Gene expression plasticity and frontloading promote thermotolerance in *Pocillopora* corals

Running title: *Pocillopora* holobiont response to heat stress

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ABSTRACT

Ecosystems worldwide are suffering from climate change. 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 changes, and would be favored in variable environments. In this study, we investigated the thermal stress response in *Pocillopora* coral colonies from two contrasting thermal regimes [more stable seawater temperatures in New Caledonia (South Pacific), more variable in Oman (Persian Gulf)] by exposing them to heat stress. We compared the physiological state, bacterial and Symbionaceae 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 presented open and colored polyps during heat stress, with no change in bacterial and Symbionaceae community composition. In contrast, they differed in their transcriptomic responses. The colonies from Oman displayed a more plastic transcriptome, but some genes had a higher basal expression level (frontloading) compared to the less thermotolerant colonies from New Caledonia. In terms of biological functions, we observed an increase in the expression of stress response genes (including induction of tumor necrosis factor receptors, heat shock proteins, and detoxification of reactive oxygen species), together with a decrease in the expression of genes involved in morpho-anatomical functions. Gene regulation (transcription factors, mobile elements) appeared to be overrepresented in the Oman colonies, indicating possible epigenetic regulation. These results show that transcriptomic plasticity and frontloading can be co-occurring processes in corals confronted to highly variable thermal regimes.

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

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 extreme climatic events 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 communities (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 cope with heat stress. More specifically, populations inhabiting in zones with more variable temperature regimes display better tolerance to heat stress. This pattern can be verified from local (Kenkel *et al.* 2013) to geographical scales (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 acclimation (i.e. intra-generational gene expression plasticity; (Barnosky *et al.* 2011; Kenkel & Matz 2016)) and/or through genetic adaptation (i.e. inter-generational

88 microevolution; (Barnosky *et al.* 2011; Dixon *et al.* 2015)). Under the former hypothesis
89 one should expect that the present-day sensitive coral populations could potentially
90 acquire tolerance to heat stress along with the ongoing climate change. According to the
91 latter hypothesis, the persistence of initially thermo-sensitive coral populations would
92 depend on the emergence of better adapted lineages through microevolution and/or on
93 the genetic rescue via gene flow from populations already adapted to heat stress
94 (Bellwood *et al.* 2004; Barnosky *et al.* 2011; Dixon *et al.* 2015). Actually, some studies
95 strongly suggest that both processes (i.e. acclimation and adaptation) are likely to co-
96 occur in wild coral populations (Hoegh-Guldberg *et al.* 2007; Reusch 2013; Palumbi *et al.*
97 2014; Torda *et al.* 2017).

98 With the recent advances of high throughput molecular methods, it is now possible to
99 discriminate both processes while providing a more precise account of the molecular
100 mechanisms underlying coral response to heat stress. In particular, recent studies
101 clearly demonstrated that coral responses to heat stress involve the fine-tuned
102 regulation of expression levels of some genes/proteins involved in several molecular
103 pathways such as metabolism, stress-response and apoptosis (Brown *et al.* 2002; Weis
104 2008; Ainsworth *et al.* 2011; Bellantuono *et al.* 2012a; Barshis *et al.* 2013; Kenkel *et al.*
105 2013; Palumbi *et al.* 2014). In this regard, two main molecular patterns having different
106 temporalities have been put forward: (1) "transcriptional plasticity", i.e. extensive
107 changes in gene expression levels according to the occurring thermal condition and (2)
108 "transcriptional frontloading", i.e. the elevation of stress related genes baseline
109 expression that preconditions organisms to subsequent (recurrent) stresses (Reaka-
110 Kudla 2005; Mayfield *et al.* 2011; Barshis *et al.* 2013; Palumbi *et al.* 2014; Hughes *et al.*
111 2017a). While such elevated constitutive gene expression levels could reflect local

112 adaptation (i.e. genetically fixed gene expression level; (Oliver & Palumbi 2010; Palumbi
113 *et al.* 2014), it could also reflect an acclimation via epigenetic processes leading to
114 constitutive gene expression (Torda *et al.* 2017). Epigenetic changes through
115 environmental priming (i.e. translation of environmental cues) may be involved in
116 adaptive evolution at such short timescales, eventually enabling transgenerational
117 plasticity (Jablonka 2017).

118 Surprisingly, frontloading and gene expression plasticity were generally discussed as
119 mutually exclusive patterns (Barshis *et al.* 2013; Dixon *et al.* 2015; Kenkel & Matz 2016)
120 although these two molecular processes most likely co-occur during coral responses to
121 heat stress. In particular, one might expect that the regulation strategy of genes
122 (plasticity versus frontloading) will greatly depend on the molecular pathways in which
123 they are involved and the energetic, physiological, and ultimately fitness cost associated
124 with gene expression. So far, frontloading has been detected for stress response genes
125 such as Heat Shock Proteins (HSPs), apoptosis and tumour suppression factors in
126 resilient coral populations under experimentally simulated heat stress inducing
127 bleaching in the common reef-building coral *Acropora hyacinthus* (Polato *et al.* 2010;
128 Barshis *et al.* 2013; Dixon *et al.* 2015; Kenkel & Matz 2016) and for metabolic genes in
129 populations pre-exposed to warm temperatures in response to long-term heat stress in
130 *Porites astreoides* (Kenkel *et al.* 2013; Palumbi *et al.* 2014). Conversely, in the latter
131 species, plasticity was observed in the expression of environmental stress response
132 genes (Riegl *et al.* 2011; Kenkel & Matz 2016), hence challenging the patterns observed
133 in *A. Hyacinthus* (Barshis *et al.* 2013; Coles & Riegl 2013). Although both strategies (i.e.
134 constitutive frontloading *versus* expression plasticity) undoubtedly exist in wild coral
135 populations, the pre-exposure conditions that foster their induction and their relative

136 effects on coral resistance to heat stress still remain unclear (but see (Hughes *et al.*
137 2003; Kenkel & Matz 2016)).

138 Importantly, scleractinian corals are composed of several symbiotic organisms including
139 the cnidarian host, the mutualist photosynthetic algae (formerly defined as belonging to
140 the genus *Symbiodinium* but now considered as different genera within the family
141 Symbionaceae (LaJeunesse *et al.* 2018)) and bacterial communities. All partners (bionts)
142 involved in a stable symbiosis effectively form the entire organism, and constitute what
143 is referred to the holobiont (Margulis & Fester 1991). A decade after this term was
144 defined, its use has been particularly popularized in reference to corals (Rohwer *et al.*
145 2002), and subsequent research has led to the hologenome theory of evolution
146 (Rosenberg *et al.* 2007; Zilber-Rosenberg & Rosenberg 2008). In this context, the
147 hologenome is defined as the sum of the genetic information of the host and its
148 symbiotic microorganisms. Phenotypes are thus the product of the collective genomes of
149 the holobiont partners in interaction with the environment, which constitute the unit of
150 biological organization and thus the object of natural selection (Zilber-Rosenberg &
151 Rosenberg 2008; Guerrero *et al.* 2013; McFall-Ngai *et al.* 2013; Bordenstein & Theis
152 2015; Theis *et al.* 2016). Additionally to the cnidarian host response, the genotype -or
153 association of genotypes- of the photosynthetic mutualist Symbionaceae symbionts
154 plays a key role in the thermotolerance of the holobiont (Hume *et al.* 2013; Mayfield *et*
155 *al.* 2014; Suggett *et al.* 2017). There is less certainty about the importance of the coral
156 bacterial community in participating to the fitness of the holobiont, although accruing
157 evidences strongly suggest their implication in coral response to environmental
158 conditions (Li *et al.* 2014; Pantos *et al.* 2015; Hernandez-Agreda *et al.* 2016), and in the
159 resistance to diseases (Sato *et al.* 2009; Cróquer *et al.* 2013; Meyer *et al.* 2016). Finally,

160 the role of the coral-associated microorganisms and their potential to modify holobiont
161 adaptability remain so far overlooked (but see (Ziegler *et al.* 2017; Torda *et al.* 2017).
162 Hence, studying how corals respond to stress implies an integrative approach to analyze
163 the response of each symbiotic protagonist.

164 With this aim, we investigated the molecular mechanisms underlying thermo-tolerance
165 of coral holobionts. We analyzed the holobiont response to stress of two coral
166 populations originating from environments with contrasting thermal regimes. We used
167 scleractinian corals from the genus *Pocillopora* as model species because they have
168 abroad spatial distribution throughout the Indo-Pacific (Veron & Stafford-Smith 2000).
169 The genus *Pocillopora* is considered to be one of the most environmentally sensitive
170 (van Woesik *et al.* 2011) but its widespread distribution clearly suggests potential for
171 acclimation and/or adaptation which may be correlated to specific differences (i.e.
172 different cryptic lineages may be adapted to different environmental conditions). In
173 particular, we focused on *Pocillopora damicornis-like* colonies from two localities with
174 contrasting thermal regimes: colonies from New Caledonia (NC) are exposed to
175 temperate and stable temperatures over the year, while those from Oman are exposed
176 to globally warmer and more seasonal fluctuating temperatures. As the *corallum*
177 macromorphology is not a discriminant character in *Pocillopora* and as the taxonomic
178 revision of this genus using molecular data reveals that some of the *Pocillopora* species
179 (Schmidt-Roach *et al.* 2014; Gélin *et al.* 2017b) are actually species complexes, we
180 identified *a posteriori* the species of the sampled colonies (mitochondrial ORF
181 sequencing and individual clustering) in order to interpret the results in a precise
182 evolutionary context. To avoid biases inherent in transplantation-based field
183 experiments resulting from environmental factors other than temperature, we

184 undertook our comparative study in a controlled environment in which we mimicked
185 ecologically realistic heat stress to compare the responses of colonies from both
186 localities. We combined a specific RNA-seq approach to study the cnidarian host
187 response, and metabarcoding analyses using ITS-2 and 16S amplicon sequencing to
188 study the dynamics of the associated algal (Symbionaceae) and bacterial community
189 compositions, respectively. According to the literature we first expected to detect
190 changes in both symbiotic algal and bacterial communities in corals from both localities
191 when exposed to heat stress. Moreover, since variable environments are expected to
192 promote the evolution of plasticity, we predicted that the cnidarian hosts from Oman
193 will display more gene expression plasticity than those from New Caledonia. However,
194 because frontloading was also found to be an alternative response to recurrent changing
195 conditions, we might also expect some degrees of constitutive high levels of gene
196 expression at least for some molecular pathways and more particularly in Oman corals.

197 MATERIAL AND METHODS

198 CORAL SAMPLING AND MAINTENANCE

199 *Pocillopora damicornis*-like colonies originating from environments characterized by
200 contrasting thermal regimes were sampled during the warmer month in two different
201 localities: (1) in Oman, Gulf of Oman, Northwestern Indian Ocean (Om; June 2014; local
202 seawater temperature during sampling 30.8°C), where corals are exposed to a globally
203 warmer and variable thermal environment, and (2) in New Caledonia, Southwestern
204 Pacific Ocean (NC; November 2014; local seawater temperature during sampling
205 27.1°C), where corals are subject to more mitigate and stable temperatures (see Table 1
206 for the temperature regime of locality). From each location, we thus sampled colonies
207 morphologically similar and occupying the same water depth niche. To account for

208 possible intra-population diversity, three colonies (>20 cm in diameter) were collected
209 in each locality, and separated by at least 10 m to decrease the probability to collect
210 members of the same genet, as some *Pocillopora* species are able to propagate by
211 asexual reproduction (Adjeroud *et al.* 2013; Gélin *et al.* 2017a; 2018). Immediately
212 following collection, a 1 cm branch tip of each colony was excised, rinsed three times in
213 filtered seawater (0.22 µm), and placed in RNAlater solution (Sigma Aldrich) for the *in*
214 *situ* microbiota analysis. The rest of the colony was fragmented into 20 branches each of
215 10 cm length and physiologically stabilized in openwater system for one week before
216 shipping (Al-Hail field stationof the Sultan Qaboos University and the Public aquarium of
217 Noumea for OM and NC localities respectively). For shipping, individual branches were
218 placed in plastic bags containing oxygenated seawater (800mL seawater and 1600mL of
219 medical oxygen), and transported by aircraft to the research aquarium of the Banyuls-
220 sur-Mer oceanographic observatory (France). The coral branches were maintained in
221 artificial seawater (Seachem Reef Salt) at 26°C, and supplied daily with *Artemia* nauplii
222 to satisfy their heterotrophic demand. The conditions in the maintenance tank were
223 controlled to mimic the natural physicochemical parameters of coral reefs (pH:8.2;
224 salinity: 36; light intensity: 150 to 250 µmol of photons/m^2/s; photoperiod: 12h
225 night/12h day;kH: 6–7.5 dkH; calcium concentration: 410–450 mg/L; inorganic
226 phosphate concentration: < 0.1 mg/L; magnesium concentration: 1300–1400 mg/L;
227 nitrate concentration: < 5 mg/L). After 3 and 7 months of acclimatization to the
228 laboratory condition (marked by growth resumption) for Om and NC colonies,
229 respectively, corals were fragmented to produce a total of ~15 to 20 clones (nubbins)
230 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 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: 100L/h in each tank). The eight tanks shared the same seawater but their temperature was monitored individually (HOBBY BiothermPro, model 10892; 500W Aqua Medic titanium heater; HOBO TidbiT v2 logger) (Supplementary Figure S1). For each locality, 5 to 8 nubbins per mother colonies were randomly placed in each tank (four tanks per locality) for two weeks at the control temperature and the following protocol was applied: three tanks were then subjected to a gradual temperature increase (stress treatment) while the fourth (control) was maintained at the control temperature to verify that the stress observed in the stressful treatment was not due to other potential confounding effects or water cues (Figure 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

255 gradually by 1°C (over 5 consecutive hours) each week until physiological collapse of the
256 corals became evident (polyps closure, bleaching or necrosis), as described by (Vidal-
257 Dupiol *et al.* 2009). Sampling was performed in the three sampling tanks just before the
258 first temperature increase (control condition) as well as each week before the next
259 temperature increase. The beginning of polyp closure was consistently observed for the
260 different colonies of the same locality at the same temperature threshold. Samples for
261 subsequent genetic and transcriptomic analyses were chosen *a posteriori*. They
262 corresponded to those sampled in each tank just before the first increase of temperature
263 (control samples), and just before the temperature that produced the first signs of
264 physiological collapse and before bleaching (stress temperature samples). Thus, for each
265 condition (control and stress) we obtained three biological replicates of each colony
266 from the three different tanks (three colonies per locality) to reach a total of 36 samples
267 (2 localities × 3 colonies× 2 experimental conditions × 3 replicates/tanks). The general
268 health of the nubbins was assessed via daily photographic monitoring (at noon prior to
269 feeding) throughout the period of the experiment.

270 DNA EXTRACTION

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

275 HOST SPECIES AND CLONEMATES IDENTIFICATION

276 As the *corallum* macromorphology is not a diagnostic criterion in *Pocillopora* genus, the
277 host species was thus identified molecularly. Thus each colony was sequenced for the
278 mitochondrial variable open reading frame (ORF) and was genotyped using 13 specific

microsatellites, as in Gélin *et al.* (Gélin *et al.* 2017b). Then each colony used in the experiment was assigned to Primary and Secondary Species Hypothesis (PSH and SSH; *sensu* Pante et al.) (Pante *et al.* 2015) following the nomenclature from Gélin *et al.* (Gélin *et al.* 2017b). Indeed, sampling *Pocillopora* colonies presenting various morphs from different locations from the Indo-Pacific, Gélin et al classified these colonies, without *a priori* based on *corallum* macromorphology ,into Species Hypotheses (*sensu* Pante et al., i.e. the species are hypotheses that can be confirmed or refuted while new data are added) (Pante *et al.* 2015) using sequence-based species delimitation methods, a first sorting allowed to define Primary Species Hypotheses (PSH) and then individual clustering based on microsatellite multilocus genotypes allowed a second sorting delimiting Secondary Species Hypotheses (SSH). Thus comparing the ORF sequences obtained in this study to those from (Gélin *et al.* 2017b), the sampled colonies were assigned to a PSH. Then, if relevant, the colonies were assigned to SSH performing clustering analysis using Structure 2.3.4 (Pritchard *et al.* 2000), as in (Gélin *et al.* 2017b). Meanwhile, the identical multi-locus genotypes (i.e. clonemates if any) were identified by microsatellite analysis using GenClone (Arnaud-Haond & Belkhir 2006) as in Gélin *et al.* 2017a).

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.

303 AMPLICON SEQUENCING

304 A bacterial 16S rDNA amplicon library was generated for each of the 42 samples (one *in*
305 *situ* condition, three control conditions and three stress conditions per colony, three
306 colonies per locality, two localities), using the 341F (CCTACGGGNGGCWGCAG) and 805R
307 (GACTACHVGGGTATCTAATCC) primers, which target the variable V3/V4 loops
308 (Klindworth *et al.* 2012). The Symbiodiniaceae assemblages were analyzed using ITS2
309 (internal transcribed spacer of the ribosomal RNA gene) amplicon libraries and specific
310 primers targeting a sequence of approximately 350 bp (ITS2-F
311 GTGAATTGCAGAACTCCGTG; ITS2-R CCTCCGCTTACTTATATGCTT) (Lajeunesse &
312 Trench 2000; Quigley *et al.* 2014). For both markers, paired-end sequencing using a
313 250 bp read length was performed on the MiSeq system (Illumina) using the v2
314 chemistry, according to the manufacturer's protocol at the Centre d'Innovation Génome
315 Québec and McGill University, Montreal, Canada.

316 BIOINFORMATIC ANALYSIS:

317 The FROGS pipeline (Find Rapidly OTU with Galaxy Solution) implemented on a Galaxy
318 platform (http://sigenae-workbench.toulouse.inra.fr/galaxy/) was used for data
319 processing (Escudié *et al.* 2017). In brief, paired reads were merged using FLASH
320 (Magoč & Salzberg 2011). After cleaning and removal of primer/adapters using cutadapt
321 (Martin 2011), *de novo* clustering was performed using SWARM (Mahé *et al.* 2014). This
322 uses a local clustering threshold with an aggregation distance (d) of 3. Chimeras were
323 removed using VSEARCH (Rognes *et al.* 2016). We filtered the dataset for singletons and
324 performed affiliation using Blast+ against the Silva database (release 128, September
325 2016) for 16S amplicons (Altschul *et al.* 1990). For ITS2 metabarcoding, the
326 Symbiodiniaceae type was assessed using Blast+ against an in-house database of

327 Symbiodiniaceae reference sequences built from sequences publicly available. An OTU
328 table in standard BIOM format with taxonomic affiliation was produced for subsequent
329 analyses.

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

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

338 TRANSCRIPTOME ANALYSIS

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

341 RNA EXTRACTION

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

348 BIOINFORMATIC ANALYSES

349 Fastq read files were processed on the Galaxy instance of the IHPE (http://bioinfo.univ-
350 perp.fr) (Giardine *et al.* 2005). Quality control and initial cleaning of the reads were

351 performed using the filter by quality program (version 1.0.0) based on the FASTX-toolkit
352 (Blankenberg *et al.* 2010). Reads having fewer than 90% of bases having a Phred quality
353 score ≤ 26 were discarded (probability of 2.5 incorrect base call out of 1000, and a base
354 call accuracy of 99.75%). Adaptors used for sequencing were removed using the
355 cutadapt program version 1.6 (Martin 2011). All paired-end reads were aligned using
356 RNAstar software under default parameters, with at least 66% of the bases being
357 required to align to the reference, and a maximum of ten mismatches per read (Dobin *et*
358 *al.* 2013). The *Pocillopora damicornis sensu lato* reference genome used in this study
359 (manuscript in preparation) consisted of a draft assembly of 25,553 contigs (352 Mb
360 total) and N50 = 171,375 bp. The resulting transcriptome served as the reference for
361 reads mapping, and a GTF annotation file was constructed using cufflink/cuffmerge
362 (Trapnell *et al.* 2010). HTseq was used to produce count files for genes (Anders *et al.*
363 2015). The DESeq2 package was used to estimate the normalized abundances of the
364 transcripts, and to calculate differential gene expression for samples between the
365 control temperature and the stress temperature for each locality (Love *et al.* 2014),
366 considering the different genotypes (three biological replicates for each genotype) and
367 using default parameters. We next analyzed genes according to their expression
368 patterns among the different colonies and temperature treatments. Genes were
369 clustered manually into six groups according to their differential expression levels:
370 common over-expressed genes, NC-specific over-expressed genes, Om-specific over-
371 expressed genes, common under-expressed genes, NC-specific under-expressed genes,
372 and Om-specific under-expressed genes. Cluster 3.0 (de Hoon *et al.* 2004) and Treeview
373 (Saldanha 2004) were used to build the heatmap.

374

375 DISCRIMINANT ANALYSIS OF PRINCIPAL COMPONENTS (DAPC):
376 Our aim was to quantify and compare the level of genome-wide transcriptome plasticity
377 between colonies from Om and NC in response to heat stress. To achieve this we
378 performed a discriminant analysis of principal components (DAPC) based on a log-
379 transformed transcript abundance matrix (containing 26,600 genes) obtained from the
380 36 samples (*i.e.* 9 control and 9 stressed replicates per locality), as described previously
381 (Kenkel & Matz 2016). Specifically, we ran a DAPC analysis using the resulting log2
382 transformed dataset for the colonies from NC and Om reared in controlled conditions as
383 predefined groups in the *adegenet* package implemented in R (Jombart *et al.* 2010). Two
384 principal components and a single discriminant function were retained. We then
385 predicted the position of stressed colonies from both localities (Om and NC) onto the
386 unique discriminant function of the DAPC.

387 We next ran a general linear model (GLM) using the DAPC scores as dependent variable,
388 and accounted for the locality of origin (NC and Om), the conditions (control and heat
389 stress), and their interaction as explanatory variables. We also considered the effect of
390 individual colonies nested within localities as random effects in the model. Our final
391 objective was to test for a potential significant effect of the interaction between the
392 locality and the condition effects, as a proxy of significant differences in the genome-
393 wide gene expression reaction norms (i.e. differences in DAPC scores between the
394 control and the heat stress treatments) between Om and NC colonies..

395 GO ENRICHMENT OF DIFFERENTIALLY EXPRESSED GENES
396 The transcriptome was annotated *de novo* using a translated nucleotide query (blastx
397 (Altschul *et al.* 1990)) against the non-redundant protein sequence database (nr). The
398 25 best hits were then used to search for gene ontology terms using the Blast2Go

399 program (Conesa *et al.* 2016). To identify the biological functions significantly enriched
400 within up or down-regulated genes, a Gene Ontology (GO) term enrichment analysis was
401 performed. Lists of GO terms belonging to significantly up-regulated and down-
402 regulated genes were compared to the GO terms of the whole expressed gene set using a
403 Fischer exact test and a FDR value of 0.05. We used REVIGO to visualize the enriched
404 biological processes (Supek *et al.* 2011).

405 RESULTS

406 HOST IDENTIFICATION

407 Among the three colonies from New Caledonia, colonies NC2 and NC3 presented
408 haplotype ORF18 and were assigned to Primary Species Hypothesis PSH05and more
409 precisely to Secondary Species Hypothesis SSH05a (Gélin *et al.* 2017b), corresponding to
410 *P. damicornis* type *β* (Schmidt-Roach *et al.* 2014) or type 5a (Pinzón *et al.* 2013), while
411 colony NC1 presented ORF09 and was assigned to PSH04, *P. damicornis* type *α*,
412 *P. damicornis* or type 4a, respectively. As for colonies from Oman, they all presented
413 ORF34 and were assigned to PSH12 (Gélin *et al.* 2017a) or type 7a (Pinzón *et al.* 2013)
414 (Supplementary Table S2), that is not part of the *P. damicornis sensu lato* species
415 complex. Thus NC colonies are phylogenetically closer from each other than from
416 colonies from Oman. These three PSHs represent three different species.

417 Furthermore, NC2 and NC3 multi-locus genotypes (MLGs) differed only from one allele
418 over 26 gene copies, and were thus part of the same clonal lineage (genet), i.e. the entity
419 that groups together colonies whose multi-locus genotypes slightly differ due to somatic
420 mutations or scoring errors. All the other colonies presented MLG that differed enough
421 not to be considered as clonemates or members of the same clonal lineage (genet).

422 ECOLOGICALLY REALISTIC HEAT STRESS

423 Our goal was to ensure that our experimental heat stress faithfully reflects a realistic
424 heat stress *in natura*. Following collection from the field, the corals from the different
425 localities were first maintained in the same controlled conditions at 26°C prior to the
426 experiment. During this period no mortality or signs of degradation/stress were
427 observed for any of the coral colonies. Two weeks before the experiment, a first
428 acclimatization to the control temperatures (27°C or 31°C for NC and Om respectively)
429 was performed. During the experimental heat stress (i.e. gradual temperature increase),
430 visual and photographic monitoring clearly indicated that the first sign of coral stress
431 (i.e. the closure of polyps) occurred at day 30 for both sampling localities, corresponding
432 to 30°C and 34°C for the NC and Om colonies, respectively. These temperatures perfectly
433 match the warmest temperature experienced by these colonies in the field (Table 1). No
434 signs of physiological collapse were observed in control corals throughout the
435 experiment indicating that all the other parameters were maintained optimal for coral
436 colonies.

437 BACTERIAL COMMUNITIES

438 Among the overall 42 samples analyzed, a total of 5,308,761 16S rDNA amplicon
439 sequences were obtained after cleaning and singleton filtering corresponding to 15,211
440 OTUs. In all samples the class Gammaproteobacteria was dominant (77.7%), particularly
441 the genus *Endozoicomonas* (44.7% of the sequences); this genus is known to be an
442 endosymbiont of numerous scleractinians (Neave *et al.* 2016b) (See Supplementary
443 Figure S3 for complete bacterial composition in each colony and replicate). The PCoA of
444 Bray-Curtis distances for all colonies showed no evident clusters based on the
445 experimental treatments (Figure 2). We observed a loose grouping based on localities

446 and colonies, except for colony NC1, which appeared to have a more specific microbiota
447 composition, as it had a different grouping associated with the first axis, which
448 explained22.3% of the variance. This could be correlated with the different species
449 hypotheses for NC1 compared to NC2 and NC3 (see above). The one-way ANOVA for
450 alpha diversity (Shannon index) revealed significant differences in the microbiota
451 diversity between localities (P <0.05) and colonies (P < 0.05), but no differences among
452 the *in situ,* control and stress conditions (P=0.885). Similar results were obtained for the
453 beta-diversity (Bray-Curtis distance) (ANOVA between localities: P<0.05; between
454 colonies: P<0.05; between conditions: P=0.554; the ANOVA results are provided in
455 Supplementary Table S4). Thus, the bacterial composition appeared to be relatively
456 specific to each colony within each locality, but no major shift was observed during the
457 transition from the natural environment to artificial seawater, nor during heat stress
458 exposure.

459 SYMBIODINIACEAE ASSEMBLAGES

460 Analysis of the Symbiodiniaceae composition was performed based on an ITS2
461 metabarcoding, which allowed intra-clade resolution.

462 Removal of OTUs having an abundance of < 1% left only 4 OTUs among all samples. Two
463 of these corresponded to type C1, while the other two corresponded to type D1a
464 according to (Baker 2003). Type D1a was highly dominant in the colonies originating
465 from Oman, whereas type C1 was almost exclusive to the corals from New Caledonia
466 (Figure 3). The Symbiodiniaceae community composition was very specific to each
467 locality, but remained stable during the transition from the natural environment to
468 artificial seawater, and during heat stress exposure.

469 HOST TRANSCRIPTOME ANALYSIS

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

472 Overall, the transcriptome sequencing of these36 samples yielded 1,970,889,548 high
473 quality Illumina paired reads of 100 bp. Globally, 40–64% of reads obtained for the Om
474 colonies, and 59–70% of reads obtained for NC colonies successfully mapped to the
475 *Pocillopora damicornis* (type β) reference genome. The apparently better alignment of
476 samples from New Caledonia most likely relies on the fact that the New Caledonia
477 colonies used in this study belong to *P. damicornis* types αor 4a (PSH04) and βor 5a
478 (PSH05), which are phylogenetically close to each other and closer from the reference
479 genome, than the Om colonies from type 7a (PSH12) that is phylogenetically more
480 distant from the reference genome. The aligned reads were assembled in 99,571 unique
481 transcripts (TCONS), representing putative splicing variants of 26,600 genes identified
482 as "XLOC" in the genome (FASTA sequences available in Supplementary File S5).

483 The hierarchical clustering analyses clearly grouped together samples belonging to the
484 same locality and species hypothesis according to their genome-wide gene expression
485 patterns, in link with the phylogenetic differences between the NC and Om haplotypes
486 (Figure 4). Within locality and species hypothesis, the transcriptomes also grouped by
487 colony, indicating that the transcriptomes were genotype-specific. For each colony, the
488 transcriptomes then grouped by condition (control or heat stress), except for New
489 Caledonia colonies NC2 and NC3 (corresponding to the same clonal lineage) that
490 clustered together when exposed to control and heat stress conditions.

491 Despite clustering of the transcriptomes by locality, as the sampling of *Pocillopora*
492 *damicornis*-like colonies actually corresponded to different species we performed
493 differential gene expression analysis for each colony independently (comparing the
494 biological triplicates for the control condition vs. triplicates for the heat stress
495 conditions). For each locality, the different colonies displayed similar patterns of
496 differential gene expression with in any case a higher number of differentially expressed
497 genes and higher fold-changes between control and heat stress condition in Om
498 compared to NC (Supplementary Figure S6). We detected 673, 479 and 265
499 differentially expressed genes for NC1, NC2 and NC3 respectively, vs. 2870, 2216 and
500 959 for Om1, Om2 and Om3. Samples were thus grouped for each locality (nine control
501 nubbins + nine heat stress nubbins) for subsequent analyses (full results of the
502 comparisons between stressed and controls (log2-foldchange and adjusted *p*-values) for
503 each colony or between the two localities are provided in Supplementary File S7). For
504 Om colonies, a total of 5,287 genes were differentially expressed between control and
505 stress conditions. This number was much lower for NC colonies with 1,460 differentially
506 expressed genes (adjusted $P < 0.05$).

507 Among differentially expressed genes genes, 848 were differentially expressed in the
508 same direction in both localities (498 over-expressed and 350 under-expressed).
509 Nevertheless, the differential expression level was significantly higher for the Om corals
510 with a mean log2-fold change of 0.9 for shared over-expressed genes in Om *vs.* 0.6 in NC,
511 and -1.2 for the shared under-expressed genes in Om *vs.* -0.8 in NC (Wilcoxon test; $P <$
512 0.0001) (Figure 6 and Supplementary Table S8).

513 Additionally, colonies from the two localities also responded specifically to heat stress.
514 In particular, 272 genes were over-expressed and 294 were under-expressed only in the
515 NC corals, whereas 2,082 were over-expressed and 2,311 were under-expressed only in
516 the Om ones when exposed to heat stress. Finally, the colonies from both localities
517 displayed antagonistic transcriptomic responses to heat stress for a small subset of
518 genes (24 over-expressed in NC but under-expressed in Om, and 22 under-expressed in
519 NC but over-expressed in Om).

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

523 DISCRIMINANT ANALYSIS OF PRINCIPAL COMPONENTS (DAPC):
524 At the overall gene expression level, our DAPC analysis clearly discriminated the
525 colonies from both localities (Figure 7). More interestingly, the GLM revealed a
526 significant interaction term between the locality and condition (control or heat stress)
527 effects (P = 0.04), hence indicating that the slope of the reaction norm was different
528 between localities. More particularly, the Om colonies responded to a greater extent
529 than the NC ones, and thus showed significantly higher gene expression plasticity in
530 response to heat stress.

531 It is worth stressing that colonies having experienced the heat stress displayed more
532 similar genome-wide expression profiles than controlled colonies (Fig. 7). Such pattern
533 was also observed in colonies of mustard hill coral *P. astreoides* experiencing a heat
534 stress compared to controls (Kenkel & Matz 2016). This apparent convergence in the
535 functional response of colonies from different habitats to heat stress might be at least

536 partly explain by the fact some common molecular pathways are turned-on when
537 colonies are facing stressful conditions although the magnitude of such responses is
538 different.

539

540 ANALYSIS OF GENE FUNCTION:
541 To investigate the functions associated with the differentially expressed genes we
542 performed a blastx annotation of transcripts followed by a Gene Ontology (GO) term
543 (biological process, molecular function, and cell compartment) (Supplementary FileS9).

544 For the 498 common over-expressed genes, 139 biological processes were enriched
545 compared to the entire set of annotated genes. The most significant biological process
546 identified in the REVIGO analysis (i.e. with lowest FDR= 2.1×10^{-68}) was response to
547 stress (Figure 8). Following this sequentially, were cellular metabolism (FDR=3.7×10^{-49}),
548 positive regulation of biological processes (FDR = 2.4×10^{-43}), cell death (FDR = $2.5\times10^{-}$
549 ^{33}), cellular localization (FDR = 8.4×10^{-25}), and pigment metabolism (FDR = 2.1×10^{-21}).
550 Among the 272genes over-expressed in the NC but not in the Om colonies in response to
551 heat stress, 38 biological processes were enriched: organic acid catabolism (FDR =
552 1.6×10^{-22}), protein transport (FDR = 1.8×10^{-16}), stress response (FDR = 4.8×10^{-13}), and
553 cellular metabolism (FDR = 3×10^{-12}) were the four most significantly enriched biological
554 processes (Figure 8). Among the 2,082 genes over-expressed in the Om but not in the NC
555 colonies in response to heat stress, 160 biological processes were enriched, the most
556 significant being ncRNA metabolism (FDR = 8.9×10^{-303}), cellular metabolism (FDR =
557 4.4×10^{-70}), carbohydrate derivative biosynthesis (FDR = 5.9×10^{-64}), and organic
558 substance transport (FDR = 2×10^{-44}).

559 For the 350 genes that were under-expressed following heat stress irrespective to the
560 locality of origin (Om or NC), 48 biological processes were enriched and grouped into
561 five biological processes: nitrogen compound transport (FDR = 5.4×10^{-89}), localization
562 (FDR = 8.1×10^{-10}), regulation of neurotransmitter levels (FDR = 1.2×10^{-8}), system
563 development (FDR = 8.8×10^{-6}), and single organism process (FDR = 4×10^{-4}). Among the
564 under-expressed genes in the NC colonies only, a single biological process (anatomical
565 structure/morphogenesis) was found to be enriched (FDR = 9×10^{-3}). Among the under-
566 expressed genes in the Om colonies, 139 biological processes were enriched, with the
567 most significant being ion transmembrane transport (FDR = 7.6×10^{-104}), single
568 multicellular organism process (FDR = 7.5×10^{-53}), regulation of biological quality (FDR =
569 6×10^{-48}), cell–cell signaling (FDR = 1.5×10^{-23}), single organism process (FDR = $1.1\times10^{-}$
570 ^{18}), multicellular organism process (FDR = 1.5×10^{-16}), biological regulation (FDR =
571 2.3×10^{-15}), response to abiotic stimulus (FDR = 6.2×10^{-13}),and localization (FDR =
572 4.6×10^{-12}).

573 The complete results for all GO term categories including molecular function are
574 available in Supplementary File S9.

575 Regarding cellular compartments, the mitochondria was the most significantly enriched
576 in the common over-expressed genes (FDR = 1.5×10^{-180}), as well as among genes over-
577 expressed in NC (FDR = 2.5×10^{-82}) while in genes over-expressed in Om corals the
578 intracellular organelle lumen was the most significantly enriched (FDR = 1×10^{-560}).

579 To investigate whether the presumably more thermotolerant colonies from Oman
580 displayed a frontloading strategy (i.e. a higher expression for some genes compared to

581 the colonies from NC) as previously described in scleractinian corals (Barshis *et al.*
582 2013), we compared the gene expression levels in control conditions between Om and
583 NC colonies for those genes that were over-expressed in NC colonies (Supplementary
584 File S10). This comparison revealed that the constitutive expression level was often
585 greater in the Om colonies. Among the 770 genes that were over-expressed in NC
586 colonies in response to thermal stress (272 specifically and 498 in common with Om),
587 484 were constitutively (i.e. in the control condition) more expressed in Om. Among
588 these genes,163 were not differentially expressed between the control and stress
589 temperatures reflecting true frontloading based on the definition of Barshis et al.
590 (2013), while 301 were over-expressed and only 20 were under-expressed during heat
591 stress. These 484 genes with higher constitutive expression in Om were submitted to GO
592 term enrichment analysis. No significant results were found for the under-expressed
593 genes. The frontloaded genes were enriched in the biological processes cellular
594 respiration (FDR = 4.4×10^{-23}), cellular component organization (FDR = 0.002),
595 homeostatic process (FDR = 0.005), cellular component organization or biogenesis (FDR
596 = 0.007), cofactor metabolism (FDR = 0.009), and stress response (FDR = 0.009), and in
597 the mitochondrion for the most significant cellular compartment (FDR = 1.6×10^{-66}).
598 Most interestingly, for genes associated with a higher basal expression level together
599 with over-expression in the Om colonies, the most enriched biological processes were
600 stress response (FDR = 1.2×10^{-26}), pigment metabolism (FDR = 5.1×10^{-24}), regulation of
601 phosphate metabolism (FDR = 3.2×10^{-15}), cellular metabolism (FDR = 2.7×10^{-11}), and
602 protein folding (FDR = 7.3×10^{-6}).

603

DISCUSSION

SPECIFIC CONTEXT OF ADAPTATION

Our aim was to compare the phenotypic plasticity in terms of transcriptomic response to heat stress of coral colonies originating from different localities displaying contrasted thermal regimes. As morphology can be misleading for species identification in scleractinians, notably in *Pocillopora* genus (Gélin et al., 2017a), we used a molecular approach to test the species relationships of our samples. The analysis of mitochondrial sequences and clustering analyses indicated that, despite similar morphologies, our samples corresponded to different species. This agrees well with previous works showing the importance of cryptic lineages and morphological plasticity in the *Pocillopora* genus (Gélin *et al.* 2017a and references herein). Oman colonies corresponded to species hypothesis PSH12 of (Gélin *et al.* 2017b),which is restricted to the Northwestern Indian Ocean. Regarding the two species hypotheses from NC, SSH05a (*P. damicornis* type β SSH05a or *P. acuta*) is found in the Pacific Ocean and PSH04 (*P. damicornis* type α or *P. damicornis sensu stricto*) is nearly exclusively found in the Pacific Ocean (very rare in the Indian Ocean, and not found yet in Red Sea) (Gélin *et al.* 2017b). It would be interesting to study whether inside each species hypothesis, different thermotolerance phenotypes are present. Conversely, the observation of a similar response to thermal stress in two different species in NC, as revealed by differential gene expression as well as DAPC analyses, could indicate either a conserved strategy or a convergence under the same ecological conditions.

AN ECOLOGICALLY REALISTIC HEAT STRESS

The heat stress applied in this study was ecologically realistic, since the first visual response (i.e. polyp closure) was observed for all colonies when the gradually increasing

628 experimental temperature reached the upper temperature they are subjected to *in*
629 *natura* (30°C and 34°C for NC and Om corals, respectively). From a biological point of
630 view this first result hence clearly supports that these colonies from two localities that
631 are experiencing two different thermal regimes *in natura* display differential ability to
632 deal with heat stress. Moreover, the accurate control of all other seawater parameters
633 allows us to consider that the holobiont response to the thermal treatment is specific to
634 heat stress and not to other possible confounding effects. Last, as we analyzed the
635 samples before the first visible signs of stress (polyp closure), any change in the
636 holobiont would therefore reflect the response to the heat stress and not homeostasis
637 breakdown after disruption of the coral integrity.

638 SYMBIOTIC COMMUNITY: BACTERIAL AND SYMBIONACEAE COMPOSITION

639 For the bacterial community, we identified significant differences between localities and
640 colonies. The microbiota composition of all samples was consistent with previous
641 studies, showing a high proportion of Gammaproteobacteria and dominance of the
642 symbiotic *Endozoicomonas* genus (Bourne & Munn 2005; Neave *et al.* 2016a; Peixoto *et*
643 *al.* 2017). However, our results clearly demonstrate that neither maintenance in the
644 experimental structure nor experimental heat stress induced major bacterial community
645 changes in coral colonies irrespective to their locality of origin. For the Symbionaceae
646 community, the ITS2 metabarcoding analysis enabled inter-clade resolution (Quigley *et*
647 *al.* 2014). Two distinct types of D1a and C1 clades dominated, representing most of the
648 sequences in the Om and NC corals, respectively. Nine ITS types (A to I) have been
649 identified in the former genus *Symbiodinium* (Baker 2003). Some Symbiodiniaceae
650 strains strongly participate to the overall holobiont fitness, with type D providing
651 tolerance to higher temperatures (Berkelmans & van Oppen 2006) and C1 enhancing

652 coral growth rates (Little *et al.* 2004). Interestingly, we found that the type D1a is
653 dominant in the more thermotolerant Om corals, which is consistent with the results of
654 previous works (Berkelmans & van Oppen 2006), however recent results shows that
655 such an association is rather linked with minimal temperatures than annual amplitude
656 of temperature changes (Brener-Raffalli *et al.* 2018).

657 Although the microbial community (both bacterial and Symbiodiniaceae) differed
658 between the NC and Om corals, the composition did not change during transition from
659 the field to the artificial seawater conditions, and remained similar during the
660 experimental temperature increase. Thus, the coral holobiont assemblage remained
661 stable over the course of the experiment. Such stability of the microbial community
662 during experimental heat stress was previously observed in the scleractinian *Acropora*
663 *millepora* (Bellantuono *et al.* 2012b) and *A. tenuis* (Littman *et al.* 2010). Thus, our study
664 conforms to the idea that microbial communities associated with scleractinian corals
665 remain unchanged when the holobionts are exposed to stressful temperatures (but see
666 (Ziegler *et al.* 2017)) but further analyses of gene expression level would be needed to
667 assess their functional responses. RNA-sequencing of eukaryotic poly-adenylated mRNA
668 would allow in principle dual analysis of Symbiodiniaceae and coral host transcripts
669 {Mayfield:2014et}, but since our RNA extraction method resulted in very few algal
670 transcripts, we only focused on the host transcriptomic response.

671 Based on these results, we investigated changes in host gene expression as the main
672 mechanism of response to heat stress in our experimental design.

673 HOST TRANSCRIPTOMIC RESPONSE

674 Given the observed stability of the microbial symbiotic community during heat stress,
675 we focused more specifically on the responses attributable to the coral host. We thus
676 compared gene expression patterns at the qualitative and quantitative levels in Om and
677 NC colonies in response to heat stress compared to the control condition. Altogether, our
678 results clearly highlight that the Oman colonies exposed to more variable thermal
679 conditions *in natura* also display, in response to heat stress, a greater plasticity in gene
680 expression levels than the NC colonies. In particular, the transcriptomic response of the
681 Oman colonies involved a larger number of genes with 73% of commonly differentially
682 expressed genes having higher fold changes compared to the NC colonies. These findings
683 are consistent with the theoretical expectations that amore variable environment
684 promotes the evolution of a greater plasticity (Lande 2009). Accordingly, a recent
685 transplantation study conducted *in natura* also identified greater transcriptomic
686 plasticity in a more thermotolerant (in-shore) population compared with an (off-shore)
687 population inhabiting a more stable thermal habitat in the mustard hill coral *P.*
688 *astreoides* (Kenkel & Matz 2016).

689 Importantly however, we also identified several genes whose expression is
690 constitutively higher in the Om colonies compared to the NC colonies by comparing the
691 expression levels in the control condition. This process recently called "frontloading"
692 (Barshis *et al.* 2013) reflects the preemptive expression of stress-response genes, hence
693 predisposing organisms to better respond to stress. It has been proposed that the
694 occurrence of plasticity *vs*. frontloading strategies may depend on the frequency of
695 stresses relative to the typical response time of organisms, with frequent stresses
696 promoting frontloading strategies whereas less frequent perturbations would result in
697 an increased plasticity (Kenkel & Matz 2016). Other conceptual considerations

698 especially in regards to the predictability of environmental variation through
699 generations should also be taken into account (Danchin 2013; Herman *et al.* 2014). The
700 frontloading is by definition more costly than plasticity since it transforms a response to
701 the environment in a constitutive function. Frontloading is therefore a strategy that
702 would be more efficient when offspring's habitat is highly predictable. On the contrary,
703 an unpredictable or less predictable offspring environment may promote plasticity to
704 enable the exploration of a wider phenotypic landscape at a lesser cost. Plasticity and
705 frontloading are often discussed as mutually exclusive responses (Barshis *et al.* 2013;
706 Kenkel & Matz 2016). However, corals are known to display a high level of variation in
707 their reproduction strategies (brooder vs. broadcast spawner) (Whitaker 2006; Baird *et*
708 *al.* 2009), timing (Fan *et al.* 2006) and pelagic larval duration (Harrison & Wallace
709 1990). Environmental predictability in terms of stress frequency and annual
710 temperature variation should be therefore limited and we hypothesized that, rather
711 than being exclusive, plasticity and frontloading often co-occur especially in the
712 population experiencing extreme environments.

713 Our results clearly support that plasticity and frontloading indeed co-occur specifically
714 in the thermotolerant Om colonies experiencing a more variable thermal environment *in*
715 *natura*. To tease apart the biological processes that are regulated via plasticity or
716 frontloading in *Pocillopora* response to heat stress, we conducted an enrichment
717 analysis. Keeping in mind that congruency between gene expression and protein levels
718 should be cautious (Mayfield *et al.* 2016), we propose a detailed discussion of the
719 response of coral colonies at the molecular level for each main biological process
720 identified (Supplementary File S11). Notably, we found differences in gene expression
721 levels in response to temperature increase between the two localities for genes involved

722 in response to heat stress (such as HSPs), detoxification of reactive oxygen species,
723 apoptosis, mitochondria energetic functioning, and symbiont maintenance with higher
724 number of differentially expressed genes for the Om corals associated to higher fold
725 changes. Our results also suggest that allocating energy in heat stress response is at the
726 expense of other crucial biological processes such as growth and reproductive functions,
727 even if we could not test experimentally fitness effect of the experimental heat stress.
728 However, the molecular mechanisms underlying such overall response to heat stress are
729 still partly unresolved. Interestingly, we also found specific gene expression patterns
730 linked with epigenetic regulation that could be involved in such mechanisms and could
731 fuel rapid adaptive evolution (Maumus *et al.* 2009; Torda *et al.* 2017; Jablonka 2017)

732 CONCLUSION:

733 Comparison of the response to an ecologically realistic heat stress of corals from the
734 same genus but pertaining to different species hypotheses thriving in two contrasting
735 thermal environments sheds light on the molecular basis of thermotolerance. We found
736 that during heat exposure, the symbiotic community composition remained stable in
737 colonies from both localities, but we identified major differences in gene regulation
738 processes in the coral, thereby underlining the role of the coral host in the response to
739 heat stress. The colonies from the locality displaying the most variable environment
740 displayed (i) a more plastic transcriptome response involving more differentially
741 expressed genes and higher fold expression changes; as well as (ii) a constitutive higher
742 level of expression for another set of genes (frontloading). In the context of climate
743 change, which is predicted to cause abnormal and rapid temperature increase (IPCC
744 2014), phenotypic plasticity and the capacity for rapid adaptation through epigenetic

745 regulation and/or genetic assimilation would increase the probability of coral survival.
746 Previous studies highlighted the importance of reef managements measures (Rogers *et*
747 *al.* 2015) and assisted evolution (van Oppen *et al.* 2015), but also underlined the
748 importance of preserving standing genetic/epigenetic variation in wild coral
749 populations (Matz *et al.* 2017). Our results also suggest that management measures
750 must include protection of naturally thermotolerant populations as they have the
751 potential to resist increasing thermal anomalies. Although the molecular mechanisms
752 we described are most likely largely shared in this group of scleractinians, the question
753 remains of the determinism of this thermotolerant phenotype and of the heritability of
754 this character. If some loci are responsible for these differences in thermotolerance, the
755 possibility of gene flow between populations or even specific lineages in the genus
756 *Pocillopora*, could deeply impact the response of these species to climate change
757 (Mumby *et al.* 2011) . It is however essential to keep in mind that even the most
758 thermotolerant corals may bleach if they are exposed to temperature significantly
759 higher to their own norm (Hughes *et al.* 2017b; Le Nohaïc *et al.* 2017).

760 ## ACKNOWLEDGEMENTS

761 We are thankful to Dr. Madjid Delghandi from the Center of Marine Biotechnology at
762 Sultan Qaboos University, the Al-Hail field station and the central laboratory of the
763 College of Agricultural and Marine Sciences for providing us with necessary equipment
764 during field work in Oman. We acknowledge Dr Gilles Le Moullac and Dr Yannick
765 Gueguen from the Centre Ifremer du Pacifique for providing us aquaculture facilities
766 during sampling. We acknowledge the Plateforme Gentyane of the Institut National de la
767 Recherche Agronomique (INRA, Clermont-Ferrand, France) for microsatellite

genotyping. We also thank the Genotoul bioinformatics platform, and the Toulouse Midi-Pyrenees and Sigenae group for providing help and computing resources (Galaxy instance; http://sigenae-workbench.toulouse.inra.fr as well as the Bio-Environnement platform (University of Perpignan) for sequencing and bioinformatics service. This project was funded by the ADACNI program of the French national research agency (ANR) (project no. ANR-12-ADAP-0016; http://adacni.imbe.fr), the Campus France PHC program Maïmonide-Israel and the DHOF program of the UMR5244 IHPE (http://ihpe.univ-perp.fr/en/ihpe-transversal-holobiont/). This work is a contribution to the Labex OT-Med (n° ANR-11-LABX-0061) funded by the ANR "Investissementsd'Avenir" programthrough the A*MIDEX project (n° ANR-11-IDEX-0001-02). This study is set within the framework of the "Laboratoire d'Excellence (LABEX)" TULIP (ANR-10-LABX-41). We would like to thank the two reviewers of PCI ecology for their constructive and helpful comments.

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.

790

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

800 # FIGURE LEGENDS

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

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

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

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

817 FIGURE 5: Heatmap and clustering of significantly differentially expressed genes
818 between the control and the heat stress condition for colonies from each locality. Each
819 gene is represented by a line.

820 FIGURE 6: Scatterplot of the log2-fold changes in gene expression in response to heat
821 stress in the Om colonies (y-axis) *vs.* the NC colonies (x-axis) for the 848 genes that were

822 over-expressed (498 genes) or under-expressed (350 genes) in colonies from both
823 localities. The line represents the y=x line depicting similar responses between colonies.

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

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

838

839 ## SUPPLEMENTARY FILES

840 Supplementary Figure S1: Experimental setup. Four tanks were used for each locality, 3
841 tanks containing the sampled colonies (one replicate per timepoint and per tank) and
842 one additional tank as a control of coral health at the control temperature during the
843 experiment.

844 Supplementary Table S2: Haplotype analysis of the six sampled colonies with
845 microsatellite genotyping for the colonies from New Caledonia.

846 Supplementary Figure S3: Bacterial class composition (for the 24 most abundant) within
847 each replicate for the Om and NC colonies, the three colonies of each locality, and three
848 experimental conditions per colony. *In situ* (dark arrows); control temperature (green
849 arrows); stress temperature (red arrows).

850 Supplementary Table S4: ANOVA results for alpha diversity (Shannon index) and beta
851 diversity (Bray-Curtis distance) between localities, colonies, or experimental conditions.

852 Supplementary File S5: List and sequences of the 26,600 genes (XLOC) generated during
853 RNAseq alignment.

854 Supplementary Figure S6: Heatmap and clustering of significantly differentially
855 expressed genes between the control and the heat stress condition for each colony from
856 the two localities. Each gene is represented by a line.

857 Supplementary File S7: DEseq2results for the log2-foldchanges, a nd adjusted *p* values
858 between stress and control conditions for each locality (sheet 1) and for each colony
859 (sheet 2).

860 Supplementary Table S8: Comparison between the log2-foldchange in Om and NC
861 colonies for genes differentially under-expressed or over-expressed in the same way in
862 colonies from both localities.

863 Supplementary File S9: GO enrichment results for biological processes, molecular
864 functions, and cellular compartments for common, New Caledonia-specific, or Oman-
865 specific over-expressed and under-expressed genes.

866 Supplementary File S10: Frontloaded genes in Oman corals among genes over-
867 expressed in New Caledonia corals.

868 Supplementary File S11: Description of the functional analysis of genes, biological
869 functions and cell compartment involved in the response to stress.

870

871

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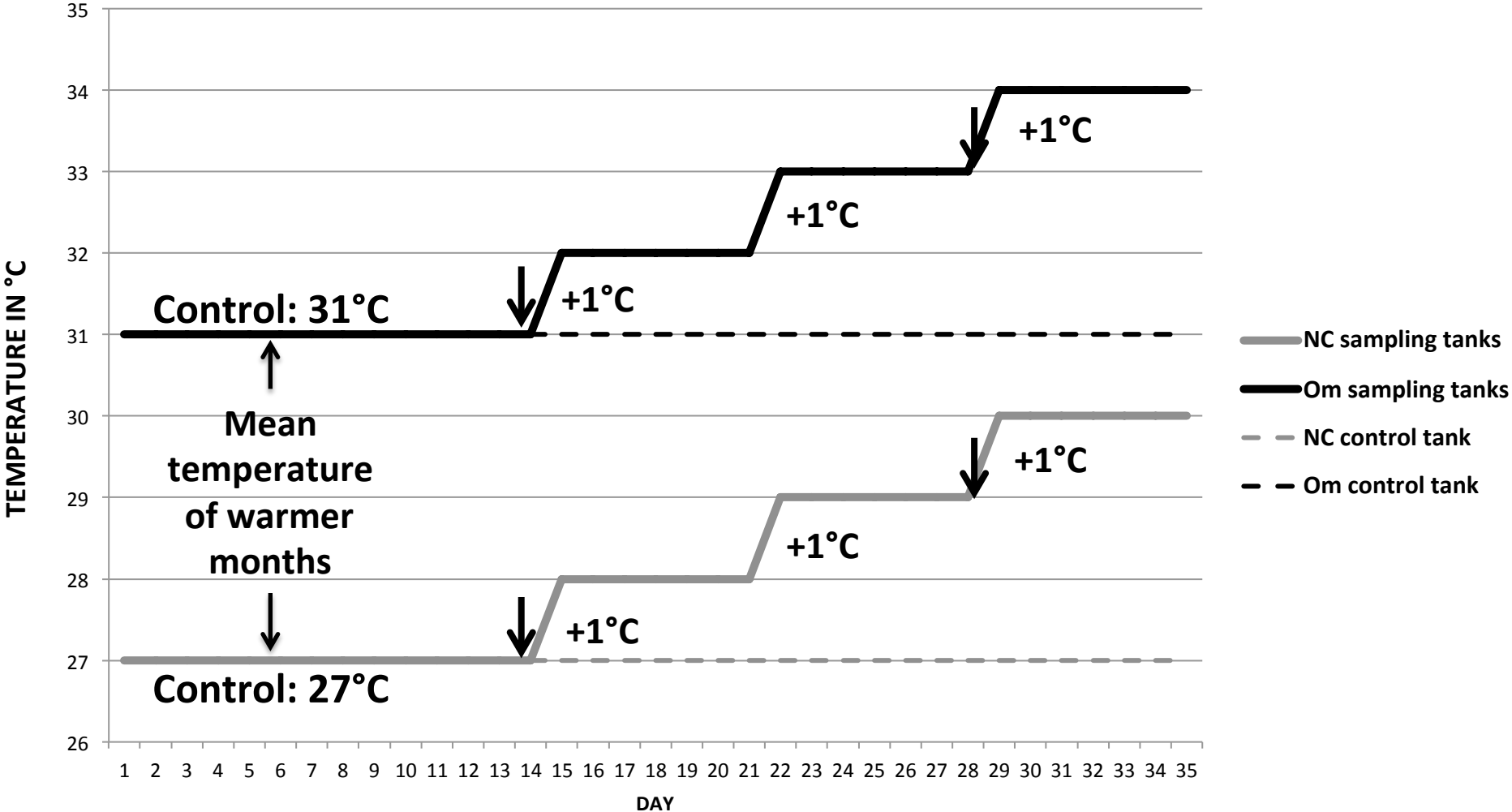
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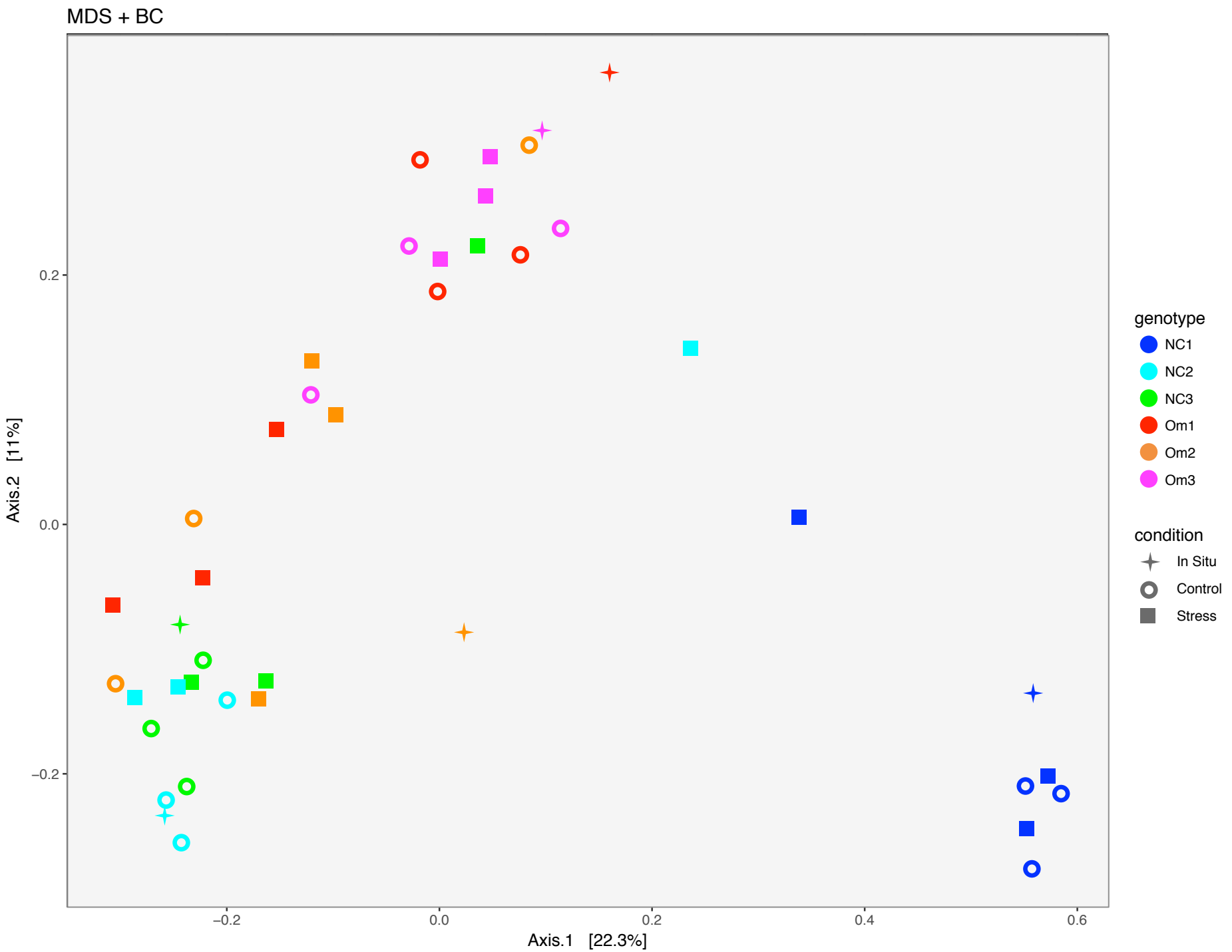
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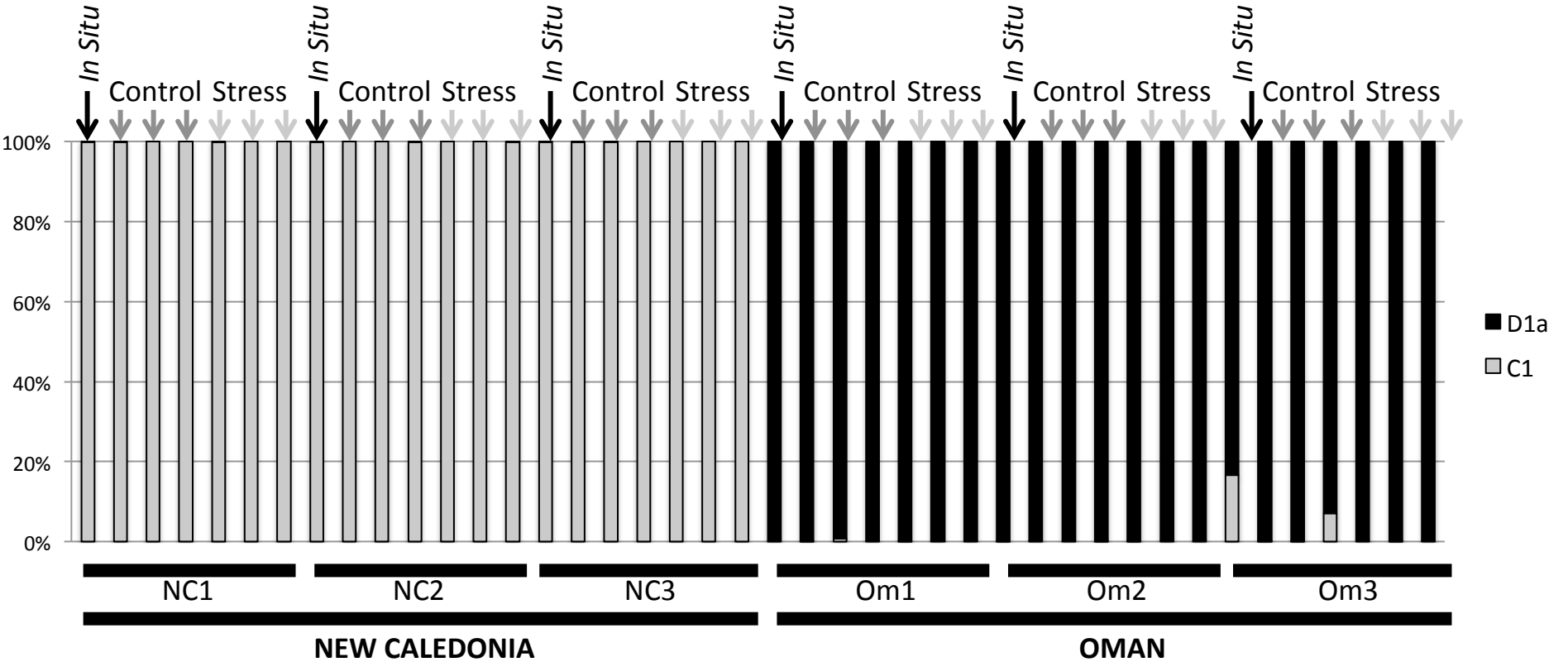
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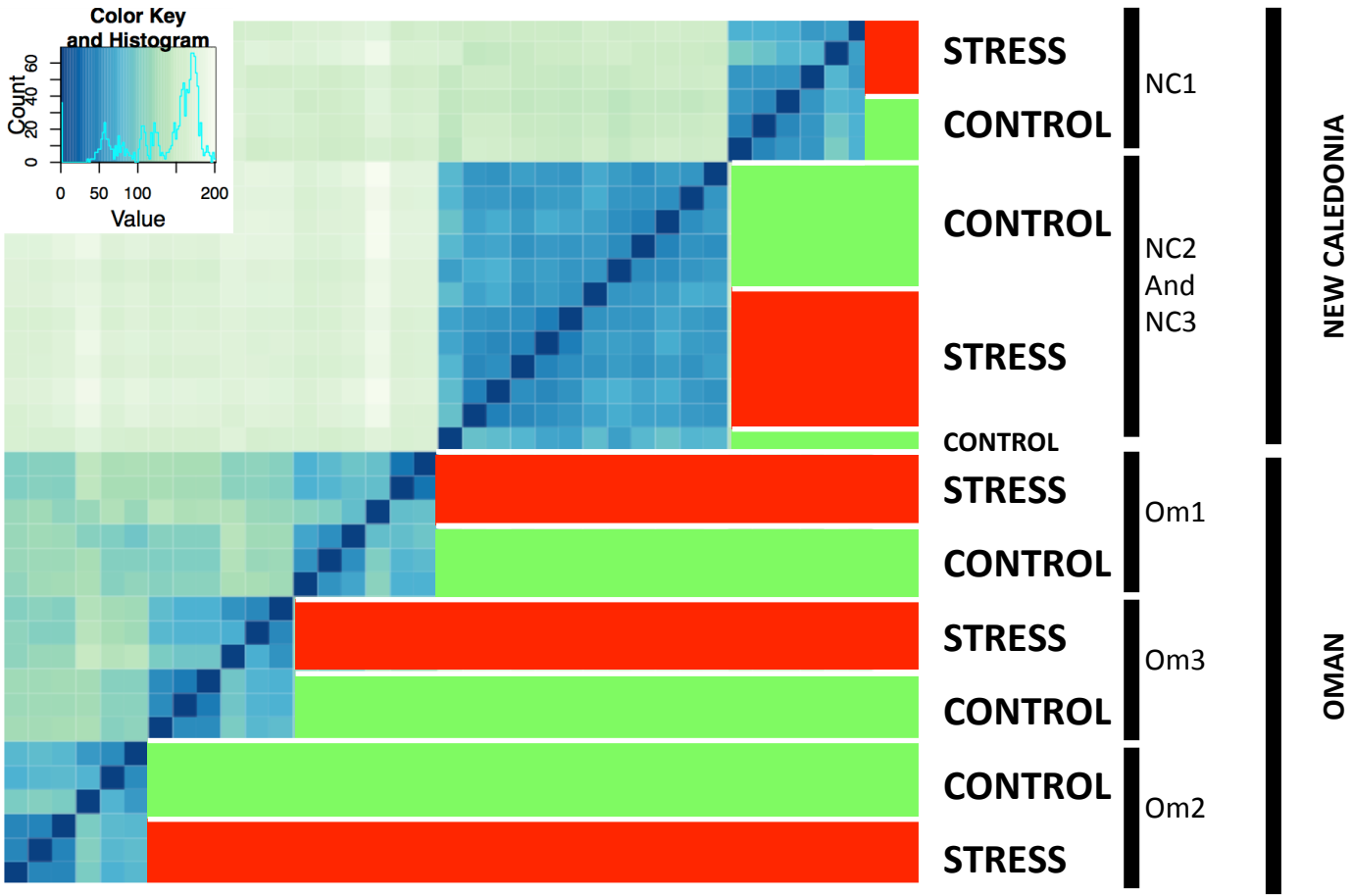
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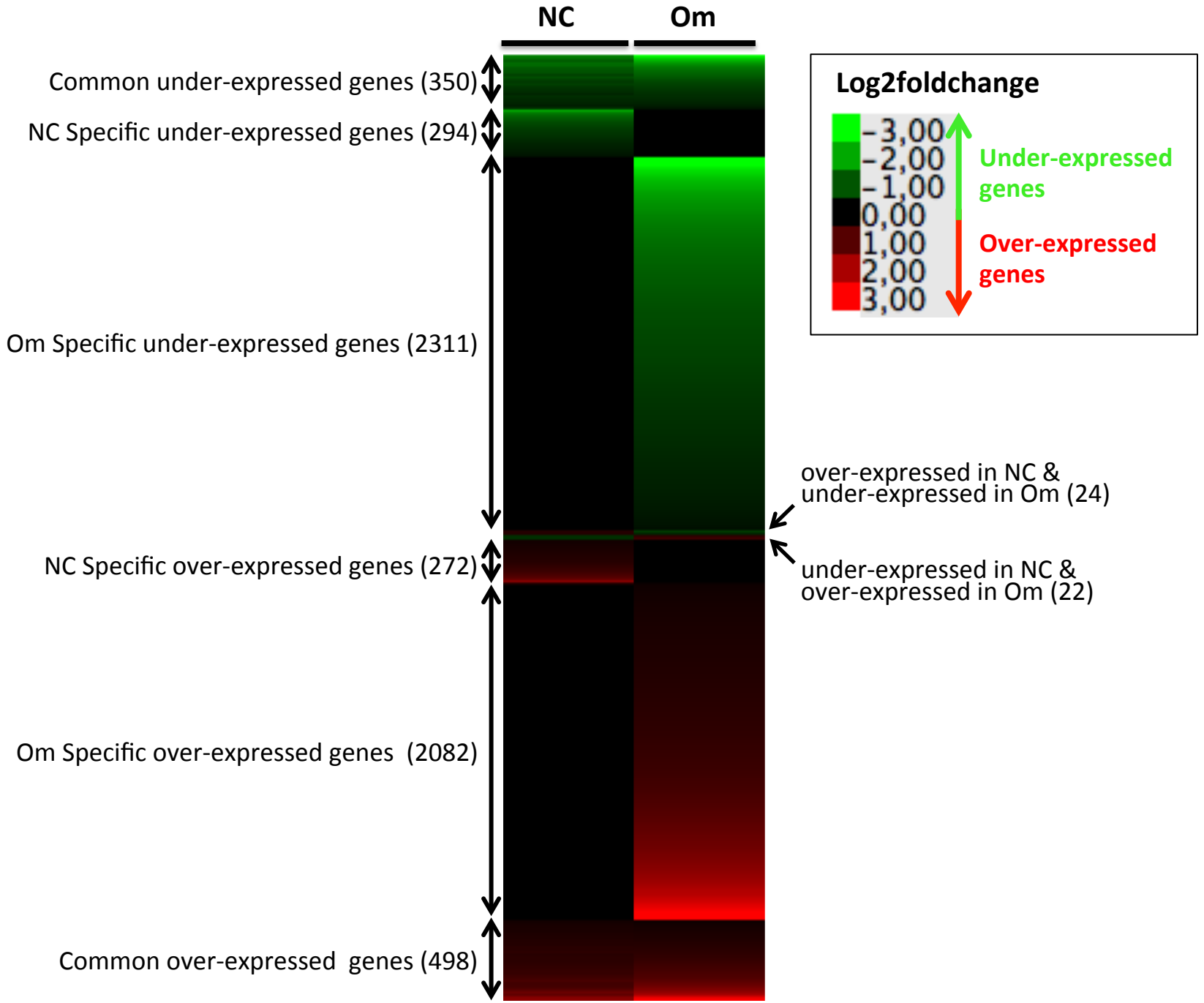
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under-expressed in Om (24)
under-expressed in NC &
over-expressed in Om (22)
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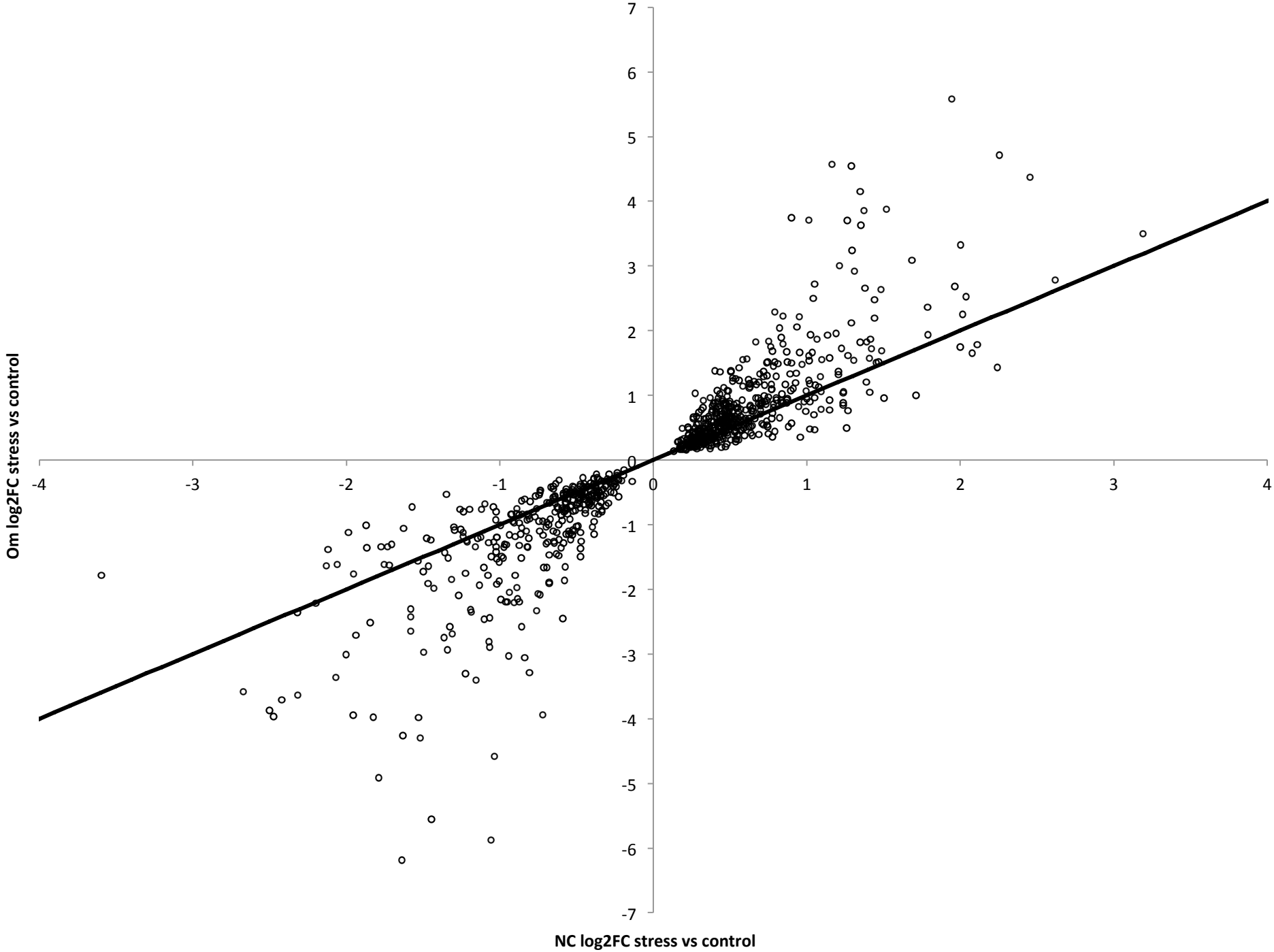
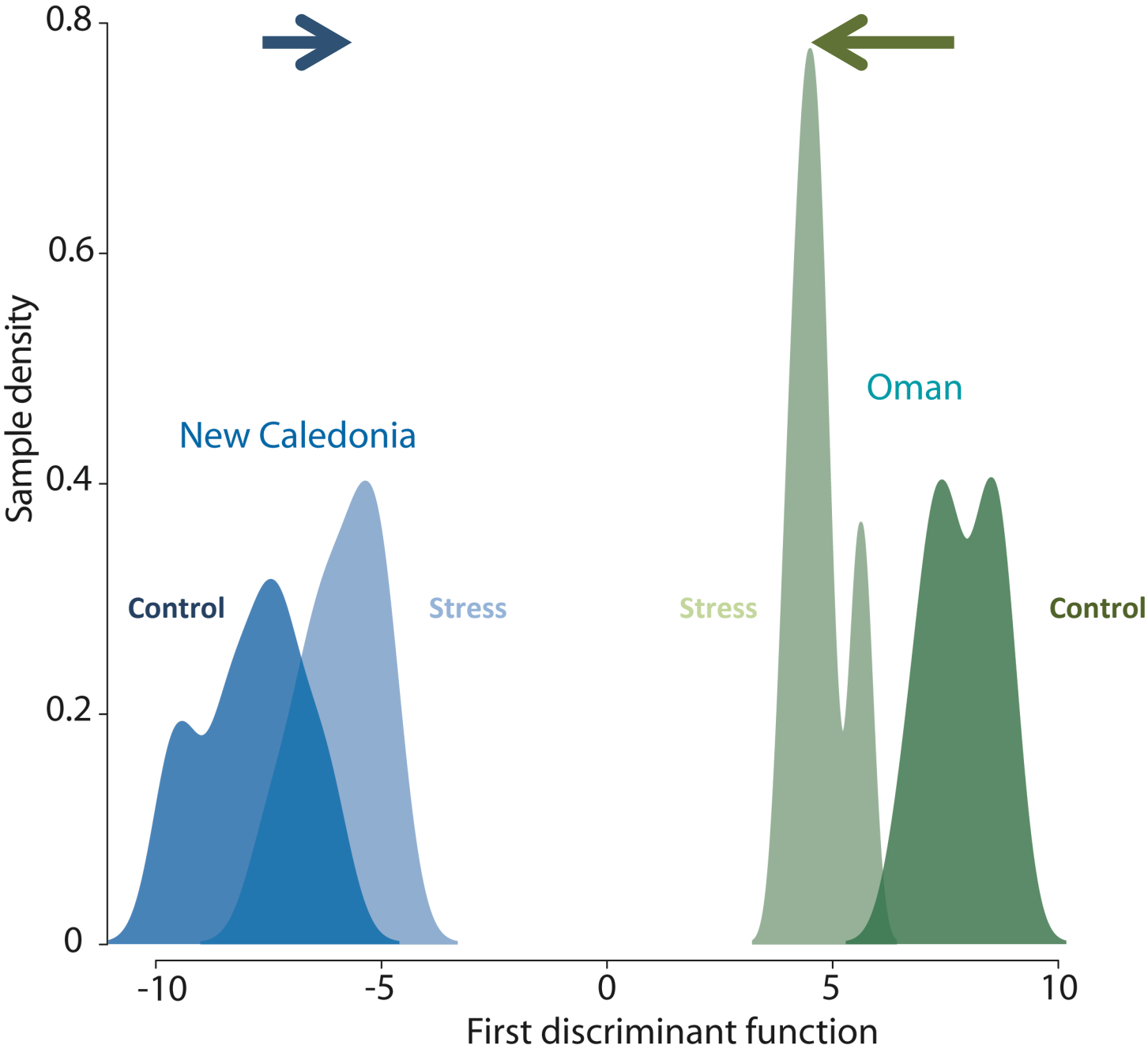
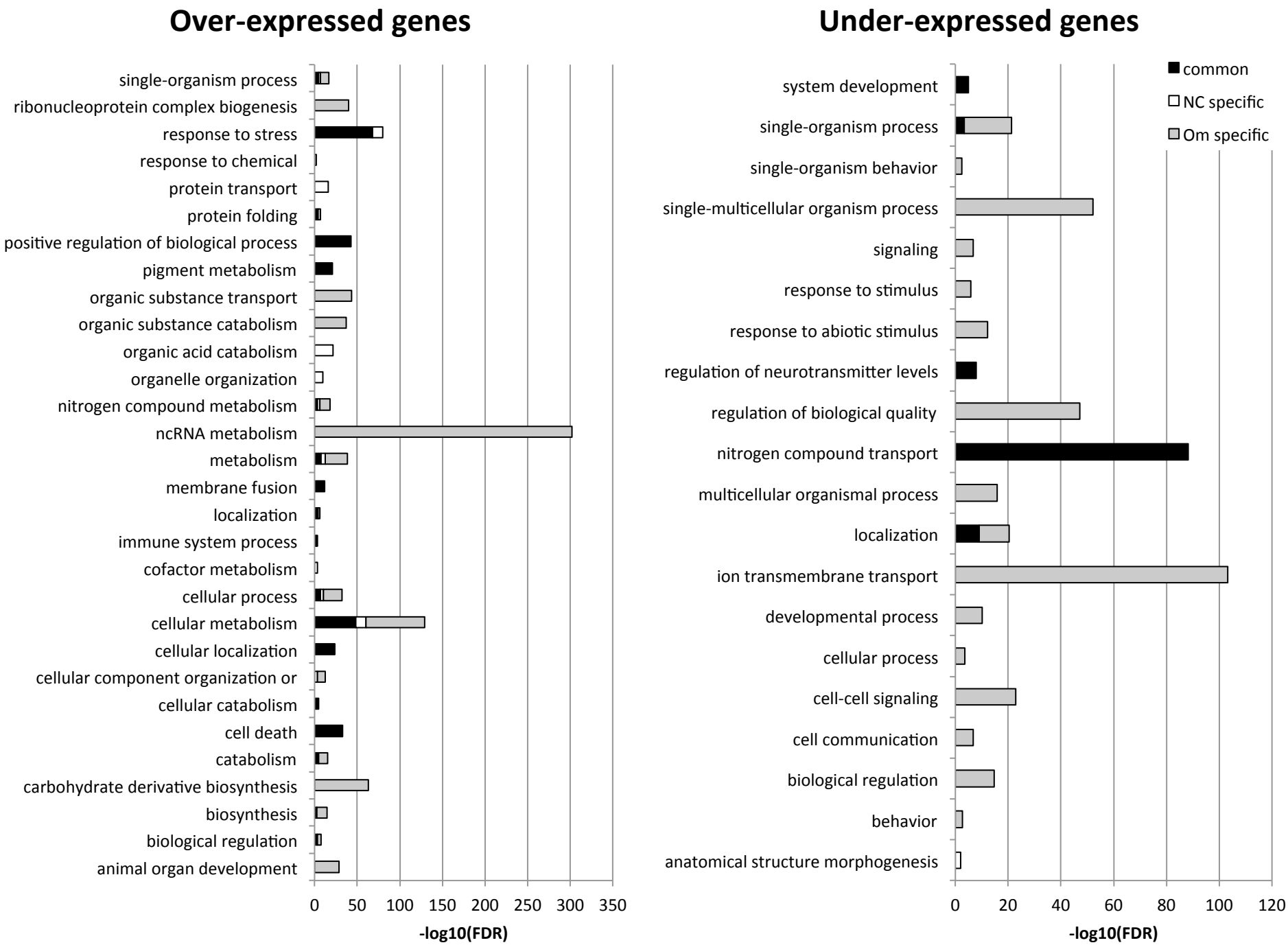
Under-expressed genes
Over-expressed genes


Om log2FC stress vs control
NC log2FC stress vs control
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-4
-3
-2
-1
0
1
2
3
4


0.8
0.6
0.4
0.2
0
Sample density
New Caledonia
Control
Stress
Oman
Stress
Control
-10
-5
0
5
10
First discriminant function


Over-expressed genes
single-organism process
ribonucleoprotein complex biogenesis
response to stress
response to chemical
protein transport
protein folding
positive regulation of biological process
pigment metabolism
organic substance transport
organic substance catabolism
organic acid catabolism
organelle organization
nitrogen compound metabolism
ncRNA metabolism
metabolism
membrane fusion
localization
immune system process
cofactor metabolism
cellular process
cellular metabolism
cellular localization
cellular component organization or
cellular catabolism
cell death
catabolism
carbohydrate derivative biosynthesis
biosynthesis
biological regulation
animal organ development
0 50 100 150 200 250 300 350
-log10(FDR)
Under-expressed genes
common
NC specific
Om specific
system development
single-organism process
single-organism behavior
single-multicellular organism process
signaling
response to stimulus
response to abiotic stimulus
regulation of neurotransmitter levels
regulation of biological quality
nitrogen compound transport
multicellular organismal process
localization
ion transmembrane transport
developmental process
cellular process
cell-cell signaling
cell communication
biological regulation
behavior
anatomical structure morphogenesis
0 20 40 60 80 100 120
-log10(FDR)