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 environments 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, histone modifications and DNA methylation) 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.

63 # INTRODUCTION

64 Earth is undergoing unprecedented global environmental changes with major effects on
65 biodiversity (Barnosky *et al.* 2011). The ongoing erosion of the most vulnerable
66 ecosystems due to current environmental degradation is particularly worrying and is
67 only a premise to what scientists have called the sixth mass extinction (Barnosky *et al.*
68 2011). In particular, climate change, ocean acidification and extreme climatic events
69 have already resulted in the irreversible degradation of more than 20% of coral reefs
70 worldwide (Bellwood *et al.* 2004; Hoegh-Guldberg *et al.* 2007). Scleractinian corals
71 constitute the biological and physical framework for a large diversity of marine
72 organisms [c.a. ~600 coral, ~2000 fish, and ~5000 mollusk species (Veron & Stafford-
73 Smith 2000; Reaka-Kudla 2005)]. Hence, the extinction or even major decrease of corals
74 would have dramatic repercussions on the overall associated communities (Hughes *et*
75 *al.* 2017a). Natural variation in thermal tolerance exists among coral populations (Oliver
76 & Palumbi 2010; Palumbi *et al.* 2014), especially along a latitudinal gradient (Polato *et al.*
77 2010; Dixon *et al.* 2015), hence providing some hope for coral survival based on their
78 capacity to cope with heat stress. More specifically, it has been shown that populations
79 inhabiting in zones with more variable temperature regimes display better tolerance to
80 heat stress from local (Kenkel *et al.* 2013) to geographical scales (Hughes *et al.* 2003;
81 Riegl *et al.* 2011; Coles & Riegl 2013).

82 It nevertheless remains unclear whether thermo-tolerance is acquired via acclimation
83 (i.e. intra-generational gene expression plasticity (Barnosky *et al.* 2011; Kenkel & Matz
84 2016)) and/or through genetic adaptation (i.e. inter-generational microevolution
85 (Barnosky *et al.* 2011; Dixon *et al.* 2015)). Actually, some studies strongly suggest that

both processes are likely to co-occur in wild coral populations (Hoegh-Guldberg *et al.* 2007; Reusch 2013; Palumbi *et al.* 2014; Hughes *et al.* 2017a; Torda *et al.* 2017).

With the recent advances of high throughput molecular methods, it is now possible to provide a more precise account of 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 (Brown *et al.* 2002; Weis 2008; Ainsworth *et al.* 2011; Barnosky *et al.* 2011; Bellantuono *et al.* 2012a; Barshis *et al.* 2013; Kenkel *et al.* 2013; Palumbi *et al.* 2014). In this regard, two main molecular patterns having different temporalities have been put forward: (1) "transcriptional plasticity", i.e. extensive changes in gene expression levels according to the occurring thermal condition and (2) "transcriptional frontloading", i.e. the elevation of stress related genes baseline expression that preconditions organisms to subsequent (recurrent) stresses (Reaka-Kudla 2005; Mayfield *et al.* 2011; Barnosky *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 (Bellwood *et al.* 2004; Hoegh-Guldberg *et al.* 2007; Oliver & Palumbi 2010; Palumbi *et al.* 2014), it could also reflect an acclimation via epigenetic processes leading to constitutive gene expression (Veron & Stafford-Smith 2000; Reaka-Kudla 2005; Torda *et al.* 2017). Epigenetic changes through environmental priming (i.e. translation of environmental cues) may be involved in adaptive evolution at such short timescales, eventually enabling transgenerational plasticity (Hughes *et al.* 2017a; Torda *et al.* 2017; Jablonka 2017).

Surprisingly, frontloading and gene expression plasticity were generally discussed as mutually exclusive patterns (Oliver & Palumbi 2010; Barshis *et al.* 2013; Palumbi *et al.* 2014; 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; Dixon *et al.* 2015; Kenkel & Matz 2016) 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 (Hughes *et al.* 2003; Riegl *et al.* 2011; Coles & Riegl 2013; Kenkel & Matz 2016), hence challenging the patterns observed in *A. hyacinthus* (Barnosky *et al.* 2011; Barshis *et al.* 2013; Coles & Riegl 2013; Kenkel & Matz 2016). Although both strategies (i.e. constitutive frontloading *versus* expression plasticity) undoubtedly exist 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; Barnosky *et al.* 2011; Dixon *et al.* 2015; Kenkel & Matz 2016)).

Importantly, scleractinian corals are composed of several symbiotic organisms including the cnidarian host, the mutualist photosynthetic algae (formerly defined as belonging to

133 the genus *Symbiodinium* but now considered as different genera within the family
134 Symbionaceae (Bellwood *et al.* 2004; Barnosky *et al.* 2011; Dixon *et al.* 2015; LaJeunesse
135 *et al.* 2018)) and bacterial communities. All symbionts involved in a stable symbiosis
136 effectively form the entire organism, and constitute what is referred to the holobiont
137 (Margulis & Fester 1991; Hoegh-Guldberg *et al.* 2007; Reusch 2013; Palumbi *et al.* 2014;
138 Torda *et al.* 2017). A decade after this term was defined, its use has been particularly
139 popularized in reference to corals (Rohwer *et al.* 2002), and subsequent research has led
140 to the hologenome theory of evolution (Rosenberg *et al.* 2007; Zilber-Rosenberg &
141 Rosenberg 2008). In this context, the hologenome is defined as the sum of the genetic
142 information of the host and its symbiotic microorganisms. Phenotypes are thus the
143 product of the collective genomes of the holobiont partners in interaction with the
144 environment, which constitute the unit of biological organization and thus the object of
145 natural selection (Zilber-Rosenberg & Rosenberg 2008; Guerrero *et al.* 2013; McFall-
146 Ngai *et al.* 2013; Bordenstein & Theis 2015; Theis *et al.* 2016). Additionally to the
147 cnidarian host response, the genotype -or association of genotypes- of the
148 photosynthetic mutualist Symbionaceae symbionts plays a key role in the
149 thermotolerance of the holobiont (Hume *et al.* 2013; Mayfield *et al.* 2014; Suggett *et al.*
150 2017). There is less certainty about the importance of the coral bacterial community in
151 participating to the fitness of the holobiont, although accruing evidences strongly
152 suggest their implication in coral response to environmental conditions (Li *et al.* 2014;
153 Pantos *et al.* 2015; Hernandez-Agreda *et al.* 2016), and in the resistance to diseases
154 (Sato *et al.* 2009; Cróquer *et al.* 2013; Meyer *et al.* 2016). Finally, the role of the coral-
155 associated microorganisms and their potential to modify holobiont response to stress
156 remain so far overlooked (but see (Ziegler *et al.* 2017; Torda *et al.* 2017). Hence,

157 studying how corals respond to stress implies an integrative approach to analyze the
158 response of each symbiotic protagonist.

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

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

192 MATERIAL AND METHODS

193 CORAL SAMPLING AND MAINTENANCE

194 *Pocillopora damicornis*-like colonies originating from environments characterized by
195 contrasting thermal regimes were sampled during the warmer month in two different
196 localities: (1) in Oman, Gulf of Oman, Northwestern Indian Ocean (Om; June 2014; local
197 seawater temperature during sampling 30.8°C), where corals are exposed to a globally
198 warmer and variable thermal environment, and (2) in New Caledonia, Southwestern
199 Pacific Ocean (NC; November 2014; local seawater temperature during sampling
200 27.1°C), where corals are subject to more mitigate and stable temperatures (see Table 1
201 for the temperature regime of locality). From each location, we thus sampled colonies
202 morphologically similar and occupying the same water depth niche. To account for
203 possible intra-population diversity, three colonies (>20 cm in diameter) were collected
204 in each locality, and separated by at least 10 m to decrease the probability to collect

205 members of the same genet, as some *Pocillopora* species are able to propagate by
206 asexual reproduction (Adjeroud *et al.* 2013; Gélin *et al.* 2017a; 2018). Immediately
207 following collection, a 1 cm branch tip of each colony was excised, rinsed three times in
208 filtered seawater (0.22 µm), and placed in RNAlater solution (Sigma Aldrich) for the *in*
209 *situ* microbiota analysis. The rest of the colony was fragmented into 20 branches each of
210 10 cm length and physiologically stabilized in openwater system for one week before
211 shipping (Al-Hail field stationof the Sultan Qaboos University and the Public aquarium of
212 Noumea for OM and NC localities respectively). For shipping, individual branches were
213 placed in plastic bags containing oxygenated seawater (800mL seawater and 1600mL of
214 medical oxygen), and transported by aircraft to the research aquarium of the Banyuls-
215 sur-Mer oceanographic observatory (France). The coral branches were maintained in
216 artificial seawater (Seachem Reef Salt) at 26°C, and supplied daily with *Artemia* nauplii
217 to satisfy their heterotrophic demand. The conditions in the maintenance tank were
218 controlled to mimic the natural physicochemical parameters of coral reefs (pH:8.2;
219 salinity: 36 psu; light intensity: 150 to 250 µmol of photons/m^2/s; photoperiod: 12h
220 night/12h day;kH: 6–7.5 dkH; calcium concentration: 410–450 mg/L; inorganic
221 phosphate concentration: < 0.1 mg/L; magnesium concentration: 1300–1400 mg/L;
222 nitrate concentration: < 5 mg/L). After 3 and 7 months of acclimatization to the
223 laboratory condition (marked by growth resumption) for Om and NC colonies,
224 respectively, corals were fragmented to produce a total of ~15 to 20 clones (nubbins)
225 from each colony (~3 cm). These were individually fixed to a support (here a p1000 tip)
226 using an epoxy adhesive. We waited for complete healing (evident as tissue extending to
227 cover the epoxy adhesive) prior to run the experiment.

228 ECOLOGICALLY REALISTIC HEAT STRESS

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

233 The experimental design comprised four tanks of 53 L per locality in which the seawater
234 was continuously recycled. The water was sterilized using UV (rate 3200 L/h) and
235 renewed twice per hour in each tank (recirculation rate: 100L/h in each tank). The eight
236 tanks shared the same seawater but their temperature was monitored individually
237 (HOBBY BiothermPro, model 10892; 500W Aqua Medic titanium heater; HOBO TidbiT
238 v2 logger) (Supplementary Figure S1). For each locality, 5 to 8 nubbins per mother
239 colonies were randomly placed in each tank (four tanks per locality) for two weeks at
240 the control temperature and the following protocol was applied: three tanks were then
241 subjected to a gradual temperature increase (stress treatment) while the fourth
242 (control) was maintained at the control temperature to verify that the stress observed in
243 the stressful treatment was not due to other potential confounding effects or water cues
244 (Figure 1). Both the control and stress temperatures were specific for each sampling
245 locality to mimic their respective natural environment. In particular, we set the control
246 temperature as the mean water temperature for the three warmer months measured at
247 the coral sampling site locality (Table 1): 31°C for the colonies from Om, and 27°C for
248 the colonies from NC. The stress treatment was ecologically realistic, i.e. reflecting a
249 naturally occurring warming anomaly, and consisted in increasing the temperature
250 gradually by 1°C (over 5 consecutive hours) each week until physiological collapse of the
251 corals became evident (polyps closure, bleaching or necrosis), as described by (Vidal-
252 Dupiol *et al.* 2009). Sampling was performed in the three sampling tanks just before the

253 first temperature increase (control condition) as well as each week before the next
254 temperature increase. The beginning of polyp closure was consistently observed for the
255 different colonies of the same locality at the same temperature threshold. Samples for
256 subsequent genetic and transcriptomic analyses were chosen *a posteriori*. They
257 corresponded to those sampled in each tank just before the first increase of temperature
258 (control samples), and just before the temperature that produced the first signs of
259 physiological collapse and before bleaching (stress temperature samples). Thus, for each
260 condition (control and stress) we obtained three biological replicates of each colony
261 from the three different tanks (three colonies per locality) to reach a total of 36 samples
262 (2 localities × 3 colonies× 2 experimental conditions × 3 replicates/tanks). The general
263 health of the nubbins was assessed via daily photographic monitoring (at noon prior to
264 feeding) throughout the period of the experiment.

265 DNA EXTRACTION

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

270 HOST SPECIES AND CLONEMATES IDENTIFICATION

271 As the *corallum* macromorphology is not a diagnostic criterion in *Pocillopora* genus, the
272 host species was thus identified molecularly. Thus each colony was sequenced for the
273 mitochondrial variable open reading frame (ORF) and was genotyped using 13 specific
274 microsatellites, as in Gélin *et al.* (Gélin *et al.* 2017b). Then each colony used in the
275 experiment was assigned to Primary and Secondary Species Hypothesis (PSH and SSH;
276 *sensu* Pante et al.) (Pante *et al.* 2015) following the nomenclature from Gélin *et al.* (Gélin

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

291 Microbial community analysis using MiSeq 16S and ITS2
292 metabarcoding

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

296 Amplicon Sequencing

297 A bacterial 16S rDNA amplicon library was generated for each of the 42 samples (one *in*
298 *situ* condition, three control conditions and three stress conditions per colony, three
299 colonies per locality, two localities), using the 341F (CCTACGGGNGGCWGCAG) and 805R
300 (GACTACHVGGGTATCTAATCC) primers, which target the variable V3/V4 loops

301 (Klindworth *et al.* 2012). The Symbiodiniaceae assemblages were analyzed using ITS2
302 (internal transcribed spacer of the ribosomal RNA gene) amplicon libraries and specific
303 primers targeting a sequence of approximately 350 bp (ITS2-F
304 GTGAATTGCAGAACTCCGTG; ITS2-R CCTCCGCTTACTTATATGCTT) (Lajeunesse &
305 Trench 2000; Quigley *et al.* 2014). For both markers, paired-end sequencing using a
306 250 bp read length was performed on the MiSeq system (Illumina) using the v2
307 chemistry, according to the manufacturer's protocol at the Centre d'Innovation Génome
308 Québec and McGill University, Montreal, Canada.

309 BIOINFORMATIC ANALYSIS:
310 The FROGS pipeline (Find Rapidly OTU with Galaxy Solution) implemented on a Galaxy
311 platform (http://sigenae-workbench.toulouse.inra.fr/galaxy/) was used for data
312 processing (Escudié *et al.* 2017). In brief, paired reads were merged using FLASH
313 (Magoč & Salzberg 2011). After cleaning and removal of primer/adapters using cutadapt
314 (Martin 2011), *de novo* clustering was performed using SWARM (Mahé *et al.* 2014). This
315 uses a local clustering threshold with an aggregation distance (d) of 3. Chimeras were
316 removed using VSEARCH (Rognes *et al.* 2016). We filtered the dataset for singletons and
317 performed affiliation using Blast+ against the Silva database (release 128, September
318 2016) for 16S amplicons (Altschul *et al.* 1990). For ITS2 metabarcoding, the
319 Symbiodiniaceae type was assessed using Blast+ against an in-house database of
320 Symbiodiniaceae reference sequences built from sequences publicly available. An OTU
321 table in standard BIOM format with taxonomic affiliation was produced for subsequent
322 analyses.

323 For community composition analysis we used the *phyloseq* R package (McMurdie &
324 Holmes 2013) to infer alpha diversity metrics at the OTU level (i.e. Shannon and Chao

325 indexes), and beta diversity (between sample similarity) from the OTU table.
326 Community similarity was assessed by Principal Coordinate Analysis (PCoA) using the
327 Bray-Curtis distance matrices.

328 To test for possible effect of corals' locality of origin and for temperature treatments on
329 the two alpha diversity metrics (i.e. Shannon and Chao) we ran a series of four
330 generalized mixed models including these two fixed factors and their interactions as
331 explanatory variables. The four models consisted in considering all factors, the two fixed
332 factors without accounting for their possible interactions, and the two factors
333 independently. In all models, we also accounted for possible effect of genotypes that
334 were considered as random factors nested within locality. All models were run using the
335 lme4 package implemented in R (Bates *et al.* 2015) and considering a Gamma
336 distribution. The best model for each alpha diversity metrics was identified based on the
337 Akaike Information Criterion (AIC (Akaike 1974)). Concerning beta diversity, we
338 performed MANOVAs to compare beta diversity metrics among the groups of samples by
339 sampling locality, genotype or treatment.

340 Corrections based on multiple testing were performed using FDR (Benjamini &
341 Hochberg 1995). For all analyses, the threshold significance level was set at 0.05.

342 TRANSCRIPTOME ANALYSIS

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

345 RNA EXTRACTION

346 Total RNA was extracted from each coral sample using TRIzol reagent (Invitrogen),
347 according to the manufacturer's protocol. The quantity and integrity of the total RNA

348 extracted was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies)(mean
349 RIN =7.5). Paired-end fragment libraries (2 × 100 bp) were constructed and sequenced
350 on an Illumina HiSeq 2000 platform at the Centre d'Innovation Génome Québec at McGill
351 University, Montreal, Canada.

352 BIOINFORMATIC ANALYSES

353 Fastq read files were processed on the Galaxy instance of the IHPE (http://bioinfo.univ-
354 perp.fr) (Giardine *et al.* 2005). Quality control and initial cleaning of the reads were
355 performed using the filter by quality program (version 1.0.0) based on the FASTX-toolkit
356 (Blankenberg *et al.* 2010). Reads having fewer than 90% of bases having a Phred quality
357 score ≤ 26 were discarded (probability of 2.5 incorrect base call out of 1000, and a base
358 call accuracy of 99.75%). Adaptors used for sequencing were removed using the
359 cutadapt program version 1.6 (Martin 2011). All paired-end reads were aligned using
360 RNAstar software under default parameters, with at least 66% of the bases being
361 required to align to the reference, and a maximum of ten mismatches per read (Dobin *et*
362 *al.* 2013). The *Pocillopora damicornis sensu lato* reference genome used in this study
363 (Vidal-Dupiol *et al.* 2019) consisted of a draft assembly of 25,553 contigs (352 Mb total)
364 and N50 = 171,375 bp. The resulting transcriptome served as the reference for reads
365 mapping, and a GTF annotation file was constructed using cufflink/cuffmerge (Trapnell
366 *et al.* 2010). HTseq was used to produce count files for genes (Anders *et al.* 2015). The
367 DESeq2 package was used to estimate the normalized abundances of the transcripts, and
368 to calculate differential gene expression for samples between the control temperature
369 and the stress temperature for each locality (Love *et al.* 2014), considering the different
370 genotypes (three biological replicates for each genotype) and using default parameters.
371 We next analyzed genes according to their expression patterns among the different

372 colonies and temperature treatments. Genes were clustered manually into six groups
373 according to their differential expression levels: common over-expressed genes, NC-
374 specific over-expressed genes, Om-specific over-expressed genes, common under-
375 expressed genes, NC-specific under-expressed genes, and Om-specific under-expressed
376 genes. Cluster 3.0 (de Hoon *et al.* 2004) and Treeview (Saldanha 2004) were used to
377 build the heatmap.

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

391 We next ran a general linear model (GLM) using the DAPC scores as dependent variable,
392 and accounted for the locality of origin (NC and Om), the treatment (control and heat
393 stress), and their interaction as explanatory variables. We also considered the effect of
394 individual colonies nested within localities as random effects in the model. Our final
395 objective was to test for a potential significant effect of the interaction between the

396 locality and the treatment effects, as a proxy of significant differences in the genome-
397 wide gene expression reaction norms (i.e. differences in DAPC scores between the
398 control and the heat stress treatments) between Om and NC colonies..

399 GO ENRICHMENT OF DIFFERENTIALLY EXPRESSED GENES

400 The transcriptome was annotated *de novo* using a translated nucleotide query (blastx
401 (Altschul *et al.* 1990)) against the non-redundant protein sequence database (nr). The
402 25 best hits were then used to search for gene ontology terms using the Blast2Go
403 program (Conesa *et al.* 2016). To identify the biological functions significantly enriched
404 within up or down-regulated genes, a Gene Ontology (GO) term enrichment analysis was
405 performed. Lists of GO terms belonging to significantly up-regulated and down-
406 regulated genes were compared to the GO terms of the whole expressed gene set using a
407 Fischer exact test and a FDR value of 0.05. We used REVIGO to visualize the enriched
408 biological processes (Supek *et al.* 2011).

409 RESULTS

410 HOST IDENTIFICATION

411 Among the three colonies from New Caledonia, colonies NC2 and NC3 presented
412 haplotype ORF18 and were assigned to Primary Species Hypothesis PSH05 and more
413 precisely to Secondary Species Hypothesis SSH05a (Gélin *et al.* 2017b), corresponding to
414 *P. damicornis* type *β* (Schmidt-Roach *et al.* 2014) or type 5a (Pinzón *et al.* 2013), while
415 colony NC1 presented ORF09 and was assigned to PSH04, *P. damicornis* type *α*,
416 *P. damicornis* or type 4a, respectively. As for colonies from Oman, they all presented
417 ORF34 and were assigned to PSH12 (Gélin *et al.* 2017a) or type 7a (Pinzón *et al.* 2013)
418 (Supplementary Table S2), that is not part of the *P. damicornis sensu lato* species

419 complex. Thus NC colonies are phylogenetically closer from each other than from
420 colonies from Oman. These three PSHs represent three different species.

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

426 ECOLOGICALLY REALISTIC HEAT STRESS

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

441 BACTERIAL COMMUNITIES

442 Among the overall 42 samples analyzed, a total of 5,308,761 16S rDNA amplicon
443 sequences were obtained after cleaning and singleton filtering corresponding to 15,211
444 OTUs. In all samples the class Gammaproteobacteria was dominant (77.7%), particularly
445 the genus *Endozoicomonas* (44.7% of the sequences); this genus is known to be an
446 endosymbiont of numerous scleractinians (Neave *et al.* 2016b) (See Supplementary
447 Figure S3 for complete bacterial composition in each colony and replicate).

448 Concerning the two alpha diversity metrics (i.e. Shannon and Chao indexes), the best
449 statistical models to explain the overall variance included the coral's locality of origin
450 only (AIC = 101.9). According to these models, colonies from Oman displayed a higher
451 Shannon index than those from New Caledonia (P = 0.04). Conversely, the effect of
452 locality was not significant for the Chao index (P > 0.05). The PCoA of Bray-Curtis
453 distances for all colonies showed no evident clusters based on the experimental
454 treatments (Figure 2). We observed a loose grouping based on localities and colonies,
455 except for colony NC1, which appeared to have a more specific microbiota composition,
456 as it had a different grouping associated with the first axis, which explained 22.3% of the
457 variance. This could be correlated with the different species hypotheses for NC1
458 compared to NC2 and NC3 (see above). These observations were confirmed by statistical
459 analyses (MANOVA between localities: P=0.003; between genotypes: P=0.001; between
460 temperature treatments: P=0.761; Supplementary Table S4). Thus, the bacterial
461 composition appeared to be relatively specific to each colony within each locality, but no
462 major shift was observed during the transition from the natural environment to artificial
463 seawater, nor during heat stress exposure.

464 SYMBIODINIACEAE ASSEMBLAGES

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

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

474 HOST TRANSCRIPTOME ANALYSIS

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

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

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

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

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

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

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

528 DISCRIMINANT ANALYSIS OF PRINCIPAL COMPONENTS (DAPC):
529 At the overall gene expression level, our DAPC analysis clearly discriminated the
530 colonies from both localities (Figure 7). More interestingly, the GLM revealed a
531 significant interaction term between the locality and condition (control or heat stress)
532 effects ($P = 0.04$), hence indicating that the slope of the reaction norm was different
533 between localities. More particularly, the Om colonies responded to a greater extent

534 than the NC ones, and thus showed significantly higher gene expression plasticity in
535 response to heat stress.

536 It is worth stressing that colonies having experienced the heat stress displayed more
537 similar genome-wide expression profiles than controlled colonies (Fig. 7). Such pattern
538 was also observed in colonies of mustard hill coral *P. astreoides* experiencing a heat
539 stress compared to controls (Kenkel & Matz 2016). This apparent convergence in the
540 functional response of colonies from different habitats to heat stress might be at least
541 partly explain by the fact some common molecular pathways are turned-on when
542 colonies are facing stressful conditions although the magnitude of such responses is
543 different.

544

545 ANALYSIS OF GENE FUNCTION:
546 To investigate the functions associated with the differentially expressed genes we
547 performed a BLASTX annotation of transcripts followed by a Gene Ontology (GO) term
548 (biological process, molecular function, and cell compartment) (Supplementary FileS9).

549 For the 498 common over-expressed genes, 139 biological processes were enriched
550 compared to the entire set of annotated genes. The most significant biological process
551 identified in the REVIGO analysis (i.e. with lowest FDR= 2.1×10^{-68}) was response to
552 stress (Figure 8). Following this sequentially, were cellular metabolism (FDR=3.7×10^{-49}),
553 positive regulation of biological processes (FDR = 2.4×10^{-43}), cell death (FDR = $2.5\times10^{-}$
554 ^{33}), cellular localization (FDR = 8.4×10^{-25}), and pigment metabolism (FDR = 2.1×10^{-21}).
555 Among the 272 genes over-expressed in the NC but not in the Om colonies in response
556 to heat stress, 38 biological processes were enriched: organic acid catabolism (FDR =

557 1.6×10^{-22}), protein transport (FDR = 1.8×10^{-16}), stress response (FDR = 4.8×10^{-13}), and
558 cellular metabolism (FDR = 3×10^{-12}) were the four most significantly enriched biological
559 processes (Figure 8). Among the 2,082 genes over-expressed in the Om but not in the NC
560 colonies in response to heat stress, 160 biological processes were enriched, the most
561 significant being ncRNA metabolism (FDR = 8.9×10^{-303}), cellular metabolism (FDR =
562 4.4×10^{-70}), carbohydrate derivative biosynthesis (FDR = 5.9×10^{-64}), and organic
563 substance transport (FDR = 2×10^{-44}).

564 For the 350 genes that were under-expressed following heat stress irrespective to the
565 locality of origin (Om or NC), 48 biological processes were enriched and grouped into
566 five biological processes: nitrogen compound transport (FDR = 5.4×10^{-89}), localization
567 (FDR = 8.1×10^{-10}), regulation of neurotransmitter levels (FDR = 1.2×10^{-8}), system
568 development (FDR = 8.8×10^{-6}), and single organism process (FDR = 4×10^{-4}). Among the
569 under-expressed genes in the NC colonies only, a single biological process (anatomical
570 structure/morphogenesis) was found to be enriched (FDR = 9×10^{-3}). Among the under-
571 expressed genes in the Om colonies, 139 biological processes were enriched, with the
572 most significant being ion transmembrane transport (FDR = 7.6×10^{-104}), single
573 multicellular organism process (FDR = 7.5×10^{-53}), regulation of biological quality (FDR =
574 6×10^{-48}), cell–cell signaling (FDR = 1.5×10^{-23}), single organism process (FDR = $1.1\times10^{-}$
575 ^{18}), multicellular organism process (FDR = 1.5×10^{-16}), biological regulation (FDR =
576 2.3×10^{-15}), response to abiotic stimulus (FDR = 6.2×10^{-13}),and localization (FDR =
577 4.6×10^{-12}).

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

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

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

603 Most interestingly, for genes associated with a higher basal expression level together
604 with over-expression in the Om colonies, the most enriched biological processes were
605 stress response (FDR = 1.2×10^{-26}), pigment metabolism (FDR = 5.1×10^{-24}), regulation of
606 phosphate metabolism (FDR = 3.2×10^{-15}), cellular metabolism (FDR = 2.7×10^{-11}), and
607 protein folding (FDR = 7.3×10^{-6}).

608 DISCUSSION

609 SPECIFIC CONTEXT OF ADAPTATION

610 Our aim was to compare the phenotypic plasticity in terms of transcriptomic response to
611 heat stress of coral colonies originating from different localities displaying contrasted
612 thermal regimes. As morphology can be misleading for species identification in
613 scleractinians, notably in *Pocillopora* genus (Gélin et al., 2017a), we used a molecular
614 approach to test the species relationships of our samples. The analysis of mitochondrial
615 sequences and clustering analyses indicated that, despite similar morphologies, our
616 samples corresponded to different species. This agrees well with previous works
617 showing the importance of cryptic lineages and morphological plasticity in the
618 *Pocillopora* genus (Gélin *et al.* 2017a and references herein). Oman colonies
619 corresponded to species hypothesis PSH12 of (Gélin *et al.* 2017b), which is restricted to
620 the Northwestern Indian Ocean. Regarding the two species hypotheses from NC, SSH05a
621 (*P. damicornis* type β SSH05a or *P. acuta*) is found in the Pacific Ocean and PSH04
622 (*P. damicornis* type α or *P. damicornis sensu stricto*) is nearly exclusively found in the
623 Pacific Ocean (very rare in the Indian Ocean, and not found yet in Red Sea) (Gélin *et al.*
624 2017b). It would be interesting to study whether inside each species hypothesis,
625 different thermotolerance phenotypes are present. Conversely, the observation of a

626 similar response to thermal stress in two different species in NC, as revealed by
627 differential gene expression as well as DAPC analyses, could indicate either a conserved
628 strategy or a convergence under the same ecological conditions.

629 ### An ecologically realistic heat stress

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

642 ### Symbiotic community: bacterial and Symbionaceae composition

643 For the bacterial community, we identified significant differences between localities and
644 colonies. The microbiota composition of all samples was consistent with previous
645 studies, showing a high proportion of Gammaproteobacteria and dominance of the
646 symbiotic *Endozoicomonas* genus (Bourne & Munn 2005; Neave *et al.* 2016a; Peixoto *et*
647 *al.* 2017). However, our results clearly demonstrate that neither maintenance in the
648 experimental structure nor experimental heat stress induced major bacterial community
649 changes in coral colonies irrespective to their locality of origin. For the Symbionaceae

650 community, the ITS2 metabarcoding analysis enabled inter-clade resolution (Quigley *et*
651 *al.* 2014). Two distinct types of D1a and C1 clades dominated, representing most of the
652 sequences in the Om and NC corals, respectively. Nine ITS types (A to I) have been
653 identified in the former genus *Symbiodinium* (Baker 2003). Some Symbiodiniaceae
654 strains strongly participate to the overall holobiont fitness, with type D providing
655 tolerance to higher temperatures (Berkelmans & van Oppen 2006) and C1 enhancing
656 coral growth rates (Little *et al.* 2004). Interestingly, we found that the type D1a is
657 dominant in the more thermotolerant Om corals, which is consistent with the results of
658 previous works (Berkelmans & van Oppen 2006), however recent results shows that
659 such an association is rather linked with minimal temperatures than annual amplitude
660 of temperature changes (Brener-Raffalli *et al.* 2018).

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

673 (Mayfield *et al.* 2014), but since our RNA extraction method resulted in very few algal
674 transcripts, we only focused on the host transcriptomic response.

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

677 HOST TRANSCRIPTOMIC RESPONSE

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

693 Importantly however, we also identified several genes whose expression is
694 constitutively higher in the Om colonies compared to the NC colonies by comparing the
695 expression levels in the control condition. This process recently called "frontloading"

696 (Barshis *et al.* 2013) reflects the preemptive expression of stress-response genes, hence
697 predisposing organisms to better respond to stress. It has been proposed that the
698 occurrence of plasticity *vs*. frontloading strategies may depend on the frequency of
699 stresses relative to the typical response time of organisms, with frequent stresses
700 promoting frontloading strategies whereas less frequent perturbations would result in
701 an increased plasticity (Kenkel & Matz 2016). Other conceptual considerations
702 especially in regards to the predictability of environmental variation through
703 generations should also be taken into account (Danchin 2013; Herman *et al.* 2014). The
704 frontloading is by definition more costly than plasticity since it transforms a response to
705 the environment in a constitutive function. Frontloading is therefore a strategy that
706 would be more efficient when offspring's habitat is highly predictable. On the contrary,
707 an unpredictable or less predictable offspring environment may promote plasticity to
708 enable the exploration of a wider phenotypic landscape at a lesser cost. Plasticity and
709 frontloading are often discussed as mutually exclusive responses (Barshis *et al.* 2013;
710 Kenkel & Matz 2016). However, corals are known to display a high level of variation in
711 their reproduction strategies (brooder vs. broadcast spawner) (Whitaker 2006; Baird *et*
712 *al.* 2009), timing (Fan *et al.* 2006) and pelagic larval duration (Harrison & Wallace
713 1990). Environmental predictability in terms of stress frequency and annual
714 temperature variation should be therefore limited and we hypothesized that, rather
715 than being exclusive, plasticity and frontloading often co-occur especially in the
716 population experiencing extreme environments.

717 Our results clearly support that plasticity and frontloading indeed co-occur specifically
718 in the thermotolerant Om colonies experiencing a more variable thermal environment *in*
719 *natura*. To tease apart the biological processes that are regulated via plasticity or

720 frontloading in *Pocillopora* response to heat stress, we conducted an enrichment
721 analysis. Keeping in mind that congruency between gene expression and protein levels
722 should be cautious (Mayfield *et al.* 2016), we propose a detailed discussion of the
723 response of coral colonies at the molecular level for each main biological process
724 identified (Supplementary File S11). Notably, we found differences in gene expression
725 levels in response to temperature increase between the two localities for genes involved
726 in response to heat stress (such as HSPs), detoxification of reactive oxygen species,
727 apoptosis, mitochondria energetic functioning, and symbiont maintenance with higher
728 number of differentially expressed genes for the Om corals associated to higher fold
729 changes. In corals, Hsp70 which displayed more intense overexpression in Om colonies,
730 is one of the most documented protein chaperones associated to heat stress response
731 (Barshis *et al.* 2013; Haguenauer *et al.* 2013). Several genes involved in oxidative stress
732 had greater differential expression levels in Om such as calmodulin, quinone
733 oxidoreductases and thioredoxin (Supplementary File S11). Reactive oxygen species
734 (ROS), generated in consequence to heat stress, play a central role in host-symbiont
735 interactions ultimately leading to bleaching as a host innate immune response to a
736 compromised symbiont (see (Weis 2008) for a review). We also identified many genes
737 involved in apoptosis, a process that has been recurrently associated with coral
738 responses to heat stress (Ainsworth *et al.* 2011; Barshis *et al.* 2013; Pratlong *et al.* 2015;
739 Maor-Landaw & Levy 2016).

740 Our results also suggest that allocating energy in heat stress response is at the expense
741 of other crucial biological processes such as growth and reproductive functions as
742 already shown in *Pocillopora* (Vidal-Dupiol *et al.* 2009; 2014), even if we could not test
743 experimentally fitness effect of the experimental heat stress. However, the molecular

744 mechanisms underlying such overall response to heat stress are still partly unresolved.
745 Interestingly, we also found specific gene expression patterns linked with epigenetic
746 regulation such as histone modifying enzymes as well as enzymes involved in DNA
747 methylation and dsRNA regulation (Supplementary File S11). We also identified
748 expression of many retro-transposons in Om. These processes could be involved in rapid
749 epigenome modifications and thus fuel rapid adaptive evolution (Maumus *et al.* 2009;
750 Torda *et al.* 2017; Jablonka 2017) .

751 ## CONCLUSION:

752 Comparison of the response to an ecologically realistic heat stress of corals from the
753 same genus but pertaining to different species hypotheses thriving in two contrasting
754 thermal environments sheds light on the molecular basis of thermotolerance. We found
755 that after heat exposure, the symbiotic community composition remained similar in
756 colonies from both localities, but we identified major differences in gene regulation
757 processes in the coral, thereby underlining the role of the coral host in the response to
758 heat stress. The colonies from the locality displaying the most variable environment
759 displayed (i) a more plastic transcriptome response involving more differentially
760 expressed genes and higher fold expression changes; as well as (ii) a constitutive higher
761 level of expression for another set of genes (frontloading). In the context of climate
762 change, which is predicted to cause abnormal and rapid temperature increase (IPCC
763 2014), phenotypic plasticity and the capacity for rapid adaptation through epigenetic
764 regulation and/or genetic assimilation would increase the probability of coral survival.
765 Previous studies highlighted the importance of reef managements measures (Rogers *et*
766 *al.* 2015) and assisted evolution (van Oppen *et al.* 2015), but also underlined the

767 importance of preserving standing genetic/epigenetic variation in wild coral
768 populations (Matz *et al.* 2017). Although the molecular mechanisms we described are
769 most likely largely shared in this group of scleractinians, the question remains of the
770 determinism of this thermotolerant phenotype and of the heritability of this character. It
771 is however essential to keep in mind that even the most thermotolerant corals may
772 bleach if they are exposed to temperature significantly higher to their own norm
773 (Hughes *et al.* 2017b; Le Nohaïc *et al.* 2017).

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795 DATA ACCESSIBILITY

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

799 AUTHORS' CONTRIBUTIONS

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

804

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

814 # FIGURE LEGENDS

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

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

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

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

831 FIGURE 5: Heatmap and clustering of significantly differentially expressed genes
832 between the control and the heat stress condition for colonies from each locality. Each
833 gene is represented by a row.

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

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

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

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

852

853 ## SUPPLEMENTARY FILES

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

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

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

864 Supplementary Table S4: MANOVA results for beta diversity (Bray-Curtis distance)
865 between localities, colonies, or experimental conditions.

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

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

871 Supplementary File S7: DESeq2 results for the log2-fold changes, and adjusted *p* values
872 between stress and control conditions for each locality (sheet 1) and for each colony
873 (sheet 2).

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

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

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

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

884

885

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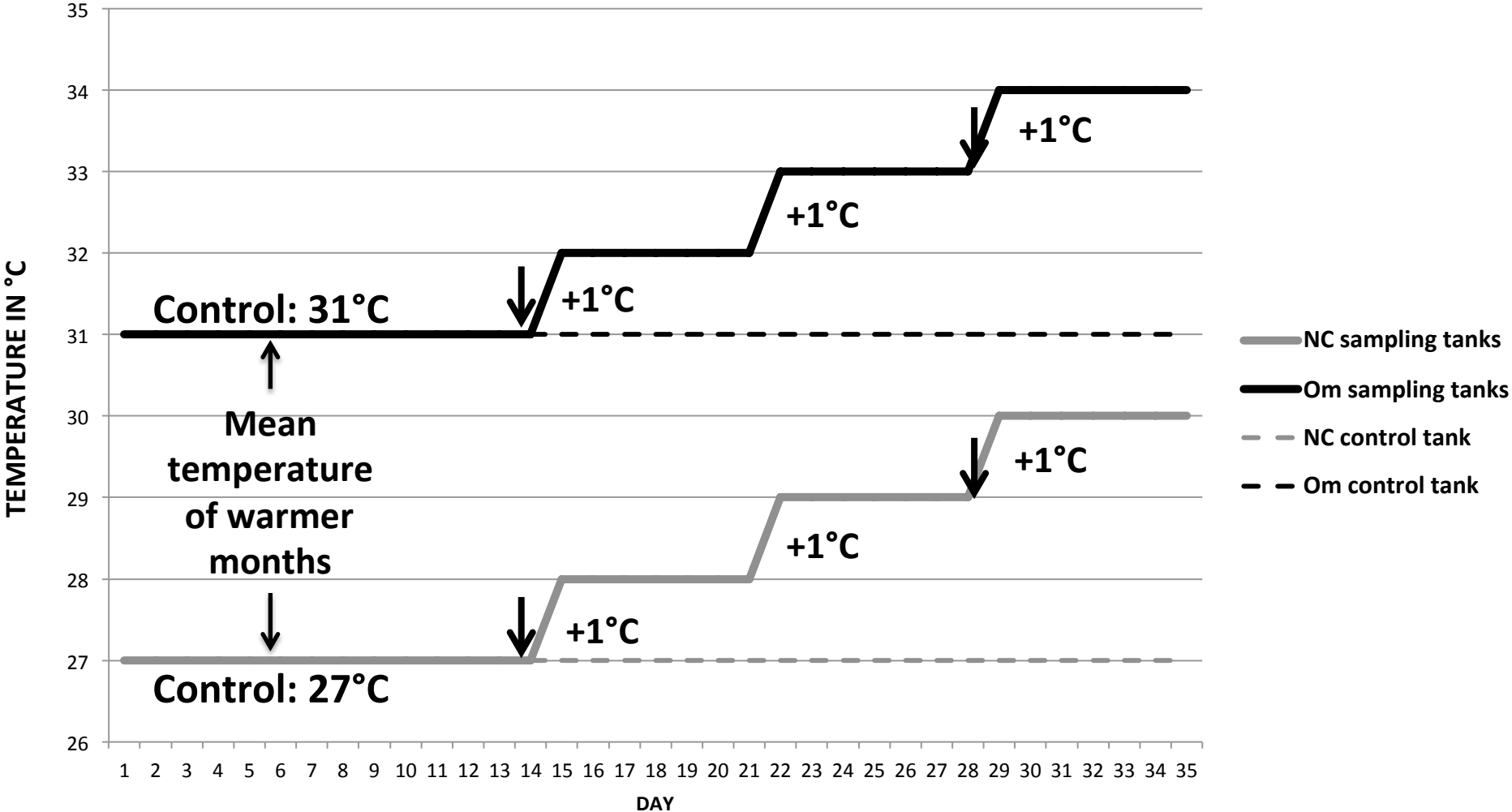
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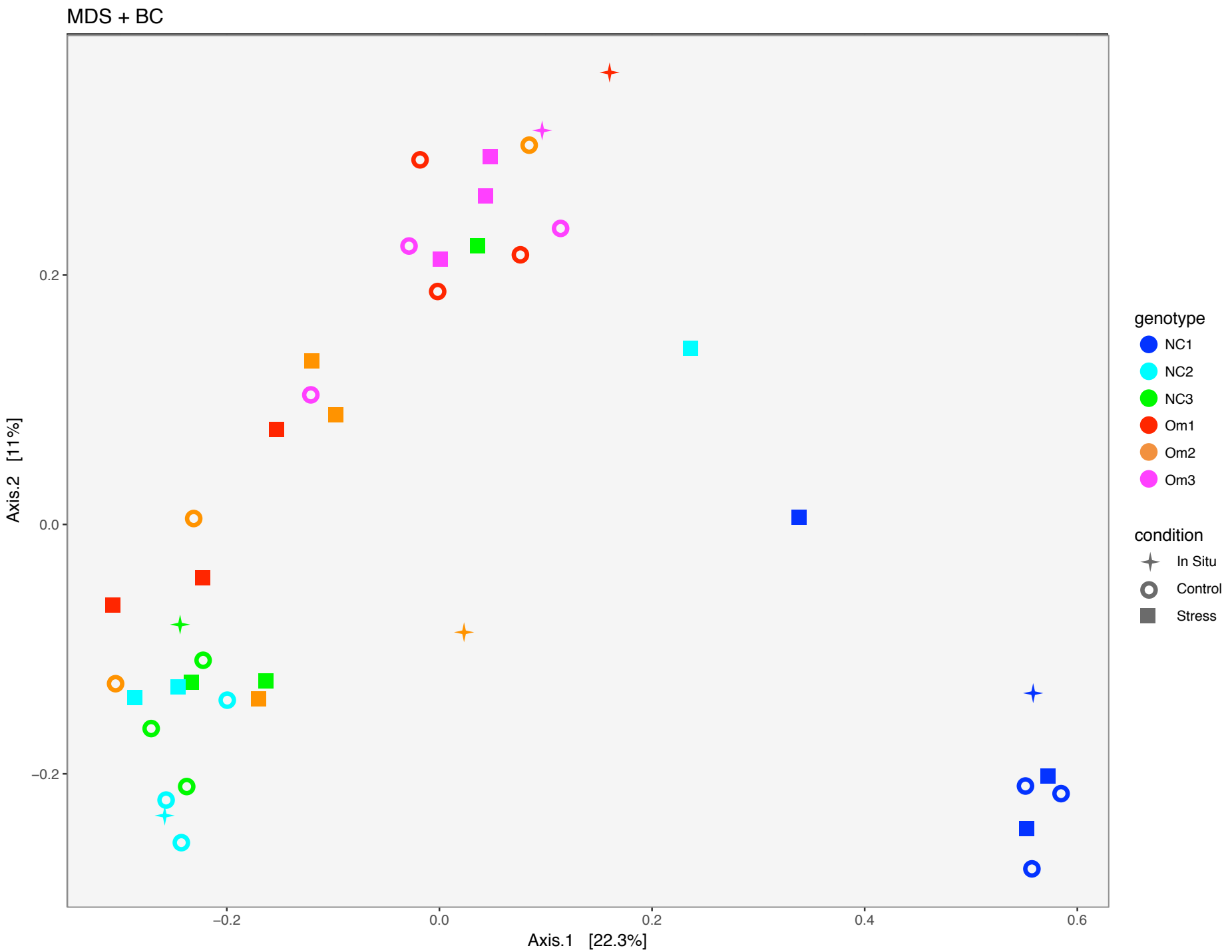
MDS + BC
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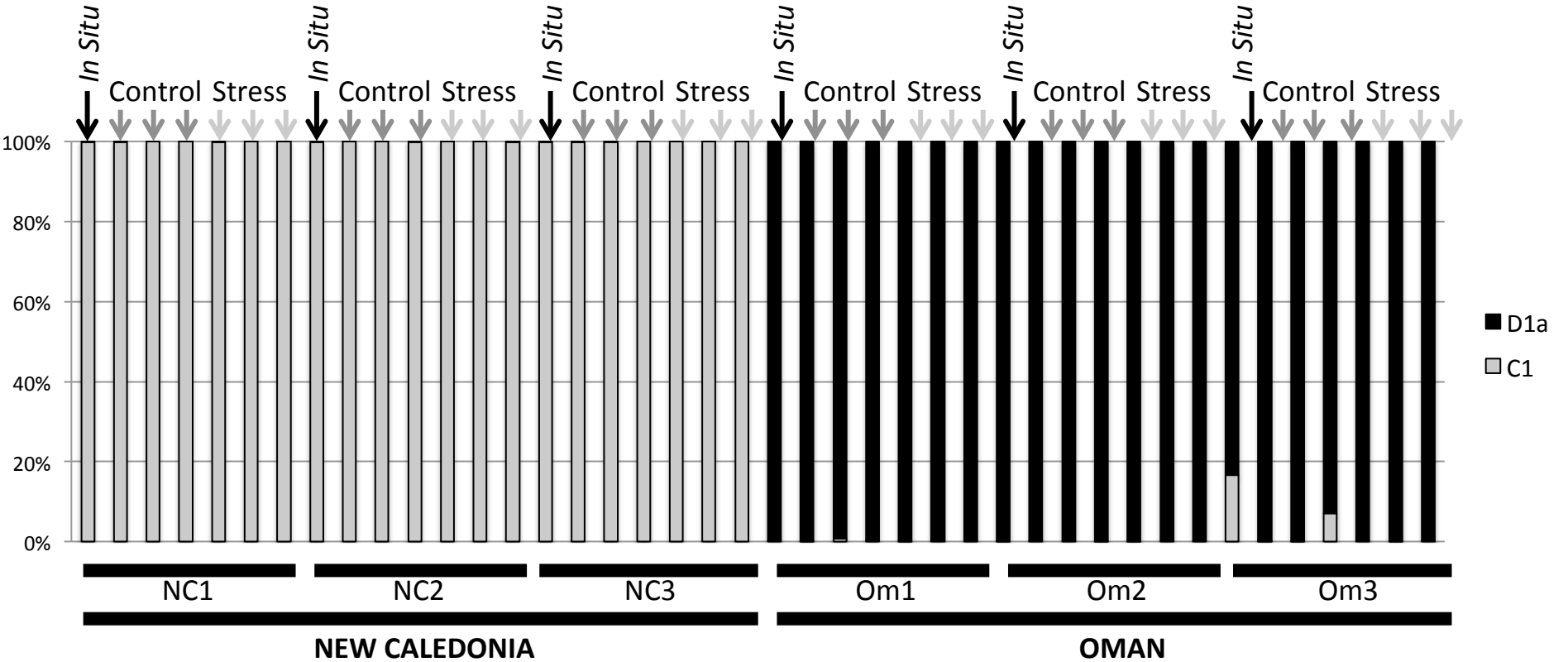
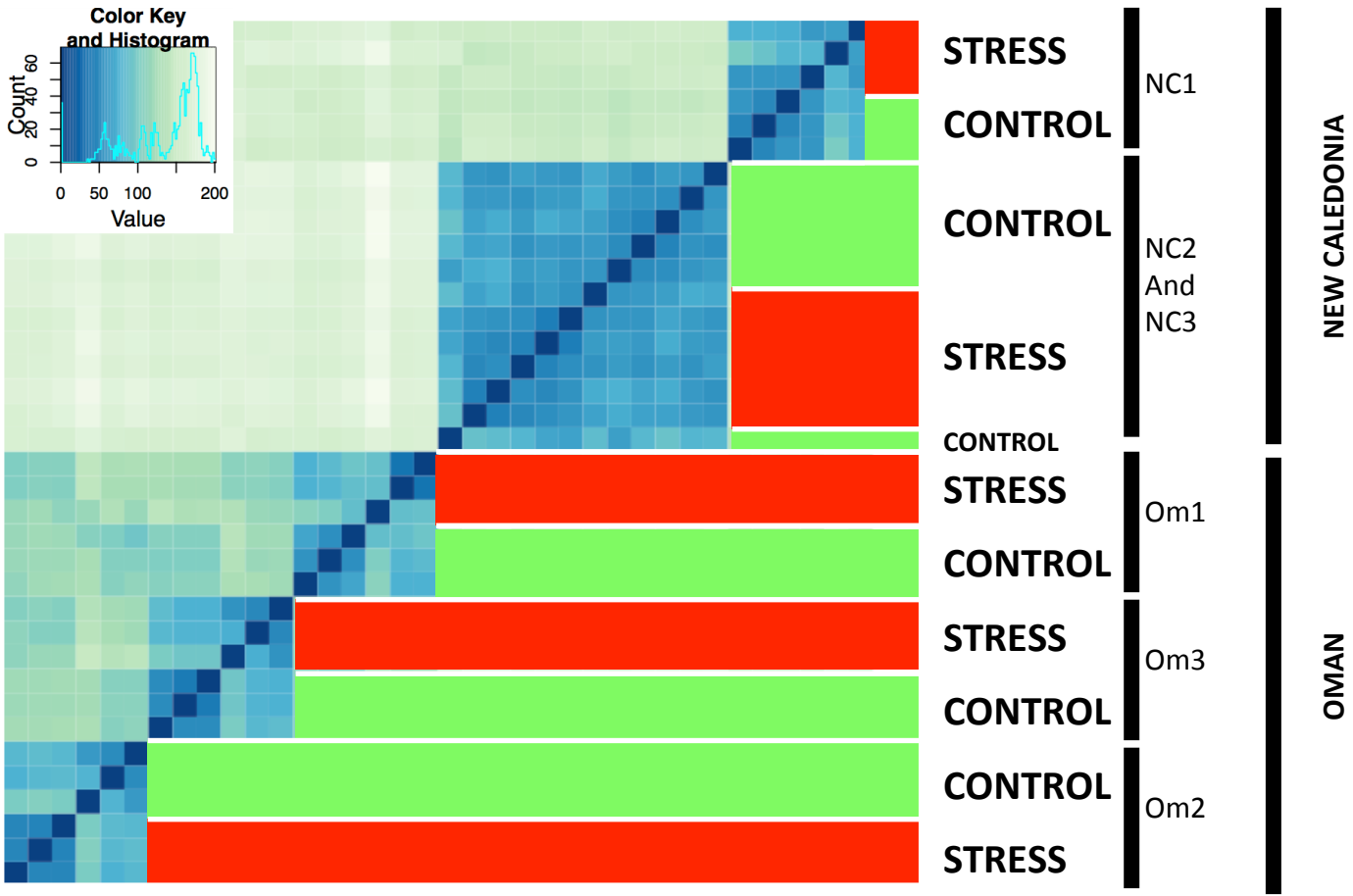
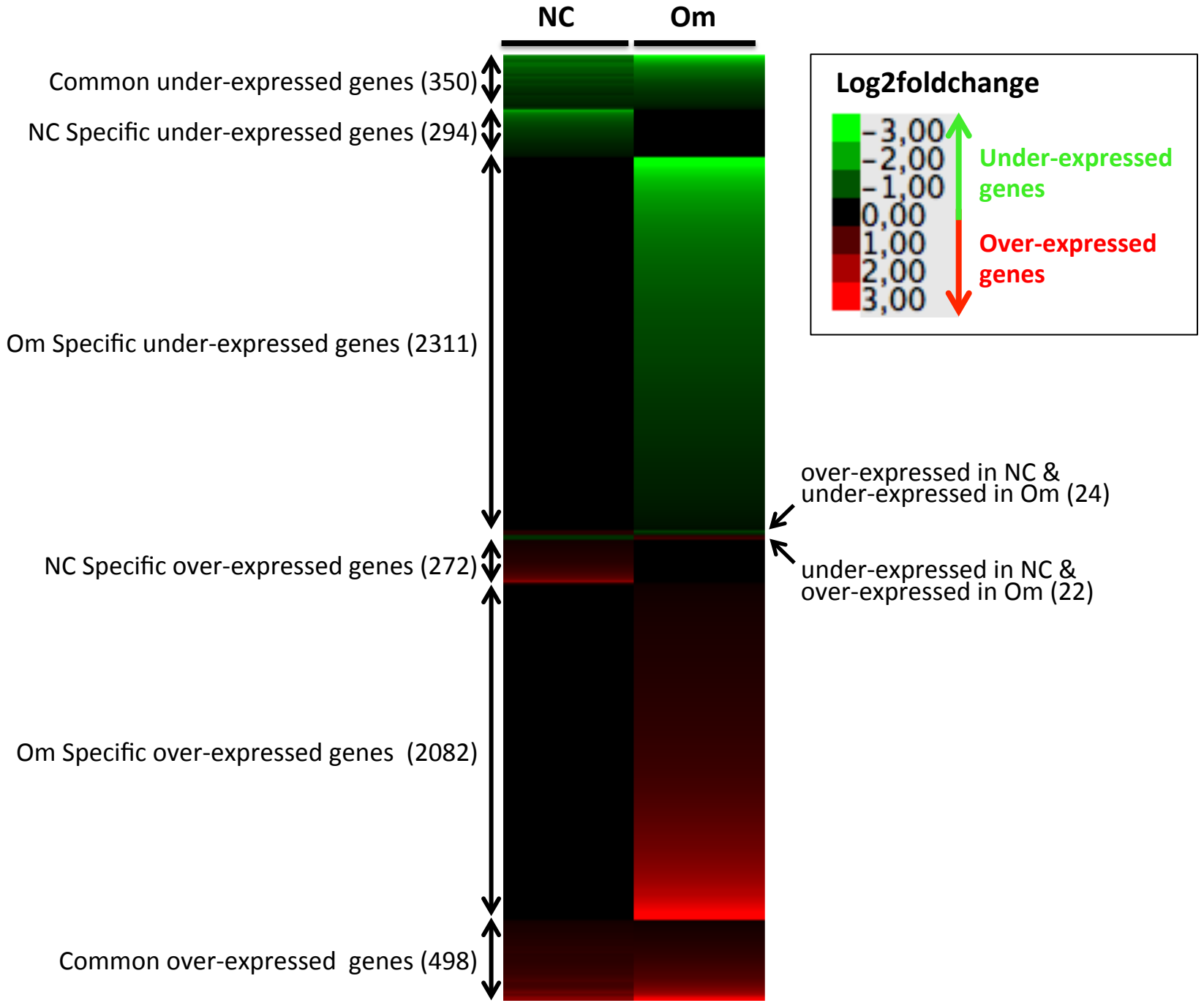
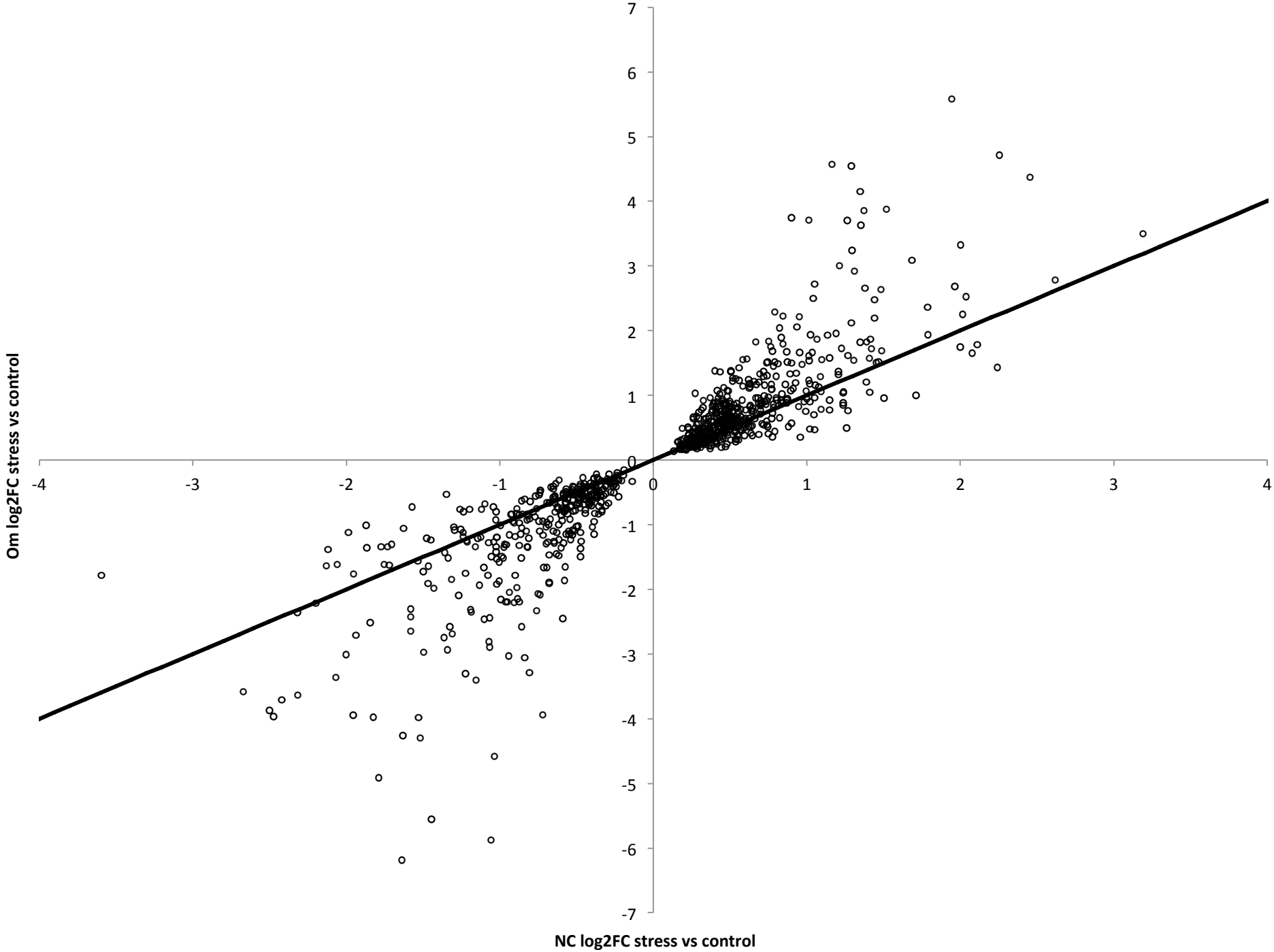
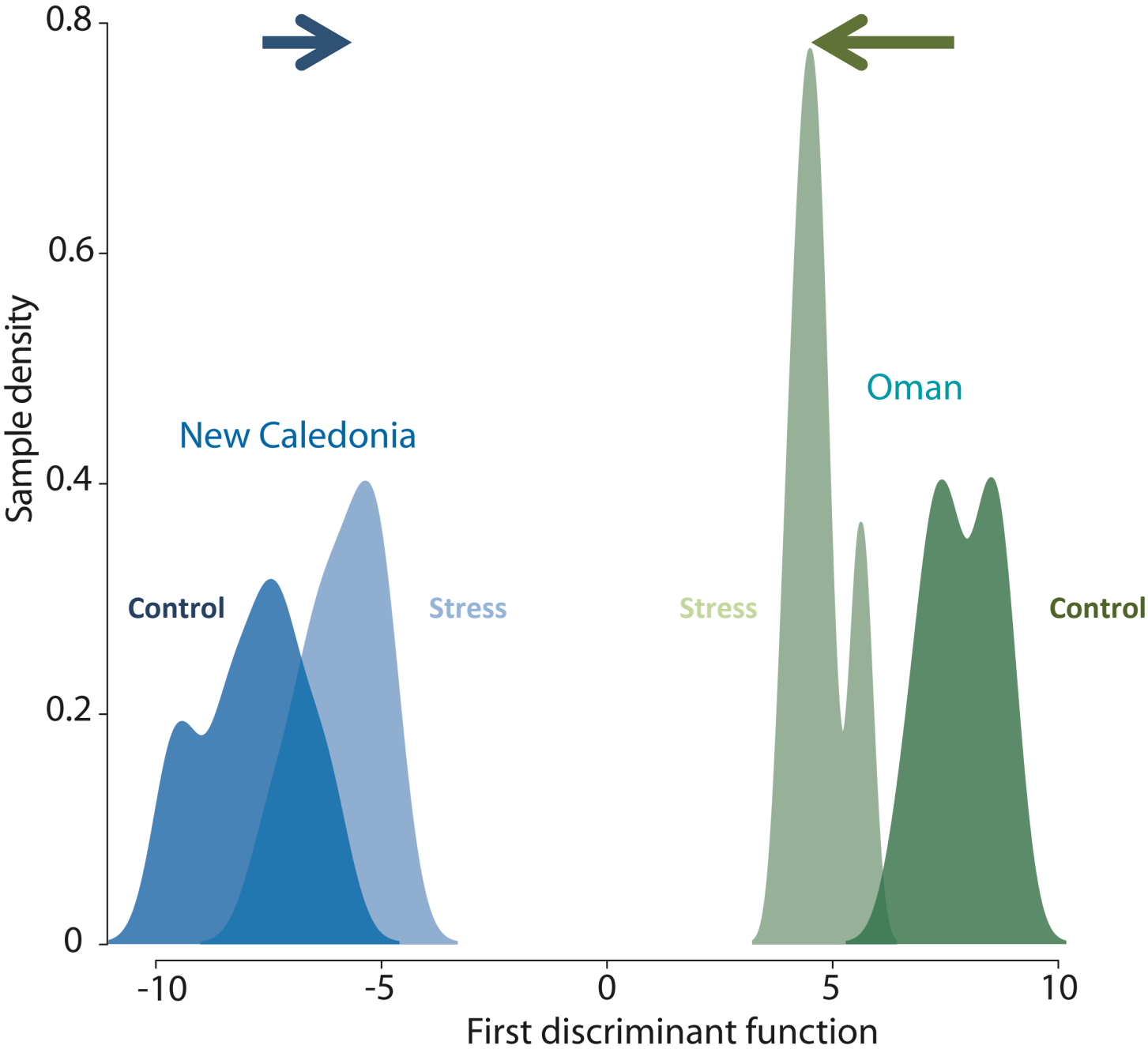
Om1
Om2
Om3
condition
In Situ
Control
Stress

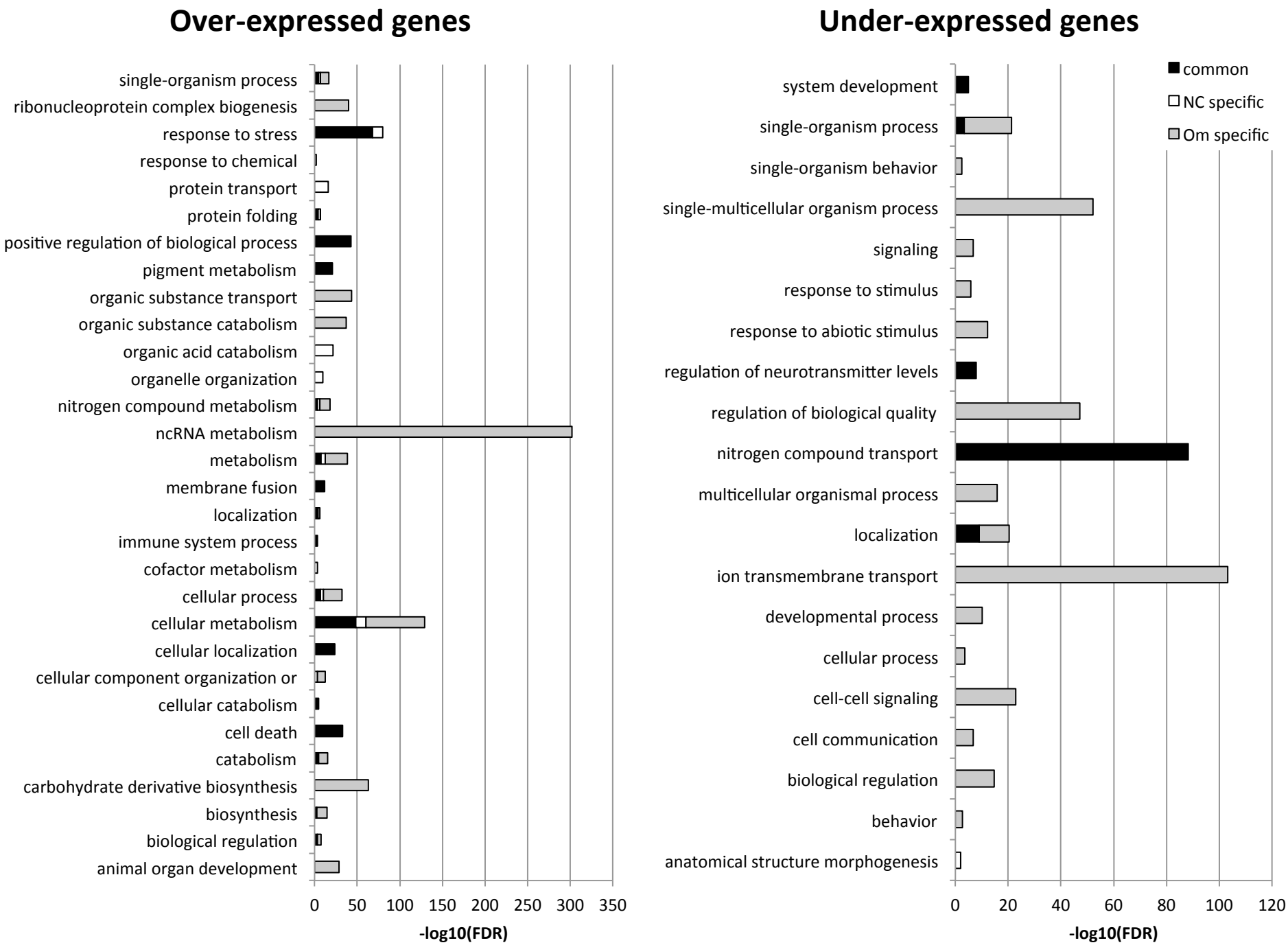

In Situ
Control
Stress
100%
80%
60%
40%
20%
0%
D1a
C1
NC1
NC2
NC3
Om1
Om2
Om3
NEW CALEDONIA
OMAN


Color Key
and Histogram
Count
0
20
40
60
0
50
100
200
Value
STRESS
CONTROL
NC1
CONTROL
STRESS
CONTROL
NC2
And
NC3
NEW CALEDONIA
STRESS
CONTROL
Om1
STRESS
CONTROL
Om3
CONTROL
STRESS
Om2
OMAN


NC
Om
Common under-expressed genes (350)
NC Specific under-expressed genes (294)
Om Specific under-expressed genes (2311)
NC Specific over-expressed genes (272)
Om Specific over-expressed genes (2082)
Common over-expressed genes (498)
over-expressed in NC &
under-expressed in Om (24)
under-expressed in NC &
over-expressed in Om (22)
Log2foldchange
-3,00
-2,00
-1,00
0,00
1,00
2,00
3,00
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)