LETTERS
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Molecular processes of transgenerational acclimation to a warming ocean

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Some animals have the remarkable capacity to acclimate across generations to projected future climate change[1–4]; however, the underlying molecular processes are unknown. We sequenced and assembled *de novo* transcriptomes of adult tropical reef fish exposed developmentally or transgenerationally to projected future ocean temperatures and correlated the resulting expression profiles with acclimated metabolic traits from the same fish. We identified 69 contigs representing 53 key genes involved in thermal acclimation of aerobic capacity. Metabolic genes were among the most upregulated transgenerationally, suggesting shifts in energy production for maintaining performance at elevated temperatures. Furthermore, immune- and stress-responsive genes were upregulated transgenerationally, indicating a new complement of genes allowing the second generation of fish to better cope with elevated temperatures. Other differentially expressed genes were involved with tissue development and transcriptional regulation. Overall, we found a similar suite of differentially expressed genes among developmental and transgenerational treatments. Heat-shock protein genes were surprisingly unresponsive, indicating that short-term heat-stress responses may not be a good indicator of long-term acclimation capacity. Our results are the first to reveal the molecular processes that may enable marine fishes to adjust to a future warmer environment over multiple generations.

Over the next century, rising ocean temperatures due to climate change will pose a serious threat to the survival of many aquatic species. To persist, populations will either need to shift their geographic distributions[5] or adapt through genetic evolution or phenotypic plasticity[6–8]. Of particular concern for marine species is that rising temperatures will reduce the capacity for oxygen supply and delivery[9,10], limiting activities essential to survival and individual fitness. Reduced aerobic scope (the capacity for oxygen uptake above resting metabolic rate) at higher temperatures can affect vital functions such as growth, swimming performance, reproduction and competitive ability[10–14]. In reef fishes, aerobic scope declines at temperatures just a few degrees above the summer average, well within the range projected to occur as a result of climate change[9,12,15]. However, aerobic capacity can be fully restored transgenerationally, when parents and their offspring both experience the same elevated temperatures (transgenerational acclimation)[1]. Understanding the molecular processes that make this transgenerational plasticity possible is important for assessing the performance of marine organisms and sustainability of their populations in a rapidly warming ocean.

We used a multi-generational rearing experiment to identify the molecular pathways associated with transgenerational thermal acclimation of metabolic traits in a common reef fish, *Acanthochromis polyacanthus*. Second-generation fish were reared developmentally (from hatching to adulthood) and transgenerationally (two generations) at two elevated temperatures (+1.5 and +3.0 °C) and in control conditions (+0.0 °C; Fig. 1a and Supplementary Methods). The full transcriptome of four to five adult fish from each of the five treatments (Fig. 1a) was sequenced and expression data were correlated to standardized metabolic traits from the same fish: routine metabolic rate (RMR), maximum metabolic rate (MMR), and net aerobic scope (MMR − RMR; NAS; Supplementary Methods). As observed in previous studies[1], developmental exposure to elevated temperatures from just after hatching into adulthood led to a reduction in aerobic scope (Fig. 1b). However, when both parents and offspring were exposed to elevated temperatures, complete restoration of aerobic scope was achieved (Fig. 1b). Of 89,543 assembled contiguous sequences (contigs), 165 had significant differential expression (adjusted $P < 0.05$) in at least one of the treatment comparisons (transgenerational and developmental treatments versus control; transgenerational versus developmental treatments). One hundred and sixty of the differentially expressed contigs had BLASTN and/or BLASTP (ref. 16) sequence matches with E-values less than 10^{-10} (Supplementary Fig. 2), of which 69 had expression that was significantly correlated to at least one of the standardized metabolic measures (RMR, MMR and NAS; Fig. 2 and Supplementary Table 1). Comparing transgenerational and developmental treatments at the same temperatures enabled us to distinguish patterns of gene expression due to transgenerational effects, compared with effects of within-generation exposure to elevated temperatures.

The 69 differentially expressed and correlated contigs represent 53 genes that are associated with transgenerational thermal

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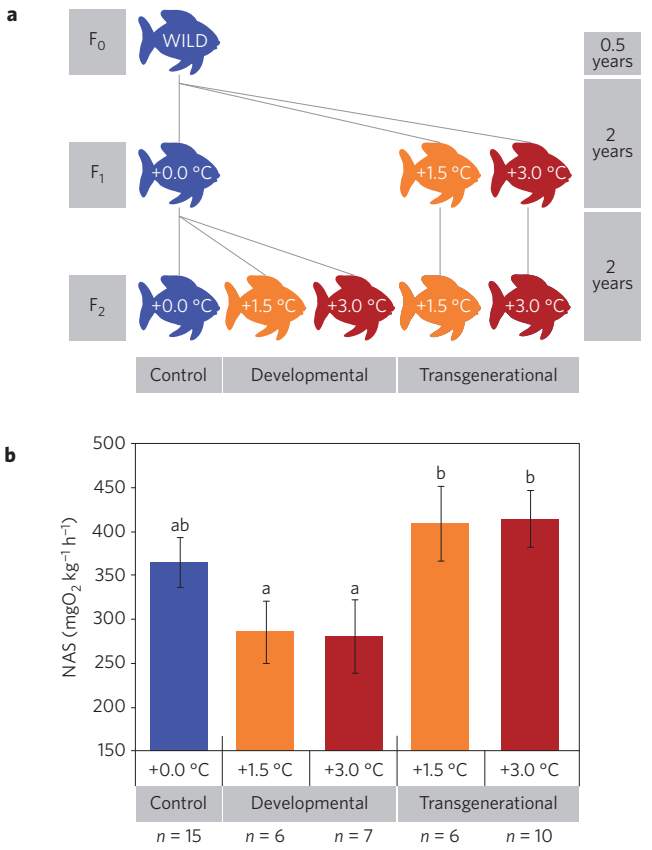

Figure 1 | Transgenerational experimental design and corresponding net aerobic scope measures. a, Experimental design tree showing the three temperature treatments (+0.0 °C, +1.5 °C and +3.0 °C) at which three generations (F_0, F_1 and F_2) of *Acanthochromis polyacanthus* were reared. Temperature treatments are colour coded and experimental duration for each generation is shown in the vertical grey bars to the right. Fish in the F_2 generation representing control, developmental and transgenerational temperature treatments are indicated by horizontal grey bars. **b**, Net aerobic scope (NAS) of fish in control, developmental and transgenerational F_2 treatments (mean ± s.e.m.). Lower case letters above bars indicate significant differences ($P < 0.05$) among treatments. Number of fish used to measure NAS for each treatment is shown beneath the grey bars.

acclimation. These genes are involved in a variety of cellular processes such as metabolism, transport, immune and stress responses, growth and development, cell cycle, cell organization, and transcriptional regulation (Fig. 2 and Supplementary Table 1). The expression profiles of these contigs were separated into three distinct groups, with the first and largest group (Fig. 2a; 46 contigs) containing contigs with expression that primarily correlated to the acclimating phenotypic trait, NAS (78%). Metabolism is the main function associated with genes in this group (lipid, protein and carbohydrate metabolism; nine, nine and five contigs each, respectively), including 79% of the most highly upregulated contigs transgenerationally relative to controls (≥1.5 $\log_2$ fold change; Supplementary Table 1). During thermal stress, the composition of lipid membranes is altered (homeoviscous adaptation)[17] and there are changes in lipid use[18] and expression of the fatty acid pathway[19]. Of the nine contigs associated with lipid metabolism, six were strongly upregulated in transgenerational treatments (representing four genes: *acsl5, adtrp, apoEb* and *pdzk1*). ApoE has a major role in triglyceride and cholesterol homeostasis, suggesting that transgenerational upregulation of lipid metabolism may be critical for improved aerobic scope. *apoE* and other apolipoproteins are also upregulated after short-term thermal challenge in fish[20,21], suggesting a link between short-term thermal stress and long-term thermal acclimation of aerobic capacity. Many of the metabolic genes in the first group (Fig. 2a) are involved in catabolism and digestion (Supplementary Table 1), suggesting that their augmented expression provides increased energy for aerobic performance in transgenerational fish (Supplementary Table 1). Supporting this hypothesis, 11 contigs are involved in the cellular transport of ions, solutes, amino acids, lipids and carbohydrates, possibly as a result of increased substrate digestion. Our results suggest that there is transgenerational regulation of lipid, protein and carbohydrate metabolism and that each may be critical for increased energy use associated with acclimation of aerobic scope across generations.

In addition to metabolic responses, 16 contigs with putative functions associated with immune responses and inflammation, apoptosis, homeostasis and stress were significantly upregulated during transgenerational thermal acclimation (Supplementary Table 1). Immune responses can be maternally imprinted in fish[22], potentially by transferring maternal idiotypic networks to juveniles at a critical stage[23]. Such imprinting, we propose, would then be augmented throughout development to establish an immune response better suited for survival under thermal stress. As chronic stress can suppress immune function and lead to increased susceptibility to disease and pathogens[24], the transgenerational augmentation of five putative immune-related contigs (*gimap8, xpnpep2, mep1b* and *natterin3*) may represent new baseline levels of immune-related genes to protect against elevated temperatures experienced across generations.

The second main group of genes (Fig. 2b) is comprised of 12 contigs, all of which had expression that was negatively correlated to standardized RMR. RMR was lower in fish exposed transgenerationally to +3.0 °C compared with controls (Supplementary Fig. 1). The high proportion of contigs in this group with putative function in organ development (two contigs; *ppdpfa* and *ptf1a*) and endothelial cell proliferation (four contigs; *nlrp14* and *timp2*) suggests that lower metabolic costs enabled these cellular processes to function at a higher level in transgenerationally acclimated fish, which is consistent with acclimation of growth rates in fish exposed transgenerationally to elevated temperatures[3,4]. In addition, this group contains five contigs related to transcriptional regulation (three genes: *rorb, ptf1a* and *rps27*), two of which enhance expression of genes involved in organogenesis (*rorb* and *ptf1a*). The third gene, *rps27*, encodes a nuclear protein induced on DNA damage[25]. Therefore, increased transgenerational expression and negative correlation to standardized RMR suggest that rps27 plays a role in maintaining DNA integrity after transgenerational exposure to elevated temperatures to restore routine metabolic function.

Although the first two groups in the heatmap (Fig. 2a,b) contained contigs with expression that was significantly elevated transgenerationally, the third group (Fig. 2c) contained 11 contigs (16% of total) with expression that was downregulated transgenerationally. Most of these contigs had expression that positively correlated to standardized RMR (64%; seven contigs) and largely matched genes with functions related to stress, homeostasis and immune responses (Supplementary Table 1). As many other stress- and immune-related genes were upregulated transgenerationally in the first two groups, the fact that genes with these functions were downregulated in the final group suggests that their expression was reduced in favour of other more beneficial genes for transgenerational acclimation.

The heatmap indicates that many contigs had higher differential expression in transgenerational compared with developmental treatments (Fig. 2); however, only three were statistically significant: cytochrome p450 2j2 (*cyp2j2*), ribosomal protein large P1 (*rplp1*), and an uncharacterized gene (Supplementary Table 1). Cyp2j2 is associated with epoxidation of arachidonic acid[26], of which the primary products formed, epoxyeicosatrienoic acids, are involved

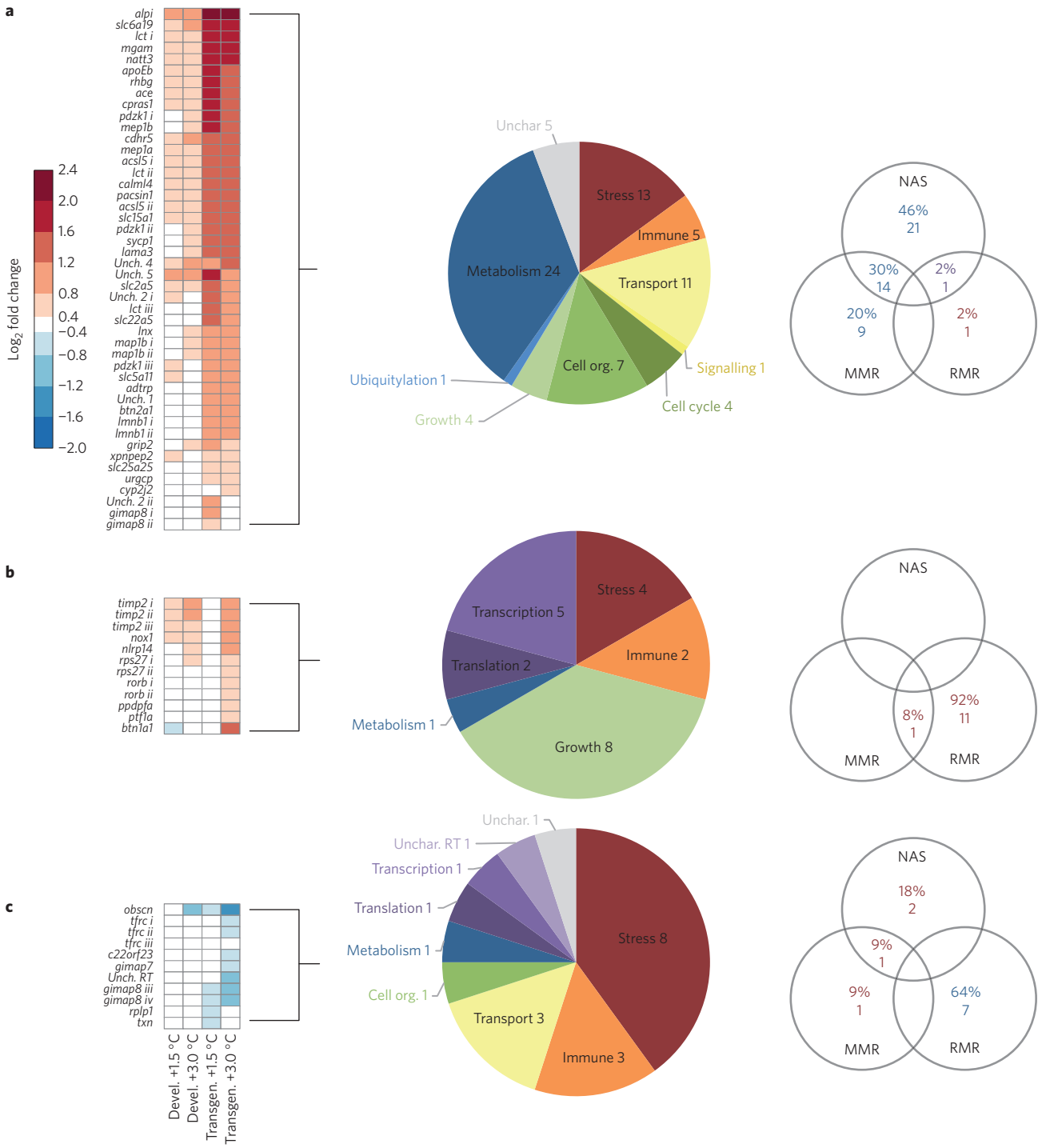

Figure 2 | Differentially expressed contigs, correlations to metabolic performance, and putative cellular function. a–c, Heatmap (left) of differentially expressed contigs (adjusted $P < 0.05$) from *Acanthochromis polyacanthus*, comparing +1.5 °C and +3.0 °C developmental (devel.) and transgenerational (transgen.) treatments with control (+0.0 °C). On the basis of expression patterns, contigs were separated into three groups (**a–c**). The associated cellular functions for each group are presented as pie charts (middle), with each contig represented by two functions with the exception of those that were uncharacterized. Numbers within pie chart sections represent the total number of contigs that correspond to that function. Venn diagrams (right) indicate the proportion of contigs with expression that positively (blue) or negatively (red) correlated to metabolic data (NAS: net aerobic scope, MMR: maximum metabolic rate, and RMR: routine metabolic rate). Purple text indicates negative NAS and positive RMR.

in a variety of processes such as vasodilation, anti-inflammation and cytoprotection. For example, cyp2j2 seems to play a cytoprotective role in animals exposed to hypoxia[27] and high-fat diets[28]. Thus, we suggest that increased transgenerational *cyp2j2* expression may play an important cytoprotective role, allowing proper cellular function after transgenerational but not developmental exposure to elevated temperatures. The ribosomal protein rplp1 plays a key role in the elongation step of protein synthesis. Therefore, rplp1 may be required in developmental treatments to increase protein translation owing to a higher rate of protein degradation during thermal stress, but is no longer required transgenerationally because of the aforementioned increases in cytoprotective gene

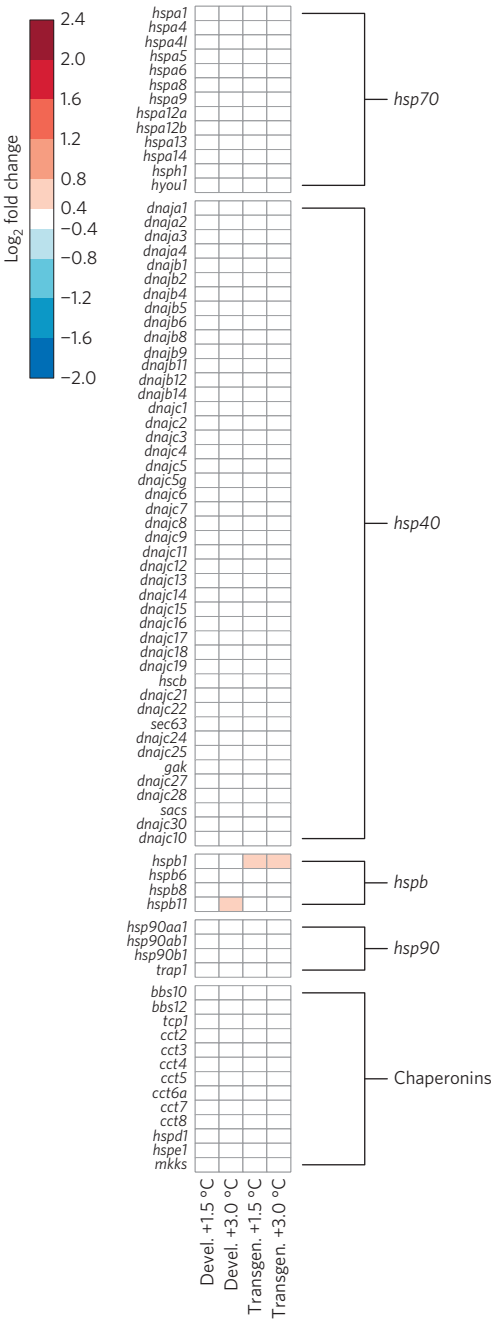

Figure 3 | HSP contig expression pattern. Heatmap of HSP expression from *Acanthochromis polyacanthus*, comparing +1.5 °C and +3.0 °C developmental (devel.) and transgenerational (transgen.) treatments with control (+0.0 °C). There were no significant differences in expression (adjusted $P < 0.05$). Expression values correspond to the contig with the best match (E-value $< 10^{-27}$) to HSP genes within our transcriptome.

expression. Importantly, there was only one contig (*btn1a1*) that was significantly differentially expressed in developmental but not transgenerational treatments (Supplementary Table 1). This suggests that there is not a different suite of genes and cellular processed engaged during developmental exposure to elevated temperatures compared with transgenerational acclimation.

A commonly used molecular measure of thermal stress has been to examine molecular chaperone expression, specifically heat-shock proteins (HSPs). Some HSPs are constitutively expressed and are involved in nascent polypeptide folding and others are expressed to help refold proteins that unfolded owing to various stressors[29]. We found no HSP genes with significantly altered expression in developmental or transgenerational *A. polyacanthus*. Of all 160 significantly differentially expressed contigs identified in this study, including contigs with expression that did and did not correlate to metabolic traits (Supplementary Fig. 2), only one matched a gene with putative chaperone function: eukaryotic translation elongation factor 1a (*eef1a*). The protein encoded by this gene, eef1a, has been shown to protect aminoacyl transfer RNA synthetases from denaturation in mammals[30], and may therefore have a more specific role in maintaining the integrity of transgenerational protein synthesis in our study. Although contigs with matches for many HSPs were found within the *A. polyacanthus* transcriptome, none was significantly differentially expressed among the five treatments (Fig. 3; adjusted $P > 0.7$). Therefore, the lack of differential HSP gene expression and limited chaperone activity suggests that other genes outlined in this study are better indicators of transgenerational thermal acclimation, at least in *A. polyacanthus*. Although HSPs may be good indicators of acute thermal stress[29,31], our results suggest that they may not be good indicators of the capacity for long-term thermal acclimation to predicted temperatures under climate change.

Acclimation of aerobic scope within two generations[1] suggests that epigenetic inheritance is involved. Future research into epigenetic mechanisms and their effect on genes identified in this study will be useful to improve our understanding of adaptive responses to rapid environmental change. In this study we identified key genes and processes involved in transgenerational thermal acclimation, including genes involved in enhanced fatty acid oxidation, protein and carbohydrate metabolism, and changes in genes involved in cytoprotection, immunity, organogenesis and cellular organization. The plasticity of these genes and their strong correlation to known acclimating phenotypic traits suggests that they may be critical in aiding reef fishes, and possibly other marine organisms, to survive in a warmer future environment.

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Author contributions

J.M.D. and P.L.M. designed and managed the fish rearing experiments. J.M.D. performed metabolism experiments. H.D.V. prepared samples for sequencing. T.Ryu assembled transcriptome. T.Ryu, T.Ravasi, L.S. and Y.G. analysed expression and assessed assembly quality. H.D.V. performed quantitative real-time-PCR expression validation. H.D.V. analysed the data. H.D.V., P.L.M., T.Ryu, J.M.D., L.v.H., M.L.B., W.L. and T.Ravasi wrote the paper and all authors read and approved the manuscript.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. RNA-seq transcriptome sequences have been deposited in GenBank under BioProject ID PRJNA255544. Correspondence and requests for materials should be addressed to T.Ravasi or P.L.M.

Competing financial interests

The authors declare no competing financial interests.