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Gene expression plasticity and frontloading promote thermotolerance in Pocilloporid corals

Running title: *Pocillopora* holobiont response to heat stress

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ABSTRACT

Ecosystems worldwide are suffering from climate change. For example, the coral reef ecosystems are globally threatened by increasing sea surface temperatures. However gene expression plasticity provides the potential for organisms to respond rapidly and effectively to environmental change, and would be favored in variable environments. In this study we investigated the thermal stress response of Pocilloporid corals from two contrasting thermal regimes (more stable seawater temperatures in New Caledonia, more variable in Oman) by exposing them to heat stress. We compared the physiological state, bacterial and *Symbiodinium* communities (using 16S and ITS2 metabarcoding), and gene expression levels (using RNA-Seq) between control conditions and heat stress (the temperature just below the first signs of compromised health). Colonies from both thermal regimes remained apparently normal and had open and colored polyps during heat stress, with no change in bacterial and *Symbiodinium* community composition. In contrast, they differed in their transcriptomic responses. The thermotolerant colonies (Oman) presented a more plastic transcriptome (more differentially expressed genes and higher fold-changes between control and heat stress conditions), but some other genes had a higher basal expression level (frontloading) compared to the thermosensitive colonies (New Caledonia). In terms of biological function we observed a trade-off between stress response (including induction of tumor necrosis factor receptors, heat shock proteins, and detoxification of reactive oxygen species) and morpho-anatomical functions. Gene regulation (transcription factors, mobile elements) appeared to be highly enriched, indicating possible epigenetic regulation. These results provide new insights into the balance between plasticity and frontloading, and the origin and evolution of these strategies.

KEYWORDS: Gene expression plasticity, Frontloading, Coral holobiont, *Pocillopora*, Heat stress.

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INTRODUCTION

Earth is undergoing unprecedented global environmental changes with major effects on biodiversity (Barnosky *et al.* 2011). The ongoing erosion of the most vulnerable ecosystems due to current environmental degradation is particularly worrying and is only a premise to what scientists have called the sixth mass extinction (Barnosky *et al.* 2011). In particular, climate change, ocean acidification and the exceptional frequency to which extreme climatic events occur have already resulted in the irreversible degradation of more than 20% of coral reefs worldwide (Bellwood *et al.* 2004; Hoegh-Guldberg *et al.* 2007). Scleractinian corals constitute the biological and physical framework for a large diversity of marine organisms [c.a. ~600 coral, ~2000 fish, and ~5000 mollusk species (Veron & Stafford-Smith 2000; Reaka-Kudla 2005). Hence, the extinction or even major decrease of corals would have dramatic repercussions on the overall associated community (Hughes *et al.* 2017a). Natural variation in thermal tolerance exists among coral populations (Oliver & Palumbi 2010; Palumbi *et al.* 2014), especially along a latitudinal gradient (Polato *et al.* 2010; Dixon *et al.* 2015), hence providing some hope for coral survival based on their capacity to acclimatize and/or adapt. More specifically, populations inhabiting in zones with more variable temperature regimes display better tolerance to heat stress. This pattern can be verified from local scale (Kenkel *et al.* 2013; Palumbi *et al.* 2014) to geographical scale (Hughes *et al.* 2003; Riegl *et al.* 2011; Coles & Riegl 2013)

Understanding the evolutionary processes underlying coral thermo-tolerance at the host level is crucial to better predict the fate of coral populations in response to climate change. In particular, it remains unclear whether thermo-tolerance is acquired via acclimatization (i.e. intra-generational gene expression plasticity; (Barnosky *et al.* 2011; Kenkel & Matz 2016) and/or through Darwinian adaptation (i.e. inter-generational microevolution; (Barnosky *et al.* 2011; Dixon *et al.* 2015). Under the former hypothesis one should expect that the present-day sensitive coral populations could potentially acquire tolerance to heat stress along with the ongoing climate change. According to the latter hypothesis, the persistence of initially thermo-sensitive coral populations would depend on the emergence of better adapted lineages through microevolution and/or on the genetic rescue via gene flow from populations already adapted to heat stress (Bellwood *et al.* 2004; Dixon *et al.* 2015). Actually, some studies strongly

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suggest that both processes (i.e. acclimatization and adaptation) are likely to co-occur in wild coral populations (Hoegh-Guldberg *et al.* 2007; Reusch 2013; Palumbi *et al.* 2014).

The recent advent of high throughput molecular methods have made it possible to go beyond the distinction between the evolutionary processes and focus further on the molecular mechanisms underlying coral response to heat stress. In particular, recent studies clearly demonstrated that coral responses to heat stress involve the fine-tuned regulation of expression levels of some genes/proteins involved in several molecular pathways such as metabolism, stress-response and apoptosis (Veron & Stafford-Smith 2000; Brown *et al.* 2002; Weis 2008; Ainsworth *et al.* 2011; Bellantuono *et al.* 2012a; Barshis *et al.* 2013; Palumbi *et al.* 2014; Kenkel & Matz 2016). In this regard, two main molecular patterns having different temporalities have been put forward. One would be "transcriptional plasticity", i.e. extensive changes in gene expression levels according to the occurring thermal condition and the other is coined "transcriptional frontloading", i.e. the elevation of stress related genes baseline expression that precondition organisms to subsequent (recurrent) stresses (Reaka-Kudla 2005; Mayfield *et al.* 2011; Barshis *et al.* 2013; Palumbi *et al.* 2014; Hughes *et al.* 2017a). While such elevated constitutive gene expression levels could reflect local adaptation (i.e. genetically fixed gene expression level; (Oliver & Palumbi 2010; Palumbi *et al.* 2014), it could also reflect an acclimation process via stable epigenetic constitutive gene expression regulation. Surprisingly, frontloading and gene expression plasticity were generally discussed as mutually exclusive patterns (Barshis *et al.* 2013; Dixon *et al.* 2015; Kenkel & Matz 2016) although these two molecular processes most likely co-occur during coral responses to heat stress. In particular, one might expect that the regulation strategy of genes (plasticity versus frontloading) will greatly depend on the molecular pathways in which they are involved and the energetic, physiological, and ultimately fitness cost associated with gene expression. So far, frontloading has been detected for stress response genes such as Heat Shock Proteins (HSPs), apoptosis and tumour suppression factors in resilient coral populations under experimentally simulated heat stress inducing bleaching in the common reef-building coral *Acropora hyacinthus* (Polato *et al.* 2010; Barshis *et al.* 2013) and for metabolic genes in populations pre-exposed to warm temperatures in response to long-term heat stress in *Porites astreoides* (Kenkel *et al.* 2013; Palumbi *et al.* 2014). Conversely, in the latter species, plasticity was observed in the expression of environmental stress response genes (Riegl *et al.* 2011; Kenkel & Matz 2016), hence

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challenging the patterns observed in *A. hyacinthus* (Barshis *et al.* 2013; Coles & Riegl 2013). Although both strategies (i.e. constitutive frontloading versus expression plasticity) undoubtedly exists in wild coral populations, the pre-exposure conditions that foster their induction and their relative effects on coral resistance to heat stress still remain unclear (but see (Hughes *et al.* 2003; Kenkel & Matz 2016)).

Importantly, scleractinian corals are composed of several symbiotic organisms including the cnidarian host, the mutualist photosynthetic algae (genus *Symbiodinium*) and bacterial communities. All partners (bionts) involved in a stable symbiosis effectively form the entire organism, and constitute what is referred to as the holobiont (Margulis & Fester 1991). A decade after this term was defined, its use has been particularly popularized in reference to corals (Rohwer *et al.* 2002), and subsequent research has led to the hologenome theory of evolution (Rosenberg *et al.* 2007; Zilber-Rosenberg & Rosenberg 2008). In this context the hologenome is defined as the sum of the genetic information of the host and its symbiotic microorganisms. Phenotypes are thus the product of the collective genomes of the holobiont partners in interaction with the environment, which constitute the unit of biological organization and thus the object of natural selection (Zilber-Rosenberg & Rosenberg 2008; Guerrero *et al.* 2013; McFall-Ngai *et al.* 2013; Bordenstein & Theis 2015; Theis *et al.* 2016). Additionally to the cnidarian host response, the genotype -or association of genotypes- of the photosynthetic mutualist *Symbiodinium* symbionts plays a key role in the thermotolerance of the holobiont (Mayfield *et al.* 2014; Hume *et al.* 2015; Suggett *et al.* 2017; Mayfield *et al.* 2018). There is less certainty about the importance of the coral bacterial community in participating to the fitness of the holobiont, although accruing evidences strongly suggest their implication in coral response to environmental conditions (Li *et al.* 2014; Pantos *et al.* 2015; Hernandez-Agreda *et al.* 2016) and in the resistance to disease (Sato *et al.* 2009; Cróquer *et al.* 2013; Meyer *et al.* 2015). Finally, the role of the coral-associated microbes and their potential to modify holobiont adaptability remain so far overlooked (but see (Ziegler *et al.* 2017; Torda *et al.* 2017)). Hence studying how corals respond to stress implies an integrative approach to analyze the response of each symbiotic protagonist.

The aim of the present study was to investigate the molecular mechanisms underlying thermo-tolerance of coral holobionts. We analyzed the holobiont response to stress of two coral populations originating from environments with contrasting thermal regimes. We used the scleractinian corals from the genus *Pocillopora* as model species because they have a broad spatial distribution throughout the

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Indo-Pacific (Veron & Stafford-Smith 2000). These coral species are considered to be one of the most environmentally sensitive (van Woesik *et al.* 2011) but their widespread distribution clearly suggests potential for acclimatization and/or adaptation which may be correlated to specific differences (i.e. different cryptic lineages may be adapted to different environmental conditions). In particular, we focused on *Pocillopora* colonies from two localities with contrasting thermal regimes: colonies from New Caledonia (NC) are exposed to temperate and stable temperatures over the year, while those from Oman are exposed to globally warmer and more seasonal fluctuating temperatures. In order to interpret the results in a precise evolutionary context we first identified the mitochondrial lineage of the colonies. To avoid biases inherent in transplantation-based field experiments resulting from environmental factors other than temperature, we undertook our comparative study in a controlled environment in which we mimicked ecologically realistic heat stress to compare the responses of colonies from both localities. We combined a specific RNA-seq approach to study the cnidarians host response, and metabarcoding analyses using ITS-2 and 16S amplicon sequencing to study the dynamics of the associated algal (*Symbiodinium* sp.) and microbial community compositions, respectively. According to the literature we first expected to detect changes in both symbiotic algal and bacterial communities in corals from both localities when exposed to heat stress. Moreover, since variable environments are expected to promote the evolution of plasticity, we predicted that the cnidarian hosts from Oman will display more gene expression plasticity than those from New Caledonia. However, because frontloading was also found to be an alternative response to recurrent changing conditions, we might also expect some degrees of constitutive high levels of gene expression at least for some molecular pathways and more particularly in Oman corals.

MATERIAL AND METHODS

CORAL SAMPLING AND MAINTENANCE

Pocillopora damicornis-like colonies originating from environments characterized by contrasting thermal regimes were sampled in two different localities: (1) in Oman (Om; June 2014), where corals are exposed to a globally warmer and variable thermal environment, and (2) in New Caledonia (NC; November 2014), where corals are subject to more mitigate and stable temperatures (see Table 1 for the temperature regime of locality). At this aim, we sampled colonies from the two geographical locations occupying the same apparent morphological and water depth niche. To account for possible intra-population diversity,

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three colonies (20 cm in diameter) were collected in each locality, and separated by at least 10 m to avoid the collection of members of the same clone or clonal lineage, as some *Pocillopora* species are able to propagate by asexual reproduction (Adjeroud *et al.* 2013). Immediately following collection, a 1 cm tip of each colony was excised, rinsed three times in filtered seawater (0.22 µm), and placed in RNAlater solution (Sigma Aldrich) for the *in situ* microbiota analysis. The remainder of the colony was fragmented into 20 branches each of 10 cm length, and these were individually placed in plastic bags containing oxygenated seawater, and transported by aircraft to the research aquarium of the Banyuls-sur-Mer oceanographic observatory (France). The coral branches were maintained in artificial seawater (Seachem Reef Salt) at 26°C, and supplied daily with *Artemia* nauplii to satisfy their heterotrophic demand. The conditions in the maintenance tank were controlled to mimic the natural physicochemical parameters of coral reefs (pH: 8.2; salinity: 36 g/L; light Intensity: 150 to 250 photons/m^2/s; photoperiod: 12h night/12h day; kH: 6–7.5 dkH; calcium concentration: 410–450 mg/L; inorganic phosphate concentration: < 0.1 mg/L; magnesium concentration: 1300–1400 mg/L; nitrate concentration: < 5 mg/L). After two months of acclimation in the lab, the coral branches were fragmented to produce several clones from each colony (~3 cm). These were individually fixed to a support (here a p1000 tip) using an epoxy adhesive. We waited for complete healing (evident as tissue extending to cover the epoxy adhesive) prior to run the experiment.

Ecologically realistic heat stress

The aim of this experiment was to compare the response to heat stress of colonies from two localities having the same physiological state, to investigate the patterns of expression of the molecular pathways involved during the stress exposure and the putative modifications of the coral microbiota.

The experimental design comprised eight tanks of 53 L (four per locality) in which the seawater was continuously recycled. The water was sterilized using UV (rate 3200 L/h) and renewed twice per hour in each tank (recirculation rate: 100 L/h in each tank). The eight tanks shared the same seawater but their temperature was monitored individually (HOBBY BiothermPro, model 10892; 500W AquaMedic titanium heater; HOBO TidbiT v2 logger). For each population, at least four nubbins per mother colonies were randomly placed in each tank (four tanks per locality) for two weeks at 26°C and the following protocol was applied: three tanks were subjected to a gradual temperature increase (stress treatment) while the

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fourth (control) was maintained at the control temperature. These controls were used to assess that the stress observed in the stressful treatment was not due to other potential confounding effects (Fig. 1). Both the control and stress temperatures were specific for each sampling locality to mimic their respective natural environment. In particular, we set the control temperature as the mean water temperature for the three warmer months measured at the coral sampling site locality (Table 1): 31°C for the colonies from Om, and 27°C for the colonies from NC. The stress treatment was ecologically realistic, i.e. reflecting a naturally occurring warming anomaly, and consisted in increasing the temperature gradually by 1°C (over 5 consecutive hours) each week until physiological collapse of the corals became evident (polyps closure, bleaching or necrosis), as described by (Vidal-Dupiol *et al.* 2009). Sampling was performed each week before the temperature increase and were chosen *a posteriori* for subsequent genetic and transcriptomic analyses. This corresponded to those sampled in each tank just before the first increase of temperature (control samples), and just before the temperature that produced the first signs of physiological collapse (stress temperature samples). Thus, for each condition (control and stress) we obtained three replicates of each colony (three colonies per locality) to reach a total of 36 samples (2 localities × 3 colonies × 2 experimental conditions × 3 replicates). The general health of the nubbins was assessed via daily photographic monitoring (at noon prior to feeding) throughout the period of the experiment.

DNA EXTRACTION

DNA was extracted from each 36 samples as well as coral tips directly collected on the six colonies *in natura* for the *in situ* condition (three in Oman, three in New Caledonia), using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's instructions. DNA was quantified by spectrophotometry (NanoDrop).

HOST HAPLOTYPE

As the *corallum* macro-morphology is not a diagnostic criterion in *Pocillopora* genus, the host species was thus identified molecularly. We analyzed the mitochondrial variable open reading frame (ORF) sequence of each colony using the FATP6.1 and RORF primers and following the protocol detailed in (Flot & Tillier 2007). The PCR products were sequenced using Sanger sequencing, and the obtained sequences were aligned with the ORF haplotypes from Gélin *et al.* (2017a) using Geneious 8.0 (Kearse *et al.*, 2012) with the MAFFT algorithm (Katoh *et al.* 2005). Each colony used in the experiment was then assigned to Primary

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Species Hypothesis (PSH) following the nomenclature from Gélin *et al.* (2017a). Furthermore, to refine the assignment with the ORF to Secondary Species Hypothesis (SSH) and to identify whether the sampled colonies are members of a same clone, they were genotyped using 13 specific microsatellites as in Gélin et al. (2017a). If relevant, the colonies were assigned to SSH. Meanwhile, the identical multi-locus genotypes were identified as in Gélin *et al.* (2017b).

Microbial community analysis using MiSeq 16S and ITS2 metabarcoding

The aim of this analysis was to investigate the composition and the dynamics of the two principal symbiotic coral communities (i.e. bacterial and algal) *in situ* and during heat stress.

Amplicon Sequencing

A bacterial 16S rDNA amplicon library was generated for each of the 42 samples (one *in situ* condition, three control conditions and three stress conditions per colony, three colonies per locality, two localities), using the 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) primers, which target the variable V3/V4 loops (Klindworth *et al.* 2012). The *Symbiodinium* assemblages were analyzed using ITS2 (internal transcribed spacer of the ribosomal RNA gene) amplicon libraries and specific primers targeting a sequence of approximately 350 bp (ITS2-F GTGAATTGCAGAACTCCGTG; ITS2-R CCTCCGCTTACTTATATGCTT) (Lajeunesse & Trench 2000; Quigley *et al.* 2014). For both markers, paired-end sequencing using a 250 bp read length was performed on the MiSeq system (Illumina) using the v2 chemistry, according to the manufacturer's protocol at the Centre d'Innovation Génome Québec and McGill University, Montreal, Canada.

Bioinformatic analysis:

The FROGS pipeline (Find Rapidly OTU with Galaxy Solution) implemented on a Galaxy platform (http://sigenae-workbench.toulouse.inra.fr/galaxy/) was used for data processing (Escudié *et al.* 2015). In brief, paired reads were merged using FLASH (Magoč & Salzberg 2011). After cleaning and removal of primer/adapters using cutadapt (Martin 2011), *de novo* clustering was performed using SWARM (Mahé *et al.* 2014). This uses a local clustering threshold with an aggregation distance (d) of 3. Chimeras were removed using VSEARCH (Rognes *et al.* 2016). We filtered the dataset for singletons and performed affiliation using Blast+ against the Silva database (release 128, September 2016) for 16S amplicons

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(Altschul *et al.* 1990). For ITS2 metabarcoding, the *Symbiodinium* type was assessed using Blast+ against an in-house database of *Symbiodinium* reference sequences built from sequences publicly available. An OTU table in standard BIOM format with taxonomic affiliation was produced for subsequent analyses.

For community composition analysis we used the *phyloseq* R package (McMurdie & Holmes 2013) to infer alpha diversity metrics at the OTU level, and beta diversity (between sample similarity) from the OTU table. Community similarity was assessed by Principal Coordinate Analysis (PCoA) using the Bray-Curtis distance matrices.

We performed one-way ANOVAs to compare alpha and beta diversity metrics among the groups of samples by sampling locality or by treatment. Corrections based on multiple testing were performed using FDR (Benjamini & Hochberg 1995). For all analyses, the threshold significance level was set at 0.05.

Transcriptome analysis

The aim of this analysis was to study the transcriptomes of the sampled colonies in response to heat stress compared with controlled conditions.

RNA extraction

Total RNA was extracted from each coral sample using TRIzol reagent (Invitrogen), according to the manufacturer's protocol. The quantity and integrity of the total RNA extracted was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies) (mean RIN = 7.5). Paired-end fragment libraries (2 × 100 bp) were constructed and sequenced on an Illumina HiSeq 2000 platform at the Centre d'Innovation Génome Québec at McGill University, Montreal, Canada.

Bioinformatic analyses

Fastq read files were processed on the Galaxy instance of the IHPE (http://bioinfo.univ-perp.fr) (Giardine *et al.* 2005). Quality control and initial cleaning of the reads were performed using the filter by quality program (version 1.0.0) based on the FASTX-toolkit (Blankenberg *et al.* 2010). Reads having fewer than 90% of bases having a Phred quality score ≤ 26 were discarded (probability of 2.5 incorrect base call out of 1000, and a base call accuracy of 99.75%). Adaptors used for sequencing were removed using the cutadapt program version 1.6 (Martin 2011). All paired-end reads were aligned using RNAstar software under default parameters, with at least 66% of the bases being required to align to the reference, and a maximum of ten mismatches per read (Dobin *et al.* 2013). The *Pocillopora damicornis* reference genome

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used in this study (manuscript in preparation) consisted of a draft assembly of 25,553 contigs (352 Mb total) and N50 = 171,375 bp. The resulting transcriptome served as the reference for reads mapping, and a GTF annotation file was constructed using cufflink/cuffmerge (Trapnell *et al.* 2010). HTseq was used to produce count files for genes (Anders *et al.* 2015). The DESeq2 package was used to estimate the normalized abundances of the transcripts, and to calculate differential gene expression for samples between the control temperature and the stress temperature for each locality (Love *et al.* 2014), considering the different genotypes (3 biological replicates for each genotype) and using default parameters. We next analyzed genes according to their expression patterns among localities and temperature treatments. Genes were clustered manually into six groups according to their differential expression levels: common over-expressed genes, NC-specific over-expressed genes, Om-specific over-expressed genes, common under-expressed genes, NC-specific under-expressed genes, and Om-specific under-expressed genes. Cluster 3.0 (de Hoon *et al.* 2004) and Treeview (Saldanha 2004) were used to build the heatmap.

Discriminant analysis of principal components (DAPC):

Our aim was to quantify and compare the level of genome-wide transcriptome plasticity between colonies from Om and NC in response to heat stress. To achieve this we performed a discriminant analysis of principal components (DAPC) based on a log-transformed transcript abundance matrix (containing 26,600 genes) obtained from the 36 samples (i.e. 9 control and 9 stressed replicates per locality), as described previously (Kenkel & Matz 2016). Specifically, we ran a DAPC analysis using the resulting log2 transformed dataset for the colonies from NC and Om reared in control conditions as predefined groups in the *adegenet* package implemented in R (Jombart *et al.* 2010). Two principal components and a single discriminant function were retained. We then predicted the position of stressed colonies from both localities (Om and NC) onto the unique discriminant function of the DAPC.

We next compared the absolute mean DAPC scores between Om and NC colonies for each experimental group (control versus heat stress) using the non-parametric Wilcoxon test. We also ran a general linear model (GLM) using the DAPC scores as dependent variable, and accounted for the locality of origin (NC and Om), the conditions (control and heat stress), and their interaction as explanatory variables. In particular, as an indicator of significant differences in the genome-wide gene expression reaction norms

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(i.e. differences in DAPC scores between the control and the heat stress treatments) between Om and NC colonies we tested for significant effects of the interaction between the localities and the condition effects.

GO ENRICHMENT OF DIFFERENTIALLY EXPRESSED GENES

The transcriptome was annotated *de novo* using a translated nucleotide query (blastx (Altschul *et al.* 1990)) against the non-redundant protein sequence database (nr). The 25 best hits were then used to search for gene ontology terms using the Blast2Go program (Conesa *et al.* 2005). Lists of significantly up-regulated and down-regulated genes were subjected to GO enrichment analysis by comparison with all expressed gene using a Fischer exact test and a FDR value of 0.05. We used REVIGO to visualize the enriched biological processes (Supek *et al.* 2011).

RESULTS

HOST HAPLOTYPE

Among the three colonies from New Caledonia, colonies NC2 and NC3 presented haplotype ORF18 and were assigned to Primary Species Hypothesis PSH05 and more precisely to Secondary Species Hypothesis SSH05a (Gelin *et al.* 2017a), corresponding to *P. damicornis* type *β* (Schmidt-Roach *et al.* 2013) also named *P. acuta* (Schmidt-Roach *et al.* 2013) or type 5a (Pinzon *et al.* 2013), while colony NC1 presented ORF09 and was assigned to PSH04, *P. damicornis* type *α*, *P. damicornis* or type 4a, respectively. As for colonies from Oman, they all presented ORF34 and were assigned to PSH12 (Gelin *et al.* 2017a) or type 7a (Pinzon *et al.* 2013)(Additional Table 4). These three PSHs represent three different species.

Furthermore, NC2 and NC3 multi-locus genotypes differed from one allele over 26 and were thus part of the same clonal lineage, i.e. the entity that groups together colonies whose multi-locus genotypes slightly differ due to somatic mutations or scoring errors.

ECOLOGICALLY REALISTIC HEAT STRESS

Our goal was to ensure that our experimental heat stress faithfully reflects a realistic heat stress *in natura*. Following collection from the field, the corals from the different localities were first maintained in the same controlled conditions prior to the experiment. During this period no mortality or signs of degradation/stress were observed for any of the coral colonies. During the experimental heat stress (i.e. gradual temperature increase), visual and photographic monitoring clearly indicated that the first sign of

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coral stress (i.e. the closure of polyps) occurred at day 30 for both sampling localities, corresponding to 30°C and 34°C for the NC and Om colonies, respectively. These temperatures perfectly match the warmest temperature experienced by these populations in the field (Table 1). No signs of physiological collapse were observed in control corals throughout the experiment indicating that all the other parameters were maintained optimal for coral colonies.

Bacterial communities

Among the overall 42 samples analyzed, a total of 5,308,761 16S rDNA sequences were obtained after cleaning and singleton filtering corresponding to 15,211 OTUs. In all samples the class Gammaproteobacteria was dominant (77.7%), particularly the genus *Endozoicomonas* (44.7% of the sequences); this genus is known to be an endosymbiont of numerous scleractinians (Neave *et al.* 2016b) (See Additional Figure 1 for complete bacterial composition in each colony and replicate). The PCoA of Bray-Curtis distances for all colonies showed no evident clusters based on the experimental treatments (Fig. 2). We observed a loose grouping based on localities and colonies, except for colony NC1, which appeared to have a more specific microbiota composition, as it had a different grouping associated with the first axis, which explained 22.3% of the variance. This could be correlated with the different species hypotheses for NC1 compared to NC2 and NC3 (see above). The one-way ANOVA for alpha diversity (Shannon index) revealed significant differences in the microbiota diversity between localities ($P < 0.05$) and colonies ($P < 0.05$), but no differences among the *in situ* control and stress conditions ($P = 0.885$). Similar results were obtained for the beta-diversity (Bray-Curtis distance) (ANOVA between localities: $P < 0.05$; between colonies: $P < 0.05$; between conditions: $P = 0.554$; the ANOVA results are provided in Additional Table 2). Thus, the bacterial composition appeared to be relatively specific to each colony within each locality, but remained stable during the transition from the natural environment to artificial seawater, and during heat stress exposure.

Symbiodinium assemblages

Analysis of the *Symbiodinium* composition was performed based on an ITS2 metabarcoding, which facilitated intra-clade resolution.

Removal of those OTUs having an abundance of < 1% left only 4 OTUs among all samples. Two of these corresponded to type C1, while the other two corresponded to type D1a according to (Baker 2003). Type

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D1a was highly dominant in the colonies originating from Oman, whereas type C1 was almost exclusive to the corals from New Caledonia (Fig. 3). The *Symbiodinium* community composition was very specific to each locality, but remained stable during the transition from the natural environment to artificial seawater, and during heat stress exposure.

HOST TRANSCRIPTOME ANALYSIS

We generated 36 transcriptomes corresponding to triplicate samples for three colonies of each locality exposed to the control (C) and stress (S) temperatures.

Overall, the transcriptome sequencing of these 36 samples yielded 1,970,889,548 high quality Illumina paired reads of 100 bp. Globally, 40–64% of reads obtained for the Om colonies, and 59–70% of reads obtained for NC colonies successfully mapped to the *Pocillopora damicornis* (type β) reference genome. The apparently better alignment of samples from New Caledonia most likely relies on the fact that the New Caledonia colonies used in this study belong to *P. damicornis* types α or 4a (PSH04) and β or 5a (PSH05), which are phylogenetically close to each other and closer from the reference genome, than the Om colonies from type 7a (PSH12) that is phylogenetically more distant from the reference genome. The aligned reads were assembled in 99,571 unique transcripts (TCONS), representing putative splicing variants of 26,600 genes identified as "XLOC" in the genome (FASTA sequences available in Additional File 3).

The hierarchical clustering analyses clearly grouped together samples belonging to the same locality and species hypothesis according to their genome-wide gene expression patterns, in link with the phylogenetic differences between the NC and Om phylotypes (Fig. 4). Within locality and species hypothesis, whenever pertinent, the transcriptomes also grouped by colony, indicating that the transcriptomes were genotype-specific. For each colony, the transcriptomes then grouped by condition (temperature treatment) excepted for New Caledonia colonies NC2 and NC3 (corresponding to the same MLL) that clustered together when exposed to controlled and heat stress conditions.

In total 5,287 genes were differentially expressed between colonies exposed to stress and control conditions (adjusted $P < 0.05$) in Om colonies, and 1,460 genes in NC colonies (full results of the comparisons between stressed and controls (log2-foldchange and adjusted *p*-values) are provided in

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Additional File 5). The heatmap generated to visualize the expression pattern of each gene in Om and NC colonies is shown in Figure 5.

Among these genes, 498 were over-expressed and 350 were under-expressed in colonies exposed to stressful conditions compared to those kept under optimal temperatures (i.e. control) irrespective to the locality of origin (NC or Om). Nevertheless, the differential expression level was significantly higher (Wilcoxon test; $P = 2.2\times10^{-16}$) for the Om corals (Fig. 6). Among the 498 over-expressed genes, 358 were more induced in Om corals than the NC ones (log2-fold change Om > NC; Fig. 6), and of the 350 under-expressed genes, 259 were more repressed in Om corals (Additional Table 6). The mean of the log2-fold change for common over-expressed genes was 0.9 for Om samples *vs.* 0.6 for NC samples (variance: 0.6 for Om; 0.2 for NC), and for the common under-expressed genes was -1.2 for Om colonies *vs.* -0.8 for NC colonies (variance: 1 for Om; 0.3 for NC).

Additionally, colonies from the two thermal regimes also responded specifically to heat stress. In particular, 272 genes were over-expressed and 294 were under-expressed only in the NC corals and 2,082 were over-expressed and 2,311 were under-expressed only in the Om ones when exposed to heat stress. Finally, the colonies from both localities displayed antagonistic transcriptomic responses to heat stress for a subset of genes: 24 were over-expressed in NC colonies but under-expressed in Om ones, while 22 were under-expressed in NC colonies but over-expressed in the Om ones.

Altogether these results revealed a greater transcriptomic response to heat stress in colonies originating from Oman compared to those from New Caledonia (4,393 differentially expressed genes for the Om corals *vs.* 566 genes for the NC ones).

Discriminant Analysis of Principal Components (DAPC):

The DAPC analysis clearly discriminated the colonies from both localities based on their overall gene expression patterns (Fig. 7). We also found that Om and NC colonies significantly differed in their gene expression plasticity in response to heat stress (Wilcoxon tests; control p-value = 0.73, stress p-value < 0.01). This result was corroborated by the significance of the interaction term between localities and temperature effects in the GLM ($P = 0.04$), which indicate that the slope of the reaction norm was different between localities. More particularly, the Om colonies responded to a greater extent than the NC ones, and thus showed significantly higher gene expression plasticity in response to heat stress.

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ANALYSIS OF GENE FUNCTION:

To investigate the functions associated with the differentially expressed genes we performed a blastx annotation of transcripts followed by a Gene Ontology (GO) term annotation to determine to which protein each gene (XLOC) corresponded most closely, and the biological process, molecular function, and cell compartment localization of the protein (Additional File 7). Differentially expressed genes associated with the control and stress conditions (adjusted p-value < 0.05) clustered into six groups according to their expression patterns in corals from both localities (see host transcriptome analysis section).

For the 498 common over-expressed genes, 139 biological processes were enriched compared with the entire set of annotated genes. The most significant biological process identified in the REVIGO analysis (i.e. with lowest FDR value: FDR = 2.1×10^{-68}) was stress response (Fig. 8). Following this sequentially, were cellular metabolism (FDR = 3.7×10^{-49}), positive regulation of biological processes (FDR = 2.4×10^{-43}), cell death (FDR = 2.5×10^{-33}), cellular localization (FDR = 8.4×10^{-25}), and pigment metabolism (FDR = 2.1×10^{-21}). Among the 272 genes over-expressed in the NC but not in the Om colonies in response to heat stress, 38 biological processes were enriched and the REVIGO analysis showed that organic acid catabolism (FDR = 1.6×10^{-22}), protein transport (FDR = 1.8×10^{-16}), stress response (FDR = 4.8×10^{-13}), and cellular metabolism (FDR = 3×10^{-12}) were the four most significantly enriched biological processes (Fig. 8). Among the 2,082 genes over-expressed in the Om but not in the NC colonies in response to heat stress, the REVIGO analysis showed that 160 enriched biological processes grouped together, with the most significant being ncRNA metabolism (FDR = 8.9×10^{-303}), cellular metabolism (FDR = 4.4×10^{-70}), carbohydrate derivative biosynthesis (FDR = 5.9×10^{-64}), and organic substance transport (FDR = 2×10^{-44}).

For the 350 genes that were under-expressed following heat stress irrespective to the locality of origin (Om or NC), 48 biological processes were enriched compared with the entire set of annotated genes (Fig. 8). The REVIGO analysis grouped these genes into five biological processes: nitrogen compound transport (FDR = 5.4×10^{-89}), localization (FDR = 8.1×10^{-10}), regulation of neurotransmitter levels (FDR = 1.2×10^{-8}), system development (FDR = 8.8×10^{-6}), and single organism process (FDR = 4×10^{-4}). Among the under-expressed genes in the NC colonies only, a single biological process (anatomical structure/morphogenesis) was found to be enriched (FDR = 9×10^{-3}). Among the under-expressed genes in the Om colonies only the REVIGO analysis grouped 139 enriched biological processes, with the most

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significant being ion transmembrane transport (FDR = 7.6×10^{-104}), single multicellular organism process (FDR = 7.5×10^{-53}), regulation of biological quality (FDR = 6×10^{-48}), cell–cell signaling (FDR = 1.5×10^{-23}), single organism process (FDR = 1.1×10^{-18}), multicellular organism process (FDR = 1.5×10^{-16}), biological regulation (FDR = 2.3×10^{-15}), response to abiotic stimulus (FDR = 6.2×10^{-13}), and localization (FDR = 4.6×10^{-12}).

Regarding other Gene Ontology annotations, the common over-expressed genes were correlated with enhancement of 41 cellular components that were associated in the REVIGO analysis with cellular compartments including the mitochondria (FDR = 1.5×10^{-180}), cells (FDR = 1.5×10^{-19}), organelles (FDR = 2.5×10^{-15}), endomembrane systems (FDR = 6.5×10^{-7}), the membrane-enclosed lumen (FDR = 2×10^{-4}), the entire membrane (FDR = 0.0019), the membrane raft (FDR = 0.009), and the membrane (FDR = 9×10^{-3}). The genes over-expressed in NC corals were associated in the REVIGO analysis with 23 cellular compartments, with the mitochondria (FDR = 2.5×10^{-82}), cells (FDR = 4.9×10^{-7}), organelles (FDR = 6.9×10^{-6}), and the envelope (FDR = $9\text{x}10^{-3}$) being the four most significant. The genes over-expressed in Om corals were associated in the REVIGO analysis with 70 cellular components, the most significant of which were the intracellular organelle lumen (FDR = 1×10^{-560}), organelles (FDR = 9.9×10^{-48}), cells (2.7×10^{-46}), the membrane-enclosed lumen (FDR = 1.2×10^{-19}), macromolecular complexes (FDR = 1×10^{-16}), the endomembrane system (FDR = 2.7×10^{-11}), the envelope (FDR = 3×10^{-9}), and the membrane (FDR = 8×10^{-4}).

The common under-expressed genes were associated in the REVIGO analysis with 13 cellular components including the plasma membrane (FDR = 1.1×10^{-44}), membrane (FDR = 2.4×10^{-11}), neurons (FDR = 3×10^{-4}), presynapse (FDR = 4×10^{-4}), and synapse (FDR = 7×10^{-3})(Fig. 8). The genes under-expressed in NC corals were associated in the REVIGO analysis with seven biological processes in cellular components amongst which the plasma membrane region (FDR = 8.5×10^{-9}), cell periphery (FDR = 1×10^{-3}), the membrane (FDR = 2×10^{-3}), and membrane part (FDR = 9×10^{-3}) were the four most significant. The under-expressed genes in Om corals only were associated in the REVIGO analysis with 26 cellular components, the most significant of which included the integral component of the plasma membrane (FDR = 3.1×10^{-81}), the periphery (FDR = 1.2×10^{-22}), the membrane (FDR =6.2×10^{-17}), the transmembrane transporter complex

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(FDR = 1.5×10^{-14}), the extracellular region (FDR = 4.5×10^{-6}), the cell (FDR = 9.5×10^{-6}), synapses (FDR = 1.9×10^{-5}), and the synapse part (FDR = 4.4×10^{-5}).

To investigate whether the supposed thermotolerant colonies in our study (i.e. the colonies from Oman) displayed a frontloading strategy, as previously described in scleractinian corals (Barshis *et al.* 2013), we compared the constitutive expression levels (i.e. in control conditions) between Om and NC colonies for those genes that were over-expressed in NC colonies (Additional File 8). This analysis showed that the basal expression level was often greater in the more thermotolerant colonies (Om). Among the 770 genes that were over-expressed in NC colonies in response to thermal stress (272 specifically and 498 in common with Om), 484 were constitutively (i.e. in the control condition) more expressed in Om than NC control conditions. Among these genes, 301 were over-expressed in the Om colonies, 20 were under-expressed in the Om colonies, and 163 were not differentially expressed between the control and stress temperature in these colonies, reflecting true frontloading based on the definition of Barshis et al. (2013). These three categories of genes (over-expressed, under-expressed, and frontloaded) were submitted to GO term enrichment analysis. No significant results were found for the under-expressed genes. The frontloaded genes were enriched in the biological processes cellular respiration (FDR = 4.4×10^{-23}), cellular component organization (FDR = 0.002), homeostatic process (FDR = 0.005), cellular component organization or biogenesis (FDR = 0.007), cofactor metabolism (FDR = 0.009), and stress response (FDR = 0.009), and in the cellular compartments including the mitochondrion (FDR = 1.6×10^{-66}), envelope (FDR = 0.0002), cell (FDR = 0.0002), and organelle (FDR = 0.0009). Most interestingly, for genes associated with a higher basal expression level and over-expression in the Om colonies, the most enriched biological processes included stress response (FDR = 1.2×10^{-26}), pigment metabolism (FDR = 5.1×10^{-24}), regulation of phosphate metabolism (FDR = 3.2×10^{-15}), cellular metabolism (FDR = 2.7×10^{-11}), and protein folding (FDR = 7.3×10^{-6}). Among the 43 over-expressed genes involved in the response to stress in the NC colonies, 23 were frontloaded in the Om colonies.

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DISCUSSION

PHYLOGENETIC CONTEXT OF ADAPTATION

Our aim was to study the evolution of adaptive abilities of coral colonies originating from different thermal regimes. As morphology can be misleading for species identification in scleractinians, we used a molecular approach to test the species relationships of our samples. The analysis of mitochondrial sequences indicated that, despite similar morphologies, our samples corresponded to different phylogroups. This agrees well with previous works showing the importance of cryptic lineages and morphological plasticity in the *Pocillopora* genus (Gelin *et al.* 2017a and references herein). Oman corresponds to species hypothesis PSH12 of (Gelin *et al.* 2017a) which is restricted to the Northwestern Indian Ocean. Regarding the two species hypothesis from NC, PSH05 (type β or 5a) is found in the Indian Ocean, the Pacific Ocean and the Red Sea and PSH04 (type α or 4a) is nearly exclusively found in the Pacific Ocean (very rare in the Indian Ocean, and not found yet in Red Sea) (see Gelin *et al.* 2017a). It would be interesting to study if inside each phylogroup, different thermotolerance mechanisms are present, which would be informative on the evolution or conservation of such strategies. Conversely, the observation of a similar response to thermal stress in two different species in NC could indicate either a conserved strategy or a convergence under the same ecological conditions.

AN ECOLOGICALLY REALISTIC HEAT STRESS

Here we characterized and compared the response to heat stress of coral colonies collected from wild populations which were exposed to two contrasting thermal regimes. The heat stress applied in this study was ecologically realistic, since the first visual response to (i.e. polyp closure) was observed for all colonies when the gradually increasing experimental temperature reached exactly the upper temperature they are subject to *in natura* (30°C and 34°C for NC and Om corals, respectively). From a biological point of view this first result hence clearly supports that these colonies from two localities and different phylogroups within the genus *Pocillopora* are experiencing to different thermal regimes *in natura* and that they display differential ability to deal with heat stress. Moreover, the accurate control of all other seawater parameters allows us to consider that the holobiont response to the thermal treatment is specific to heat stress and not to other possible confounding effects. Any change in the holobiont is therefore a response to the heat stress and not because of disruption of the coral integrity.

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SYMBIOTIC COMMUNITY: BACTERIAL AND *SYMBIODINIUM* COMPOSITION

For the bacterial community we identified significant differences between localities and genotypes. The microbiota composition of all samples was consistent with previous studies, showing a high proportion of Gammaproteobacteria and dominance of the symbiotic *Endozoicomonas* genus (Bourne & Munn 2005; Neave *et al.* 2016a; Peixoto *et al.* 2017). However, our results clearly demonstrate that experimental heat stress did not induce bacterial community changes in coral colonies irrespective to their locality of origin. For the *Symbiodinium* community the ITS2 metabarcoding analysis enabled intra-clade resolution (Quigley *et al.* 2014). Two distinct types of D1a and C1 clades dominated, representing most of the sequences in the Om and NC corals, respectively. Nine ITS types (A to I) have been identified in the genus *Symbiodinium* (Baker 2003). Some *Symbiodinium* strains strongly participate to the overall holobiont fitness, with type D providing tolerance to higher temperatures (Berkelmans & van Oppen 2006) and C1 enhancing coral growth rates (Little *et al.* 2004). Interestingly, we found that the *Symbiodinium* type D1a is dominant in the more thermotolerant Om corals, which is consistent with the results of previous works (Berkelmans & van Oppen 2006).

Although the microbial community (both bacterial and *Symbiodinium*) differed between the NC and Om corals, the composition did not change during transition from the field to the artificial seawater conditions, and remained stable during the experimental temperature increase. Thus, the coral holobiont assemblage remained stable over the course of the experiment. Such stability of the microbial community during experimental heat stress was previously observed in the scleractinian *Acropora millepora* (Bellantuono *et al.* 2012b) and *A. tenuis* (Littman *et al.* 2010). Thus, our study comforms to the idea that microbial communities associated with scleractinian corals remain unchanged when the holobionts are exposed to stressful temperatures (but see (Ziegler *et al.* 2017)) but further analyses of gene expression level would be needed to assess their functional responses.

HOST TRANSCRIPTOMIC RESPONSE

Given the observed stability of the microbial symbiotic community during heat stress, we focused more specifically on the responses attributable to the coral host. We thus compared gene expression patterns at the qualitative and quantitative levels in Om and NC colonies in response to heat stress compared to the control condition. Altogether, our results clearly highlight that the Oman thermotolerant

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colonies exposed to more variable thermal conditions *in natura* also display, in response to heat stress, a greater plasticity in gene expression levels than the NC thermosensitive colonies. In particular, the transcriptomic response of the Oman colonies involved a larger number of genes and among the set of genes that were differentially expressed similarly in both localities, the absolute difference in expression level of 73% of these genes being significantly higher in the Om colonies. These findings are consistent with the theoretical expectations that a more variable environment promotes the evolution of a greater plasticity (Lande 2009). Accordingly, a recent transplantation study conducted *in natura* also identified greater transcriptomic plasticity in a more thermotolerant (in-shore) population compared with an (off-shore) population inhabiting a more stable thermal habitat in the mustard hill coral *Porites astreoides* (Kenkel & Matz 2016) .

Importantly however, we also identified several genes whose expression is constitutively higher in the Om colonies compared to the thermosensitive NC colonies (i.e. comparing the expression levels in the control condition); a process recently called "frontloading" (Barshis *et al.* 2013). Frontloading reflects the preemptive expression of stress-response genes hence predisposing organisms to better respond to stress endured in the past. It has been proposed that the occurrence of plasticity *vs*. frontloading strategies may depend on the frequency of stresses relative to the typical response time of organisms, with frequent stresses promoting frontloading strategies whereas less frequent perturbations result in an increased plasticity (Kenkel & Matz 2016). Surprisingly, plasticity and frontloading are often discussed as exclusive responses (Barshis *et al.* 2013; Kenkel & Matz 2016). We hypothesized that, rather than being exclusive, plasticity and frontloading often co-occur and might also mainly depend on the regulatory pathways in which genes are involved. In particular, each strategy is expected to be selected for, only if it provides a more efficient response and a better fitness despite possible trade-offs. Our results clearly support that plasticity and frontloading indeed co-occur, specifically in the thermotolerant Om colonies experiencing a more variable thermal environment *in natura*. To tease apart the biological processes that are regulated via plasticity or frontloading in *Pocillopora* response to heat stress, we conducted an enrichment analysis. In the next section we detail the response of coral colonies at the molecular level for each main biological process identified, keeping in mind that congruency between gene expression and protein levels should be cautious (Mayfield *et al.* 2016). RNA-sequencing of eukaryotic poly-adenylated mRNA would allow in

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principle dual analysis of *Symbiodinium* and coral host transcripts (Mayfield *et al.* 2014), but since our RNA extraction methods resulted in very few, we only focused on the host transcriptomic response.

FUNCTIONAL ANALYSES:

RESPONSE TO STRESS:

HSPs:

Heat shock proteins (HSPs) are ubiquitous stress-induced chaperone molecules involved in protein folding and protein damage repair in most organisms (Feder & Hofmann 1999). As such, several HSPs were found to be over-expressed in colonies from both localities, whichever their thermal regime, in response to heat stress. Among these, the over-expression of Hsp70 genes was significantly more intense in the Om colonies compared to NC ones. Hsp70 is one of the most documented protein chaperones in coral heat stress response (Barshis *et al.* 2013; Haguenauer *et al.* 2013), but other forms of HSPs such as Hsp60 (Brown *et al.* 2002), Hsp90 (Carpenter *et al.* 2010), and Hsp40 (Maor-Landaw & Levy 2016) are also known to be involved in the response of these organisms. Accordingly we found that Hsp105, Hsp75, Hsp90, and Hsp71 were over-expressed only in Om corals and Hsp60 and Hsp71 showed higher basal expression levels in the Om corals. We also found that chaperones of the Hsp40 family (also known as DNAj) were also regulated in response to heat stress; three of these were over-expressed in colonies from both localities with a higher basal expression level in the Om corals, whereas five were over-expressed only in the Om corals. These proteins seem to be implicated in protein folding during early heat stress in the scleractinian *Stylophora pistillata*, and are highly expressed prior to bleaching and tissue peeling (Maor-Landaw *et al.* 2014). We also found specific Om over-expression of the pyrexia gene, which codes for a transient receptor-activated cation channel, and had been shown to protect flies from high temperatures (Lee *et al.* 2005). Together these results indicate that most genes involved in the heat shock protein pathways are plastically regulated by corals when exposed to stress with a greater response in colonies inhabiting thermally variable environment. However few HSP genes are also frontloaded in these pre-exposed colonies, in particular Hsp60 which gene is encoded in the mitochondria. This pattern is likely to reflect a more general higher mitochondrial activity in colonies accustomed to more variable and/or warmer temperatures.

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Reactive Oxygen Species (ROS) detoxification and DNA repair:

One of the first consequences of heat stress is the production of reactive oxygen species (ROS), which might cause protein, membrane and DNA damages (Kowaltowski & Vercesi 1999; Yakovleva *et al.* 2009; Jena 2012). These oxidative molecules are produced in excess by the symbionts and mitochondria during the photosynthesis and the respiration processes, respectively. Cells produce several detoxification enzymes to limit cellular damages (Weis 2008). In particular, under-expression of the calcium-binding messenger protein calmodulin is a sign of response to oxidative stress (Schallreuter *et al.* 2007; Voolstra *et al.* 2009). This protein had more isoforms that were differentially expressed in response to heat stress in Om compared to NC corals, together with one calcium calmodulin-dependent kinase. The Quinone oxidoreductases proteins are involved in the generation of ROS (Porté *et al.* 2009) and were also specifically under-expressed in Om corals in response to heat stress. Conversely, the thioredoxin gene was over-expressed in colonies from both localities, but with a higher fold change in Om corals. This enzyme detoxifies oxidized molecules and is often implicated in coral response to heat stress (DeSalvo *et al.* 2010; Maor-Landaw *et al.* 2014). Finally, polyamine oxidase is a key component of the oxidative burst in plants, and is involved in the induction of apoptosis (Yoda *et al.* 2006). Two genes, one under-expressed in Om corals and one over-expressed in NC corals, were found to be regulated in our experiment. Overall, the molecular pathway underlying ROS detoxification hence appears to be plastically regulated, with greater plasticity in the thermo-tolerant Om colonies than in the thermo-sensitive NC colonies.

Several genes coding for DNA repair enzymes including DNA excision repair, DNA ligase, and DNA polymerase were over-expressed in colonies from both localities when exposed to heat stress. However, among these genes, three showed higher basal expression levels and two were frontloaded in the Om colonies.

Apoptosis:

Among the genes differentially regulated in colonies when exposed to heat stress, many are also involved in apoptosis, a process that has been recurrently associated with coral responses to heat stress (Ainsworth *et al.* 2011; Pratlong *et al.* 2015). Among these, TNFR and TRAF genes code for receptors and receptor-associated components of the tumor necrosis factor (TNF), the latter being central in the apoptosis pathway (Ashkenazi & Dixit 1998). Five TNFR and 11 TRAF genes were specifically over-

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expressed in the Om stressed colonies, indicating a more intense regulation of the apoptosis pathway in these thermotolerant colonies. Furthermore, four TNFs were found to be specifically under-expressed in the Om corals, whereas TNFAIP3 was specifically over-expressed in response to heat stress. It is worth stressing that the TNFAIP3 gene (TNF alpha-induced protein3) is known to inhibit the NFkB process (inflammatory response) together with apoptosis (Opipari *et al.* 1990). Moreover, Caspase8 and Caspase3 were specifically over-expressed in the Om stressed corals. Caspases constitute the effector core of the apoptotic process (Nicholson & Thornberry 1997), and Caspase3 has commonly been implicated in the heat stress response in corals (Maor-Landaw & Levy 2016). This further suggests that the thermotolerant Om colonies are capable of greater regulation of apoptosis than the thermosensitive NC ones when exposed to warm temperature. Another set of 10 TNFR genes and 3 TRAF genes were found to be over-expressed in colonies from both localities, but with a higher expression level in the Om corals both in the control and the stress conditions for 10 of the 13 genes irrespective to the treatment (i.e. stress and control temperature). Fem-1 homolog B, which is involved in the apoptotic process as a death receptor-associated protein, was also constitutively higher expressed in the Om compared to the NC colonies. Together these results indicate that part of the apoptosis pathway is frontloaded in the thermotolerant Om colonies. These results echoes with previous studies showing that TNFR genes are frontloaded in the common reef-building coral *Acropora hyacinthus* (Barshis *et al.* 2013) and hence strengthen the idea that frontloading such biological pathway is an adaptive response to variable and/or warmer temperatures in corals.

Energetic metabolism:

Mitochondrial functions:

The mitochondria are central to many important biological processes of their cell hosts including energy-generating respiration and can be the source of several oxidative molecules involved in apoptosis. As such, heat stress is expected to promote severe changes in the expression levels of genes involved in the mitochondrial function in all living organisms (Guderley & St-Pierre 2002). Accordingly, our enrichment analysis highlighted several mitochondrial genes (either mitochondria- or nuclear-encoded). Among those, some of them were specifically or more intensively regulated in Om corals, including genes coding for 2-oxoglutarate dehydrogenase (which catalyzes the conversion of 2-oxoglutarate to succinyl-CoA and

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CO_2 in the Krebs cycle), and several monocarboxylate transporters for lactate or pyruvate, the latter being essential for the regulation of energy metabolism in the thermosensitive sea anemone *Aiptasia* (Halestrap & Meredith 2004; Lehnert *et al.* 2014). These results corroborate with the general higher gene expression plasticity observed in colonies from a population experiencing a more variable thermal regime (Om) compared to colonies from a population exposed to narrower thermal amplitudes (NC).

Nevertheless, several other genes that participate in energy metabolism displayed a frontloaded pattern in the Om colonies such as the succinate dehydrogenase [ubiquinone] flavo mitochondrial-like and the citrate synthase genes. Interestingly, the expression of the latter gene is often used as a reliable indication of organisms' aerobic capacity and mitochondrial density (e.g. (Rivest & Hofmann 2014; Hawkins & Warner 2017)). Thus, it is likely that the observed frontloading pattern of these mitochondrial genes (encoded in the mitochondrial genome) reflects a greater mitochondrial density in the Om compared to the NC colonies. According to the literature related to non-phototrophic symbiotic ectotherms such increase in mitochondrial density as an adaptive response to heat stress appears counterintuitive since the opposite pattern is generally observed (Pörtner 2002). However, elevated mitochondrial activity on cnidarian hosts have already been found to be associated with thermal-tolerance in corals and sea anemones (Dixon *et al.* 2015; Hawkins & Warner 2017). Hawkins & Warner (2016) suggest that such specific pattern might be at least partly explained by a decrease in symbiotic algae density resulting from heat stress. Energy metabolism in scleractinian corals is highly dependent of their symbiotic algae, which are the major source of glucose generated by photosynthesis. A partial loss of their symbiotic algal may force cnidarian hosts to increase their energy metabolism, hence requiring an increase in their mitochondrial contents, and to use heterotrophic sources of energy that can be obtained from feeding or from the use of metabolic reserve such as lipids. In this context we also observed a high level of regulation of lipid metabolism in the Om corals. The apolipo A (a constituent of lipoprotein associated with lipid dissolution) and dLp HDL-BGBP precursor (which has lipid transporter activity) genes were specifically over-expressed in the Om corals, and could facilitate the transport and assimilation of lipids.

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Symbiont regulation:

Perturbation of energy metabolism and a decrease in the efficiency of energy production by symbiotic algae can be linked to the modulation of genes associated with symbiont maintenance. In our experiment three Rab-11 isoforms were found to be over-expressed in colonies when exposed to stress, including: one being over-expressed in colonies from both localities but with a higher fold change in the Om corals; one that occurred in NC corals while showing frontloading expression in Om corals; and one induced only in the Om corals. This recycling regulatory protein has been shown to regulate phagosomes containing *Symbiodinium* cells in the *Aiptasia-Symbiodinium* model system (Chen *et al.* 2005). Calumenin was also over-expressed in both Om and NC colonies in response to heat stress, but had a higher fold change in Om corals. This protein is known to be a signal intermediate for sym32, a signaling protein involved in symbiont recognition by the coral host (Reynolds *et al.* 2000; Schwarz & Weis 2003). In addition, three lectins exhibited differential expression in the Om corals: one was over-expressed (with a log2-fold change of 4.1) and two were under-expressed (log2-fold change of -2.8 and -2.5). It is worth stressing that lectins are key proteins involved in symbiont recognition (Wood-Charlson *et al.* 2006) and that a breakdown of this recognition may lead to the disruption of the symbiosis (Vidal-Dupiol *et al.* 2009). Thus although we did not expressly quantify the density of *Symbiodinium* algae in colonies during our experiment, our results strongly suggest that *Symbiodinium* were at least partly excluded as a response to heat stress prior to bleaching process. These results hence corroborate with those found relative to a shift in both the greater involvement in energy metabolism and resources use shift.

Cnidocytes:

We observed a higher level of regulation of genes involved in the functioning of cnidocytes (also known as nematocytes) in Om corals. These ectodermal cells are specific to cnidarians, and are involved in environment sensing, defense, and predation. Three cytosolic phospholipase A2 (cPLA2) genes were specifically over-expressed in Om corals. In cnidocytes, the corresponding proteins are involved in venom efficacy by promoting prey lysis (Argiolas & Pisano 1983) (Nevalainen *et al.* 2004). One MAC perforin domain-containing gene had a higher basal expression level in Om corals. This gene encodes for a membrane attack complex/perforin-domain containing protein, and is found in gland cells and nematocytes in the sea anemone *Nematostella*, suggesting a potential role in prey killing (Miller *et al.* 2007). Conversely, we found under-expression in Om corals of a gigantoxin homolog, which is a cytolysin

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(actinoporin) found abundantly in cnidocytes (Hu *et al.* 2011) (Frazão *et al.* 2012). Several polycystic kidney disease gene (PKD1 and 2) isoforms were also found to be under-expressed in the Om corals. These proteins are localized in tentacles in Hydra, and seem to be involved in cnidocyte discharge (McLaughlin 2017). These changes could have been promoted by notch signaling (with over-expression of one notch homolog 2); in cnidarians, this pathway is particularly important for cnidogenesis as shown in *Nematostella vectensis* (Marlow *et al.* 2012). Together these results clearly indicate that the Om colonies are better adapted to switch to heterotrophic nutrition in response to heat stress. Such switch could be linked to higher levels of resistance to heat stress via a decrease in *Symbiodinium* cell numbers, as described in other scleractinian corals (Hughes & Grottoli 2013; Aichelman *et al.* 2016).

To summarize, we found that Om colonies displayed a more plastic transcriptomic response for many genes involved in fundamental biological pathways such as stress-response pathways (e.g. HSPs, apoptosis, DNA repair, ROS management) in response to heat stress. Moreover, several genes were found to be frontloaded especially in the apoptosis as well as in the energy metabolic pathway. The latter pattern could be explained by a higher regulation of metabolic genes but could also reflect in part an increase in mitochondrial activity as already documented in other symbiotic cnidarians in response to heat stress (Rivest & Hofmann 2014). Higher plasticity and frontloading are two strategies that are costly. As expected, we found strong evidence for possible trade-offs with other critical life history traits such as growth and reproduction. In the next sections we will discuss the transcriptomic response of both Om and NC colonies in response to stress at molecular pathways associated with central fitness traits.

MORPHO-ANATOMIC PROCESSES AND REPRODUCTION:

SKELETON AND MUSCLE CELLS:

Some enzymes potentially involved in the calcification and muscle contraction were under-expressed in response to heat stress in Om corals, in particular, a single chain carbonic anhydrase and four carbonic anhydrase genes (1, 2, 7, and 12). Carbonic anhydrases catalyze the interconversion of CO_2 and water to bicarbonate and protons, enabling maintenance of the acid-base balance (Tashian 1989). In scleractinian corals, carbonic anhydrases are also involved in biomineralization processes involved in skeleton formation (Bertucci *et al.* 2013). Two hephaestin genes were also under-expressed following heat stress in Om corals. These copper-dependent ferroxidases were identified in the skeleton of *Acropora millepora*

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(Vulpe *et al.* 1999), where they can be involved in the incorporation and regulation of iron in the carbonate skeleton (Ramos-Silva *et al.* 2013). Several myosin related proteins similar to myosin light chain kinase or myosin regulatory light polypeptide 9 were found to be specifically under-expressed in the Om corals. Actin-binding LIM partial, potentially involved in Z-disk activity of muscles, and several genes having the LIM domain were also found to be under-expressed in Om corals. In eumetazoans including cnidarians, these proteins are involved in regulation of muscle activity (for a review, see (Leclère & Röttinger 2016). In addition, the genes for the acetyl choline receptor "43 kDa receptor-associated of the synapse-like" and the "calcium-activated potassium channel subunit beta-like" proteins, both of which down-regulate muscle contraction, were over-expressed in Om colonies, which is in accordance with a global decrease in functioning of the contractile apparatus. The observed down-regulation of skeleton formation and muscle contraction strongly suggest that mounting a higher –and most likely better– transcriptomic response to heat stress is associated with a decrease energy allocation to crucial fitness traits and particularly to growth in scleractinian corals.

NERVOUS SYSTEM:

Many genes involved in nervous system functioning were differentially regulated, with some being over-expressed (e.g. neuronal acetylcholine receptor and VWFA, which regulate the calcium dependent voltage chain), and others being under-expressed, including a proton myo-inositol cotransporter, SCO-spondin (axon guidance), sodium- and chloride-dependent GABA and taurine transporters (neurotransmission), sodium-dependent phosphate transport 2B, and synaptotagmin. Many transcription factors that could be involved in morpho-anatomic integrity, including numerous forkhead box homologs implicated in cell growth, proliferation, and differentiation were over-expressed in Om corals. All these may be involved more globally in the regulation of morpho-anatomic processes (muscle contraction, cnidocytes).

CIRCADIAN CLOCK / REPRODUCTION:

Several photoreceptors were differentially expressed in the corals. A cryptochrome gene was frontloaded in Om colonies. These flavoproteins are found in plants, animals, insects, and cnidarians, and in corals it has been shown that cryptochrome can synchronize the circadian clock and the reproductive system (Reitzel *et al.* 2013). Three melatonin receptors were also found to be regulated in response to heat stress in Om corals (two over-expressed and one under-expressed). As in vertebrates, in which melatonin is well characterized, in cnidarians it is also involved in the circadian clock (Peres *et al.* 2014). Similarly, two

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under-expressed and three over-expressed opsin-like genes were identified. The protein is a homolog of the photoreceptor melanopsin, and in *Acropora* is involved in circadian cycle regulation (Vize 2009).

Taken together, changes in morpho-anatomic regulation and perturbations in circadian cycles and reproduction could also reflect a trade-off mechanism in the stress response of Om colonies, which is consistent with greater plasticity, and intensive and not fully specific gene regulation activity. We believe that our study contribute to a better comprehension of the biological processes involved in coral thermotolerance. In particular we highlighted the molecular pathways that were regulated either via frontloading or plasticity or both. We also detected clear transcriptomic evidence that allocating energy in heat stress response to better cope with higher temperatures is at the expense of other crucial biological processes such as growth and reproductive functions. However, the molecular mechanisms underlying such global response to heat stress are still partly unresolved. Interestingly we also found specific gene expression patterns linked with epigenetic regulation that could give new insights into our comprehension of such mechanisms and these are exposed and discussed in the next and last section.

EPIGENETIC PROCESSES:

Two histone acetyltransferase genes were over-expressed in Om corals. These enzymes, which are also found in *Nematostella*, are involved in epigenetic gene regulation by modifying the nucleosome structure, and thus the transcription of genes (Sterner & Berger 2000; Karmodiya *et al.* 2014). Several histone–lysine N-methyltransferases were also identified, five of which were under-expressed (four PRDM6 and one setd3 isoform) and three of which were over-expressed in Om corals. As with histone acetyltransferase, these enzymes are involved in gene expression regulation through their influence on chromatin structure (Huang 2002; Vervoort *et al.* 2016). One lymphoid-specific helicase-like (HELLS) gene was frontloaded in Om corals. This protein is known to participate in epigenetic processes, and interacts with DNMT1 (cytosine methyl-transferase) for the maintenance of *de novo* methylation (Myant *et al.* 2011; Dabe *et al.* 2015). We identified under-expression of two SID-1 transmembrane family member genes involved in dsRNA regulation (Li *et al.* 2015). Many reverse transcriptase homologues, including RNA-directed DNA polymerase from a jockey-like mobile element, were differentially expressed or more intensely regulated during heat stress in the Om corals. These reverse transcriptases are typical of the retro-transposon activity known to be activated or less controlled during general stress (Wessler

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1996). The loss of inhibition of retro-transposon activity may reflect a trade-off with more specific functions, but could also be a "SOS-like" survival mechanism. Indeed, in a threatening environment a high transposition activity may induce mutations in polyps with potential genomic changes, which could fuel rapid adaptive evolution (Maumus *et al.* 2009).

CONCLUSION:

Comparison of the response to an ecologically realistic heat stress of corals from the same genus but pertaining to different phylogroups thriving in two contrasting thermal environments sheds light on the molecular basis of thermotolerance. We found that during heat exposure the symbiotic community composition was stable in colonies from both thermal environments, but we identified in coral hosts major differences in gene regulation processes. The more thermotolerant colonies displayed (i) a more plastic transcriptome response involving more differentially expressed genes and higher fold expression changes; and (ii) a constitutive and higher level of expression for a range of genes (frontloaded genes). In the context of climate change, which is predicted to cause abnormal and rapid temperature increase (Change 2014), phenotypic plasticity and the capacity for rapid adaptation through epigenetic regulation and/or genetic assimilation could increase the probability of coral survival. Previous studies highlighted the importance of reef managements measures (Rogers *et al.* 2015) and assisted evolution (van Oppen *et al.* 2015), but also underlined the importance of preserving standing genetic/epigenetic variation in wild coral populations (Matz *et al.* 2017). Our results also suggest that management measures must include protection of natural thermotolerant populations having the potential to resist increasing thermal anomalies. Although the molecular mechanisms we described are most likely largely shared in this group of scleractinians, the question remains as to whether these more thermotolerant genotypes are secured in different phylogroups as was the case here, or whether they also exist inside more closely related phylogroups where they could circulate via long range gene flow. (Mumby *et al.* 2011). It is however essential to keep in mind that even the most thermotolerant reefs may bleach if they are exposed to temperature significantly higher to their own norm (Hughes *et al.* 2017b; Le Nohaïc *et al.* 2017).

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Data Accessibility

The datasets generated and analyzed during the current study have been submitted to the SRA repository under bioproject number PRJNA399069 (to be released upon publication).

Authors' contributions

JVD, MA, DA, GM, and ET were involved in the study concept and design. KBR, LF, MC, MA, PR and JVD were involved in the collection of samples. All authors were involved in data acquisition and analyses. KBR, JVD, GM, OR and ET drafted the manuscript, and all authors contributed to critical revisions and approved the final manuscript.

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TABLES

Table 1: Sea Surface Temperature (SST) regimes to which the colonies sampled in this study (i.e. Oman and New Caledonia) are exposed in their natural environments. Thermal regime descriptors were compiled from weekly mean sea surface temperature data collected from the Integrated Global Ocean Services System Products Bulletin (i.e. IGOSS: http://iridl.ldeo.columbia.edu/SOURCES/.IGOSS/) for quadrats of 1° longitude X 1° latitude and from 1982 to the year of sampling (2013-2014).

	New Caledonia	Oman
Mean SST (°C)	24.8	27.9
Variance (°C)	2.7	9.5
Min SST (°C)	22.6	22.1
Max SST (°C)	27.1	33.2
Mean SST of 3 warmer months (°C)	26.8	31.3
Mean SST of 3 cooler months (°C)	22.8	23.8

FIGURE LEGENDS

FIGURE 1: The ecologically realistic heat stress experiment: from mean temperatures of the warmer months *in natura* to a pre-bleaching physiological state. The arrows represent points at which nubbins were collected for analyzing the microbial composition and the transcriptomic response of the host.

FIGURE 2: Principal coordinate analysis plot for Bray-Curtis distances of the bacterial composition of each colony in each experimental condition. Different colors represent different colonies, the circles represent the *in situ* conditions, the triangles represent the control conditions, and the squares represent the stress conditions.

FIGURE 3: Composition of the *Symbiodinium* community in each colony *in situ* and in controlled and stressful experimental conditions.

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FIGURE 4: Hierarchical clustering analyses performed using DESeq2 rlog-normalized RNA-seq data for the 36 transcriptomes: two conditions (control and heat stress); three replicates per condition for each colony; three3 colonies per locality; and two localities [Oman (Om, i.e. thermotolerant) and New Caledonia (NC; thermosensitive)]. The color (from white to dark blue) indicates the distance metric used for clustering (dark blue corresponds to the maximum correlation values).

FIGURE 5: Heatmap for significant differential gene expression in at least one comparison. Each gene is represented by a line.

FIGURE 6: Scatterplot of the log2-fold changes in gene expression in response to heat stress in the Om colonies (y-axis) *vs.* the NC colonies (x-axis) for the 848 genes that were over-expressed (498 genes) or under-expressed (350 genes) in colonies from both localities. The line represents the y=x line depicting similar responses between colonies.

FIGURE 7: Colony level gene expression variation in response to heat stress, based on DAPC analysis. The x axis is the first discriminant function of the DAPC along which the overall gene expression difference between colonies at both experimental conditions (stress and control) and from both localities (NC and Om) was maximized. This indicates the degree of similarity between the transcriptomes. The density plots obtained for NC and Om colonies are represented in blue and green, respectively. Dark and light density plots correspond to samples from the control and stress experimental conditions. The arrows above the density plots represent the direction of the mean change in the gene expression profiles.

FIGURE 8: Summary of the GO enrichment analysis following REVIGO synthesis. Each enriched biological process is represented by a bar proportional to the log10(FDR). The colors correspond to the three categories of genes (common: black; Om-specific: grey; NC-specific: white) that were over-expressed (left panel) or under-expressed (right panel).

ADDITIONAL FILES

Additional Figure 1. Bacterial class composition (for the 24 most abundant) within each replicate for the Om and NC colonies, the three colonies of each locality, and three experimental conditions per colony. *In situ* (dark arrows); control temperature (green arrows); stress temperature (red arrows).

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Additional Table 2: ANOVA results for alpha diversity (Shannon index) and beta diversity (Bray-Curtis distance) between localities, colonies, or experimental conditions.

Additional File 3: List and sequences of the 26,600 genes (XLOC) generated during RNAseq alignment.

Additional Table 4: Haplotype analysis of the six sampled colonies with microsatellite genotyping for the colonies from New Caledonia.

Additional File 5: DEseq2results for the log2-foldchanges, and adjusted *p* values between stress and control conditions for each locality.

Additional Table 6: Comparison between the log2-foldchange in Om and NC colonies for genes differentially under-expressed or over-expressed in the same way in colonies from both localities.

Additional File 7: GO enrichment results for biological processes, molecular functions, and cellular compartments for common, New Caledonia-specific, or Oman-specific over-expressed and under-expressed genes.

Additional File 8: Frontloaded genes in Oman corals among genes over-expressed in New Caledonia corals.

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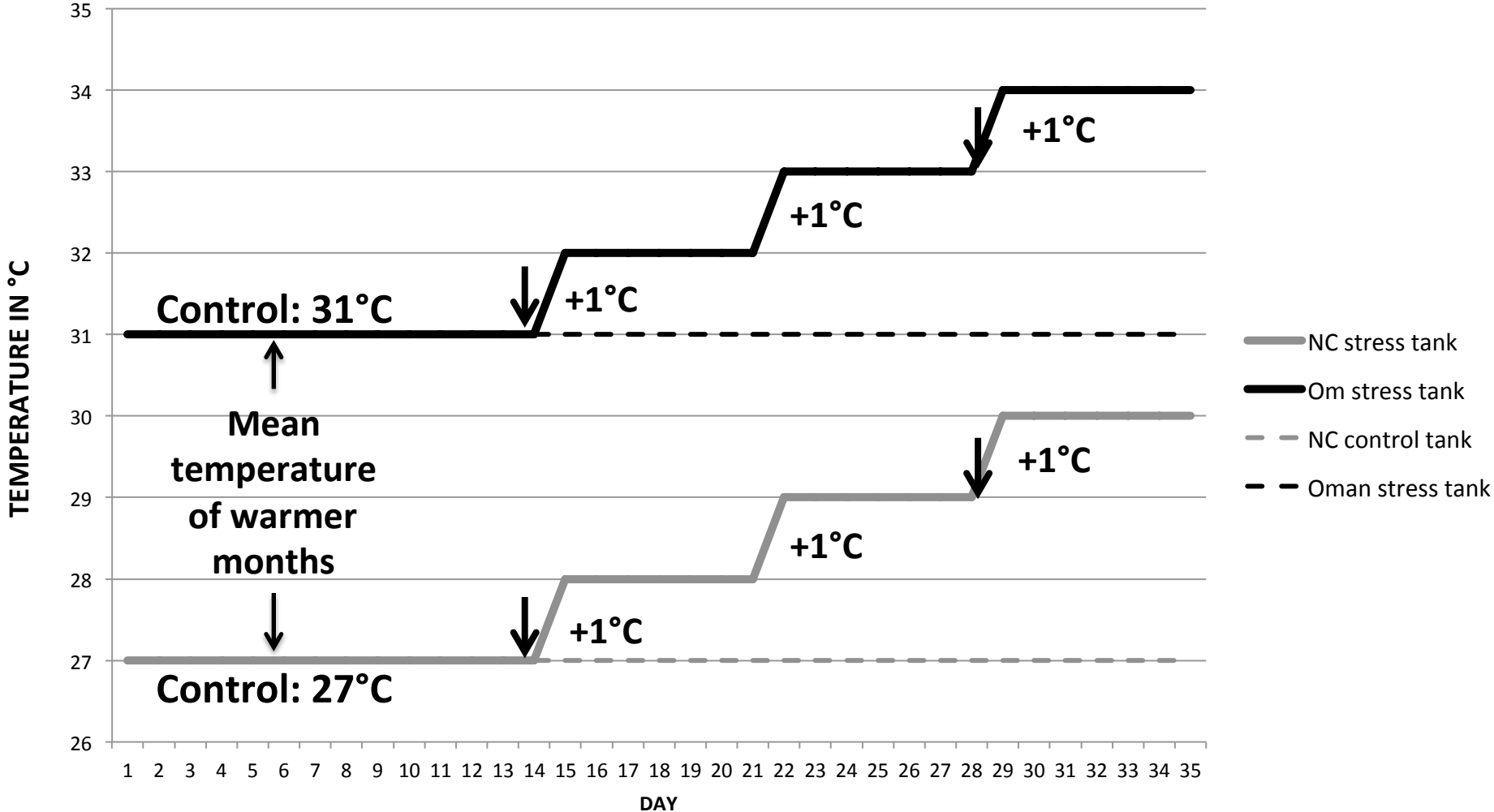
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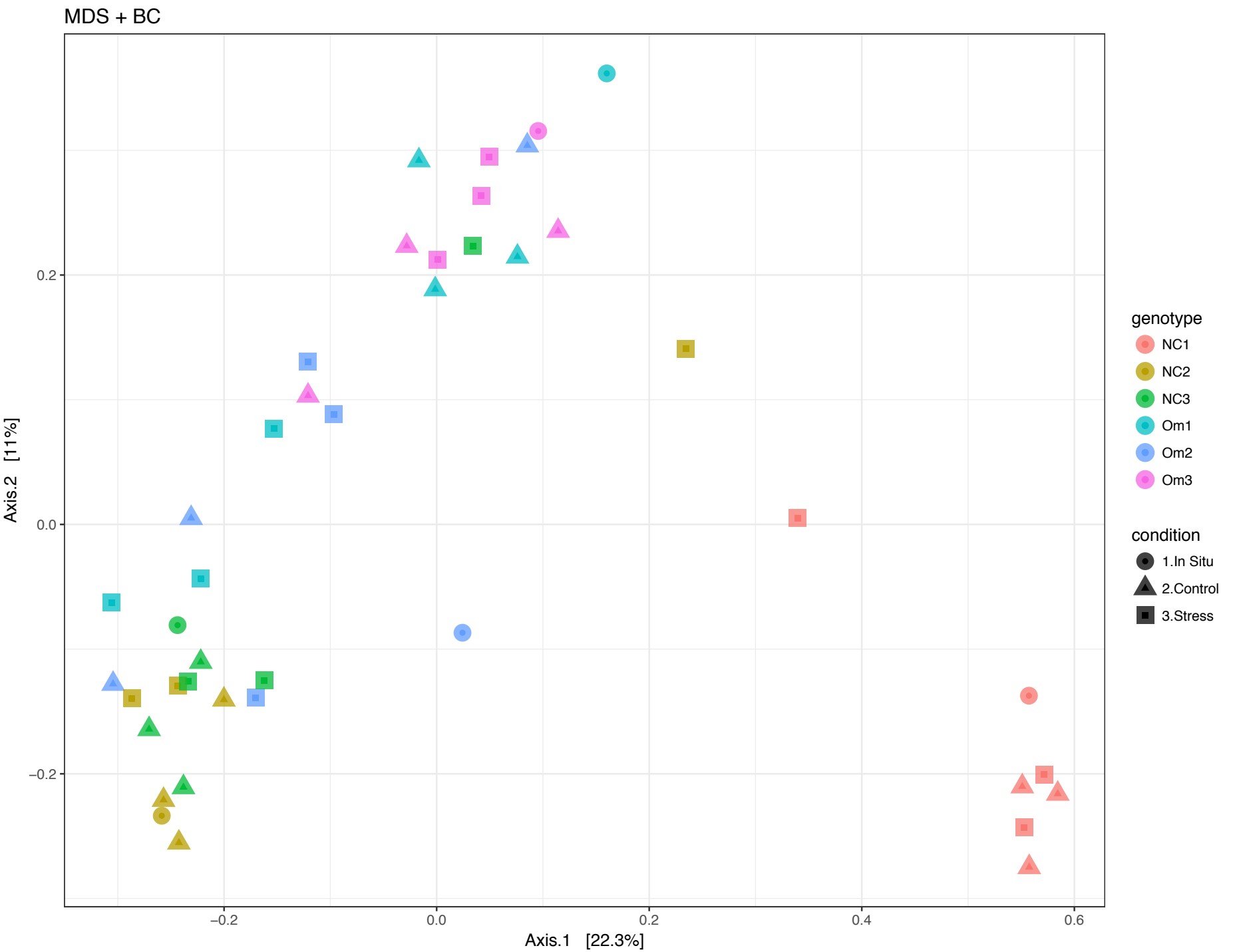
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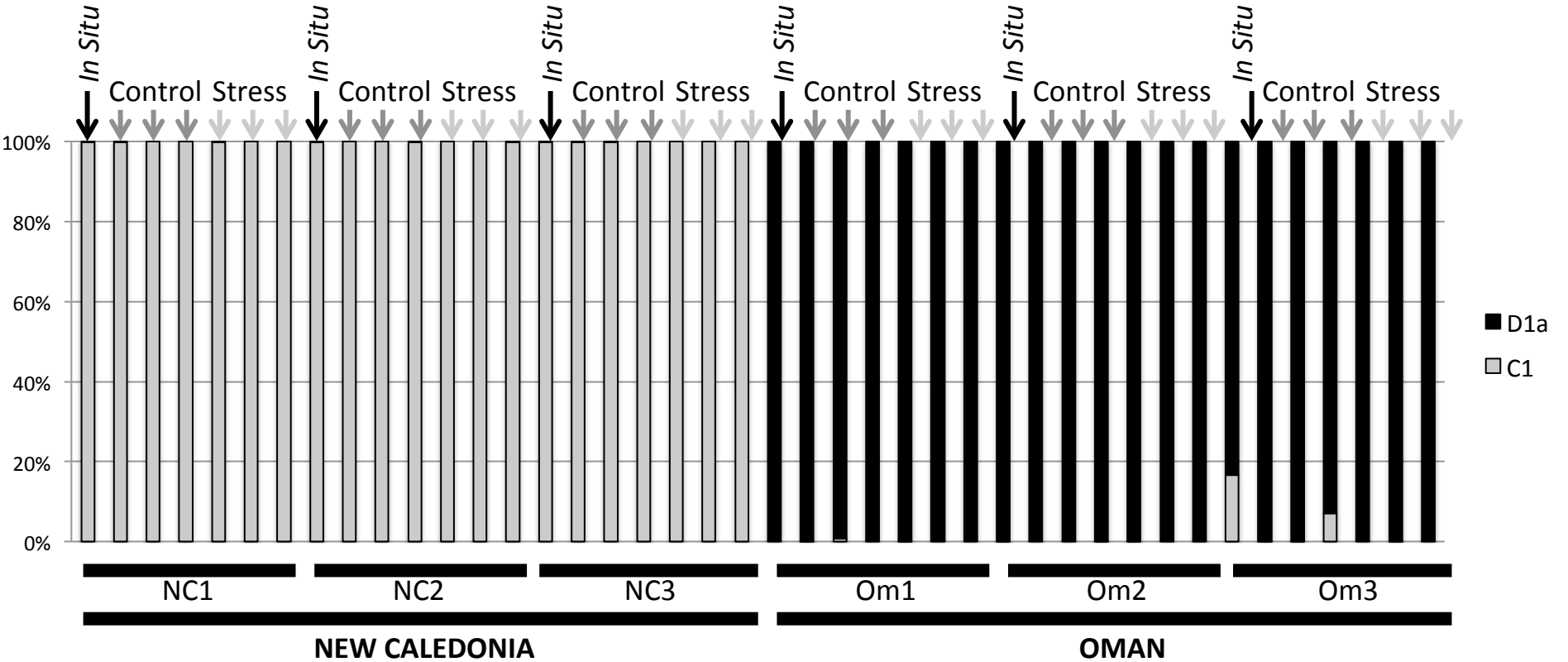
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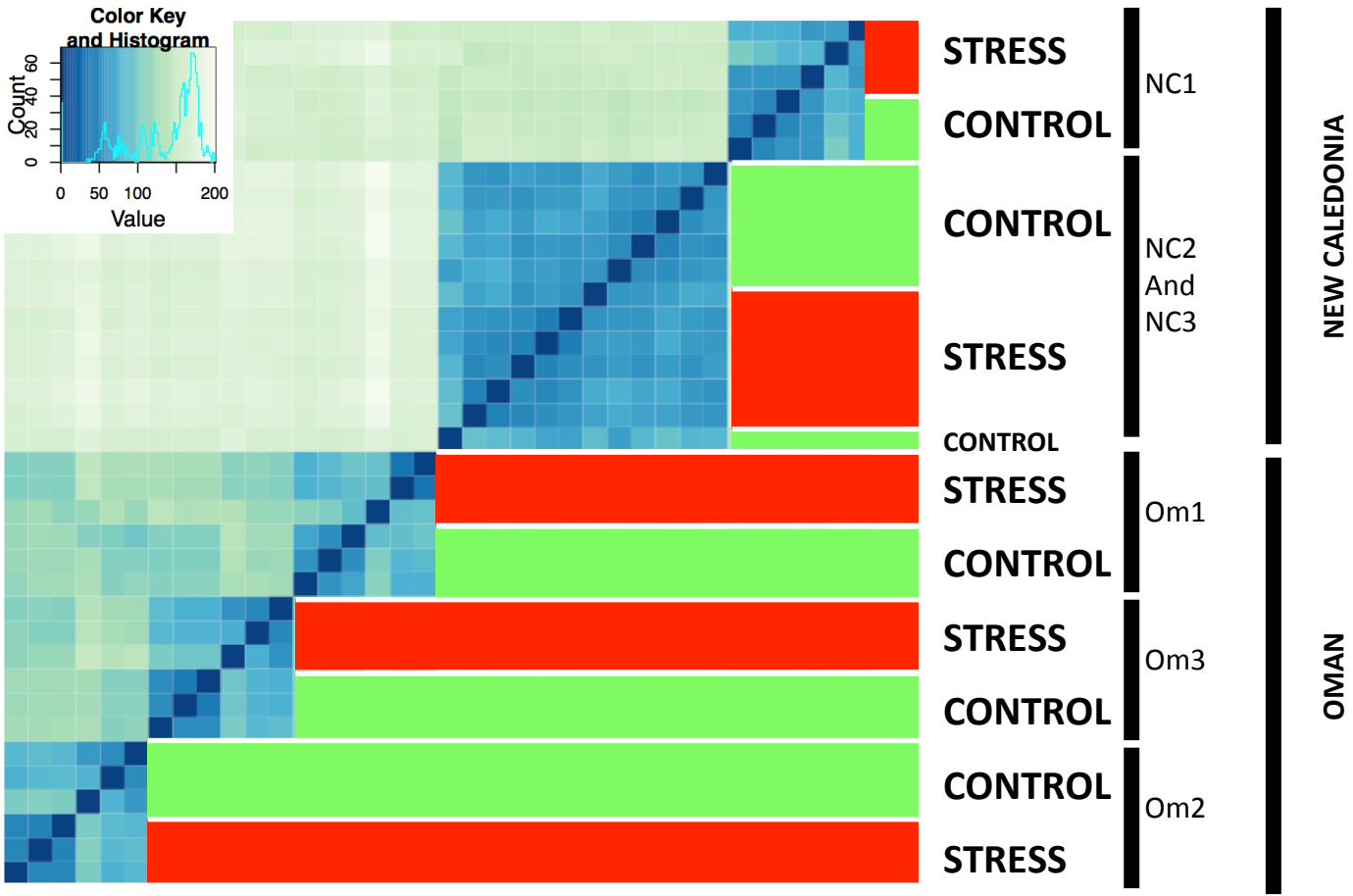
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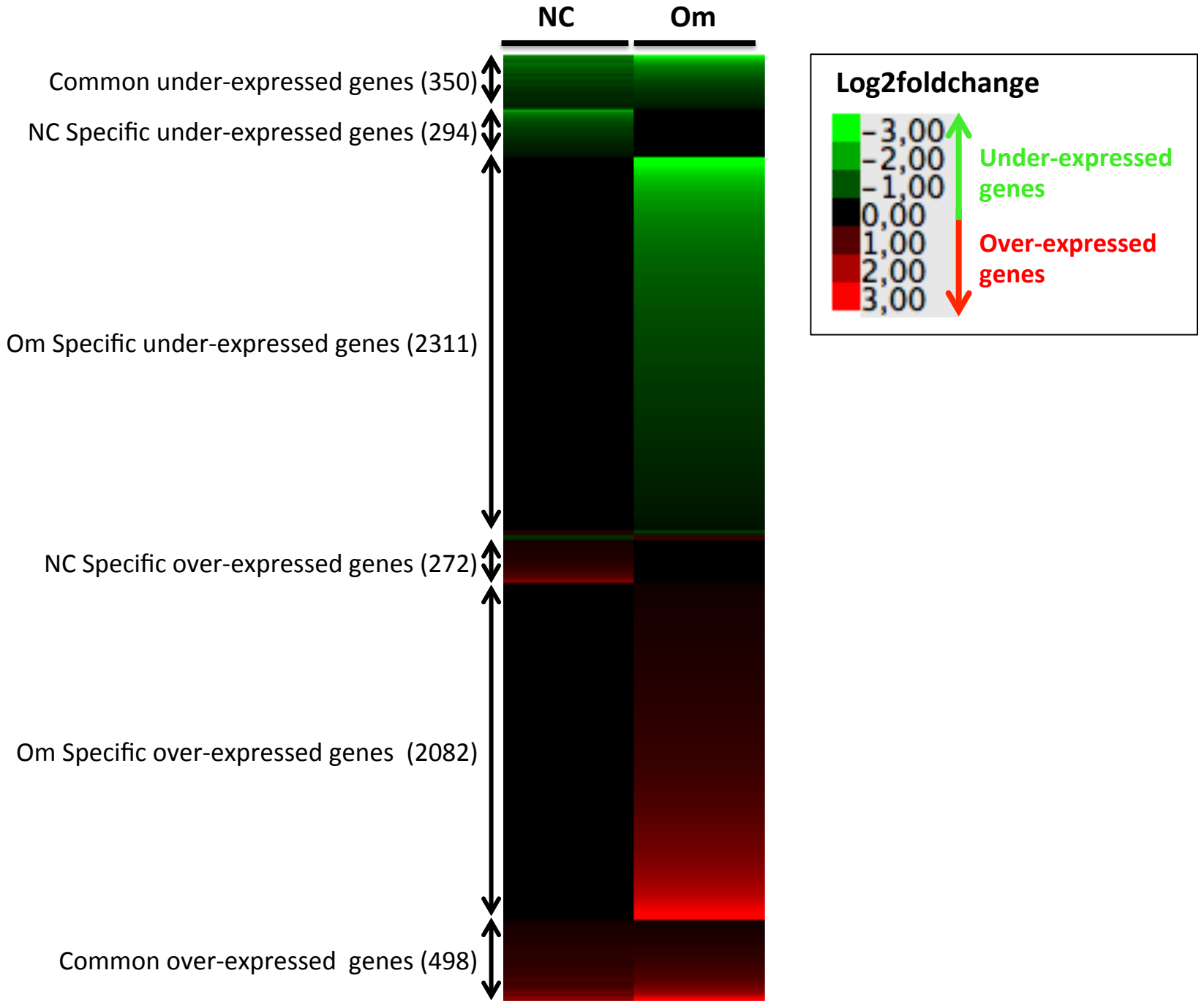
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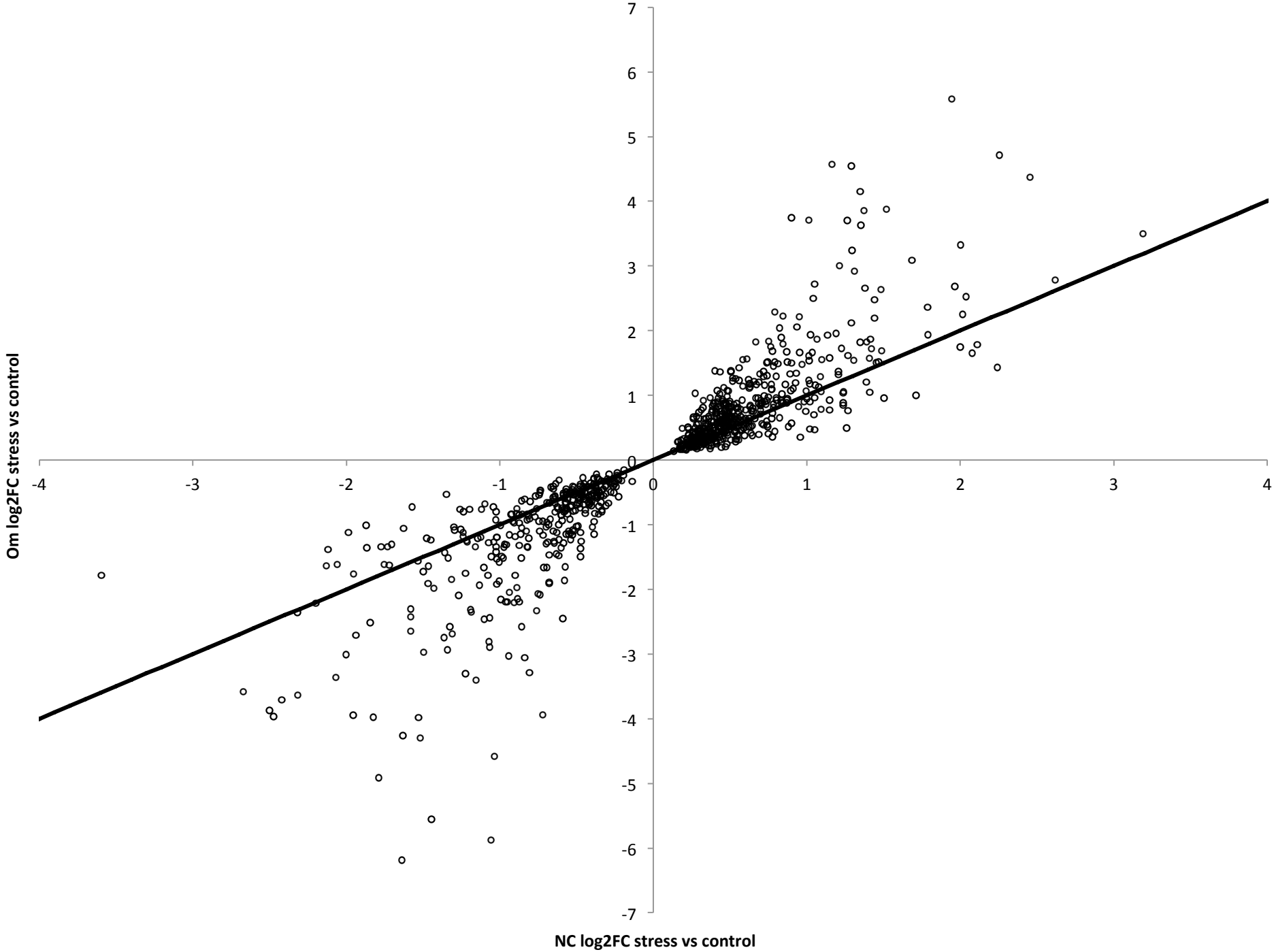
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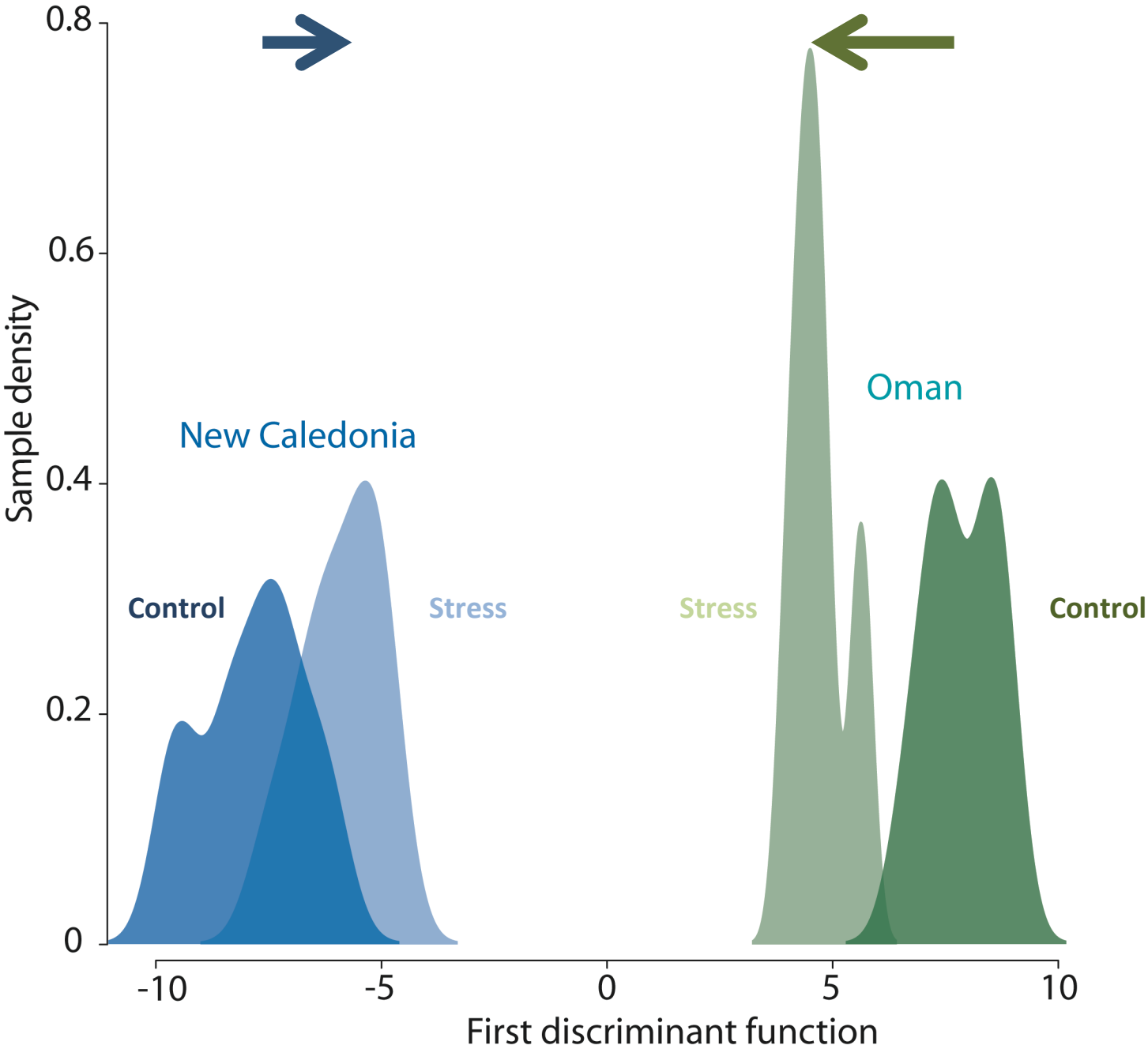
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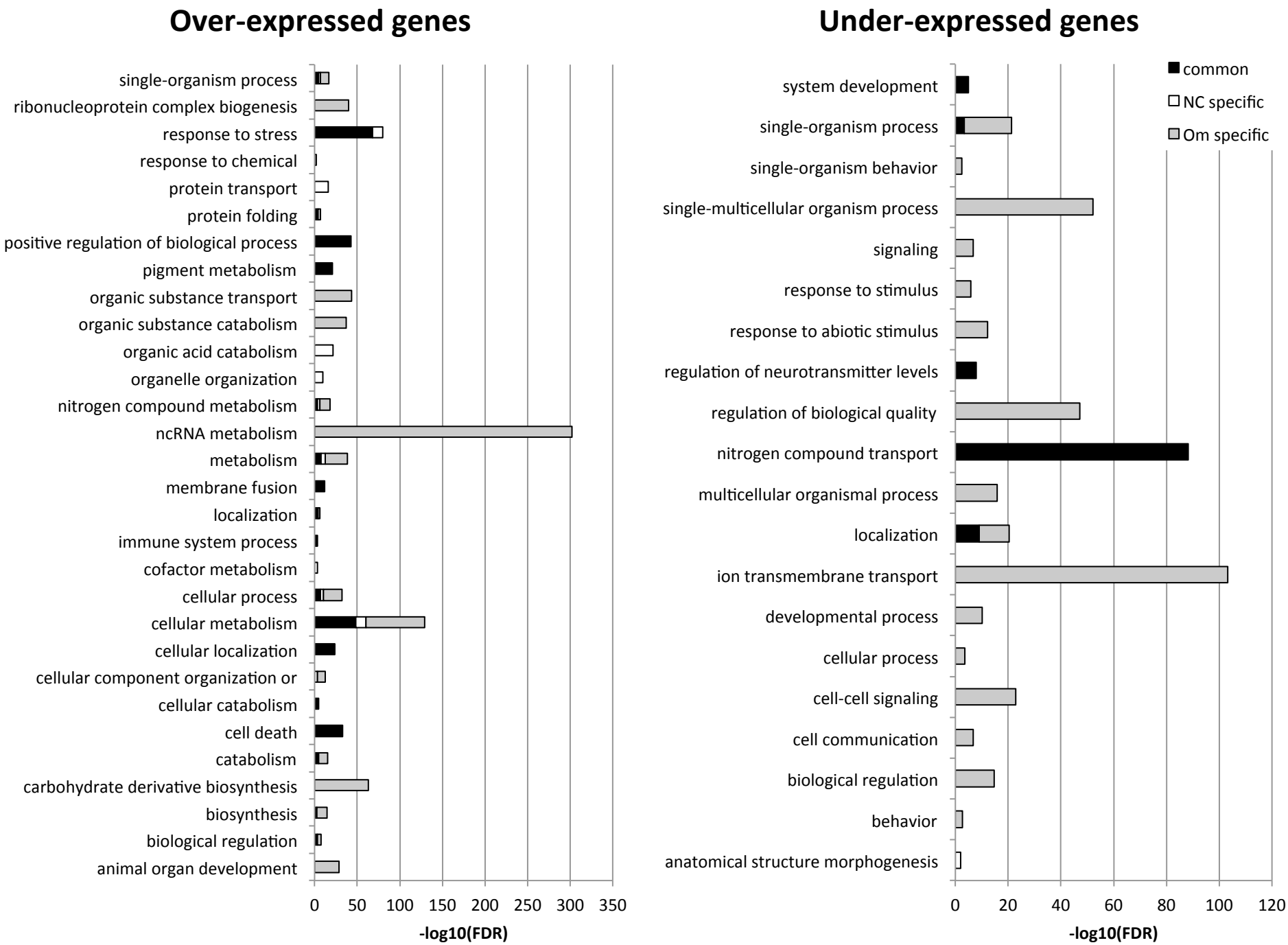
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