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Matching maternal and paternal experiences underpin molecular thermal acclimation

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Abstract

The environment experienced by one generation has the potential to affect the subsequent one through non-genetic inheritance of parental effects. Since both mothers and fathers can influence their offspring, questions arise regarding how the maternal, paternal and offspring experiences integrate into the resulting phenotype. We aimed to disentangle the maternal and paternal contributions to transgenerational thermal acclimation in a reef fish, *Acanthochromis polyacanthus*, by exposing two generations to elevated temperature (+1.5°C) in a fully factorial design and analysing the F2 hepatic gene expression. Paternal and maternal effects showed not only common but also parent-specific components, with the father having the largest influence in shaping the offspring's transcriptomic profile. Fathers contributed to transcriptional transgenerational response to warming through transfer of epigenetically controlled stress–response mechanisms while mothers influenced increased gene expression associated with lipid metabolism regulation. However, the key to acclimation potential was matching thermal experiences of the parents. When both parents were exposed to the same condition, offspring showed increased expression of genes related to structural RNA production and transcriptional regulation, whereas environmental mismatch in parents resulted in maladaptive parental condition transfer, revealed by translation suppression and endoplasmic reticulum stress. Interestingly, the offspring's own environmental experience had the smallest influence on their hepatic transcription profiles. Taken together, our results show the complex nature of the interplay among paternal, maternal and offspring cue integration, and reveal that acclimation

potential to ocean warming might depend not only on maternal and paternal contributions but importantly on congruent parental thermal experiences.

KEYWORDS
climate change, coral reef fish, gene expression, non-genetic inheritance, transcriptomics, transgenerational plasticity

1 | INTRODUCTION

Parents can affect the phenotypes of their offspring through a range of environmentally induced non-genetic inheritance mechanisms (Bell & Hellmann, 2019; Bonduriansky & Day, 2009; Salinas et al., 2013). As a consequence, the phenotype of the current generation can be influenced by the environmental experiences of the previous ones as a result of environmentally induced parental effects. The term 'parental effects' has long been used as a synonym of maternal effects, as mothers were thought to be the most likely parent responsible for the transmission of non-genetic material to the offspring (Crean & Bonduriansky, 2014; Mousseau & Fox, 1998), mainly through egg provisioning of nutrients, hormones and mitochondria. However, both females and males have been found to be responsible for epigenetic mechanisms of inheritance, such as DNA methylation, histone modifications and small non-coding RNAs (Immler, 2018), and fathers have increasingly been recognized as important in the transmission of environmentally induced parental effects (Rutkowska et al., 2020; Stein & Bell, 2014; Tariel et al., 2020).

The existence of both maternal and paternal effects raises the question of how they are combined in their offspring's phenotype (Bell & Hellmann, 2019). Perceived environmental cues could simply have additive effects and integrate to modulate the strength of the offspring's phenotypic response. In sheepshead minnow *Cyprinodon variegatus*, for example, offspring growth is highest when paternal, maternal and offspring temperature match (Chang et al., 2021). Conversely, maternal and paternal effects could interact with each other in more complex ways (Lehto & Tinghitella, 2020; Moschilla et al., 2022). In the freshwater snail *Physa acuta*, for example, offspring are bigger only when the mother is exposed to predatory risk, but not when both parents are (Tariel et al., 2020), while in the beetle *Tribolium castaneum*, offspring are more starvation tolerant if one but not both parents experienced cold stress (Gilad & Scharf, 2019). Males and females can indeed influence their progeny phenotypes in independent ways, either because of differences in transmission mechanisms between sexes or because of asymmetrical investments in reproduction and parental care. As a consequence, the same environmental cue can result in parents-specific effects, like in predator-exposed mother and father sticklebacks (*Gasterosteus aculeatus*) inducing different behaviours and brain transcriptional responses in their offspring (Hellmann et al., 2020), or paternal effects in immunity transfer and gene expression being dominant compared to the maternal ones in sex-role-reversed pipefish (*Syngnathus typhle*; Beemelmanns & Roth, 2016). Finally, how parental cues are integrated into the offspring's phenotype can also depend on the offspring's own environmental experience, as well as on its sex, as daughters and sons may be differently affected or affected more prevalently by one parent only (Metzger & Schulte, 2016; Schwanz et al., 2020; Seebacher et al., 2023). To shed light on the complexity of non-genetic inheritance, elaborate experimental designs are needed, in order to disentangle the different parental effects of mothers and fathers and further our understanding of their potential role in acclimation to environmental change.

If adaptive, non-genetic inheritance might enable acclimation to persistent environmental changes such as global warming (Donelan et al., 2020; Donelson et al., 2018) through environmentally induced parental effects. In a coral reef fish, the spiny chromis *Acanthochromis polyacanthus* (Bleeker 1855), for instance, studies have shown that parental developmental exposure to elevated temperature can provide transgenerational acclimation to ocean warming and restoration of aerobic scope in offspring through molecular changes in lipid and carbohydrate metabolism, as well as immune system and transcriptional regulation (Bernal et al., 2021, 2022; Bonzi et al., 2023; Donelson et al., 2012; Ryu et al., 2018; Veilleux et al., 2015). The relative importance of fathers and mothers in such acclimation process, however, is unknown but has implications on adaptive potential. Parental pairs, for instance, might be formed by individuals differently exposed to marine heatwaves during development (Frölicher et al., 2018), resulting in mothers and fathers having divergent past thermal experiences and thus potentially imparting different parental effects. Indeed, marine heatwaves have been reported with increased frequency in the home range of this species, with duration and occurrence varying from 1 year to the next (Huang et al., 2024), which increases the likelihood of parental pairs composed of individuals with different developmental thermal experiences. Understanding how these multiple signals from maternal, paternal and personal experiences are integrated into the resulting phenotype will help make predictions about population acclimation potential in a changing world.

In this study, we investigate the molecular processes underlying paternal and maternal effects of ocean warming in *A. polyacanthus* and how they interact with each other, as well as the thermal experience of the offspring. We used a fully factorial, split clutch design where we developmentally exposed *A. polyacanthus* females, males or both sexes, as well as their offspring to either control or elevated (+1.5°C) temperature (Figure 1), and then analysed the offspring hepatic gene expression profiles. Since in this damselfish, females and males are not sexually dimorphic, have the same ecology and form monogamous pairs jointly providing parental care for the demersal eggs and the fry (Robertson, 1973), we expected

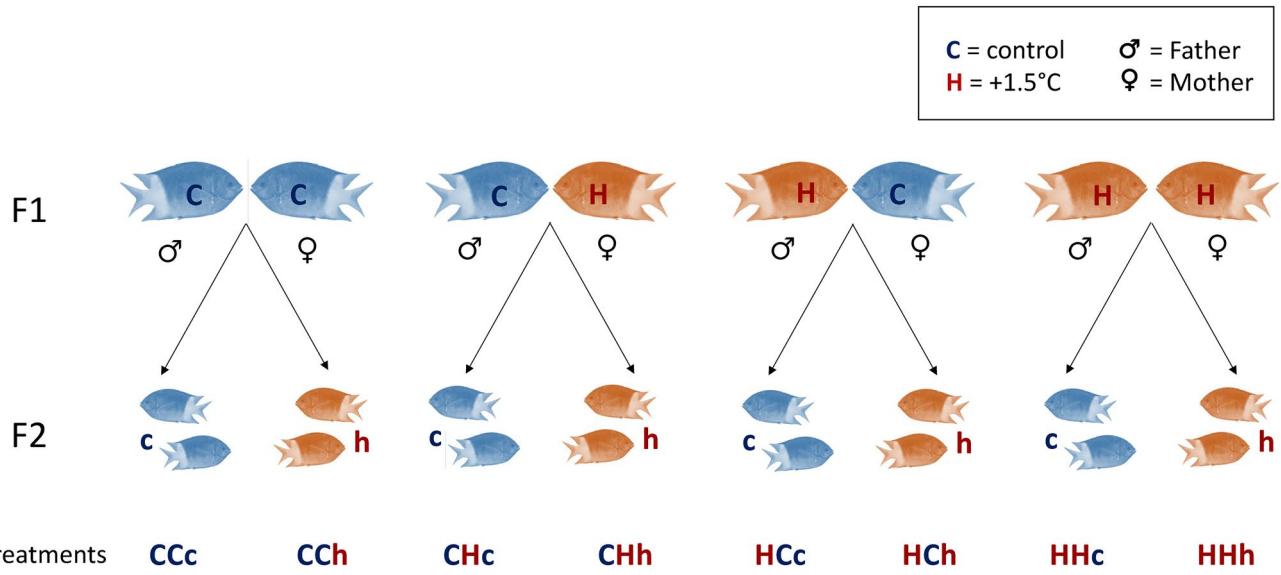

FIGURE 1 Experimental design to investigate paternal and maternal effects of warming in *A. polyacanthus*. F1 males and females developed either at present-day control temperature (in summer 28.5°C with ±0.6°C diurnal variation) or at +1.5°C elevated temperature (in summer 30.0°C with ±0.6°C diurnal variation). At 1.5 years of age, F1 were paired in reciprocal sex crosses of the two thermal treatments. F2 were split after hatching into present-day control or elevated temperature, where they developed for 80 days. Treatment code: first capital letter = thermal treatment of the F1 father; second capital letter = thermal treatment of the F1 mother; third lowercase letter = thermal treatment of F2 offspring.

a similar influence of mothers and fathers on the offspring phenotype. However, using fish from this same experiment, Spinks et al. (2021) found parent-specific effects in life history traits and physiological performance, with mothers producing larger eggs and better quality offspring when exposed to warming, possibly a sign of developmental plasticity of their reproductive system, while fathers sired fewer and poorer-quality offspring if raised at elevated temperature. There was also a non-additive interaction between maternal and paternal effects on offspring swimming performance, with fish from mismatched parents swimming faster than offspring of parents that developed at the same temperature, potentially indicating maladaptive energy resource allocations (Spinks, 2021). Here we aim to: (1) tease apart the molecular processes regulating the individual maternal and paternal effects associated with acclimation to elevated temperature in juvenile fish, (2) investigate the interaction between the different parental experiences and thermal acclimation to elevated temperature in juvenile fish, and (3) unravel how the paternal, maternal and offspring experiences of warming integrate in the resulting juvenile hepatic transcriptional phenotype. Overall, with this study, we aim to establish key molecular processes and the relative importance of each parent in transgenerational acclimation to ocean warming.

2 | MATERIALS AND METHODS

2.1 | Experimental design

To investigate the parental contributions to thermal acclimation, we analysed the liver gene expression of 80-day-old F2 *A. polyacanthus* offspring produced in the experimental set-up described in Spinks et al. (2021, 2022). Briefly, wild adult spiny chromis damselfish (F0 generation) were collected from the Palm Islands region (18°37′ S, 146°30′ E) and nearby Bramble Reef (18°22′ S, 146°40′ E) of the central Great Barrier Reef (GBR), Australia, paired and housed with seasonally cycling water temperature in the Marine and Aquaculture Research Facility of the James Cook University in Townsville, Australia.

In the Austral summer of early 2016, newly hatched F1 offspring from six breeding pairs were split into two thermal treatments, present-day control and elevated temperature. Different from most past experiments on ocean warming, we included both seasonal (minimum 23.2°C in winter, maximum 28.5°C in summer) and diurnal (3:00 AM −0.6°C, 3:00 PM +0.6°C) cycles that are representative of the Palm Islands region of the GBR in the two temperature treatments, to better mimic natural temperature variation and more accurately predict *A. polyacanthus* responses to ocean warming. The elevated thermal treatment followed the same cycles but with an increase of 1.5°C, chosen to match the projections for ocean warming by the end of the century in a low CO_2 emission scenario (Fox-Kemper et al., 2021). Moreover, this average temperature increase already occurs during heatwaves on the GBR (Frölicher et al., 2018). F1 fish developed and were reared in each of the two thermal treatments until they reached maturity at approximately 1.5 years of age. At this time, all fish were moved to present-day control temperature and F1 breeding pairs were formed between non-sibling fish, so that reciprocal sex crosses of the developmental temperatures were created. Reproduction for all pairs occurred at control temperature to ensure that observed phenotypic effects of developmental warming were not

confounded by the exposure to elevated temperature during sperm production and oocyte maturation (Pankhurst et al., 1999; J. Donelson, personal communication), nor embryogenesis, since eggs are left with the parents until hatching to allow parental care. This resulted in F1 breeding pairs consisting of four pair combinations of males and females: (1) both sexes developed at present-day control temperature (CC), (2) males developed at elevated temperature and females developed at control temperature (HC), (3) males developed at control temperature and females developed at elevated temperature (CH) or (4) both sexes developed at elevated temperature (HH; Figure 1; Table S1).

In the Austral summer of 2017–2018 F1 breeding pairs produced the F2 generation. The first clutch of each parental pair (three to five clutches per parental treatment in total) was split into two thermal treatments, either present-day control or elevated temperature, which followed the above-mentioned seasonal and diurnal cycles, with two replicate tanks of 20 fish each per thermal treatment per clutch. This resulted in a fully factorial, split clutch design of a total of eight different final treatments: (1) CC offspring raised at control temperature (CCc); (2) CC offspring raised at elevated temperature (CCh); (3) HC offspring raised at control (HCc) or (4) at elevated temperature (HCh); (5) CH offspring raised at control (CHc) or (6) at elevated temperature (CHh); (7) HH offspring raised at control (HHc) or (8) at elevated temperature (HHh; Figure 1). F2 fish were reared in the described thermal treatments until 80 days post-hatching (dph), when they were sexed by urogenital papilla external examination, and two males and two females per clutch (12 to 20 individual fish per treatment) were euthanized by cervical dislocation, measured for length, weighed for mass and dissected. Livers were immediately snap frozen in liquid nitrogen and stored at −80°C for subsequent RNA extraction. During rearing, an average of approximately 11% natural mortality occurred (Table S2). All samples were collected between 9:00 AM and 12:00 PM. All procedures were performed in accordance with relevant guidelines and were conducted under James Cook University's animal ethics approval A1990, A2210 and A2315.

2.2 | RNA sequencing and gene expression analysis

Samples were processed as described in Bonzi et al. (2023). Briefly, total RNA was isolated from homogenized whole livers using a *mir*Vana miRNA Isolation kit. Liver was chosen because of its major role in metabolism, and to allow comparison more easily with previous works (Bernal et al., 2018, 2021; Veilleux et al., 2015). Isolated RNA was checked for quality and quantity, and mRNA-focused libraries were prepared using the Illumina TruSeq stranded mRNA Library Preparation Kit. Libraries were paired-end sequenced with Illumina HiSeq 4000 (150 bp) at the King Abdullah University of Science and Technology Bioscience Core Lab.

FastQC (Andrews, 2010) was used to quality check the raw reads before and after Trimmomatic v0.39 (Bolger et al., 2014) quality trimming and adapter removal, using a sliding window of 4:15 and retaining paired-end reads with minimum length of 40 bp. Trimmed reads were then mapped against the *Acanthochromis polyacanthus* genome (ENSEMBL ASM210954v1) using HiSAT2 v2.2.1 (Kim et al., 2019), and featureCounts (Liao et al., 2014) from the Subread v2.0.2 package was used to calculate gene counts, in read pair counting mode, allowing for multi-mapping fractional computation.

The resulting count matrix was then imported in R v.3.6.3 (R Core Team, 2020), where DESeq2 package v1.26.0 (Love et al., 2014) was used to detect differentially expressed genes across treatments. Raw counts were variance-stabilizing transformed (VST) using the *vst* function, and principal component analyses (PCAs) and heatmaps of the sample-to-sample distances were run to check for outliers and batch effects. Based on the resulting plots, four outlier samples were excluded from further analyses, leaving a total of 124 samples (11–20 samples/treatment; Table S3). We applied likelihood ratio tests (LRTs) to determine which variables (e.g. family, sex and size) and interactions between treatments were significant. We found family effects and the two-way interaction between maternal and paternal developmental thermal environments to be significant, while the other two-way interactions (paternal: offspring thermal environments and maternal: offspring thermal environments), the three-way interaction (paternal:maternal:offspring thermal environments), offspring sex and offspring size were found not to be significant (i.e. they returned no differentially expressed genes) and were therefore excluded from the final design. Then, we performed differential gene expression analyses using two approaches: (a) we ran LRTs comparing the full model to reduced models where we removed either one of the main terms or the paternal:maternal interaction term to obtain the differentially expressed genes (DEGs; padj <0.05) due to the overall effects of paternal, maternal and offspring's own thermal exposure, as well as the effects of the interaction between paternal and maternal thermal environments; (b) we used Wald tests to run pairwise comparisons between the final eight treatments (CCc, CCh, HCc, HCh, CHc, CHh, HHc and HHh), as well as between offspring from the four different parental combinations (CC, HC, CH and HH), regardless of their own thermal experience, followed by log2 Fold Change (log2FC) shrinkage by apeglm method (Zhu et al., 2019). The threshold cut-offs chosen to identify significant DEGs in the pairwise comparisons were false discovery rate (FDR)-adjusted *p*-value <.05 (Benjamini & Hochberg, 1995), $|\log2FC| \geq 0.3$ to reduce false positives and a mean expression of >10 reads (baseMean). Finally, LRT-identified DEGs due to parental treatment interaction were clustered using the *degPatterns* function from the DEGreport R package v1.22.0 (Pantano, 2021) to identify similar expression profiles. The function was run on the VST processed count matrix of such genes with default settings, except for cluster outlier removal (reduce = TRUE).

Functional enrichment analyses of differentially expressed genes and expression clusters were performed in OmicsBox v2.0.36

(OmicsBox, 2019) with the Fisher's exact test (FDR < 0.05). For gene sets with non-significant GO enrichment, genes were manually grouped based on function and pathway membership.

3 | RESULTS

3.1 | Overall influence of parental and offspring thermal environments

3.1.1 | Parental exposure to warming had stronger effects than the offspring's own exposure

Paternal and maternal exposures to elevated temperature caused the differential regulation of 6684 and 5877 genes in their offspring, respectively (Figure 2; Tables 1 and S4; Tables S5 and S6, respectively). Roughly half (3914) of the differentially expressed genes (DEGs) due to the parental exposure to elevated temperature were common to both parents (Figure 2) and are involved in metabolism, translation, gene expression, ncRNA processing, T cell activation and telomere organization (Figure 3; Table S7). A total of 2121 DEGs were instead exclusive to the paternal thermal exposure (Figure 2; Table S5), and 1304 DEGs were exclusive to the maternal thermal exposure (Figure 2; Table S6). Conversely, the offspring's own exposure to warming resulted in 1579 DEGs (Figure 2), mainly involved in oxidoreductase activity, metabolism, DNA replication and vacuolar transport (Figure 3; Table 1; Table S8).

3.1.2 | Offspring gene expression did not differ depending on offspring gender or interaction between parental and offspring thermal environments

We did not find any offspring sex-specific parental effects, or differential gene expression between male and female offspring. Testing the interaction between the two generations' thermal treatments resulted in no DEGs, indicating that the gene expression patterns in the offspring were not influenced by the match or mismatch of their thermal experience with the parental environment.

3.1.3 | Paternal warming exposure altered how maternal exposure affected offspring's gene expression, and vice versa

Maternal and paternal exposures to warming interacted to shape the expression of 4592 genes (Figure 2; Table S9). For this

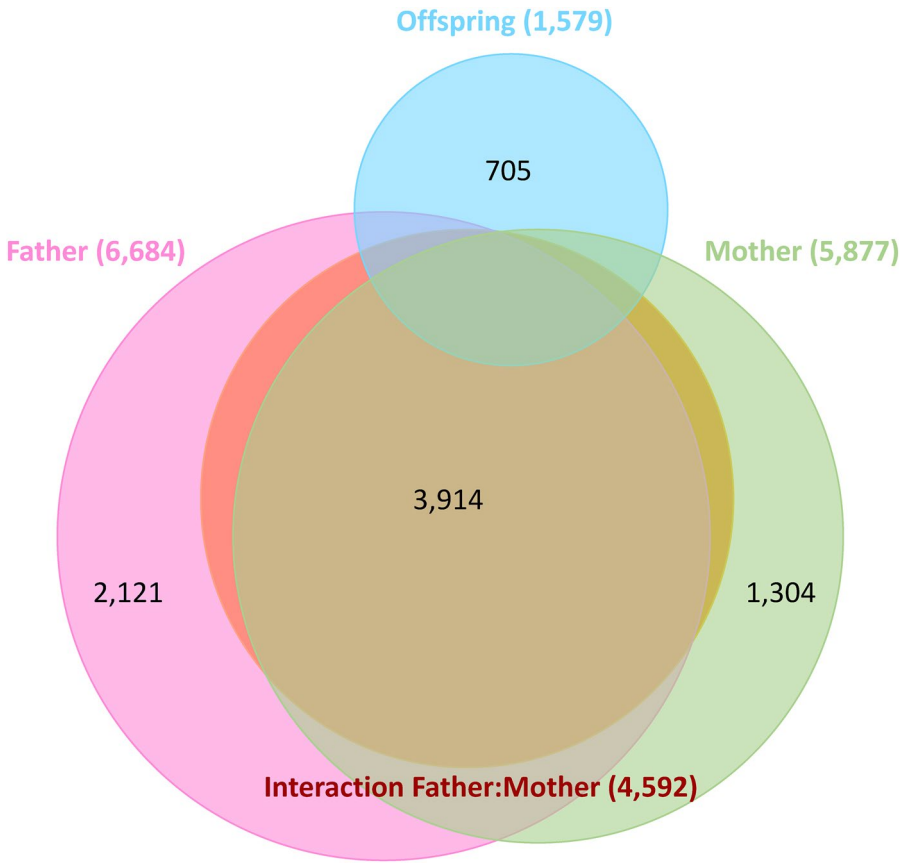

FIGURE 2 Venn diagram of differentially expressed genes attributable to the paternal, maternal and offspring developmental thermal experiences and to the interaction between the paternal and the maternal ones, identified by likelihood ratio tests.

TABLE 1 Overview of tests used to distinguish paternal, maternal and offspring warming exposure effects. Only three representative enriched GO terms are shown. Full enriched GO term lists for the differentially expressed genes (DEGs) can be found in Table S4.

Test	Factor/pairwise comparison tested	# DEGs	Enriched GO terms	FDR
Likelihood ratio test	Offspring's exposure	1579	GO:0008152 metabolic process	2.41E-05
			GO:0006261 DNA-templated DNA replication	1.96E-04
			GO:0007034 vacuolar transport	2.16E-03
	Paternal exposure	6684	GO:0003735 structural constituent of ribosome	8.27E-21
			GO:0006915 apoptotic process	7.07E-05
			GO:0010608 post-transcriptional regulation of gene expression	4.33E-03
	Maternal exposure	5877	GO:0032555 purine ribonucleotide binding	2.03E-11
			GO:006629 lipid metabolic process	1.11E-05
			GO:0042110 T cell activation	4.39E-03
	Paternal:maternal interaction – cluster A	1861	GO:0005783 endoplasmic reticulum	9.81E-07
			GO:006629 lipid metabolic process	3.01E-04
			GO:0016485 protein processing	2.62E-03
	Paternal:maternal interaction – cluster B	1533	GO:0003735 structural constituent of ribosome	4.94E-54
			GO:0010467 gene expression	1.27E-23
			GO:0004129 cytochrome-c oxidase activity	2.68E-02
Pairwise comparisons	HH vs. CC	↑ 593	GO:0042254 ribosome biogenesis	3.84E-17
			GO:0034470 ncRNA processing	3.63E-16
			GO:0010467 gene expression	5.59E-10
		↓ 653	GO:0016491 oxidoreductase activity	4.54E-41
			GO:0070279 vitamin B6 binding	1.52E-07
			GO:0043401 steroid hormone-mediated signalling pathway	6.44E-07
	HC vs. CC	↑ 1621	GO:0033554 cellular response to stress	1.16E-02
			GO:0042981 regulation of apoptotic process	2.91E-02
			GO:0006281 DNA repair	2.98E-02
		↓ 1281	GO:0003735 structural constituent of ribosome	2.47E-46
			GO:0071526 semaphorin–plexin signalling pathway	3.42E-05
			GO:0070603 SWI/SNF superfamily-type complex	3.36E-02
	CH vs. CC	↑ 258	GO:0006869 lipid transport	7.85E-06
			GO:0033036 macromolecule localization	4.79E-02
		↓ 357	GO:0004364 glutathione transferase activity	3.48E-04
			GO:0003735 structural constituent of ribosome	1.80E-03
			GO:0000723 telomere maintenance	2.47E-03
	HC vs. CH	↑ 1350	GO:0008152 metabolic process	4.81E-04
			GO:0016491 oxidoreductase activity	5.13E-03
			GO:0006950 response to stress	4.77E-02
		↓ 896	GO:0044267 protein metabolic process	3.40E-05
			GO:0005524 ATP binding	3.08E-04
			GO:0003735 structural constituent of ribosome	1.81E-02
	HH vs. HC	↑ 1830	GO:0003735 structural constituent of ribosome	1.95E-42
			GO:0003899 DNA-directed 5′-3′ RNA polymerase activity	2.78E-19
			GO:0034470 ncRNA processing	1.85E-16
		↓ 2036	GO:0005783 endoplasmic reticulum	1.06E-06
			GO:0006629 lipid metabolic process	1.40E-03
			GO:0051082 unfolded protein binding	1.65E-03

TABLE 1 (Continued)

Test	Factor/pairwise comparison tested	# DEGs	Enriched GO terms	FDR
	HH vs. CH	↑ 950	GO:0034470 ncRNA processing	2.77E-15
			GO:0042254 ribosome biogenesis	2.93E-15
			GO:0003899 DNA-directed 5′-3′ RNA polymerase activity	2.65E-10
		↓ 957	GO:0051082 unfolded protein binding	1.00E-07
			GO:0005783 endoplasmic reticulum	1.06E-08
			GO:0006629 lipid metabolic process	3.18E-04
	CCh vs. CCc	↑ 60	GO:0010801 negative regulation of peptidyl-threonine phosphorylation	3.54E-02
		↓ 48	No enriched GO terms	NA
	HHh vs. HHc	↑ 10	No enriched GO terms	NA
		↓ 24	No enriched GO terms	NA
	HCh vs. HCc	↑ 60	No enriched GO terms	NA
		↓ 80	GO:0030029 actin cytoskeleton organization	3.82E-03
	CHh vs. CHc	↑ 56	No enriched GO terms	NA
		↓ 29	No enriched GO terms	NA

reason, the contributions of mothers and fathers were analysed taking into consideration not only their independent effects but also how their outcomes changed depending on the combination of the paternal and maternal experiences in different parental pairs.

3.2 | Paternal thermal molecular signatures

The general but exclusive contribution of the father's thermal history to patterns of gene expression in the offspring is shown by the 2121 DEGs which were only differentially expressed because of the development of fathers at elevated temperature (Figure 2; Table S5). Processes involved in protein modification, RNA processing, transcription and post-transcriptional regulation of gene expression were overrepresented in this set of genes (Table S10). For example, genes encoding for several translation initiation factors (*eif2ak2*, *eif4ebp1* and *eif5a*), as well as the poly(A)-specific ribonuclease (*prna*) that degrades mRNA poly(A) tails, trinucleotide repeat containing adaptors 6B and 6C (*tnrc6b*, *tnrc6c*), involved in miRNA-mediated gene silencing and pre-mRNA processing factor 6 (*prpf6*), a component of the spliceosome, were all exclusively differentially regulated in offspring because of the paternal exposure to warming. Other differentially expressed genes were involved in negative regulation of cell population proliferation and apoptosis, such as PYD and CARD domain containing (*pycard*), a key mediator of caspase-mediated apoptosis, caspases 3, 8 and 9 (*casp3*, *casp8*, *casp9*), as well as members of the BCL2 family, like BCL2-associated agonist of cell death (*bad*), BCL2-modifying factor (*bmf*) and BCL2-interacting protein 3 like (*bnip3l*; Figure 3; Table S10).

3.2.1 | Exposure of fathers to warming caused upregulation of stress response and repair pathways and downregulation of genes involved in immune system and adipogenesis

To understand which functional pathways in the offspring were upregulated and which were downregulated due to the paternal experience of warming, we compared offspring of fathers exposed to warming and mothers raised at control (HC) to offspring where both parents were exposed to control conditions (CC), regardless of the offspring's own developmental temperature. This pairwise comparison resulted in 2902 DEGs (Figure 4; Table S11), where the upregulated genes in HC offspring showed enrichment for transcription, based on genes such as transcription factors *hnf1b*, srf, *gata-4*, *-5* and *-6*, as well as deacetylation of proteins, like histone deacetylases 3 and 7 (*hdac3*, *hdac7*), regulation of apoptotic process and response to stress and DNA repair (Table S12). The gene encoding for damage-specific DNA-binding protein 2 (*ddb2*), involved in DNA repair and histone ubiquitination, was upregulated in these offspring, as well as DNA polymerases beta and epsilon (*polb*, *pole*), the FA core complex-associated protein 24 (*faap24*), crucial in DNA damage response, and the DNA mismatch repair protein genes *mlh1* and *msh6*. On the other hand, the downregulated genes were related to translation, with more than 50 genes coding for structural components of ribosomes, semaphorin–plexin signalling and chromatin remodelling via SWI/SNF complex, such as AT-rich interaction domains 1A and 1B (*arid1a*, *arid1b*), CECR2 histone acetyl-lysine reader (*cecr2*) and SWI/SNF-related matrix-associated actin-dependent regulators of chromatin subfamily A member 5 and subfamily E member 1 (*smarca5*, *smarce1*; Table S12).

Among these DEGs, a subset of 128 genes was found to be similarly upregulated or downregulated in offspring of parents that

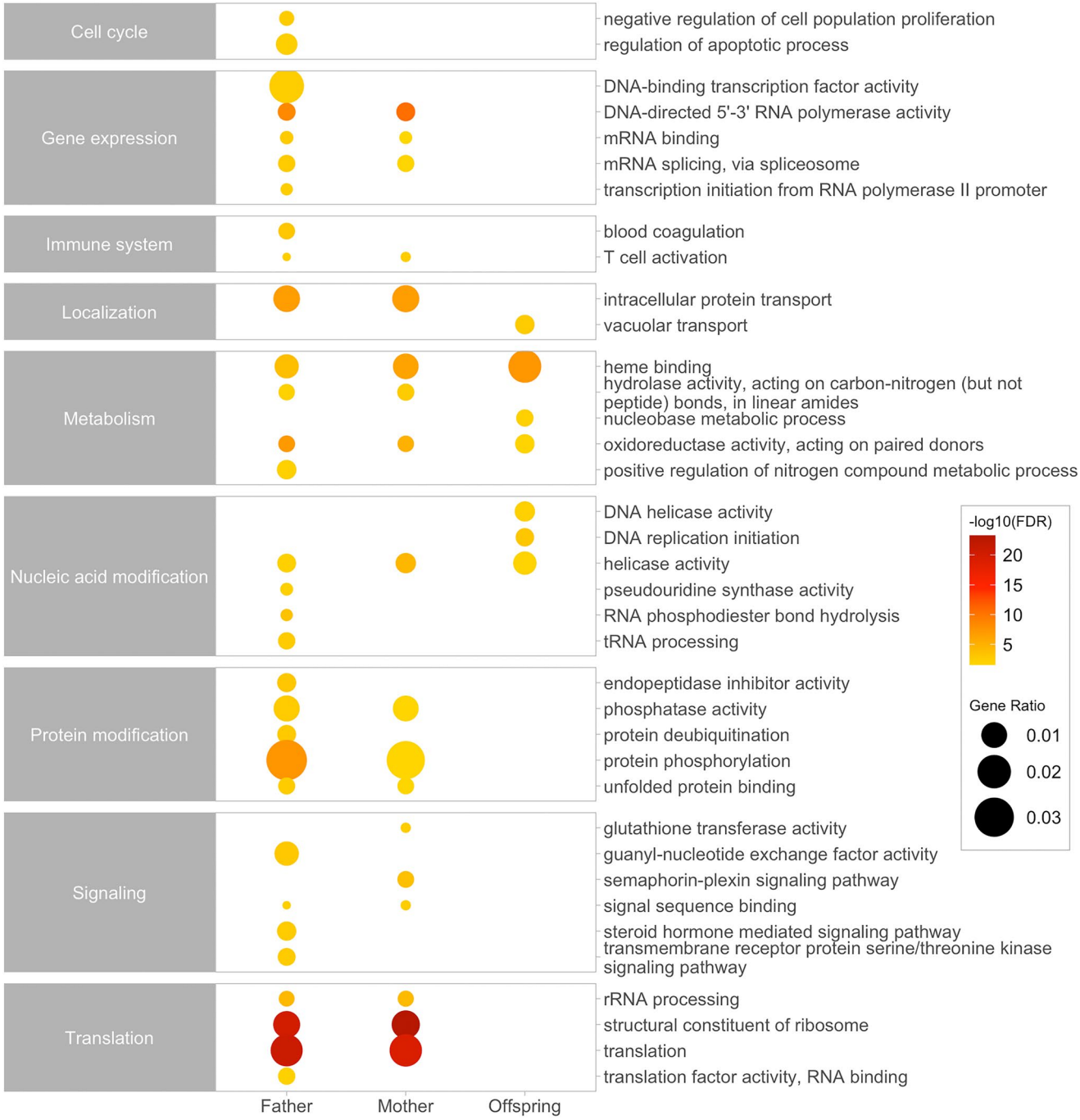

FIGURE 3 Functional enrichment analysis of differentially expressed genes attributable to the father, mother and offspring developm ental thermal experiences. The colour of the circles represents the enrichment significance with darker coloration increasing in significance, and size of circles is proportional to the number of genes underlying the enriched function.

were both exposed to warming (HH) compared to CC offspring, but not in offspring from control fathers and mothers exposed to warming (CH) compared to CC offspring (Figure 5a; Table S11). This consistency in patterns of gene expression between HC versus CC and HH versus CC provides evidence for a paternal effect independent of the maternal experience. The downregulated genes were mainly involved in inflammation and innate immune system, such as several complement system components (*c3*, *c4*, *c7*, *crp* and *mfap4*), immunoglobulins (*btn3a2*) and cytokines (*ccl3*), as well as adipogenesis (*agt*, *bmp1*, *id3*, *lifr*, *ncor2* and *stat5b*) and fatty acid metabolism (*abhd5*, *cyp3a4*, *ephx2*, *gpx4* and *prkag1*). Other shared DEGs were instead related to apoptosis, transcription regulation, chromatin organization (*loxl2*, *ncor2*, *prdm9* and *rela*) and epigenetic regulation of gene expression, through histone acetylation (*kansl3* and *naa40*) and miRNA-mediated gene silencing (*tnrc6b* and *tnrc6c*).

3.2.2 | Exposure to elevated temperature in both fathers and offspring resulted in changes in immune response, RNA processing and transcription

Finally, to investigate the effects of paternal exposure to warming when the offspring develop at elevated temperature themselves, we analysed the transcriptional changes in HC offspring that developed at elevated (HCh) versus control temperature (HCc). We found 140 DEGs, with 126 exclusive to this pairwise comparison compared to offspring from different parental combinations (Figure 6; Table S13). Among the downregulated genes in HCh offspring, we found genes involved in actin cytoskeleton organization (Table S4) and immune response (*ccl25*, *coro1a*, *lcp2* and *tlr7*). RNA processing (*adarb1*, *ddx17*, *pnpt1* and *srsf7*) and autophagy-related genes were instead upregulated, while other of these DEGs are involved in transcription regulation (*bcl9l*, *chd6*, *creb1*, *klf6* and *nr1d1*), metabolism, proteolysis and signal transduction.

3.3 | Maternal thermal molecular signatures

The exposure of mothers to elevated temperature resulted, in general, in protein and purine ribonucleotide binding, dipeptidase activity as well as catalytic activity acting on a nucleic acid, due to genes coding for helicases (*ddx54*, *dhx15*, *dhx29*, *dhx30* and *dhx38*), among the overall 1304 offspring genes exclusively differentially expressed because of the maternal thermal experience (Figure 2; Tables S6 and S14).

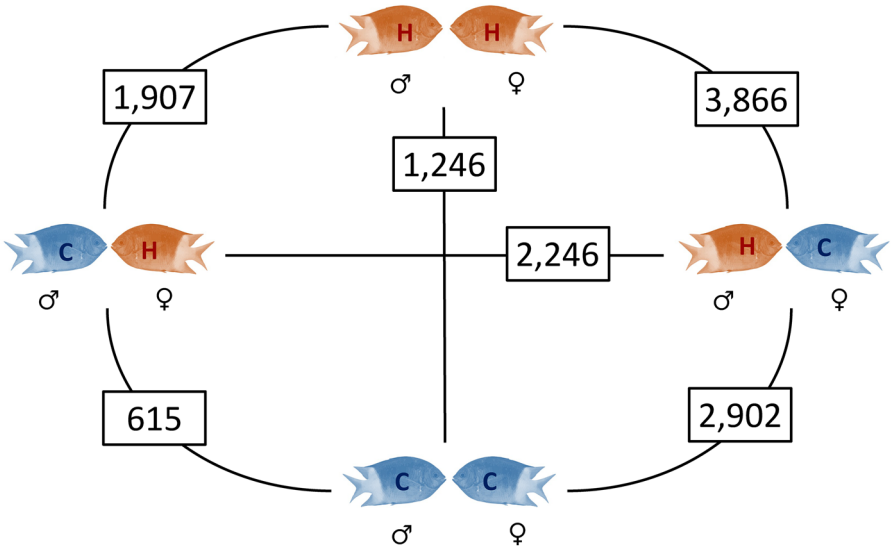

FIGURE 4 Numbers of genes differentially expressed in offspring from different parental treatments, regardless of their own developmental thermal environment. 'C' stands for control temperature and 'H' stands for +1.5°C.

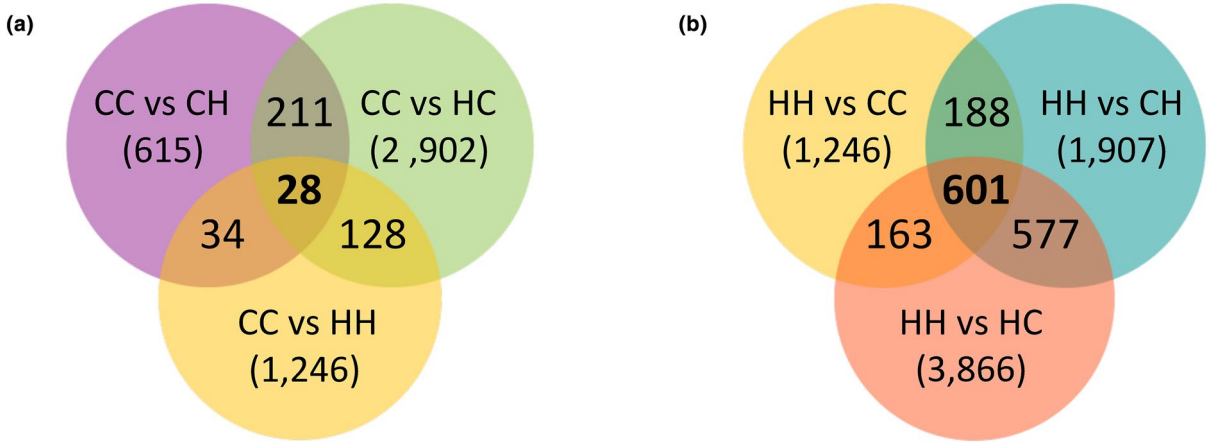

FIGURE 5 Venn diagrams of differentially expressed genes in pairwise comparisons between (a) CC and (b) HH versus other parental thermal treatment offspring. In the intersections are reported the numbers of DEGs concordantly shared between the different pairwise comparisons. The first letter stands for the paternal thermal environment, the second for the maternal one; 'C' = control temperature and 'H' = +1.5°C.

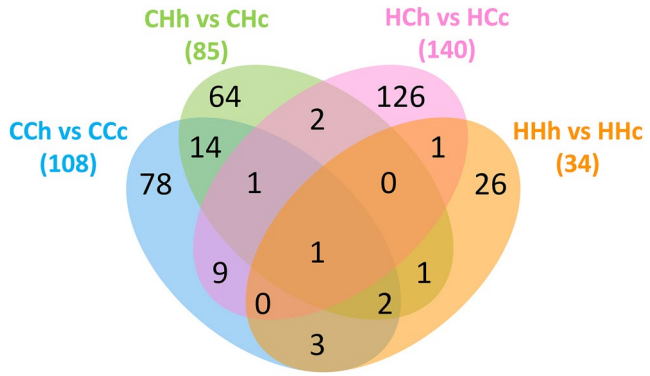

FIGURE 6 Venn diagram of differentially expressed genes in pairwise comparisons between offspring from the same parental treatments (first capital letter represents the paternal thermal environment and second capital letter represents the maternal thermal environment) developed at elevated (h) versus control temperatures (c; lowercase letter). 'C/c' stands for control temperature and 'H/h' for +1.5°C.

3.3.1 | Exposure of mothers to warming caused upregulation of lipid transport and downregulation of genes with glutathione transferase and helicase activities

Offspring born from elevated temperature mothers and control fathers (CH) differentially expressed 615 genes compared to CC offspring, regardless of their own developmental temperature (Figure 4; Table S15). 'Lipid transport' GO term was enriched for the upregulated genes in CH offspring, mainly due to several upregulated apolipoproteins (*apoa4*, *apob*, *apoc2* and *apoe*), while the downregulated genes were related to processes such as glutathione transferase activity, in particular genes encoding for glutathione S-transferases alpha 3, mu 3 and omega 1 (*gsta3*, *gstm3*, *gsto1*), structural constituent of ribosome and helicase activity (Table S16).

Among the CH versus CC DEGs, 34 genes overlap between CH versus CC and HH versus CC comparisons, representing the maternal signature independent of the paternal or offspring thermal experiences (Figure 5a; Table S15). These genes are involved in immune and inflammatory responses (*cd276*, *gpank1*, *itk* and *nlrc5*), lipid (*fabp3*, *lipe* and *pc*) and retinol (*cyp2d15* and *dhrs13*) metabolism, as well as hepatocyte apoptotic process (*krt18* and *pik3cg*).

3.3.2 | Exposure to elevated temperature in both mothers and offspring resulted in upregulation of genes related to glucose and lipid metabolism

To understand how offspring from mothers exposed to warming respond when they experience elevated temperature themselves, we compared CH offspring raised at elevated temperature (CHh) to CH offspring raised at control (CHc). We found 64 unique DEGs compared to offspring from other parental pairs (Figure 6; Table S17), mainly involved in glucose metabolism (*aldoc* and *enpp1*) and metabolism of lipids and lipoproteins, such as apolipoproteins A4 and E (*apoa4*, *apoe*), responsible for lipid transport among organs, hydroxyacid oxidase 2 (*hao2*), involved in fatty acid oxidation, and a regulator of lipid biosynthesis and apolipoprotein secretion from hepatocytes, oxysterol-binding protein-like 10 (*osbpl10*), all upregulated in offspring raised at elevated temperature.

3.4 | Direct differences between offspring of father alone or mother alone exposed to warming

Finally, we compared offspring from father alone (HC) versus mother alone (CH) exposed to warming to identify the differences between paternal and maternal effects, when one parent only experienced warming. The comparison returned 2246 DEGs (Figure 4; Table S18). Compared to CH offspring, offspring from HC parents, regardless of their own thermal treatment, upregulated genes involved in processes like oxidoreductase activity, included several cytochrome genes (*cyb561*, *cybb*, *cyp2d17*, *cyp2f2*, *cyp2j2*, *cyp2j6*, *cyp26b1* and *cyp4b1*) and response to stress, with several genes involved in DNA damage response (*brcc3*, *faap24*, *fan1*, *mlh1*, *mlh3*, *mutyh*, *nudt1*, *ogg1*, *pif1*, *polb*, *pole* and *rad18*). On the other hand, CH offspring compared to HC offspring upregulated genes with functions related to protein metabolism, structural constituent of ribosome and ATP binding, including the gene coding for insulin receptor (insr) and genes with lipid transport functions, such as ATP-binding cassette transporters (*abca1*, *abca3*, *abcc4*, *abcd1* and *abcd2*; Table S19).

3.5 | Interaction effects between maternal and paternal exposures to warming

3.5.1 | Offspring from mismatched parents showed different gene regulation compared to offspring from matched parents

Among the 4592 genes interested in the interaction between the two parental exposures, two clusters of genes were differentially regulated depending on the match or mismatch between the paternal and the maternal thermal environments (Figure 7). Specifically, 1861 DEGs belong to a cluster expressed at higher levels in offspring from mismatched parents (CH and HC; Cluster A; Figure 7; Table S20). Many of these genes show functions related to protein modification and transport, especially in the endoplasmic reticulum (ER), including members of the calnexin/calreticulin cycle and ERAD pathway involved in ER protein folding and in the response to ER stress. For example, both calnexin (*canx*) and calreticulin (*calr*) belong to this cluster, as well as protein disulfide isomerase family A members 3 and 4 (*pdia3*, *pdia4*), signal sequence receptor subunit 1 (*ssr1*), torsin family 1 member A (*tor1a*), VAMP-associated protein B and C (*vapb*) and zinc finger

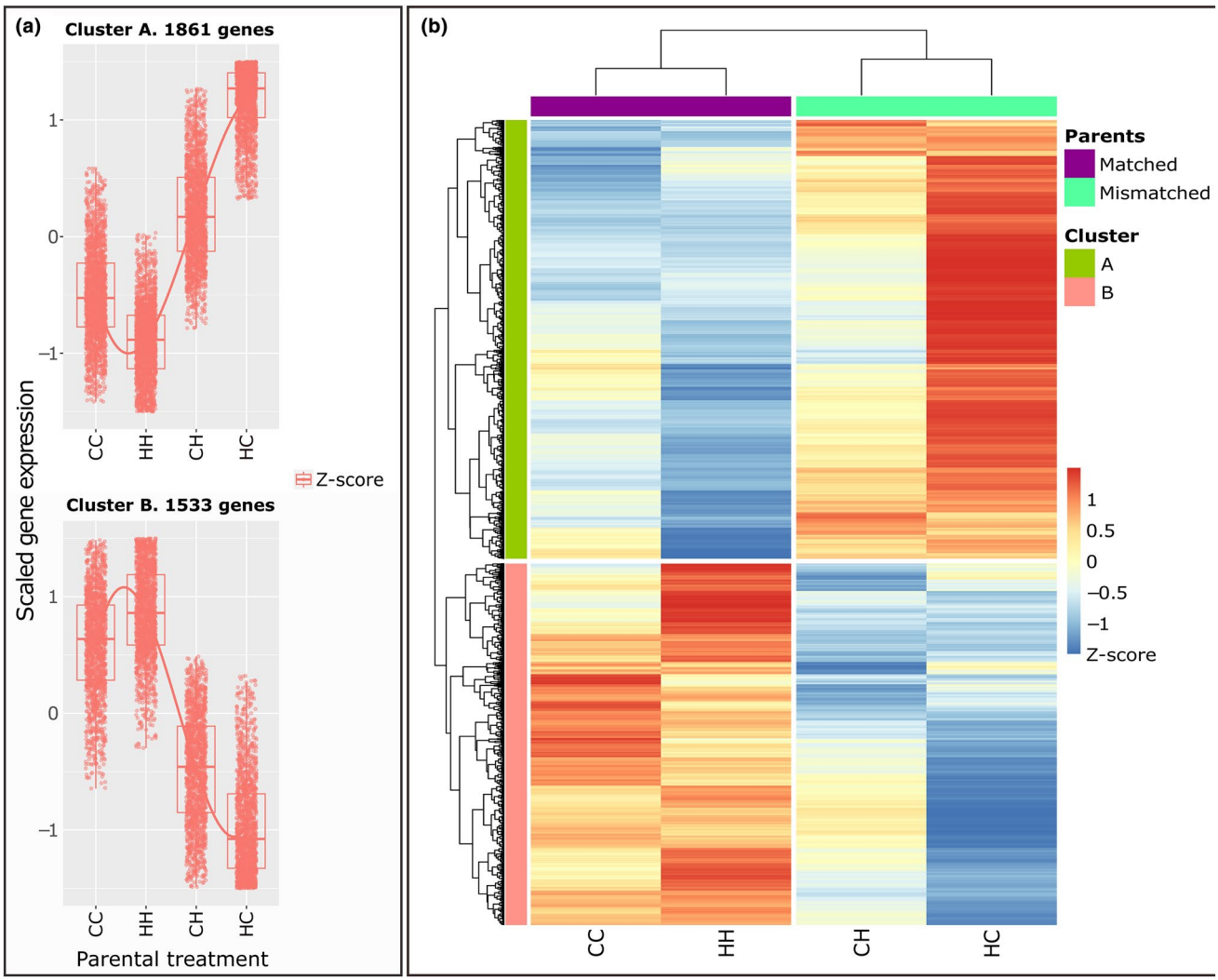

FIGURE 7 The two largest clusters of differentially expressed genes due to the interaction between parental treatments, showing expression differences between offspring from matching (CC and HH) and mismatching (CH and HC) parents. DEGs were clustered based on their scaled expression profiles (Z-score). (a) Box plots and median expression are shown for each parental treatment combination. (b) Heatmap of Z-score values. In the parental treatment, the first letter stands for the paternal thermal environment and the second for the maternal one, where 'C' = Control and 'H' = +1.5°C.

and BTB domain containing 17 (*zbtb17*), all involved in the unfolded protein response (UPR) stress response pathway. Other functions that characterize this cluster of genes are cell redox homeostasis, and lipid biosynthesis and metabolism (Table S20). Conversely, among the 1533 genes that followed the opposite pattern and were expressed at higher levels in offspring from matched parents (CC and HH; Cluster B; Figure 7; Table S21), we found 68 genes coding for ribosome components and involved in translation and protein synthesis, as well as genes encoding for histones (*h1*, *h2a*, *h2a1c*, *h2afx*, *h2afy2*, *h2b*, *h2bc21*, *h33*, *h4*, *hist1h1b* and *hist2h2l*) and proteins involved in gene expression, DNA-directed 5′-3' RNA polymerase activity (*polr1e*, *polr2c*, *pol32d*, *polr3c*, *polr3d*, *polr3e* and *polr3f*), transcription by RNA polymerase III and cytochrome-c oxidase activity (*cox42*, *cox5b*, *cox6b1*, *cox8a*, *cox8b* and *cx6c1*; Table S21).

3.5.2 | Offspring from parents that were both exposed to warming upregulated genes involved in ncRNA processing and gene expression

Accordingly, pairwise comparisons between offspring from different parental thermal histories showed that HH offspring were more different to offspring from mismatched parents, rather than from CC offspring (Figure 4). Overall, 601 HH genes were commonly DE and represent the transcriptional signature of biparental exposure to warming (Figure 5b; Table S22). Among those, the upregulated genes are involved in ribosome biogenesis, ncRNA processing, especially rRNA (*dimt1*, *fbl*, *nop2*, *rrp1*, *rrp36*, *utp14a* and *utp6*) and tRNA (*dus2*, *elac2*, *trmt6* and *tsen34*), gene expression and DNA-directed 5′-3' RNA polymerase activity and transcription. The downregulated ones, on the other hand, include nuclear

steroid receptors (*nr1d2*, *nr3c1*, *rora* and *rorb*), and several genes with oxidoreductase activity functions (Table S23).

3.6 | Offspring with different parental thermal exposures showed distinct responses to elevated temperature

Strikingly, there was little overlap between the three parental exposure combinations, with only 28 genes always concordantly differentially expressed in offspring with either one or both parents raised at elevated temperature compared to CC offspring (Figure 5a; Table S24). Accordingly, distinct parental thermal histories differently shaped the offspring response to developmental warming, with mostly unique sets of genes expressed by offspring when raised at elevated temperature (Figure 6). Interestingly, the smallest number of DEGs, 34, was found for the comparison between offspring raised at elevated temperature versus at control when born from HH parents (Table S25). Twenty-six of these genes were unique to this comparison, with functions related to oxidoreductase activity, iron ion homeostasis and heme biosynthesis (Table S25).

4 | DISCUSSION

Here, we show that hepatic transcriptional responses to ocean warming in juveniles of a coral reef fish primarily depended on the thermal exposure of their parents (maternal or paternal), with the offspring's own experience of warming instead eliciting the smallest transcriptional response. This meets our expectations of parental influence to be strongest in early life (Yin et al., 2019), especially when the predictability of the environment is high between one generation to the next (Bell & Hellmann, 2019; Leimar & McNamara, 2015), as is the case of *A. polyacanthus* with limited dispersal due to direct juvenile development without a pelagic larval stage (Robertson, 1973). Surprisingly, paternal thermal exposure to elevated temperature had a stronger effect on differential gene expression in offspring compared with maternal exposure. However, it was the consistency between the two parental experiences that was key to offspring's thermal acclimation potential at the transcriptional level. While different parental thermal histories uniquely shaped how offspring responded to warming during their own developmental period, these parental effects did not interact with the sex nor the environment of the offspring, and therefore had a carry-over rather than an anticipatory nature, contrasting with what has been found in some other fish species (Chang et al., 2021; Shama et al., 2014), but not surprising given the overall small effects of the offspring experience and the widespread nature of parental carry-over effects across taxa (Bonduriansky & Crean, 2018; Uller et al., 2013). Strikingly, the smallest transcriptional response to warming experienced in the F2 generation was found in offspring from parents where both the mother and father were exposed to elevated temperature, suggesting limited transcriptional adjustments needed by these offspring to respond to a warmer environment.

Among the differentially expressed genes due to the parental thermal experience, approximately half was exclusively due to either the father or the mother. Similarly, specific molecular signatures in *A. polyacanthus* brains were found to be maternally and paternally inherited in response to elevated CO_2 exposure (Monroe et al., 2021). Parent-specific effects are usually expected when the reliability of the environmental information transmitted by one parent is greater than the other one (Bell & Hellmann, 2019; Burke et al., 2020). However, since *A. polyacanthus* is not sexually dimorphic, both sexes have the same ecology, and they provide joint parental care (Kavanagh, 2000; Nakazono, 1993; Robertson, 1973); these parent-specific effects are likely due to other differences between the two sexes, notably the way information is transmitted by sperm and oocyte and/or by unbalanced parental care effort by one of the parents (Bell & Hellmann, 2019; Perez & Lehner, 2019). For example, maternal and paternal contributions can differ not only because of maternal provisioning but different epigenetic factors might also be independently transferred by mothers and fathers, like in sticklebacks following exposure to higher temperatures (Fellous et al., 2021). Additionally, despite *A. polyacanthus* females and males showing equal parental care in the wild, in captivity, males have been previously observed to be the most involved in brood care (Pankhurst et al., 1999). While we have not measured parental care effort here, the importance of paternal contributions has been increasingly recognized (Crean & Bonduriansky, 2014; Immler, 2018; Rando, 2012). In fact, we observed a larger effect of the paternal experience compared to the maternal one, as also seen in the marine tubeworm *Galeolaria caespitosa* exposed to warming (Guillaume et al., 2015). Therefore, our results show that 80-day-old *A. polyacanthus* exhibit parent-specific intergenerational effects that influence distinct molecular pathways in offspring livers, and where paternal information is the strongest driver of gene expression change.

The father-specific effects due to the paternal exposure to warming involved the activation of stress-related gene expression pathways, like apoptosis and suppression of innate immunity response, as well as epigenetic gene expression regulatory mechanisms through histone modifications and microRNA (miRNA)-mediated gene silencing. Chromatin modifications and miRNA production could represent epigenetic defence mechanisms against stress-induced genome mutations, potentially transferred from the male germline to the offspring (Immler, 2018). Differential histone regulation has been found to be influenced by the paternal phenotype in *A. polyacanthus* exposed to simulated ocean acidification (Monroe et al., 2021) and in stickleback sperm following warming (Fellous et al., 2021). Sperm-born miRNAs from stressed fathers, on the other hand, alter mice offspring behaviour and metabolism (Gapp et al., 2014; Rodgers et al., 2013), and stressed zebrafish show changes in sperm miRNA levels (Ord et al., 2020). Here, when fathers alone were exposed to elevated temperature, their offspring additionally showed downregulation of several ribosomal proteins and translation-related genes, indicating an inhibition of the protein synthesis machinery, likely

because of cellular stress, and linked to trade-offs in energy investment (Sokolova et al., 2012; Spriggs et al., 2010). In support of these findings, juveniles from fathers exposed to warming and control mothers were shorter compared to offspring from other parental combinations (Spinks et al., 2022), suggesting impaired growth possibly due to redirection of energy towards thermal stress response processes. Interestingly, offspring were also lighter compared to control, not only when the father alone was exposed to warming but also when both parents were (Spinks et al., 2022). Notably, adipogenesis and fatty acid metabolism-related genes were downregulated in offspring from both those parental combinations. The paternal exposure to warming therefore might interfere with offspring lipid storage and overall energy homeostasis, since lipids, and in particular, fatty acids, are the main fish metabolic energetic source necessary for growth, reproduction and swimming (Tocher, 2003). Hence, the fathers' development at elevated temperature causes changes in offspring gene expression regulation of pathways linked to stress, which might result in the reallocation of energy expenditure from macromolecular biosynthesis (e.g. protein synthesis) and metabolism to repair mechanisms (e.g. DNA repair), with some pathways potentially epigenetically controlled through histone modifications and miRNA-mediated gene silencing.

Compared to the paternal effects, maternal thermal exposure appeared to be less influential to 80-day-old *A. polyacanthus*, but likewise stressful, especially for offspring from pairs where only the mother experienced warming. Indeed, maternal exposure alone caused downregulation of glutathione S-transferases, whose dysregulation is commonly associated with liver disease forms in mammals (Kirpich et al., 2011), and differential regulation of genes involved in immune and inflammatory responses, as well as hepatocyte apoptosis, possibly indicating long-term detrimental effects caused by mothers' exposure to warming. Therefore, similar to the stress-related pathways elicited by paternal exposure, maternal experience of warming in *A. polyacanthus* seems to be causing some maladaptive parental effects, especially when fathers were raised at control temperature. Nevertheless, maternal exposure to elevated temperature also caused some potentially beneficial effects. Mothers' development at elevated temperature resulted in changes in the expression of offspring genes involved in lipid and retinol metabolism, both linked to energy metabolism regulation (Klyuyeva et al., 2021). Apolipoproteins, for example, key regulators of cholesterol and triglyceride transport (Dominiczak & Caslake, 2011), were upregulated, especially when offspring from mother-only exposed pairs were raised at elevated temperature themselves. Transgenerational thermal acclimation through improved aerobic performance in *A. polyacanthus* has been linked in the past to increases in expression of genes involved in lipid metabolism (Bernal et al., 2021; Veilleux et al., 2015), notably with the transgenerational upregulation of apolipoproteins (Veilleux et al., 2015). However, while in previous studies both parents have been equally exposed to the same temperature, our results suggest that this metabolic adjustment, possibly needed to sustain the increased energy demand at elevated temperatures, might primarily be a maternally derived effect. Therefore, these maternally influenced changes in lipid and energy metabolism gene regulation, together with paternally inherited epigenetic stress-response transcriptional mechanisms, which were found in offspring whenever mothers and fathers, respectively, were raised at elevated temperature, could represent adaptive parental effects, potentially involved in the transgenerational acclimation to warming previously reported for this species (Bernal et al., 2021; Donelson et al., 2012). We hypothesize that the maternally acquired metabolism adjustments might allow offspring to meet thermally induced rises in energy demand, while the activation of paternally derived epigenetic repair mechanisms could help protect the integrity of the DNA at elevated temperatures. Overall, because of this combination of both negative and beneficial parental effects, different traits appear to have independent acclimation potentials, and while transgenerational exposure to environmental stressors such as ocean warming might enhance tolerance to such stressors, this could come at the cost of reducing overall fitness, for example, increasing disease susceptibility through immune response suppression. Ultimately, such trade-offs and the balance between costs and benefits of adaptive plasticity (Chevin et al., 2010; DeWitt et al., 1998; Murren et al., 2015) will determine the organisms' overall ability to adapt to environmental changes.

The exposure of both parents to elevated temperatures resulted in the upregulation in their offspring of genes involved in gene expression regulation and production of regulatory RNAs responsible for protein synthesis. Interestingly, increased peptide biosynthesis ability has been linked to transgenerationally acquired acclimation to warming in sticklebacks (Shama et al., 2016). Moreover, offspring from parents that were both raised at elevated temperature also exhibited the smallest transcriptional response to their own experience of warming, potentially a sign of increased tolerance to thermal stress. Indeed, studies have shown that increased thermal tolerance is often accompanied by smaller gene expression changes in response to warming (Barley et al., 2021). Heightened tolerance to warming in the crustacean *Tigriopus californicus*, for example, was associated with decreased gene expression plasticity, that is, smaller transcriptional changes, across generations (Kelly et al., 2017). Although thermal acclimation surely encompasses many different mechanisms other than transcriptional changes, here we hypothesize that increased protein synthesis regulation together with a small number of DEGs in response to their own exposure to warming might indicate increased thermal tolerance in offspring from parents that were both exposed to elevated temperature. Hence, biparental exposure to warming might result in adaptive parental effects, as opposed to the negative parental effects found in offspring where one parent only was exposed to elevated temperature. Indeed, offspring from mismatched parents showed transcriptional signs of endoplasmic reticulum stress and concomitant energy reallocation towards stress response and repair mechanisms. Moreover, downregulation of genes encoding for cytochrome C oxidase subunits might suggest a reduced

mitochondrial respiratory capacity for these offspring compared to controls and to offspring where both parents were exposed to elevated temperature. Swimming performance of offspring from this same experiment similarly exhibits non-additive paternal and maternal effects, with fish born from mismatched parents showing maladaptive swimming speed compared to controls and offspring from parents with a matched exposure to elevated temperature (Spinks, 2021). Such non-additive parental effects have also been seen in predator-induced sticklebacks, where offspring brain gene expression profiles differed depending if one or both parents had been predator exposed (Hellmann et al., 2020), and single-parent effects on daughter mate choice were reversed when both parents were exposed to predation (Lehto & Tinghitella, 2020). Here, our transcriptional results showing activation of pathways related to stress in mismatched parents suggest that biparental exposure to warming might be necessary for the transgenerational acclimation to increased temperature previously observed in this species (Bernal et al., 2021; Donelson et al., 2012; Veilleux et al., 2015). On the contrary, breeding pairs composed of individuals of different ages that developed at different thermal regimes, for example, during or not a marine heatwave, will instead transfer to their offspring maladaptive stress condition transcriptional signatures, which might override the previously reported beneficial adaptive benefits of biparental exposure to warming.

The results of our study are limited by the fact that we only measured gene expression changes in one tissue only. Fitness and performance measurements and multi-tissue analysis would provide a more complete picture of the phenotypic responses to warming. Nevertheless, hepatic gene expression analysis allows for the investigation of the transcriptional processes involved in the metabolism changes linked to transgenerational aerobic acclimation of this species (e.g. Donelson et al., 2012). The relevance of our findings, moreover, is corroborated by the fact that they are generally in agreement with the phenotypic and fitness results found in offspring from this same experiment by Spinks et al. (2021, 2022), correlating transcriptional responses with phenotypic and performance traits. Another potential limitation is that we measured gene expression changes at one time point only, when offspring were 80 days old. Gene expression is expected to change throughout development, with the personal experience, for example, likely overriding the parental effects over time (Yin et al., 2019). Nevertheless, 80 dph is a crucial life stage in this species, when the post-brooding dispersal usually occurs (Kavanagh, 2000). Investigating this time point therefore allows us to understand the transcriptional changes in place at this crucial developmental stage, and their main drivers. Future studies integrating multi-tissue transcriptional analyses, phenotypic traits, additional molecular mechanisms and time points will, however, provide a more complete picture of maternal and paternal effects in transgenerational acclimation.

Environmentally induced parental effects, if adaptive, might allow organisms to acclimate across generations to a rapidly warming world. Understanding the relative importance of fathers and mothers and how their thermal experiences are integrated into the offspring phenotype is crucial to making better predictions about species persistence potential. Here, we show that warming induces a combination of shared, and, more strikingly, also father- and mother-specific molecular signatures, some of which are likely beneficial to the offspring. Our transcriptional results suggest that the key to transgenerational thermal acclimation potential might lie in the consistency between the paternal and maternal thermal experiences. Exposure of both parents to elevated temperature caused increased expression of genes involved in transcriptional regulation and protein synthesis in the next generation, resulting in small transcriptional adjustments needed to the offspring if raised at elevated temperature. However, if only one parent experienced warming, fish manifested changes in gene expression linked to energy trade-offs towards stress response and repair mechanisms, suggesting carry-over effects of a suboptimal condition of parents that developed at elevated temperature. Therefore, mismatched thermal experiences of mothers and fathers might interfere with the adaptive potential of parental effects, at least at hepatic transcriptional level in 80-day-old juveniles. In the race between thermal acclimation and climate change, parental experience will likely play a crucial role. Ultimately, how beneficial parental effects are will depend on the balance between the costs and benefits of plasticity, and on the consistency between the past environmental experiences of mothers and fathers.

AUTHOR CONTRIBUTIONS

L.C.B., J.M.D. and R.K.S. designed the experiment and collected the samples. L.C.B. and R.K.S. managed the fish rearing. L.C.B. prepared the samples for sequencing, analysed the sequencing data and wrote the first draft of the manuscript, with input from C.S. J.M.D., R.K.S., P.L.M. and T.R. secured the funding. All authors read, provided comments and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

RNAseq data for all individuals can be found under the BioProject PRJNA998209.

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