beneficial, while a mismatch leads to a reduced performance, with silver spoon or parental buffering effects potentially augmenting or dampening any mismatch effects; 3) clerodanoid exposure increases parental investment in offspring due to an enhanced mate attractiveness, leading to positive effects for the offspring.

Materials and Methods

Set-Up of Insect Rearing and Plant Cultivation

Adults of *A. rosae* were collected in May 2019 from meadows adjacent to farmland in Germany at two locations (population A: 52°02'48.0"N 8°29'17.7"E, population B: 52°03'54.9"N 8°32'22.2"E). Adults from each population were split between two cages (total of 4 cages) and maintained for one generation at room temperature and 16 h: 8 h light:dark on potted plants of white mustard (*Sinapis alba*) and Chinese cabbage (*Brassica rapa* var. *pekinensis*). F1 adults were used to set up a total of six cages with males and females from different populations in four cages and two cages with virgin females only (Fig. 1). Larvae of the final instar, the eonymphs, were placed into individual cups containing soil for pupation. Emerged adults were kept individually in Petri dishes and provided with a honey:water mixture (1:50). This sawfly species is haplodiploid, i.e. virgin females produce male offspring (Naito and Suzuki 1991). For mating, pairs of non-sib F2 females and males (N = 20 per treatment level) were placed together and allowed to mate at least once. Afterwards, mated as well as virgin females were placed individually into boxes (25 x 15 x 10 cm) with a middle-aged leaf of 6-8 week old cabbage plants supplied with water for oviposition and a honey:water mixture in a climate chamber (20 °C:16 °C, 16 h: 8 h light:dark, 70% r.h.). The boxes were checked and topped up with honey water daily, and an additional leaf was added if the first leaf showed signs of wilting. Females

were removed from the boxes after one week and their offspring used to set up the experimental generations (Fig. 1).

Plants of *B. rapa* and *S. alba* were grown from seeds (Kiepenkerl, Bruno Nebelung GmbH, Konken, Germany) in a greenhouse (20 °C, 16 h: 8 h light:dark, 70% r.h.) and a climate chamber (20 °C, 16 h: 8 h light:dark, 70% r.h.). Plants of *Ajuga reptans* used for clerodanoid supply were grown from seeds (RHS Enterprise Ltd, London, UK) in the greenhouse and transferred outside in late spring once about 2 months old. Middle-aged leaves were picked from non-flowering *A. reptans* when plants were approximately 8 months old.

Experimental Set-Up and Measurements of Life-History Data

The leaves of *B. rapa* offered to F2 females were checked daily for hatching larvae. Hatched larvae were individually placed into Petri dishes (5.5 cm diameter) with moistened filter paper and *B. rapa* leaf discs cut from 7-10 week old plants. Leaf discs were provided *ad libitum* (except during starvation periods) and replaced daily. Each dish was checked daily for the presence of exuviae to track the larval instar. Larvae from each box (parental pair) in both the F3 and F4 generation were split equally between one of two treatments, no starvation (N) or starvation (S). Starvation individuals were twice starved for 24 h, first the day after moulting into 2nd instar (L2) and second on the day of moulting into 4th instar (L4) to minimize early mortality whilst mimicking the food deprivation larvae may experience when occurring in high densities (Riggert 1939).

Eonymphs were placed in individual cups with soil for pupation. On the day of emergence, adults were weighed (Sartorius AZ64, M-POWER Series Analytical Balance, Göttingen, Germany), kept individually in Petri dishes and provided with honey:water mixture. For the F3 adults, pairs of non-sib females and males reared under the same larval starvation treatments (N or S) were assigned to one of two

reproductive environment treatments (C- or C+). C+ individuals were exposed to a leaf section (1 cm^2) of *A. reptans* for 48 h prior to mating, giving individuals time to take up clerodanoids (preprint Paul et al. 2021). Mated F3 females (2-9 days old) from each of these four treatments (NC-, NC+, SC-, SC+) and C- virgin females (NC-, SC-) were then placed in individual breeding boxes, as described above for the F2. The experimental generations (F3-F4) were reared to test the effects of parental (F3) and offspring (F4) larval starvation, and their interaction with the adult reproductive environment on the offspring phenotype (F4). In both generations, larval, pupal, and total development time (from larva to adult) as well as the adult body mass at the day of emergence were taken for each individual. In total 358 F3 larvae (177 females, 181 males) and 486 F4 larvae (282 females, 202 males) reached adulthood, out of the 607 and 688 larvae, respectively, that were reared in each generation. Sample sizes for the F3 and F4 generation are given in Fig. 1. Experimental rearing and consumption assays were all carried out in a climate chamber (20 °C:16 °C, 16 h: 8 h light:dark, 70% r.h.).

Consumption Assays

Consumption assays were performed with F4 larvae at the start of L3 to measure the relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested food (ECI). Each L3 larva was weighed (= initial body mass) (ME36S, accuracy 0.001 mg; Sartorius, Göttingen, Germany) and provided with four fresh discs cut from middle-aged leaves (surface area of 230.87 mm^2 per disc). After 24 hours, larvae were weighed again (= final body mass) and the leaf disc remains were scanned (Samsung SAMS M3375FD, resolution 640 x 480). The total area of leaf consumed (mm^2) was then calculated as the difference between the average leaf area and the remaining leaf area.

Statistical Analyses

All data were analyzed using R 4.0.2 (2020-06-22). We set $\alpha = 0.05$ for all tests and checked model residuals for normality and variance homogeneity. Stepwise backwards deletion using Chi-squared ratio tests (package:MASS; version 7.3-53.1) was employed to reach the minimum adequate model (Crawley 2012). Posthoc analyses were carried out using the package 'multcomp' (Hothorn et al. 2008; version 1.4-13). Post data entry, raw data were visually inspected thrice, all variables plotted and outliers and possible anomalies in the data (e.g. strings of similar values) interrogated (package:pointblank; version 0.6.0). Intragenerational effects of starvation on life-history traits of F3 individuals were tested as described in S1.

In *A. rosae*, usually 6 instars for female and 5 instars for males are found (Sawa et al. 1989). Due to observations made during the experiment (no *a priori* hypothesis), we tested in the F4 generation whether the likelihood of an additional larval instar (7 for females and 6 for males) differed based on offspring larval starvation treatment (OS: N or S) using a binomial generalized linear mixed model (package: lme4), where the predictor was OS and parental pair was included as a random effect. The effects of the parental larval starvation treatment (PS: N or S), parental clerodanoid exposure (PC: C- or C+), OS and their interaction on larval, pupal and total developmental time as well as adult mass of F4 individuals were assessed in separate linear mixed models (lmm), with parental pair included as a random effect (controlling for non-independence of sibling larvae). Female and male data were analyzed separately to enable model convergence.

RGR, RCR, and ECI of F4 larvae were analyzed using glmm (package lmerTest; version 3.1-3; Kuznetsova et al. 2017). We excluded PC as a predictor variable from the consumption assay analysis due to the low number of individuals in certain treatments (Fig. 1) and analyzed male and female data separately as above. To assess variation in RGR, the change in larval body mass [final mass — initial mass]

was used as the response variable and initial larval body mass, PS, OS, and the interactions between all the predictors. To assess RCR, we used the total area of consumed leaf material as the response variable and initial larval body mass, PS, OS, and both their three-way and two-way interactions as the predictors. Finally, for ECI the change in larval body mass was taken as the response variable and the total area of consumed leaf material, PS, OS, and the interactions between all the predictors. Parental pair was included in all three models as a random effect.

Sample Collection, Library Preparation, Sequencing and Alignment for RNAseq

A total of 24 male larvae (L4, 9 d old), comprising six biological replicates per treatment level (F3/F4: N/N, N/S, S/N, S/S, all C-, Fig. 2), were collected to investigate the effects of parental and offspring starvation treatment on gene expression in larvae of the offspring generation (F4). Individuals were chosen in a way that maximized the equal spread of siblings across treatments and frozen at -80 °C prior to extraction. RNA was extracted with an Invitrogen PureLink™ RNA Mini Kit (ThermoFisher Scientific, Germany), including a DNase step (innuPREP DNase I Kit, analyticJena, Jena, Germany). RNA quality was assessed on a bioanalyzer 2100 (Agilent, CA, United States) and Xpose (PLT SCIENTIFIC SDN. BHD, Malaysia). Library preparation (Ribo-Zero for rRNA removal) and sequencing (NovaSeq6000 and S4 Flowcells, Illumina, CA, United States) were provided by Novogene (Cambridge, UK). Sequence quality before and after trimming was assessed using FastQC (v. 0.11.9; Andrews 2010). After short (< 75 bp), low quality (Q < 4, 25 bp sliding window), and adapter sequences were removed using Trimmomatic (Bolger et al. 2014), more than 98% of reads remained. Reads were mapped to the annotated genome of *A. rosae*, version AROS v.2.0 (GCA_000344095.2) with RSEM v1.3.1 (Li and Dewey 2011), which implemented mapping with STAR v2.7.1a (Dobin et al. 2013).

Differential expression analysis was conducted with DESeq2 (version 1.28.1; Love et al. 2014). RSEM results were passed to DESeq2 for gene-level analyses using Tximport (version 1.16.1; Soneson et al. 2015). Prior to analysis, genes with zero counts in all samples and those with low counts (< 10) in less than a quarter of samples (6) were excluded. Model fitting was assessed by plotting dispersion estimates of individual gene models and outlier samples were inspected using principle component analysis of all expressed genes and pairwise-distance matrices between samples. DESeq2 allows for both the normalization of read counts (accounting for differences in library depth/sequencing depth, gene length, and RNA composition) and the shrinkage of gene-wise dispersion (accounting for differences in dispersion estimates between genes) whilst modelling a negative binomial glm of predictors for each gene to correct for overdispersion (Love et al. 2014). Expression was modelled based on the entire dataset, with the four levels representing the combination of parental and offspring starvation treatments (F3/F4): N/N, N/S, S/N, and S/S. Differential expression was assessed in four pairwise comparisons between the treatments: 1) N/N vs N/S and 2) S/N vs S/S were used to assess the effects of offspring starvation treatment for individuals whose parents experienced the same starvation treatment, whereas 3) N/N vs S/N and 4) N/S vs S/S were used to assess the effects of differing parental starvation treatment on individuals that experienced the same offspring starvation treatment. Significance was based on a Wald Test and shrunken LFC values with apeglm (version 1.10.1; Zhu et al. 2019). P-values were adjusted for multiple testing using Benjamini-Hochberg (Benjamini and Hochberg 1995) procedure with a false discovery rate (FDR) of 0.05. Afterwards, significantly differentially expressed genes (relatively up- or downregulated) were extracted and filtered with a p-adjust of < 0.05. Among these significantly differentially expressed genes we searched for the terms linked to genes with known roles in stress and/or starvation response, using the keywords: “heat shock protein” (and “hsp”),

“cytochrome P450”, “octopamine” and “tyramine” in the putative gene names. Venn diagrams (Venn.Diagram, version 1.6.20) were used to depict the relationship between the differentially expressed genes for each pairwise comparison. The expression of the significantly expressed genes of each comparison was visualized in a heatmap using normalized counts scaled per gene (scale=“row”) (pheatmap, version 1.0.12).

Unmapped reads were inspected to check for the differential expression of genes not present in the reference genome. These were extracted from the RSEM-produced BAM file using samtools view (Li et al. 2009) and converted to fastq (using bamtools bamtofastq; Barnett et al. 2011), and *de novo* assembled using Trinity (default settings) (Grabherr et al. 2011). This assembly was done jointly with reads from additional samples (preprint Paul et al. 2021) to optimize coverage. The unmapped reads were mapped back to this reference and expected read counts extracted using eXpress (Roberts et al. 2011). Transcripts with >10,000 mapped reads were identified using BLASTN (default settings, limited to one match per gene and $1e^{-1}$) against the NCBI nucleotide database. Differential expression of unmapped reads in the larval samples was carried out as for mapped reads (see above).

For characterization of differential expression at the pathways level, KEGG (Kyoto Encyclopedia of Genes and Genomes) terms were assigned to the predicted genes in the *A. rosae* genome using the KEGG Automatic Annotation Server (KAAS; Moriya et al. 2007) (S2). Of the annotated genes for which we had read counts, 65% were assigned to at least one KEGG pathway. A gene set enrichment analysis was then performed on the normalized counts using GAGE (Luo et al. 2009) and pathview (Luo and Brouwer 2013) in R, applying an FDR-adjusted *P*-value cut-off of < 0.05 to identify differentially expressed pathways. We derived the non-redundant significant gene set lists, meaning those that did not overlap heavily in their core genes, using esset.grp and a *P*-value cut-off for the overlap between gene sets of $10e^{-10}$.

Results

Life-History Traits

In the parental generation (F3), larval starvation had trait- and sex-specific effects on life-history, leading to a prolonged total development time for both sexes and a lower body mass in females (for details see S1). In the offspring generation (F4), both females (X^2_1 = 11.06, P < 0.001) and males (X^2_1 = 29.25, P < 0.001) were significantly more likely to have an additional larval instar prior to the eonymph stage if they were starved than if they were not starved (Fig. 2A, B). The exposure of parents to clerodanoids (PC) had no influence on any of the life-history parameters measured in the offspring, whereas the effect of parental (PS) and offspring larval starvation treatment (OS) were trait- and sex-specific. There was an interactive effect of PS and OS on both female (PS * OS: X^2_1 = 10.37, P = 0.001) and male (PS * OS: X^2_1 = 4.89, P = 0.027) total development time, such that intra-generational starvation led to a longer development time for both sexes (Fig. 2C, D; S3A, S3B). Larval development of F4 females was only affected by OS (OS: X^2_1 = 319.40, P < 0.001; S4A), while there was an interactive effect of PS and OS on male larval development time (PS * OS: X^2_1 = 6.60, P = 0.010; S3B, S4B). The reverse occurred for pupal developmental time with an interactive effect of PS and OS on females (PS * OS: X^2_1 = 24.57, P < 0.001, S3A, S4C), but only a significant effect of OS on males (OS: X^2_1 = 17.23, P < 0.001; S4D). Both PS (X^2_1 = 4.02, P = 0.045) and OS (X^2_1 = 12.95, P < 0.001) independently influenced female adult mass (PS * OS: n.s.), with F4 females having a lower mass if they had experienced starvation during development, but those females whose parents were starved had a higher overall mass than when parents had not starved (Fig. 2E). In contrast, F4 male adult mass was significantly affected by the interaction between PS and OS (PS *OS: X^2_1 = 4.58, P = 0.030, Fig. 2F). Male offspring of starved parents that themselves were not starved during

development (S.N) had a higher mass than offspring that were starved (S.S) (pairwise comparison: z = -3.47, P = 0.003, Table S3B).

Consumption

OS had a significant interactive effect on the relationship between change in mass and initial body mass (i.e. RGR) in offspring larvae in both females (initial body mass * OS: $F_{1,60.75}$ = 5.96, P = 0.017) and males (initial body mass * OS: $F_{1,39.54}$ = 7.32, P = 0.009). The change in body mass of starved larvae increased more steeply with an increase in initial larval body mass compared to non-starved larvae for both sexes (Fig. 3A, B), indicating a higher RGR in starved larvae. There was no effect of PS on RGR either independently or interactively for both sexes. For RCR in females, there was a significant interaction effect of initial larval body mass and PS on the area of leaf consumed (initial body mass * PS: $F_{1,62}$ = 6.15, P = 0.015), such that the leaf area consumed increased with initial body mass for individuals from non-starved parents but did not change for individuals from starved parents (Fig. 3C). Thus, larger female larvae consumed more than smaller larvae when their parents were not starved but not when their parents were starved. For males, initial body mass had a significant positive effect on the leaf area consumed (initial body mass: $F_{1,41.45}$ = 21.97, P < 0.001) across all treatments (Fig. 3D); i.e. there was no effect of either OS or PS on RCR. Regarding ECI, leaf area consumed and OS independently influenced change in body mass for both females (leaf area consumed: $F_{1,54.12}$ = 7.17, P = 0.009; OS: $F_{1,54.77}$ = 11.36, P = 0.001) and males (leaf area consumed: $F_{1,44}$ = 31.55, P < 0.001; OS: $F_{1,44}$ = 6.92, P = 0.011). The ECI was higher when larvae were not starved than when they were starved, but under both conditions consuming more leaf material led to a higher body mass increase for larvae (Fig. 3E, F).

Gene Expression

Sequencing of the 24 larvae resulted in a total of 1.4 billion reads with an average of 58 million reads per sample (SE: ±2 million reads) and an average GC content of 43%. Prior to normalization, the average number of mapped reads per sample was 33,675,600, equating to an average of 91% reads per sample aligned to the reference genome (range 85-93%).

Offspring starvation had the strongest effect on gene expression (Figs 4, 5, S5). The two comparisons between starved and non-starved larvae revealed 4727 (N.S vs N.N) and 5089 (S.N vs S.S) significantly differentially expressed genes (LFC > 0, adjusted $p < 0.05$) and a large number of these overlapped between the two comparisons (4002). In contrast, there were far fewer significantly differentially expressed genes in the two comparisons in which the larvae had the same starvation treatment, namely 155 (S.N vs N.N) and 75 (N.S vs S.S). In the comparison between starved and non-starved larvae from non-starve parents, there was evidence of regulation in some genes known to be associated with stress response. There was significant downregulation of putative heat shock proteins (eight downregulated and one upregulated) and upregulation of putative cytochrome P450 genes (21 genes upregulated, six downregulated) as well as upregulation of one octopamine and one tyramine. When parental starvation but not offspring starvation treatments differed, there was far less differential expression of such genes with only one or two cytochrome P450 genes being differentially expressed (S6, S7).

While the majority of reads mapped to the reference genome, 247,997,896 reads did not map; we assembled these into 334,717 transcripts. Of those genes that we assembled *de novo*, 803 were putatively annotated against the NCBI nt database and of these a large portion were mitochondrial (S8). DE analysis of unmapped reads identified differentially expressed genes between the treatment pairs in a pattern mirroring the genes from the reference [1523 (N.S vs N.N), 1860 (S.N vs S.S), 149 (S.N vs N.N) and 34 (N.S vs S.S)]. Many of these were *A. rosae* genes linked to the

mitochondria, particularly in the comparisons between starved and non-starved offspring individuals.

The KEGG pathway level analysis showed that across all four comparisons the ribosomal pathway was the most consistently differentially regulated, being upregulated when offspring were starved and downregulated when they were not. Interestingly, ribosomal regulation was affected not only by differences in offspring starvation treatment but also by differences in parental starvation treatment. The pathway was upregulated in starved larvae when compared to larvae that were not starved (offspring treatment differed) and to larvae that were starved but whose parents were also starved (parental treatment differed), with the reverse trend for non-starved larvae (Table 1; S9). The other significantly differentially expressed KEGG pathways only occurred between individuals that differed in offspring but not parental larval starvation treatments, mirroring the gene expression results. These pathways belong to the four main categories: metabolic breakdown and protein processing (pancreatic secretion, proteasome and protein processing in the endoplasmic reticulum, ER), immune response (phagosome and antigen processing), energy production and central metabolic processes (TCA/citrate cycle, glycolysis, thyroid hormone signalling pathway), and ECM-receptor interaction. There was generally a downregulation of these processes in larvae that were starved compared to non-starved individuals.

Discussion

We investigated the influence of intergenerational effects on offspring phenotype in the holometabolous insect *A. rosae*, which realizes distinct niches during its life-cycle. Our results revealed that offspring starvation interacted with parental starvation in a trait-and sex-specific manner, affecting the offspring phenotype. Trait-specific and sex-specific effects of an intergenerational treatment have been revealed in

previous studies (Zizzari et al. 2016; Le Roy et al. 2017; Wilson et al. 2019; Yin et al. 2019), suggesting that context-dependent effects of intergenerational starvation, usually in interaction with intragenerational treatment effects, may be common. Effects may be trait-specific because of differential selection on traits, whereby advantageous intergenerational effects are more often found for life-history traits than for reproductive traits (Yin et al. 2019). Similar to trait-specific effects, sex-specific differences can occur as a result of sex-specific directional selection on life-history traits (Tarka et al. 2018). We discuss our results in this context in detail below.

In our study, larval starvation led to an additional instar in *A. rosae* larvae of both sexes. Increasing the number of larval instars is a potentially adaptive strategy that allows individuals to recover from starvation via the prolongation of developmental time, e.g. by catch-up growth (Hector and Nakagawa 2012). Such intraspecific variation in the number of larval instars is common in insect species and can occur in response to various biotic and abiotic factors (Esperk et al. 2007). Intraspecific variability in instar numbers in response to food availability and quality can be in the range of one to three instars, including tenthredinid species (Esperk et al. 2007), but has not been described, to our knowledge, in the tenthredinid species *A. rosae*.

In line with the increased larval instar numbers, offspring of *A. rosae* that were starved had a longer development time in both sexes. Interestingly, there was a trend for faster development times in offspring that experienced a similar environment as their parents, which was at least significant for female pupal development time and may be suggestive of positive effects of matching parental and offspring environmental conditions (Monaghan 2008), i.e. a match-mismatch scenario. Similar positive effects of matching parental and offspring dietary conditions have also been found in a number of species in response to food availability (Hibshman et al. 2016; Raveh et al. 2016). However, other life history data of *A. rosae* did not reveal this

match-mismatch pattern, indicating a complex interplay between different intergenerational effects.

Adult mass showed a different pattern than development time, with female offspring having a higher adult mass when their parents were starved as larvae irrespective of their own starvation conditions, indicative of enhanced parental provisioning to offspring of starved parents, i.e. parental buffering. Similar effects have also been observed in the vinegar fly, *Drosophila melanogaster*, where females reared on poor food had larger offspring than females reared on standard food (Valtonen et al. 2012). Life-history theory predicts that under hostile conditions there is a shift towards fewer but better provisioned offspring (Roff 1992), although we did not examine the number of offspring produced here. As neither larval developmental time for female *A. rosae* nor their consumption (RGR and ECI) were affected by the parental starvation treatment, other physiological effects (discussed below for gene expression) may have mediated this kind of buffering. In contrast to females, only male offspring of starved parents differed significantly in adult mass, with starved sons weighing less. Thus, investment in body mass may be particularly low in males under repeatedly poor environmental conditions across generations, indicating "parental suffering" rather than buffering (i.e. negative parental effects). The differential investment in sexes in dependence of the parental vs. offspring starvation may be related to the haplodiploidy of this sawfly species. For example, larger eggs are usually fertilized to become females while smaller eggs remain unfertilized and become males in the haplodiploid thrip, *Pezothrips kellyanus,* and such differences could increase under parental starvation conditions (Katlav et al., 2021). Overall, there was no clear indication of silver spoon effects for any of the measured life-history traits, but in match-mismatch designs such effects cannot be entirely ruled out (Engqvist and Reinhold 2016).

Consumption assays revealed a different pattern than expected from the body mass patterns. Considering the larger body mass of female offspring from starved parents, we would have expected such individuals to have a higher growth rate, consume more leaf material, and/or have a higher consumption efficiency. Our results showed that parental starvation had no effect on any consumption trait except on RCR in females, where offspring of non-starved parents consumed more leaf material than that of starved parents, contrary to our expectation. Additionally, offspring that experienced starvation exhibited a steeper RGR than non-starved larvae, suggesting compensatory growth (Hector and Nakagawa 2012). Combining this compensatory growth with a longer development period potentially allows starved individuals to become larger and to overcome size-based selection, which can be an important determinant of individual fitness for many insect species (Beukeboom 2018). While such compensatory growth has been seen in many species, it can also pose costs to individuals (Arendt et al. 2001; Dmitriew and Rowe 2007; Auer et al. 2010). We found that individuals that were starved had a lower ECI than non-starved individuals, which may suggest a physiological cost of compensatory growth. Adjustments in consumption patterns to different starvation regimes have been revealed to be expressed in the next generation (McCue et al. 2017). However, patterns may differ depending on the developmental stage in which an individual is facing starvation, which needs to be further explored in *A. rosae*. Since we conducted the consumption assays for all larvae at the same developmental stage, we were able to disentangle the effect of starvation from effects due to differences in ontogeny (Nicieza and Álvarez 2009), in contrast to earlier studies on this sawfly (Paul et al. 2019).

Unlike starvation and in contrast to our expectation, clerodanoid uptake, which is known to enhance mate attractiveness in *A. rosae* (Amano et al. 1999; preprint Paul and Müller 2021), had no effect on the measured traits in offspring of both sexes.

This is surprising, because partner attractiveness has been shown to affect investment into offspring or/and offspring traits across a wide variety of species (Robart and Sinervo 2019), including having multigenerational consequences (Gilbert et al. 2012). In other species, mating with more attractive partners can lead to direct effects for the partner, but not necessarily for the offspring. For example, in the field cricket, *Gryllus firmus*, mating with more attractive males led to a higher number of eggs laid by females but offspring did not show any fitness benefits (Kelly and Adam-Granger 2020). In *A. rosae*, parental clerodanoid exposure may affect other offspring traits, such as immunity (Bozov et al. 2015), lifespan (Zanchi et al. 2021), or traits exhibited in adulthood, e.g. mating success (Amano et al. 1999; preprint Paul and Müller 2021), that were not measured in our study. Parental effects that occur via differential allocation may also be context-dependent.

Similar to the findings for life-history and consumption, transcriptome analysis of male larvae of the F4 generation also revealed stronger intra- than intergenerational effects of larval starvation on gene expression, in line with our hypothesis. When F4 larvae were starved, about half of the genes were differentially expressed compared to non-starved larvae. As typical stress response indicators, genes putatively encoding heat shock proteins and cytochrome P450s were differentially expressed, being both up- and down-regulated in *A. rosae* in response to starvation. Regulation of heat shock proteins in response to starvation has been reported elsewhere, including in larvae of Lepidoptera and Hymenoptera (Farahani et al. 2020; Wang et al. 2012). These proteins act to stabilize and protect other proteins in the face of both abiotic and biotic stresses (Sørensen et al. 2003; Farahani et al. 2020). Cytochrome P450s are best known for their role in metabolism of xenobiotics by insects (Feyereisen 2012). However, they were also found to be more highly expressed in *D. melanogaster* flies selected for starvation resistance compared to controls (Doroszuk et al. 2012). In addition, genes putatively encoding the monoamines octopamine and

tyramine were upregulated in starved larvae of *A. rosae*, both of which play a key role in modulating behavioral and physiological processes in invertebrates and can be involved in starvation resistance (Li et al. 2016).

Furthermore, starvation of the offspring led to differential gene expression of pathways involved in metabolism, including pancreatic secretion, which was also found in starved larvae of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Jiang et al. 2019). In starved larvae of *A. rosae*, differential regulation of metabolic pathways may explain why these larvae showed an overall lower ECI compared to non-starved larvae. Moreover, expression of metabolic genes likely changes with the duration of starvation. For example, in *D. melanogaster* a 16 h starvation period led to a downregulation particularly of the endopeptidase gene family (Moskalev et al. 2015), but extended periods of starvation may induce an activation of proteolysis genes. However, little is known about long-term effects, including cross-generational, on gene expression patterns in response to starvation. Interestingly, many of the genes differentially expressed in starved vs non-starved individuals of *A. rosae* linked to the mitochondria, reflecting wholescale disruption of homeostasis caused by starvation (Gilbert 2012).

Interestingly, parental starvation also caused differential expression in about 1 % of genes of their offspring, indicating that subtle intergenerational imprints on gene expression can occur in *A. rosae*. When only parental, but not offspring, treatment differed, offspring from starved parents displayed a downregulation of the ribosomal pathway compared to offspring of non-starved parents. In contrast, when only offspring treatment differed, larval starvation caused a comparative enrichment of genes in this pathway. These results indicate that larval starvation can have significant direct effects on the regulation of the ribosomal pathway, but that these effects may be buffered to some degree via parental starvation. Larvae that were starved but whose parents were not starved may have been more physiologically

stressed than starved larvae from starved parents, as indicated by the enrichment of putative ribosomal pathway genes. Differential regulation of the ribosomal pathway has been observed in a wide variety of taxa in response to thermal stress (Paraskevopoulou et al. 2020; Schwanz et al. 2020; Srikanth et al. 2020), demonstrating the broad role of this pathway in stress response. Importantly, the differential expression of genes involved in stress and other physiological and metabolic responses may have energetic costs, which may lead to a prolonged development time of starved larvae in *A. rosae*.

Alteration in ribosomal expression may be an important mechanism mediating intergenerational effects of starvation (Aldrich and Maggert 2015; Bughio and Maggert 2019). In *Caenorhabditis elegans* starvation led to the generation of small RNAs, and small RNA-induced gene silencing persisted over up to three generations (Rechavi et al. 2014). Such mechanisms may also explain the intergenerational changes in gene expression observed in *A. rosae* in the present study; future work comparing small RNA expression in relation to starvation could address this. Further work could also utilize tissue-specific gene expression to determine where gene expression changes are most prominent. For example, larvae of the soldier fly, *Inopus falvus* (Diptera: Stratiomyidae), differentially express numerous genes in the salivary glands in response to starvation stress (Etebari et al. 2020). Other tissues of interest in relation to starvation responses may be different sections of the gut.

In summary, our findings highlight that intragenerational starvation effects were somewhat stronger than the intergenerational effects of starvation across life-history, consumption, and gene expression patterns. We found some evidence for parental effects including match-mismatch and parental buffering, while there was no clear evidence for silver spoon effects. The parental reproductive environment left no signature on the measured offspring traits. Overall, due to different trajectories and environments experienced during different stages, distinct niches are realized, which

are expressed in a diversity of phenotypes. In terms of wider consequences these results suggest that periods of starvation may make individuals even more robust when facing another food shortage in the next generation and may thus lead to enhanced damage of crops.

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Data availability

All data in this manuscript will be deposited online on Zenodo.

Raw Reads: SRA ID = PRJNA716060. BioSample accession numbers = SAMN18393262-SAMN18393285

Author contributions

Conceptualization and funding acquisition: CM; methods development/experimental design: SCP, CM; data collection: SCP, data validation and analysis: life history data: SCP, consumption assay data: SCP, PS, gene expression data: SCP, ABD; data visualization: SCP, PS; writing original draft: SCP, CM, PS; reviewing and editing: SCP, PS, ABD, CM.

Supplemental Material

S1. Effects of Starvation on Life-History Traits in F3 Individuals

S2. KEGG Term Analysis

S3. Results of Posthoc Analyses for F4 Generation

S4: Influence of Parental and Offspring Larval Starvation Treatments on Developmental Times of *Athalia rosae* in F4 Generation

S5. Percentage of Differentially Expressed Genes

S6. Numbers of Significantly Up- or Downregulated Genes

S7. Mapped reads, DE output.

S8. Unmapped reads, DE output.

S9. KEGG pathway results.

Literature Cited

Aldrich, J. C., and K. A. Maggert. 2015. Transgenerational inheritance of diet-induced genome rearrangements in *Drosophila*. Plos Genetics 11:e1005148.

Amano, T., R. Nishida, Y. Kuwahara, and H. Fukami. 1999. Pharmacophagous acquisition of cleodendrins by the turnip sawfly (*Athalia rosae ruficornis*) and their role in the mating behavior. Chemoecology 9:145-150.

Andrews, S. 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data. Available online at http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Arendt, J., D. S. Wilson, and E. Stark. 2001. Scale strength as a cost of rapid growth in sunfish. Oikos 93:95-100.

Auer, S. K., J. D. Arendt, R. Chandramouli, and D. N. Reznick. 2010. Juvenile compensatory growth has negative consequences for reproduction in Trinidadian guppies (*Poecilia reticulata*). Ecology Letters 13:998-1007.

Badyaev, A. V., and T. Uller. 2009. Parental effects in ecology and evolution: mechanisms, processes and implications. Philosophical Transactions of the Royal Society B-Biological Sciences 364:1169-1177.

Barnett, D. W., E. K. Garrison, A. R. Quinlan, M. P. Stromberg, and G. T. Marth. 2011. BamTools: a C++ API and toolkit for analyzing and managing BAM files. Bioinformatics 27:1691-1692.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society B 57:289-300.

Beukeboom, L. W. 2018. Size matters in insects - an introduction. Entomologia Experimentalis et Applicata 166:2-3.

Boggs, C. L., and K. Niitepõld. 2016. Effects of larval dietary restriction on adult morphology, with implications for flight and life history. Entomologia Experimentalis et Applicata 159:189-196.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120.

Bozov, P., T. Girova, N. Prisadova, Y. Hristova, and V. Gochev. 2015. Antimicrobial activity of neo-clerodane diterpenoids isolated from Lamiaceae species against pathogenic and food spoilage microorganisms. Natural Product Communications 10:1797-1800.

Bughio, F., and K. A. Maggert. 2019. The peculiar genetics of the ribosomal DNA blurs the boundaries of transgenerational epigenetic inheritance. Chromosome Research 27:19-30.

Burton, T., and N. B. Metcalfe. 2014. Can environmental conditions experienced in early life influence future generations? Proceedings of the Royal Society B-Biological Sciences 281:20140311.

Cornwallis, C. K., and E. A. O'Connor. 2009. Sperm: seminal fluid interactions and the adjustment of sperm quality in relation to female attractiveness. Proceedings of the Royal Society B-Biological Sciences 276:3467-3475.
Crawley, M. J. 2012, The R book, John Wiley & Sons.
Cunningham, E. J. A., and A. F. Russell. 2000. Egg investment is influenced by male attractiveness in the mallard. Nature 404:74-77.
Dmitriew, C., and L. Rowe. 2007. Effects of early resource limitation and compensatory growth on lifetime fitness in the ladybird beetle *(Harmonia axyridis*). Journal of Evolutionary Biology 20:1298-1310.
Dobin, A., C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut et al. 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15-21.
Doroszuk, A., M. J. Jonker, N. Pul, T. M. Breit, and B. J. Zwaan. 2012. Transcriptome analysis of a long-lived natural *Drosophila* variant: a prominent role of stress- and reproduction-genes in lifespan extension. BMC Genomics 13:167.
English, S., and A. M. G. Barreaux. 2020. The evolution of sensitive periods in development: insights from insects. Current Opinion in Behavioral Sciences 36:71-78.
Engqvist, L., and K. Reinhold. 2016. Adaptive trans-generational phenotypic plasticity and the lack of an experimental control in reciprocal match/mismatch-experiments. Methods in Ecology & Evolution 7:1482-1488.
Esperk, T., T. Tammaru, and S. Nylin. 2007. Intraspecific variability in number of larval instars in insects. Journal of Economic Entomology 100:627-645.
Etebari, K., K. R. Lindsay, A. L. Ward, and M. J. Furlong. 2020. Australian sugarcane soldier fly's salivary gland transcriptome in response to starvation and feeding on sugarcane crops. Insect Science 27:708-720.
Farahani, S., A. R. Bandani, H. Alizadeh, S. H. Goldansaz, and S. Whyard. 2020. Differential expression of heat shock proteins and antioxidant enzymes in response to temperature, starvation, and parasitism in the Carob moth larvae, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae). Plos One 15:e0228104.
Feyereisen, R. 2012. Insect CYP genes and P450 enzymes, Pages 236–316 *in* I. G. Lawrence, ed. Insect Molecular Biology and Biochemistry. San Diego, USA, Academic Press.
Franzke, A., and K. Reinhold. 2013. Transgenerational effects of diet environment on life-history and acoustic signals of a grasshopper. Behavioral Ecology 24:734-739.
Gilbert, L. I. 2012, Insect Molecular Biology and Biochemistry. Amsterdam, Elsevier.
Gilbert, L., K. A. Williamson, and J. A. Graves. 2012. Male attractiveness regulates daughter fecundity non-genetically via maternal investment. Proceedings of the Royal Society B-Biological Sciences 279:523-528.
Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology 29:644-U130.
Groothuis, T. G. G., and B. Taborsky. 2015. Introducing biological realism into the study of developmental plasticity in behaviour. Frontiers in Zoology 12:(Suppl 1): S6.
Hebets, E. A., J. Wesson, and P. S. Shamble. 2008. Diet influences mate choice selectivity in adult female wolf spiders. Animal Behaviour 76:355-363.
Hector, K. L., and S. Nakagawa. 2012. Quantitative analysis of compensatory and catch-up growth in diverse taxa. Journal of Animal Ecology 81:583-593.
Hibshman, J. D., A. Hung, and L. R. Baugh. 2016. Maternal diet and insulin-like signaling control intergenerational plasticity of progeny size and starvation resistance. Plos Genetics 12:e1006396.
Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346-363.
Jiang, T., L. Ma, X. Y. Liu, H. J. Xiao, and W. N. Zhang. 2019. Effects of starvation on respiratory metabolism and energy metabolism in the cotton bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Journal of Insect Physiology 119:103951.
Kelly, C. D., and É. Adam-Granger. 2020. Mating with sexually attractive males provides female *Gryllus firmus* field crickets with direct but not indirect fitness benefits. Behavioral Ecology and Sociobiology 74:80.
Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software 82:1-26.

Le Roy, A., I. Loughland, and F. Seebacher. 2017. Differential effects of developmental thermal plasticity across three generations of guppies (*Poecilia reticulata*): canalization and anticipatory matching. Scientific Reports 7:4313.

Li, B., and C. N. Dewey. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth et al. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078-2079.

Li, Y., J. Hoffmann, F. Stephano, I. Bruchhaus, C. Fink, and T. Roeder. 2016. Octopamine controls starvation resistance, life span and metabolic traits in *Drosophila*. Scientific Reports 6:35359.

Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:550.

Luo, W. J., and C. Brouwer. 2013. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. Bioinformatics 29:1830-1831.

Luo, W. J., M. S. Friedman, K. Shedden, K. D. Hankenson, and P. J. Woolf. 2009. GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinformatics 10:161.

McCue, M. D., J. S. Terblanche, and J. B. Benoit. 2017. Learning to starve: impacts of food limitation beyond the stress period. Journal of Experimental Biology 220:4330-4338.

Miyatake, T. 2001. Effects of starvation on death-feigning in adults of *Cylas formicarius* (Coleoptera: Brentidae). Annals of the Entomological Society of America 94:612-616.

Monaghan, P. 2008. Early growth conditions, phenotypic development and environmental change. Philosophical Transactions of the Royal Society B-Biological Sciences 363:1635-1645.

Moriya, Y., M. Itoh, S. Okuda, A. C. Yoshizawa, and M. Kanehisa. 2007. KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Research 35:W182-W185.

Moskalev, A., S. Zhikrivetskaya, G. Krasnov, M. Shaposhnikov, E. Proshkina, D. Borisoglebsky, A. Danilov et al. 2015. A comparison of the transcriptome of *Drosophila melanogaster* in response to entomopathogenic fungus, ionizing radiation, starvation and cold shock. BMC Genomics 16(Supple 13):S8.

Naguib, M., A. Nemitz, and D. Gil. 2006. Maternal developmental stress reduces reproductive success of female offspring in zebra finches. Proceedings of the Royal Society B-Biological Sciences 273:1901-1905.

Naito, T., and H. Suzuki. 1991. Sex determination in the sawfly, *Athalia rosae* Ruficornis (Hymenoptera) - occurence of triploid males. Journal of Heredity 82:101-104.

Nicieza, A. G., and D. Álvarez. 2009. Statistical analysis of structural compensatory growth: how can we reduce the rate of false detection? Oecologia 159:27-39.

Paraskevopoulou, S., A. B. Dennis, G. Weithoff, and R. Tiedemann. 2020. Temperature-dependent life history and transcriptomic responses in heat-tolerant versus heat-sensitive *Brachionus* rotifers. Scientific Reports 10:13281.

Paul, S. C., A. B. Dennis, L. J. Tewes, J. Friedrichs, and C. Müller. 2021. Consequences of pharmacophagous uptake from plants and conspecifics in a sawfly elucidated using chemical and molecular techniques. bioRxiv preprint. doi: 10.1101/2021.02.09.430406

Paul, S. C., and C. Müller. 2021. Fighting over defence chemicals disrupts mating behaviour. bioRxiv preprint. doi: 10.1101/2021.02.12.430958

Paul, S. C., R. Putra, and C. Müller. 2019. Early life starvation has stronger intra-generational than transgenerational effects on key life-history traits and consumption measures in a sawfly. Plos One 14:e0226519.

Perez, M. F., and B. Lehner. 2019. Intergenerational and transgenerational epigenetic inheritance in animals. Nature Cell Biology 21:143-151.

Pilakouta, N., S. Jamieson, J. A. Moorad, and P. T. Smiseth. 2015. Parental care buffers against inbreeding depression in burying beetles. Proceedings of the National Academy of Sciences of the United States of America 112:8031-8035.

Ratikainen, II, and H. Kokko. 2010. Differential allocation and compensation: who deserves the silver spoon? Behavioral Ecology 21:195-200.

Raveh, S., D. Vogt, and M. Kolliker. 2016. Maternal programming of offspring in relation to food availability in an insect (*Forficula auricularia*). Proceedings of the Royal Society B-Biological Sciences 283:20152936.

Rechavi, O., L. Houri-Ze'evi, S. Anava, W. S. S. Goh, S. Y. Kerk, G. J. Hannon, and O. Hobert. 2014. Starvation-induced transgenerational inheritance of small RNAs in *C. elegans*. Cell 158:277-287.

Regalado, J. M., M. B. Cortez, J. Grubbs, J. A. Link, A. van der Linden, and Y. Zhang. 2017. Increased food intake after starvation enhances sleep in *Drosophila melanogaster*. Journal of Genetics and Genomics 44:319-326.

Riggert, E. 1939. Untersuchungen über die Rübenblattwespe *Athalia colobri* Christ (*A. spinarum* F.). Zeitschrift für angewandte Entomologie 26:462-516.

Robart, A. R., and B. Sinervo. 2019. Females increase parental care, but not fecundity, when mated to high-quality males in a biparental fish. Animal Behaviour 148:9-18.

Roberts, A., C. Trapnell, J. Donaghey, J. L. Rinn, and L. Pachter. 2011. Improving RNA-Seq expression estimates by correcting for fragment bias. Genome Biology 12:R22.

Roff, D. A. 1992, The evolution of life histories: Theory and analysis. New York, Chapman & Hall.

Saastamoinen, M., N. Hirai, and S. van Nouhuys. 2013. Direct and trans-generational responses to food deprivation during development in the Glanville fritillary butterfly. Oecologia 171:93-104.

Sánchez-Tójar, A., M. Lagisz, N. P. Moran, S. Nakagawa, D. W. A. Noble, and K. Reinhold. 2020. The jury is still out regarding the generality of adaptive 'transgenerational' effects. Ecology Letters 23:1715-1718.

Sawa, M., A. Fukunaga, T. Naito, and K. Oishi. 1989. Studies on the sawfly, *Athalia rosae* (Insecta, Hymenoptera, Tenthredinidae) I. General biology. Zoological Science 6:541-547.

Schwanz, L. E., J. Crawford-Ash, and T. Gale. 2020. Context dependence of transgenerational plasticity: the influence of parental temperature depends on offspring environment and sex. Oecologia 194:391-401.

Soneson, C., M. Love, and M. Robinson. 2015. Differential analyses for RNA-seq: transcript-level estimates improve gene level inferences F1000Research 4:1521.

Sørensen, J. G., T. N. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat shock proteins. Ecology Letters 6:1025-1037.

Srikanth, K., J.-E. Park, S. Y. Ji, K. H. Kim, Y. K. Lee, H. Kumar, M. Kim et al. 2020. Genome-wide transcriptome and metabolome analyses provide novel insights and suggest a sex-specific response to heat stress in pigs. Genes 11:540.

Taborsky, B. 2017. Developmental plasticity: preparing for life in a complex world. Advances in the Study of Behavior 49:49-99.

Tarka, M., A. Guenther, P. T. Niemelä, S. Nakagawa, and D. W. A. Noble. 2018. Sex differences in life history, behavior, and physiology along a slow-fast continuum: a meta-analysis. Behavioral Ecology and Sociobiology 72:132.

Uller, T., S. Nakagawa, and S. English. 2013. Weak evidence for anticipatory parental effects in plants and animals. Journal of Evolutionary Biology 26:2161-2170.

Valtonen, T. M., K. Kangassalo, M. Pölkki, and M. J. Rantala. 2012. Transgenerational effects of parental larval diet on offspring development time, adult body size and pathogen resistance in *Drosophila melanogaster*. PloS One 7:e31611.

van de Pol, M., L. W. Bruinzeel, D. Heg, H. P. van der Jeugd, and S. Verhulst. 2006. A silver spoon for a golden future: long-term effects of natal origin on fitness prospects of oystercatchers (*Haematopus ostralegus*). Journal of Animal Ecology 75:616-626.

Vega-Trejo, R., M. D. Jennions, and M. L. Head. 2016. Are sexually selected traits affected by a poor environment early in life? BMC Evolutionary Biology 16:263.

Wang, H., K. Li, J. Y. Zhu, Q. Fang, G. Y. Ye, H. Wang, K. Li et al. 2012. Cloning and expression pattern of heat shock protein genes from the endoparasitoid wasp, *Pteromalus puparum* in response to environmental stresses. Archives of Insect Biochemistry and Physiology 79:247-263.

Wang, Y., O. Kaftanoglu, C. S. Brent, R. E. Page, and G. V. Amdam. 2016. Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (*Apis mellifera* L.). Journal of Experimental Biology 219:949-959.

Wolf, J. B., and M. J. Wade. 2009. What are maternal effects (and what are they not)? Philosophical Transactions of the Royal Society B-Biological Sciences 364:1107-1115.

Yin, J. J., M. Zhou, Z. R. Lin, Q. S. Q. Li, and Y. Y. Zhang. 2019. Transgenerational effects benefit offspring across diverse environments: a meta-analysis in plants and animals. Ecology Letters 22:1976-1986.

Zanchi, C., L. K. Lo, R. R, I. Moritz, J. Kurtz, and C. Müller. 2021. Survival of the sawfly *Athalia rosae* upon infection by an entomopathogenic fungus and in relation to clerodanoid uptake. Frontiers in Physiology.

Zhu, A. Q., J. G. Ibrahim, and M. I. Love. 2019. Heavy-tailed prior distributions for sequence count data: removing the noise and preserving large differences. Bioinformatics 35:2084-2092.

Zizzari, Z. V., N. M. van Straalen, and J. Ellers. 2016. Transgenerational effects of nutrition are different for sons and daughters. Journal of Evolutionary Biology 29:1317-1327.

Figures

Figure 1:

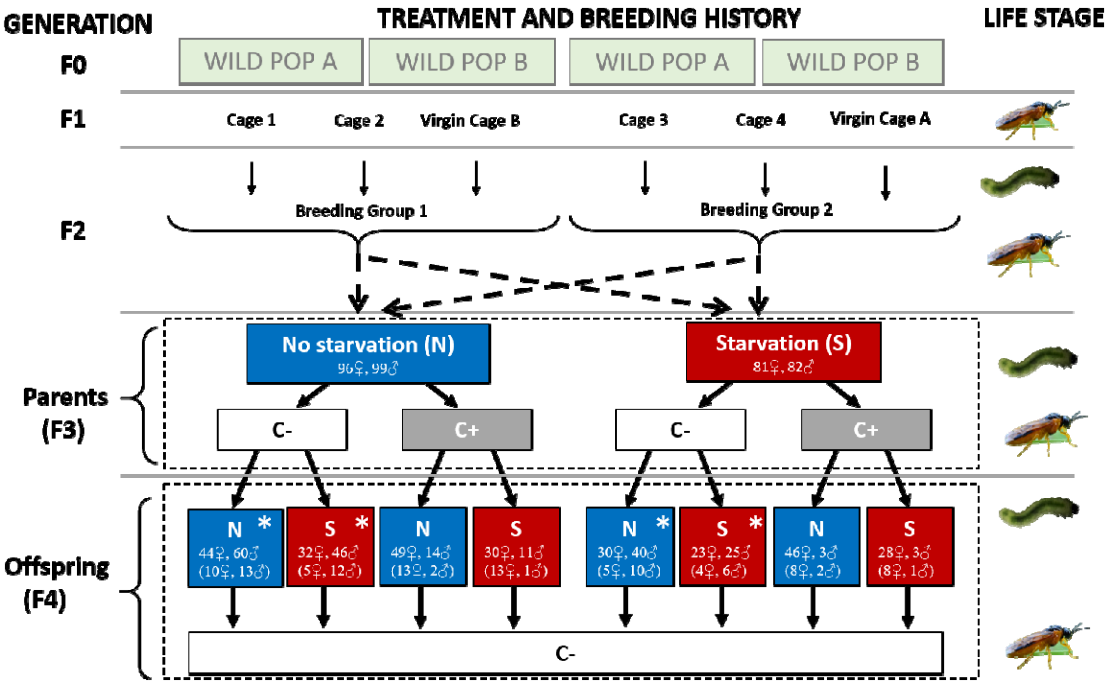

Figure 1: Full experimental design showing outline of breeding structure and experimental treatments from F0 to F4 generation in *Athalia rosae*; * indicates individuals taken for RNASeq analysis. Sample sizes split by sex are given in boxes for F3 and F4 generation. Numbers in brackets refer to sample sizes for consumption assay (please note that individuals were pooled across C+ and C- treatments).

Figure 2

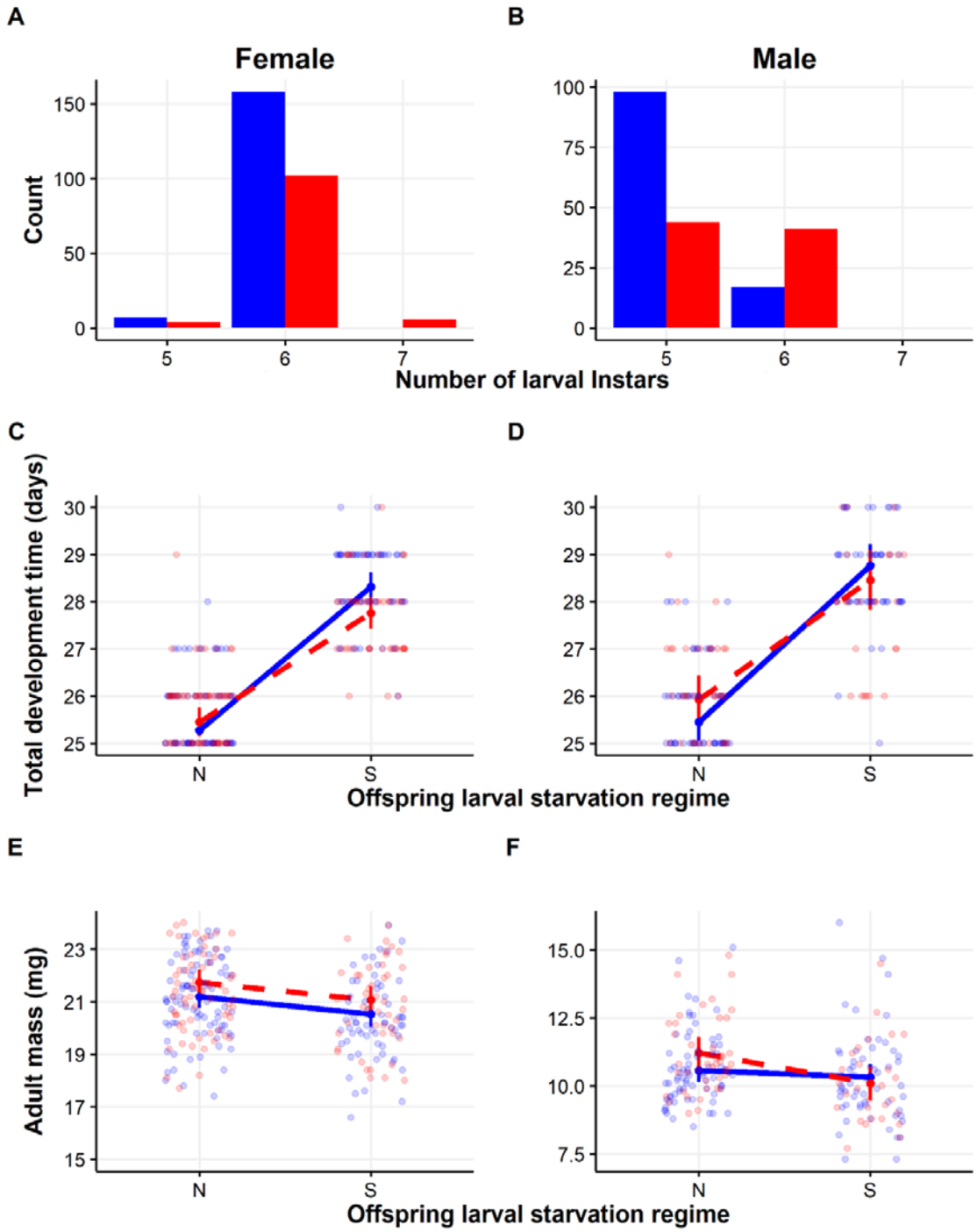

Figure 2: Number of larval instars in F4 in dependence of offspring starvation treatment (blue: non starved, red: starved) and influence of offspring larval starvation treatments (N = no starvation, S = starvation) and parental larval starvation treatments (blue solid line = no starvation, red dashed line = starvation) on offspring total development time (C, D) and adult body mass (E, F) of *Athalia rosae*. Data are

plotted separately for females (A, C, E) and males (B, D, F); individuals were sexed on emergence. Points are model predictions with associated confidence intervals and colors of points and lines correspond to parental starvation treatment. Raw data are plotted in transparent colors in the background.

Figure 3

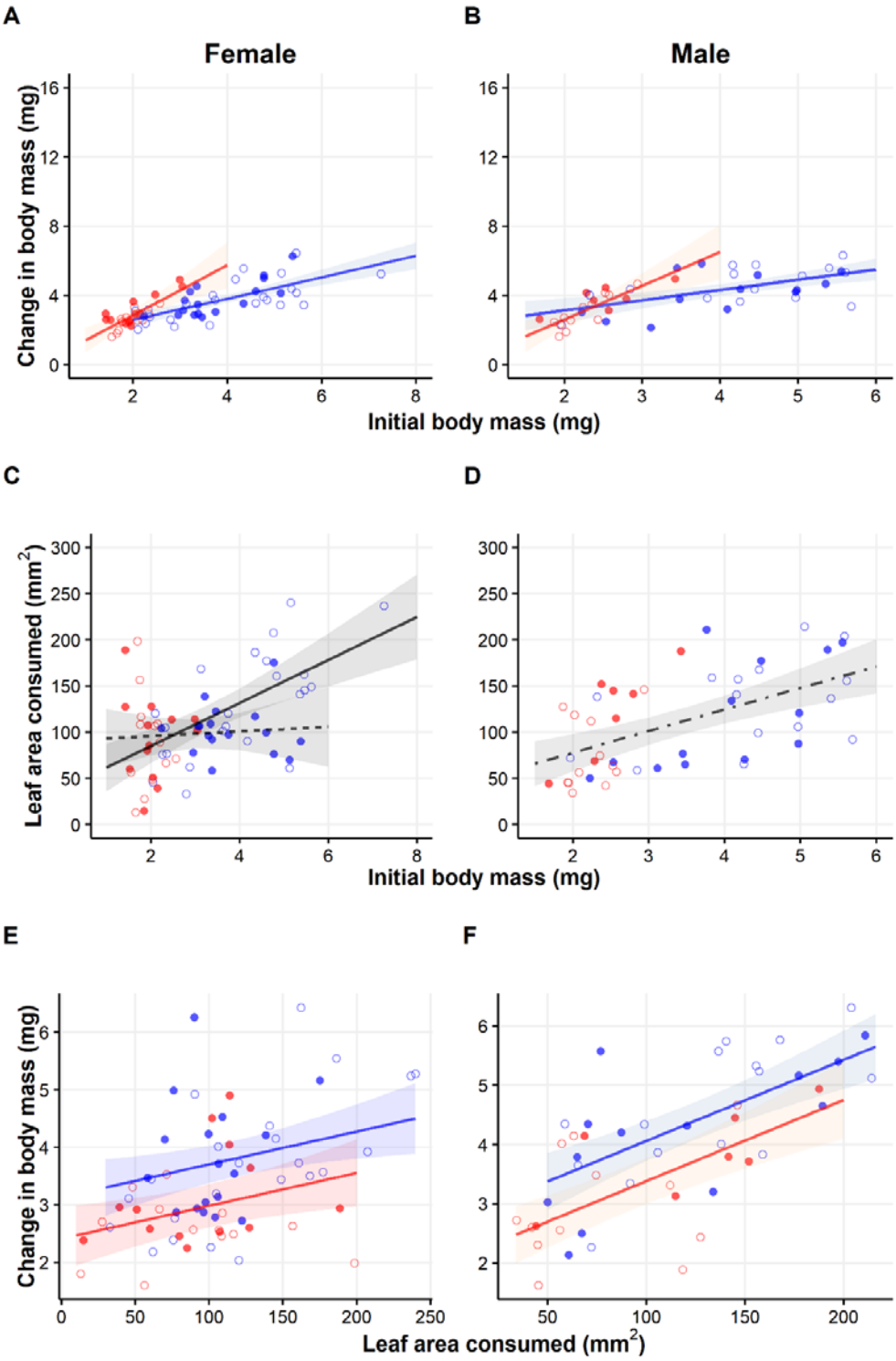

Figure 3: Influence of offspring (blue line = no starvation, red line = starvation) and parental larval starvation treatments (solid black line = no starvation, dashed black line = starvation) in F4 on relative growth rate, RGR (A, B); relative consumption rate, RCR (C, D); and efficiency of conversion of ingested food, ECI (E, F) of *Athalia*

rosae. Note that neither offspring starvation treatment nor parental starvation treatment had an effect on RCR in males (dot-dash line). Data are plotted separately for females (A, C, E) and males (B, D, F); individuals were sexed on emergence. Lines depict model predictions with shaded regions showing the 95% confidence interval range. Raw data representing offspring larval starvation treatment (blue = no starvation, red = starvation) and parental larval starvation treatment (open circles = no starvation, filled circles = starvation) are plotted in the background.

Figure 4

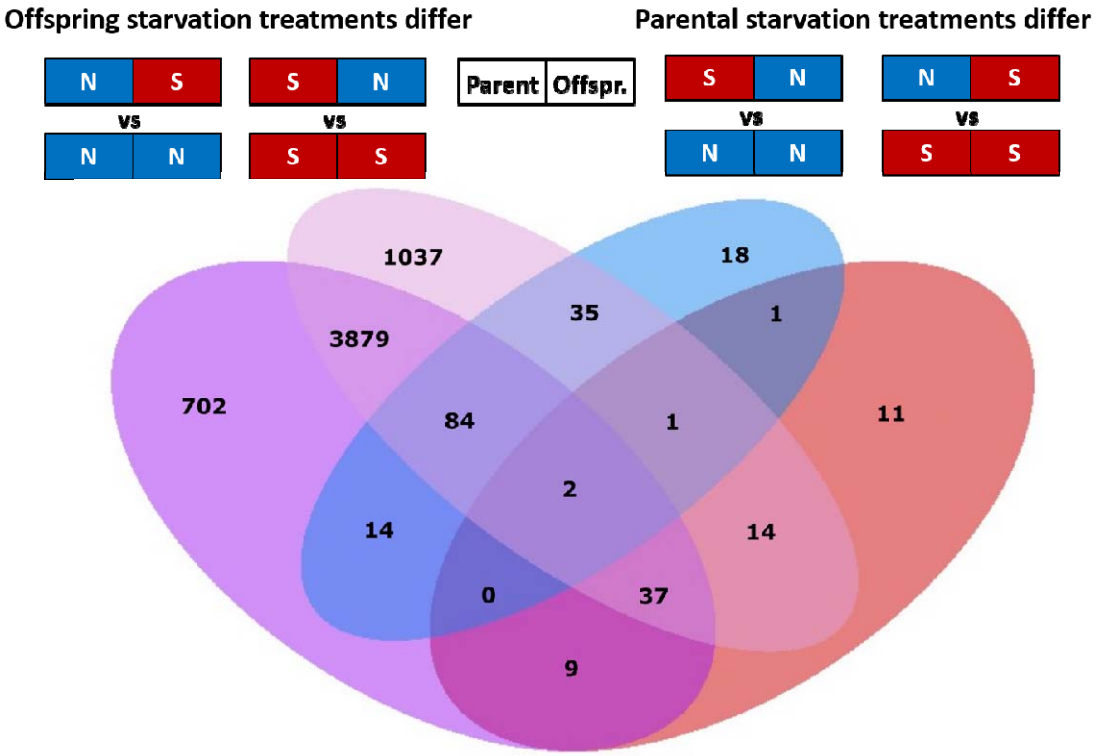

Figure 4: Venn diagram illustrating the number of unique and shared significantly differentially expressed genes (padjust < 0.05) in male offspring larvae (F4) of *Athalia rosae* resulting from each of the four pairwise comparisons, (N = no starvation and S = starvation; left box F3, right box F4).

Figure 5

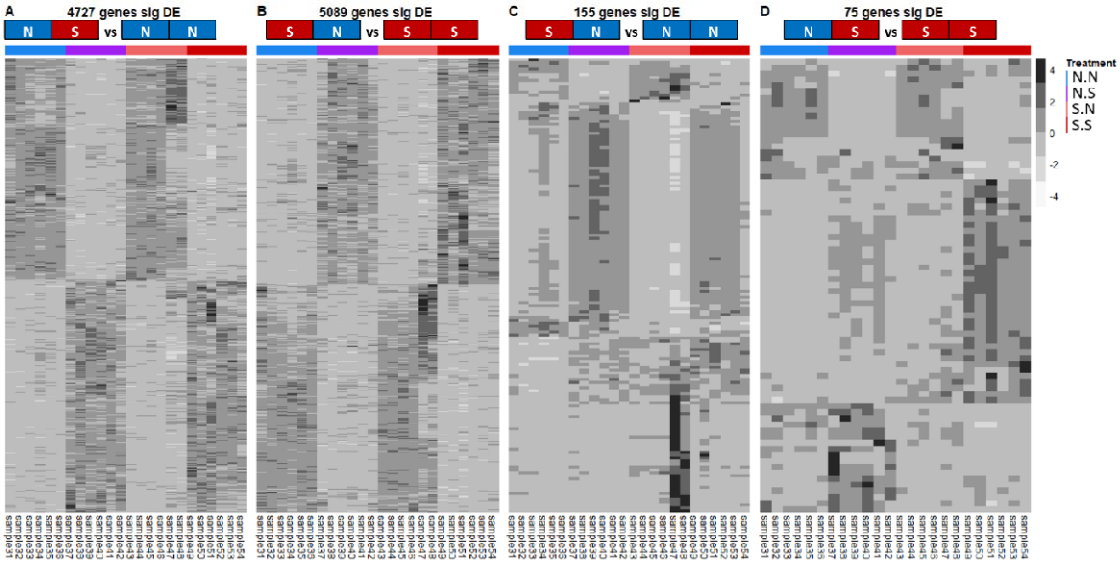

Figure 5: Heatmaps showing the expression (normalized counts) of genes in male offspring larvae (F4) of *Athalia rosae* across all samples for those genes that were significantly differentially expressed (padjust < 0.05) when offspring starvation treatment differed (A, B) or parental starvation treatment differed (C, D), (N = no starvation and S = starvation; left box F3, right box F4). Plotted values are *z*-scores computed from normalized counts post clustering.

Table 1. Significantly differentially expressed KEGG gene sets/pathways male offspring larvae (F4) of *Athalia rosae* that differed in either their own or their parent's (F3) larval starvation regime (N = no starvation and S = two periods of starvation for 24 hours during larval development; left box F3, right box F4). The combination listed first (on top) is the 'treatment' the one listed second (underneath) is the 'control' in each comparison, meaning that pathways are up- or downregulated in 'treatment' relative to 'control'. Larvae undergoing starvation (S) were subjected to two periods of starvation for 24 hours during larval development, one in L2 and one in L4.

KEGG pathway	Offspring starvation treatment differs				Parental starvation treatment differs			
	N	S	S	N	S	N	N	S
	vs		vs		vs		vs	
	N	N	S	S	N	N	S	S
Ribosome	↑ up		↓ down		↓ down		↑ up	
Pancreatic secretion	-		↓ down		-		-	
Protein processing in endoplasmic reticulum	↓ down		↑ up		-		-	
Proteasome	↓ down		↑ up		-		-	
Phagosome	-		↑ up		-		-	
Citrate (TCA) cycle	↓ down		-		-		-	
Glycolysis / Gluconeogenesis	↓ down		-		-		-	
Antigen processing and presentation	↓ down		↑ up		-		-	
Protein export	↓ down		-		-		-	
ECM-receptor interaction	-		↑ up		-		-	
Thyroid hormone synthesis	-		↑ up		-		-	