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1 BRIEF NOTE

2 **Contrasting DNA methylation responses of inbred fish lines to different**
3 **rearing environments**

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9 **Keywords:** Environmental enrichment, epigenetic variation, mangrove killifish,
10 RRBS, genotype by environment interaction

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22 **Abstract**

23 Epigenetic mechanisms can generate plastic phenotypes that can become locally
24 adapted across environments. Disentangling genomic from epigenomic variation
25 is challenging in sexual species due to genetic variation among individuals, but it
26 is easier in self-fertilising species. We analysed DNA methylation patterns of two
27 highly inbred strains of a naturally self-fertilising fish reared in two contrasting
28 environments to analyse the obligatory (genotype-dependent), facilitated
29 (partially depend on the genotype) or pure (genotype-independent) nature of the
30 epigenetic variation. We found higher methylation differentiation between
31 genotypes than between environments. Most methylation differences between
32 environments common to both strains followed a pattern where the two
33 genotypes (inbred lines) responded to the same environmental context with
34 contrasting DNA methylation levels (facilitated epialleles). Our findings suggest
35 that, at least in part, DNA methylation could depend on the dynamic interaction
36 between the genotype and the environment, which could explain the plasticity of
37 epigenetically-mediated phenotypes.

38 **Introduction**

39 Epigenetic modifications are one of the potential molecular mechanisms to
40 explain phenotypically plastic responses within genotypes ^{1, 2}. This is because
41 epigenetic markers can be altered by environmental variation and shape gene
42 expression without changing nucleotide sequences ³, and ultimately affect
43 phenotypic variation ^{1, 4, 5}. In phenotypic plasticity studies, the genome and
44 epigenome are often experimentally confounded ⁶ and an implicit assumption is
45 made that they react to environmental variation following similar norms of
46 reaction. However, this may not necessarily be the case and genomes and
47 epigenomes may respond differently to environmental change, thereby
48 generating additional phenotypic variation ^{3, 7}.

49 To what extent epigenetic modifications act independently from genomic
50 variation is key to understanding the potential role of epigenetics in evolution ^{3, 7},
51 ⁸, as epigenetic variation completely under genetic control would not contribute
52 any additional adaptive value ³. Richards ⁹ classified epigenetic variation in
53 obligatory, facilitated or pure epialleles, based on their degree of autonomy from
54 the underlying genotype. Obligatory epialleles would be fully dependent on
55 genetic variation and should show no variation across environmental change ¹⁰,
56 whereas facilitated and pure epigenetic variation would differ in their degree of
57 autonomy from the genotype (from partially depend to independent) ¹⁰, acting as
58 potential intermediaries between environmental conditions and genome
59 responses.

60 Among the epigenetic modifications, DNA methylation is the best studied,
61 and plays an important role in the pre-transcriptional control of several biological
62 processes, such as cell differentiation and genomic imprinting ^{11, 12}. While

63 correlations among DNA methylation patterns, environmental conditions and
64 phenotypic traits have been widely investigated^{8, 13, 14}, the relative contributions
65 of the genetic background and environmental variation to DNA methylation
66 plasticity are still unclear^{4, 15}. Studies in humans and model organisms suggest
67 that DNA methylation is influenced by the genotype, the environment and also by
68 their interaction^{15, 16}, but quantifying their relative influences is particularly
69 challenging in natural populations with high levels of genetic variation¹⁷. In
70 particular, there is little information on the basis of DNA methylation plasticity
71 beyond model organisms³, specially in teleost fishes, for which most of the
72 studies are focused on well known organisms such as zebrafish or salmonids¹⁸.

73 The self-fertilising hermaphroditic mangrove killifish (*Kryptolebias*
74 *marmoratus*)¹⁹ has naturally inbred lines²⁰ which inhabit mangroves with
75 markedly variability in habitat quality subject to tidal variation, ranging from
76 temporary pools to mangrove leaf litter and crab burrows^{21, 22}. The species
77 displays considerable plasticity in behaviour²³ and reproduction (mixed-mating
78 with different degrees of self-fertilisation and outcrossing)²⁴, both between and
79 within self-fertilising lines²⁵, and it has been suggested that regulation of gene
80 expression through DNA methylation could play role in its plastic response to
81 environmental variation²⁶⁻²⁸.

82 Inbred organisms provide a uniquely opportunity to detangle genetic from
83 epigenetic variation^{29, 30}. Here, we investigated the relative roles of the genotype
84 and the rearing environment (with or without physical enrichment) in DNA
85 methylation plasticity of two genetically different and highly inbred self-fertilising
86 lines of *Kryptolebias marmoratus*. We hypothesised that if DNA methylation was
87 mostly autonomous and shaped by environmental change, a higher number of

88 different epialleles would be found between environments, regardless of the
89 genetic background, than if DNA methylation was mostly under genetic control,
90 where most of the epigenetic differences would occur between genotypes.

91 **Results**

92 We compared DNA methylation patterns in the brain of fish from two highly inbred
93 mangrove killifish lines (DAN and R, originally sampled in Belize mangroves but
94 maintained under laboratory conditions for at least 20 generations) reared under
95 physically enriched (with log and plants) or impoverished conditions (barren) for
96 10 months. By using Reduced Representation Bisulphite Sequencing (RRBS) we
97 identified 5.5 million cytosine sites, of which 139,908 CpG sites fulfilled the
98 minimum coverage requirement, representing 1.2% of the total number of
99 cytosines of the mangrove killifish genome. This result is similar to recent RRBS
100 studies in other fish (1% in rainbow trout¹³; 1.5-2% in guppies³¹).

101 The majority of cytosines surveyed mapped gene bodies (71.32%) or
102 intergenic regions (19.10%), while only 2.54% were located on putative
103 promoters. Linear models using the PCA scores for 1064 DMCs and 194 DMRs
104 identified between genotypes and environments revealed that genotypes
105 explained more of the variance for PC1 (54% of overall variation for both DMCs
106 and DMRs) than environment (Figure S2; Table S1). When predictors were
107 analysed individually, differences between genotypes also corresponded to a
108 higher number of DMCs (817 vs 594, four DMCs shared) and DMRs (43 vs 17,
109 no DMR shared) than differences between environments (Figures 1 and S2).
110 Within genotypes, 357 and 3632 DMCs (25 and 373 DMRs) were identified
111 between environments, for DAN and R, respectively. An additional analysis on
112 three different subsets of six randomly selected R individuals (to match the

113 number of DAN individuals) was carried out to assess possible biases due to
114 differences in sample sizes. This additional analysis identified similar number of
115 DMCs between lines, suggesting that the difference between lines was not due
116 to sampling bias (Table S2). Unsupervised hierarchical clustering revealed
117 distinctive methylation profiles between groups, except for comparisons between
118 environments, where one and two individuals from the poor environment
119 clustered with individuals from the enriched environment for DMCs and DMRs
120 respectively (Figures 1 and S3).

121 Twenty-five annotated DMCs and four DMRs between environments were
122 shared across genotypes, potentially representing environmentally-affected
123 DMCs, independently of genetic background. Of these, based on the direction of
124 methylation across environments, 22 out 25 DMCs were classified as potentially
125 facilitated, with methylation scores following a genotype-specific pattern under
126 similar environments (Table 1). This pattern was supported by the PCA results
127 based on the DMCs methylation scores, which indicated different methylation
128 profiles between environments (PC1 explaining 55.8% of variation), as well as
129 genotypes (PC2 explaining 22.4% of variation) (Figure 2a). PC1 loadings were
130 significantly influenced by the environment ($t = 1.63$, $df = 1$, $p = 0.003$) and the
131 interaction between genotype and environment ($t = -11.25$, $df = 1$, $p < 0.001$), while
132 PC2 loadings were only significantly influenced by the genotype ($t = -1.64$, $df = 1$,
133 $p < 0.001$) (Table 2a). Methylation differences (with a lower threshold of 20%) for
134 the facilitated DMCs ranged from 20.2% to 48.6% (Table 1). The potentially
135 facilitated DMCs were mostly hypermethylated on enriched environments with
136 respect to poor environments for DAN fish, while the opposite pattern was found
137 for R fish (Table 1; Figures 2c and S4). The four DMRs between environments

138 and shared by genotypes were also classified as facilitated, following the same
139 methylation pattern found on the facilitated DMCs (Figure S5; Table S3)

140 Only three of the annotated DMCs within or neighbouring gene bodies
141 were considered pure (Table 1; Figure S6). Average methylation differences for
142 pure DMCs ranged from 25.4% to 34.37% (Table 1). The PCA only using pure
143 DMCs showed a different pattern from the facilitated DMCs, with the PC1
144 separating environments explaining 72.38% of the variation, and the PC2 partially
145 differentiating genotypes explaining 18.85% of the variation (Figure 2b). PC1
146 loadings were significantly affected by the environment ($t = -2.81$, $df = 1$, $p < 0.001$)
147 and the genotype ($t = -2.28$, $df = 1$, $p = 0.008$), while PC2 loadings were only
148 significantly influenced by genotype ($t = 0.29$, $df = 1$, $p = 0.003$) (Table 2b, Figures
149 2d and S6).

150 Molecular network analysis revealed a highly connected network linked by
151 genetic interactions and co-expression interactions, that was composed by 23
152 input annotated DMCs (the uncharacterised LOC108245430 and *ubald1* with no
153 identified connections were removed) and 20 neighbouring genes (Figure S7).
154 Centrality parameters, such as average degree (mean=10.55; $SD \pm 5.89$),
155 closeness (mean=0.53 \pm 0.06), and radiality (mean=0.77 \pm 0.06) (Table S4),
156 suggested that any alteration of the expression of the genes contained in the
157 network might have major effects on genetic interactions and gene expression
158 levels.

159 Twelve of the 15 most connected genes within the network (>10
160 connections), were input genes (i.e. genes affected by DMCs between
161 environments and shared by genotypes). Gene ontology analysis showed that
162 some of these genes are involved on important cellular and metabolic processes

163 in zebrafish, such as regulation of transcription by RNA polymerase and gene
164 expression (*myc*), RNA modification (*trit1*), intracellular calcium content (*ryr3*),
165 and lipid metabolism (*sorcs2*), as well as pathways related to angiogenesis and
166 stress response (*ryr3* and *myc*) (Table S5).

167 **Discussion**

168 The potential adaptive role of epigenetically-mediated plasticity depends on the
169 relationship between the genome, the epigenome and the environment ^{6, 7}. By
170 using two naturally inbred strains of the mangrove killifish reared under
171 contrasting environmental conditions, we have identified significant methylation
172 differences among genotypes and environments, with different levels of
173 autonomy from the genetic background.

174 Environmental enrichment in fish affects brain structures ³²⁻³⁴, however few
175 studies have investigated the molecular mechanisms underlying these changes
176 ^{35, 36} and whether it varies across different genetic backgrounds. *Kryptolebias*
177 *marmoratus* populations are composed by naturally inbred lines living in highly
178 variable habitats ^{21, 25}, which display remarkable phenotypic variation (e.g. in
179 reproductive output ^{24, 37}, behaviour ^{23, 38, 39} or sexual differentiation ³⁷), even
180 under identical environmental conditions ²⁵. Thus, the strains we used here were
181 previously shown to display different sex-ratios in response to temperature
182 variation ²⁰ as well as differences in gene expression in response to parasitic
183 infection ⁴⁰, suggesting a potential combination of genetic and non-genetic
184 mechanisms in mediating phenotypic variation ²⁹. Our results indicate that
185 genotypes have an overriding influence on brain DNA methylation patterns, and
186 that their effect is greater than that caused by environmental enrichment. We only
187 found a few DMCs that could be considered facilitated or pure epialleles,

188 supporting the idea that environmentally-induced autonomous DNA methylation
189 may be limited². Yet, the DNA methylation patterns of these putative independent
190 epialleles indicated that DNA methylation outcomes could depend on specific
191 combinations of the genotype and environmental conditions, although we cannot
192 fully discard the potential contribution of heritable epigenetic states independent
193 of the genotypes⁷ and/or brain cell heterogeneity.

194 The large differences in number of DMCS we found between lines could
195 be explained by their genetic differences⁴¹. Studies in model organisms indicate
196 that DNA methylation, and potentially other layers of chromatin organisation, are
197 strongly influenced by genomic variants⁴²⁻⁴⁴. For example, the spontaneous
198 mutation in a gene related to methyltransferase1 activity, increased in 40% the
199 methylation differences among inbred lines of *Arabidopsis thaliana*⁴⁵ and in
200 humans, 25% of variation in neonates' methylomes can be explained by their
201 genotype, while the remaining 75% is related to interactions between the
202 genotype and maternal factors (i.e. smoking, age, intrauterine environment)¹⁵.

203 Most of the DMCs observed between environments and common to both
204 genotypes were located in gene bodies and were highly integrated within a gene
205 network of genetic interactions and co-expression. Recent evidence in plants⁴⁶
206 indicates that gene body methylation can reduce erroneous transcription, and in
207 oyster⁴⁷ and zebrafish⁴⁸ there seems to be a positive correlation between gene
208 body methylation, gene expression and transcriptional regulation. Here, some of
209 the genes affected by the DMCs found in gene bodies were related to the
210 regulation of RNA polymerase activity and gene expression patterns (myc and
211 trit1)^{47, 48}, suggesting that these changes in methylation could be involved in
212 biological and cellular processes.

213 DNA methylation is a good candidate for mediating phenotypic plasticity,
214 given its responsiveness to environmental change, effects on downstream
215 phenotypes, and transgenerational stability^{3, 4, 49}. Our results, suggest that, at
216 least in part, DNA methylation patterns are influenced by a dynamic interaction
217 between genotypes and the environment. Further research to investigate whether
218 the patterns found here might influence transcription is warranted to assess the
219 generality of our results, that might provide a potential mechanistic explanation
220 for the genotype-by-environment patterns often observed in phenotypically
221 plastic responses^{6, 50}.

222

223 **Methods**

224 We used hermaphrodite fish from two highly inbred strains (R and DAN) of
225 *Kryptolebias marmoratus* originally collected from Belize^{51, 52} and kept in the
226 laboratory conditions (25-27 °C, 16-18‰ ppm salinity under a 12h light:12h dark
227 photoperiod), for at least 20 selfing generations⁵³. The R (also called 50.91) strain
228 was collected in Belize (Twin Cayes) in the early 1990s while the DAN (*Dan06*)
229 strain was also collected from Belize in the early 2000s⁵¹. These selfing lines had
230 previously shown different DNA methylation responses to environment
231 (temperature) variation²⁰.

232 We compared brain methylation of fish reared under enriched and impoverished
233 conditions, as previous studies had shown environmental enrichment can affect
234 behavioural flexibility⁵⁴, brain size and cognition⁵⁵, and induce epigenetic
235 modifications during early development⁵⁶. We used two different habitats with
236 different levels of environmental enrichment: 1) a physically enriched habitat,

237 where individual fish were placed in contiguous rectangular tanks (9cm depth x
238 12cm width x 8cm length) filled with 400ml of brackish water with one perforated
239 artificial log (3cm depth x 4 cm width x 4cm length) and three artificial plants to
240 simulate a complex habitat, and 2) a barren habitat (hereafter called poor) with
241 the same tank conditions but without physical enrichment (Figure S1). Tanks
242 were separated by opaque screens to prevent visual contact between individuals.
243 For both strains, five initial lab-reared hermaphrodite progenitors of similar size
244 (mean=3.8cm, sd= ± 0.12) and age (mean=417.3 days' post hatchling, sd= ± 13.4)
245 were chosen. Eggs from these progenitors were maintained individually in
246 circular plastic pots containing 100ml of brackish water and checked daily (Figure
247 S1). Upon hatching, individual alevins were randomly assigned to treatment tanks
248 (enriched and poor), with one fish in each tank. Hatching success was of 90%.
249 The initial experimental set up consisted of 29 R fish (18 in enriched habitat, 11
250 in poor habitat) and 21 DAN fish (10 in enriched, 11 in poor). Fish were
251 maintained under standard laboratory conditions as above and fed three times a
252 week with live brine shrimp (1ml for the first two months post-hatching, and 2ml
253 for the rest of the time). Fish were maintained in the experimental tanks for 10
254 months before being euthanized for brain methylation analysis. At 7 months post
255 hatching all fish in the experiment had laid at least one egg indicating that they
256 were all sexually mature self-fertilising hermaphrodites.

257 (a) Genome-wide DNA methylation data

258 Fish were euthanized using tricaine methane-sulfonate (MS-222) following Home
259 Office Schedule 1 and their brains kept in molecular biology grade ethanol (99%)
260 for DNA extraction. Brain DNA was extracted from 22 individuals for epigenetic
261 analysis (six DANs: three from each environment; 16 Rs: six from poor, ten from

262 enriched environment) using Qiagen DNeasy Blood and tissue kit (Qiagen). Fish
263 were genotyped for 23 microsatellites ⁵⁷. Genetic differences were identified
264 between inbred lines ($F_{ST}=1.00$, Table S6), but not within lines. All individuals
265 tested were homozygotes and identical within each line for all the markers
266 analysed (Table S6).

267 Bisulphite converted genomic DNA libraries were prepared using
268 Diagenode Premium Reduced Representation Bisulphite Sequencing (RRBS) kit
269 according to manufacturer's indications and sequenced on an Illumina NextSeq
270 500 platform using a 1x75pb single-end run, with PCR fully methylated and
271 unmethylated spike controls added.

272 Quality assessment was performed using FastQC ⁵⁸. TrimGalore! ⁵⁹ was
273 used to trim low-quality base calls and adapters. Trimmed reads were aligned to
274 the *Kryptolebias marmoratus* reference genome (ASM164957v1,
275 GCA_00164975.1: source NCBI) prior in-silico bisulphite conversion using
276 Bismark v0.17.0 ⁶⁰, which was also used for cytosine methylation calls. Only
277 methylation within CpG context ⁶¹, with a minimum coverage of 10 reads in each
278 sample across the 22 individuals sequenced ⁶² was considered for subsequent
279 analysis. Samples were divided into four experimental groups: "DAN enriched",
280 "DAN poor", "R enriched", "R poor". Mapped reads were processed using
281 SeqMonk ⁶³. After quality filtering, approximately 273 million reads were retained,
282 averaging 12 million reads per sample. Of those ~ 62.9 % were uniquely mapped
283 reads to the reference genome (Table S7). Overall bisulfite conversion was
284 99.6%.

285 (b) Differentially methylated cytosines and regions

286 To identify differentially methylated cytosines (DMCs) across experimental
287 groups, we used logistic regression on quantitated normalised data with $p < 0.01$
288 after multiple testing correction (Benjamini-Hochberg) and $>20\%$ minimal CpG
289 methylation difference ($|\Delta M|$), using R bridge in SeqMonk. We also performed t-
290 tests across experimental group replicates, to generate a more conservative list
291 of DMCs, only considering those shared by both statistical approaches. To
292 identify differently methylated regions (DMRs), we performed a genome-wide
293 unbiased DMR detection using tiling windows of 1000bp on windows with at least
294 five CpGs with ≥ 10 reads across all individuals.

295 We used the scores of methylation for DMCs and DMRs between
296 genotypes and environments for principal component analysis (PCA) using
297 ggfortify package⁶⁴ in R v. 3. 4. 3 (R Core Team 2014). To test for the effect of
298 the genotype, environment and their interaction on the methylation scores, we
299 used linear models with the scores for the first two PCA axis ($>70\%$ of the total
300 variation) as a function of genotype, environment and their interaction. We then
301 individually compared DMCs and DMRs between genotypes, followed by a
302 comparison between environments. Subsequently, a comparison within each
303 genotype between environments was carried out to identify potential
304 environment-dependent DMCs and DMRs. From these comparisons, we
305 identified annotated DMCs and DMRs shared between genotypes, which should
306 represent commonly affected DMCs regardless of the genetic background.

307 We classified the DMCs and DMRs shared across genotypes between
308 environments as facilitated, when displaying different directions of variation (non-
309 parallel) on methylation scores across genotypes in the same environment (i.e.
310 hypermethylated in an environment for one strain and hypomethylated in the

311 other), or pure when displaying the same direction of variation (parallel) across
312 genotypes and environments (i. e. hypermethylated or hypomethylated for both
313 genotypes in the same environment) ⁹.

314 (c) Molecular network analysis and centrality metrics

315 To identify potential functional implications of variation in DNA methylation for the
316 annotated DMCs identified across genotypes between environments, we built a
317 functional gene network using GeneMANIA ⁶⁵. To identify central genes ⁶⁶ within
318 the molecular network, we used NetworkAnalyzer ⁶⁷ plugin into Cytoscape v.
319 3.7.1 ⁶⁷. Panther GO terms ⁶⁸) was used to identify biological process and
320 pathways for the most connected genes (>10 connections) within the network.

321 **Ethics**

322 Work was carried out under Swansea University Animal Ethics Committee permit
323 STU_BIOL_30484_110717192024_3.

324 **Data accessibility**

325 Sequences are accessible from <https://www.ncbi.nlm.nih.gov/bioproject/506827>.

326

327 **Authors' contributions**

328 SC, WMBF designed the experiment. WMBF, NB, DRB performed the
329 experiment. WMBF, CGL, DRB analysed the data. WMBF, SC wrote the
330 manuscript with participation of all authors.

331 **Competing interests**

332 The authors have no competing interests.

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336

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552

553 **Figure legends**

554 **Figure 1.** Heat map illustrating percentage of methylation for all differentially
555 methylated cytosines (DMCs) identified (a) between genotypes, (b) between
556 environments, (c) between environments for DAN strain, and (d) between
557 environments for R strain (logistic regression $q < 0.01$ and $|\Delta M| > 20\%$, and t.test
558 $p < 0.01$) using unsupervised hierarchical clustering. Rows represent a unique
559 CpG site and columns individual fish.

560 **Figure 2.** Principal component analysis (PCA) and reaction norms of epialleles
561 across genotypes and environments. PCAs were based on individual scores of
562 methylation across either (a) facilitated or (b) pure annotated DMCs. Dark yellow
563 for DAN individuals on enriched environments; light yellow for DAN genotype on
564 poor environments; dark green for R individuals on enriched environments; light
565 green for R genotype on poor environments. Each reaction norm represents the
566 change on averaged methylation scores (in percentage) for (c) facilitated and (d)
567 pure epialleles annotated DMCs across environments. Different colours
568 represent the genotypes (yellow for DAN; green for R). Different shapes (d)
569 represent different annotated DMCs. Epialleles were classified according to
570 Richards (2006). Detailed information for each annotated DMCs methylation
571 score across genotypes is available at Table 1.

572

573

574 **Table 1.** Methylation differences averaged (percentage) for differentially methylated cytosines (DM
575 enriched), shared between genotypes (DAN, R) which overlap annotated genes (refe
576 GCA_00164975.1). Epiallele classification (pure or facilitated) followed [9]. Positive and negative
577 decreased methylation in enriched and poor environments, respectively. Q-value is the p-value a
578 (FDR=0.05).

Gene symbol	Entrez gene name	Epiallele classification	Meth diff DAN	Q-value
acvr2a	activin A receptor type 2A	P	34.37	0.007
col25a1	collagen type XXV alpha 1 chain	F	43.61	0.005
dmap1	DNA methyltransferase 1 associated protein 1	F	26.99	<0.001
foxp4	forkhead box P4	F	22.50	<0.001
gpc5	glypican 5	F	31.82	0.01
mipol1	mirror-image polydactyly 1	F	35.85	<0.001
necab2	N-terminal EF-hand calcium binding protein 2	F	20.25	0.01
neo1	neogenin 1	F	20.25	<0.001
nudcd1	NudC domain containing 1	F	39.76	<0.001
ramp3	receptor activity-modifying protein 3-like	P	-27.12	0.037
ryr3	ryanodine receptor 3	P	-30.48	0.003
sorcs2	sortilin-related VPS10 domain containing receptor 2	F	36.81	0.008
trit1	tRNA isopentenyltransferase 1	F	20.38	<0.001

trmt44	tRNA methyltransferase 44	F	23.98	<0.001
ubald1	UBA like domain containing 1	F	36.76	0.019
zeb2	zinc finger E-box binding homeobox 2	F	31.83	<0.001
znf516	zinc finger protein 516	F	31.51	<0.001
zranb3	zinc finger RANBP2-type containing 3	F	41.41	<0.001
LOC108234847	adhesion G protein-coupled receptor L3-like	F	48.57	<0.001
LOC108240988	non-muscle caldesmon-like	F	32.49	0.005
LOC108243470	protein-methionine sulfoxide oxidase mical2b-like	F	33.35	<0.001
LOC108243852	receptor-type tyrosine-protein phosphatase N2-like	F	37.97	0.014
LOC108245430	uncharacterized protein	F	42.94	<0.001
LOC108247402	spectrin beta chain, non- erythrocytic 1-like	F	40.73	0.006
LOC108251479	transcriptional regulator Myc- B-like	F	30.49	0.010

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580

581 **Table 2.** Linear model of principal component scores for mangrove killifish
 582 epialleles shared between genotypes (R, DAN) and environments (poor,
 583 enriched).

	t-values	Prop. of variance (%)	df	p-value
<i>(a) Facilitated epialleles</i>				
<u>PC1 scores</u>				
Genotype	1.63	0.06	1	0.80
Environment	1.58	12.41	1	0.003
Genotype x Environment	-11.25	68.08	1	<0.001
<u>PC2 scores</u>				
Genotype	-1.64	91.35	1	<0.001
Environment	8.28	0.21	1	0.49
Genotype x Environment	10.29	0.35	1	0.38
<i>(b) Pure epialleles</i>				
<u>PC1 scores</u>				
Genotype	2.28	13.18	1	0.008
Environment	-2.81	59.28	1	<0.001
Genotype x Environment	0.98	0.09	1	0.80
<u>PC2 scores</u>				
Genotype	0.29	37.96	1	0.003
Environment	-1.82	1.14	1	0.56
Genotype x Environment	-2.97	0.67	1	0.65

584