Check for updates

1
2 MR. DANIEL NEWHOUSE (Orcid ID : 0000-0002-7694-0475)
3 DR. MARGARIDA BARCELO-SERRA (Orcid ID : 0000-0002-2752-0896)
4 DR. CHRISTOPHER BALAKRISHNAN (Orcid ID : 0000-0002-0788-0659)
5
6
7 Article type : Original Article
8
9
10 **Corresponding author mail id:** newhoused12@students.ecu.edu
11 **Title:** Parent and offspring genotypes influence gene expression in early life
12
13 **Running Title**: Parental effects on offspring gene expression
14
15 **Authors & Affiliations**:
16 Daniel J. Newhouse[1*], Margarida Barcelo-Serra[2], Elaina M. Tuttle[2], Rusty A. Gonser[2],
17 Christopher N. Balakrishnan[1]
18
19 [1]East Carolina University, [2]Indiana State University
20 [*]Corresponding Author
21 **Abstract**
22 Parents can have profound effects on offspring fitness. Little, however, is known about the
23 mechanisms through which parental genetic variation influences offspring physiology in natural
24 systems. White-throated sparrows (*Zonotrichia albicollis*, WTSP) exist in two genetic morphs,
25 tan and white, controlled by a large polymorphic supergene. Morphs mate disassortatively,
26 resulting in two pair types: tan male x white female (TxW) pairs, which provide biparental care
27 and white male x tan female (WxT) pairs, which provide female-biased care. To investigate how
28 parental composition impacts offspring, we performed RNA-seq on whole blood of WTSP

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/MEC.15205

This article is protected by copyright. All rights reserved

Author Manuscript

nestlings sampled from nests of both pair types. Parental pair type had a large effect on nestling gene expression, with 881 genes differentially expressed (DE) and seven correlated gene co-expression modules. The DE genes and modules expressed at higher levels in WxT nests with female-biased parental care function in metabolism and stress-related pathways resulting from the overrepresentation of proteolysis and stress-response genes (e.g. SOD2, NR3C1). These results show that parental genotypes and/or associated behaviors influence nestling physiology, and highlight avenues of further research investigating the ultimate implications for the maintenance of this polymorphism. Nestlings also exhibited morph-specific gene expression, with 92 differentially expressed genes, comprising immunity genes and genes encompassed by the supergene. Remarkably, we identified the same regulatory hub genes in these blood-derived expression networks as were previously identified in adult WTSP brains (EPM2A, BPNT1, TAF5L). These hub genes were located within the supergene, highlighting the importance of this gene complex in structuring regulatory networks across diverse tissues.

Keywords

Transcriptome, parental effects, early life stress, nestling, RNAseq, ornithology

Author Manuscript

Introduction

Parents can have profound impacts on offspring development and fitness. Parental effects can manifest throughout the developmental period, both pre- and post-natally (reviewed in Meaney 2001, Lupien et al. 2009) and can be mediated through parental behaviors, genetics and physiology during early development (Trivers 1972). Parents play a substantial role in establishing the early life environment of offspring. For example in birds, parental decisions on nest placement, incubation behavior, and nest defense could strongly impact developmental conditions of the egg. These parental behaviors will impact exposure to sunlight, humidity, temperature, and other environmental impacts of the eggs, which can influence developmental physiology (e.g. Nord & Nilsson 2011). In addition to parental behaviors, prenatal effects often arise via physiological maternal effects. Developing offspring are susceptible to the maternally created environment (e.g. maternal hormones, immune state, nutrition), which influence

This article is protected by copyright. All rights reserved

offspring physiology (Mousseau & Fox 1998, Jacquin et al. 2012; reviewed in Gluckman et al. 2008, Wolf & Wade 2009, Cottrell & Secki 2009).

During the post-natal stage, provisioning plays a prominent role in offspring development, with the quality and quantity of food items crucial for offspring development (Royle 2012, van Oers et al. 2015, Griebel et al. 2019). Similar to the prenatal stage, parental behaviors could also have strong impacts on offspring physiology. In many species, offspring are left alone during parental foraging trips, increasing environmental exposure (Lloyd and Martin 2004) and predation risk (Lima 2009). Parental separation can also increase offspring anxiety (Millstein & Holmes 2007). Siblings must also compete to optimize food intake, body temperature regulation, and preening (Mock & Parker 1997). Thus, this postnatal environment, largely mediated through parental effects, can be a potential source of early life stress (ELS) in offspring, which may result in life-long fitness effects (reviewed in Monaghan 2014).

ELS has broad effects on organisms, including impaired neural development, neuroendocrine signaling, behavior, and physiology (McEwen 2007, Monaghan 2014). For example, ELS is associated with impaired neuroendocrine function and corresponding impaired hypothalamic-pituitary-adrenal (HPA) development, which leads to an increased stress response sensitivity later in life (e.g. Heim et al. 2008, Spencer et al. 2009, Crespi et al. 2012, Spencer 2017). ELS can exacerbate behavioral alterations as organisms develop and mature including symptoms of anxiety and depression in the postnatal environment (Noguera et al. 2017) and result in impaired behavior as reproductive adults (e.g. Krause et al. 2009, reviewed in Bolton et al. 2017). While the organismal effects of ELS are well studied, the genetic underpinnings are relatively underexplored.

Much of the genetic work in the context of ELS has focused on gene regulatory impacts, particularly in mammalian biomedical models (reviewed in Szyf et al. 2007, Szyf 2009, Silberman et al. 2016, Alyamani & Murgatroyd 2018). In particular, the quality of parental care can have strong impacts on offspring health resulting from epigenetic modifications (Liu et al. 1997, Meaney 2001, Weaver et al. 2004). These gene regulation studies primarily use changes in DNA methylation as an indicator of ELS (Murgatroyd et al. 2009, Kinnally et al. 2011, Lewis & Olive 2014) and recent work has expanded these approaches into non-mammalian organisms (e.g. Rubenstein et al. 2016, Moghadam et al. 2017, Pértille et al. 2017, Gott 2018, Sheldon et al.

Author Manuscript

This article is protected by copyright. All rights reserved

88 2018). DNA methylation studies of ELS investigate changes to the structure of DNA, but are
89 often limited in the functional implications of ELS (i.e. transcription and translation). In general,
90 these modifications are thought to alter transcriptional activity of genes in the modified genomic
91 region (Berger 2007, Lowdon et al. 2016). Indeed, several studies have also taken candidate gene
92 approaches to investigating gene expression in the context of ELS (Marco et al. 2014, Diaz-Real
93 et al. 2017, Anastasiadi et al. 2018, Reshetnikov et al. 2018). However, very few studies assess
94 genome-wide transcription under ELS (Moghadam et al. 2017), particularly in the context of
95 parental effects (but see: Weaver et al. 2006).

96 In this study, we examined the white-throated sparrow (*Zonotrichia albicollis*, WTSP) to
97 assess the role of parental genotype on offspring gene expression. WTSPs exist in two plumage
98 morphs, tan (T) and white (W), that are found in both sexes and in roughly equal frequencies
99 (Lowther 1961). These morphs are genetically determined by alternative alleles of a supergene, a
100 group of linked genes that are inherited together, show limited recombination, and maintain
101 complex behavioral traits (i.e. WTSP morphs; Schwander et al. 2014, Taylor & Campagna 2016).
102 The WTSP supergene resulted from a complex chromosomal rearrangement comprising multiple
103 inversions (hereafter referred to as "inversion" or "inverted"). This inversion contains ~1,100
104 genes on chromosome two, termed ZAL2^{m} (Throneycroft 1975, Thomas et al. 2008, Romanov et
105 al. 2009, Tuttle et al. 2016). W morphs are nearly always heterozygous for the inversion
106 (ZAL2/ZAL2^{m}) and T morphs are always homozygous (ZAL2/ZAL2; Thorneycroft 1966, 1975).

107 This unusual polymorphism in WTSPs influences hormonal profiles and the behavior of
108 both sexes, and thus has the potential to influence pre- and post-natal environments for the
109 offspring of different morphs. W morph males maintain higher levels of testosterone during the
110 pre-laying, incubation, and brooding stages and oestradiol during the laying and brooding stages
111 (Horton et al. 2014). Only oestradiol has been shown to differ between adult female morphs
112 during the breeding season and is higher in W morph females during the pre-laying and laying
113 stages (Horton et al. 2014). These genetic and hormonal differences also translate into striking
114 behavioral differences. W morphs of both sexes, for example, are highly territorial and sing
115 frequently whereas T morphs are far less territorial and aggressive (Lowther 1962, Kopachena &
116 Falls 1993, Tuttle 2003, Horton & Holberton 2010, Horton et al. 2014). More importantly from
117 the perspective of offspring, males of each morph also differ in paternal investment (Knapton &

Author Manuscript

This article is protected by copyright. All rights reserved

118 Falls 1983, Horton et al. 2014). W morph males are promiscuous and provision nestlings very
119 little. T morph males defend their within-pair paternity through mate guarding and are highly
120 paternal. Females tend to provision at intermediate levels, but T morph females may compensate
121 for unassisted care from W morph males and provision more than W morph females (Knapton &
122 Falls 1983). A final wrinkle in this complex mating system is that morphs nearly always mate
123 with the opposite morph (98.5%, Tuttle et al. 2016), resulting in two stable pair types: T male x
124 W female (TxW) and W male x T female (WxT) (Lowther 1961, Tuttle 2003, Tuttle et al. 2016).
125 Because males differ in paternal investment, this results in two distinct parental care strategies.
126 TxW pairs provide biparental care and WxT pairs provide female-biased parental care. In this
127 study we examined gene expression profiles of offspring from both pair-types in order to assess
128 the physiological consequences of variation in parental genotype.
129

130 **Methods**

131 *Field based sample collection*

132 All nestling whole blood samples in this study came from a breeding population of
133 WTSPs at the Cranberry Lake Biological Station in northern New York, USA (SUNY-ESF,
134 44.15ºN, 74.78ºW) and were collected during the 2015 breeding season. We only used samples
135 collected during the first clutch (June 7 - June 15, 2015), as WTSP males may increase paternal
136 investment in replacement broods (Horton et al. 2014). We collected ~80μL blood in capillary
137 tubes via brachial venipuncture on days 5-7 post-hatch. Approximately 60μL blood was
138 preserved in Longmire's lysis buffer (Longmire et al. 1992) for genotyping and ~20μL was
139 immediately placed in RNAlater. Within six hours of collection, samples were placed
140 temporarily into liquid nitrogen, before being shipped overnight on dry ice to -80ºC storage until
141 RNA extraction. All animal sampling protocols were approved by the Indiana State University
142 Institutional Animal Care and Use Committee (IACUC 562158-1:ET/RG, 562192-1:ET/RG).
143

144 *Molecular sexing & genotyping*

145 Nestling DNA was extracted from erythrocytes using the DNA IQ® magnetic extraction
146 system (Promega Corp, Madison, WI USA). To determine sex and morph, we used PCR to
147 fluorescently label and amplify a region of the chromo-helicase-DNA-binding gene, and a region

Author Manuscript

This article is protected by copyright. All rights reserved

148 of the vasoactive intestinal peptide following Griffiths et al. (1998) and Michopolous et al.
149 (2007). The PCR products were run and analyzed on an ABI PRISM™ 310 genetic analyzer.
150

151 *RNA extraction, library preparation, & sequencing*

152 We sampled a total of 45 nestlings for RNA extraction, but due to issues with RNA
153 quality after extraction (RNA concentration=0ng/ul or RIN < 7), only 32 were used for
154 sequencing. These samples represent 23 nestlings from eight TxW pairs and nine nestlings from
155 three WxT pairs. The 23 nestlings from TxW nests included 12 female, 11 male, 12 T morph,
156 and 11 W morph individuals. The nine nestlings from WxT nests included six female, three male,
157 three T morph, and six W morph individuals.

158 We removed RNAlater and homogenized whole blood tissue samples with Tri-Reagent
159 (Molecular Research Company). Total RNA was purified with a Qiagen RNeasy mini kit
160 (Valencia, CA, USA), followed by DNase treatment and further purification. We quality assessed
161 RNA with an Agilent Bioanalyzer (RIN > 7) (Wilmington, DE, USA). Both library preparation
162 and sequencing were performed at the University of Illinois Roy J. Carver Biotechnology Center.
163 A library was prepared for each RNA sample using the Illumina HT TruSeq (San Diego, CA,
164 USA) stranded RNA sample prep kit. Libraries were distributed into four pools with equimolar
165 concentrations and quantitated via qPCR. Each of the pools was sequenced on an individual lane
166 of an Illumina HiSeq 2500 using the Illumina TruSeq SBS sequencing kit v4 producing 100-
167 nucleotide single-end reads.
168

169 *Creation of masked reference genome*

170 The WTSP reference genome was generated from a male T morph individual (Tuttle et al.
171 2016). Thus, the reference genome does not contain any sequence data from the ZAL2^{m}
172 inversion. To avoid any potential bias in mapping reads derived from W morph individuals onto
173 a T morph genome, we generated a masked reference genome for this study. To do so, we used
174 previously published whole genome sequences from three W morph adults (Tuttle et al. 2016).
175 Reads were adapter trimmed with *Trim Galore!* v0.3.8
176 (https://github.com/FelixKrueger/TrimGalore) and aligned to the WTSP reference genome with
177 *bwa mem* v 0.7.10-r789 using default parameters (Li 2013). We converted and sorted the

This article is protected by copyright. All rights reserved

Author Manuscript

178 resulting SAM alignment to BAM format with *samtools view* and *samtools sort*, respectively
179 (*samtools* v1.2, Li et al. 2009). We then merged all genomic scaffolds corresponding to the
180 ZAL2^m inversion, as identified in Tuttle et al. (2016), with *samtools merge*. We called SNPs
181 within the inversion using *samtools mpileup* and *bcftools call* v 1.2 (Li et al. 2009, Li 2011). We
182 only kept SNPs that were heterozygous in each of the three individuals with *SnpSift* v 4.3p
183 (Cingolani et al. 2012) and used these SNPs to mask the reference genome with *bedtools*
184 *maskfasta* v 2.21.0 (Quinlan & Hall 2010).
185

186 *Quality control, read mapping, differential expression, & gene ontology*

187 We trimmed Illumina sequencing adapters from each of the 32 libraries with *Trim*
188 *Galore!* v0.3.8 which uses *Cutadapt* v1.7.1 (Martin 2011). Trimmed reads were then mapped to
189 the masked reference genome with *STAR* v2.5.3a using default parameters (Dobin et al. 2013).
190 The mapping results were then quantified and assigned gene IDs with *htseq-count* v0.6.0 (Anders
191 et al. 2015) specifying '-s reverse' and '-i gene'. We then removed lowly expressed genes by
192 summing the counts for each gene across all 32 samples, dividing by 32 to obtain the study
193 average, and removing genes with an average read count of < 5.

194 All statistical analyses were performed with R v3.5.0 (R Core Team 2013). We first
195 identified outlier samples based on visual inspection of sample distance in a dendrogram within
196 *WGCNA* (Horvath 2011). Two samples, one T female and one T male representing an entire
197 TxW nest, were identified as outliers and removed from all future analyses (Figure S1). Using
198 the remaining 30 samples, we normalized reads accounting for sequencing depth and assessed
199 differential expression with *DEseq2* (Love et al. 2014). We performed variance stabilizing
200 transformation of reads in *DEseq2* and performed PCA and hierarchical clustering based on
201 Euclidean distance of gene expression profiles with *pcaExplorer* v2.6.0 (Marini & Binder 2016).
202 Differential expression analyses utilized pairwise comparisons between nestling morph and pair
203 type (i.e. parental morphs). We controlled for sex in morph comparisons and sex, morph, and
204 nest ID for pair type comparisons. To include nest ID in the pair type comparison, we followed
205 the "individuals nested within groups" guide in the *DEseq2* manual. We did not include nestling
206 age in analyses, as most samples were 6 days old (n=21), limiting comparisons with nestlings
207 aged Day 5 (n=3) or Day 7 (n=6). Network analysis (see below) did not reveal any effect of age

This article is protected by copyright. All rights reserved

208 on variables of interest (morph, pair type; data not shown). We also tested for an interaction
209 between nestling morph and pair type utilizing a grouping variable as outlined in the *DEseq2*
210 manual. *DEseq2* determines differential expression with a Wald test followed by Benjamini &
211 Hochberg (1995) FDR correction. Genes were considered differentially expressed (DE) if the
212 FDR corrected p-value was < 0.10. Details for each model run, including the R code used, are in
213 this project's GitHub repository.
214 We next tested for gene ontology (GO) enrichment among DE genes with *GOrilla* (Eden
215 et al. 2007, 2009). For each *DEseq2* comparison, we ordered the list of genes based on ascending
216 FDR values, excluding any genes in which *DEseq2* did not assign a FDR value. The WTSP
217 genome is not completely annotated, so any loci without a gene symbol were excluded from GO
218 analyses (n=1,926). *GOrilla* places greater weight on genes located at the top of the list (i.e. DE
219 genes), while accounting for the contribution of each gene in the given comparison. GO
220 categories were considered significantly enriched if the FDR corrected p-value <0.05. *GOrilla*
221 does not support WTSP annotation; so, all analyses were based on homology to human gene
222 symbols.
223
224 *Weighted gene co-expression network analysis (WGCNA)*
225 We used the *WGCNA* package in R (Zhang & Horvath 2005, Langfelder & Horvath
226 2008) to identify modules of genes with highly correlated expression patterns in our dataset.
227 *WGCNA* identifies modules of co-regulated genes blind to the experimental design. These
228 modules are then correlated with external traits, offering a systems-level view into how
229 conditions impact transcriptional networks. Within these networks, we can then perform GO
230 analyses as described above and identify network hubs, which are the most highly connected
231 genes within that network. To create networks, we first exported variance stabilizing transformed
232 (vst) read counts from *DEseq2*, removed genes with an average vst < 5 averaged across all 30
233 samples, and imported the subsequent list of 8,982 genes into *WGCNA*. To build the co-
234 expression matrix, we chose a soft thresholding power (β) value of 12, at which the network
235 reaches scale-free topology (Figure S2). We generated a signed network with minimum module
236 size of 30 genes and merged highly correlated modules (dissimilarity threshold = 0.20, which
237 corresponds to $R^2 = 0.80$). We then correlated the eigengene, which is the first principal

This article is protected by copyright. All rights reserved
Author Manuscript

238 component of a module, of these merged modules with external traits (pair type, nestling morph,
239 nestling sex, nest ID). Modules with $p < 0.05$ were considered significantly correlated with a
240 given trait. For all morph-specific results, we tested for an enrichment of inversion genes with a
241 chi-squared test using a Fisher's exact test ($p < 0.05$).
242 To visualize the interaction of genes within a module, we generated the intramodular
243 connectivity (IM) score for each gene, which represents the interconnection of module genes. We
244 exported all IM scores for modules of interest and imported into *VisAnt* v5.51 (Hu et al. 2013) for
245 visualization. To maximize network clarity, we only plotted the top 300 interactions based on IM
246 scores. Thus, we only visualized the most connected genes. To identify hub genes, we visualized
247 the Degree Distribution (DD) for the network and selected the most connected genes above a
248 natural break in the distribution. This resulted in one to nine hub genes per module.
249 To understand the biological function of modules correlated with traits of interest, we
250 performed a target vs background GO analysis in *GOrilla*. For each module, we tested the
251 assigned genes for each module against the entire list of 8,982 genes used for the *WGCNA*
252 analysis. GO categories were significant with a FDR corrected p-value < 0.05.
253
254 **Results**
255 *Sequencing results*
256 We sequenced each sample to an average depth of 29.4 million reads (range = 16.2-58.5
257 million reads). The 32 libraries were distributed into four pools in equimolar concentration. One
258 pool contained only four samples, which corresponded to the four samples with lowest RNA
259 concentrations. This pool was sequenced to an average depth of 56.17 million reads per library.
260 The remaining three pools were sequenced to an average depth of 25.62 million reads per library.
261 Samples mapped to our masked genome at an average rate of 91.08% (range = 88.19%-92.87%)
262 (Table S1). A total of 8,982 genes had count values ≥ 5 across all samples, which included 641
263 located in the W morph inversion. Samples did not segregate by pair type or morph in PCA or
264 hierarchical clustering (Figures S3, S4).
265
266 *Differential Expression – Morph*

Author Manuscript

This article is protected by copyright. All rights reserved

267 Ninety-two genes were differentially expressed between morphs. Sixty-five of these
268 genes (71%) were located in the inversion, representing a significant enrichment (χ^2=553.73,
269 df=1, p<0.00001) (Table S2). The inversion represents only 641 out the 8,892 genes (7%)
270 sampled here. Additionally, expression of 59 of these 92 genes was higher in W morph nestlings,
271 including several innate immune related genes (e.g. IFIT5, IL20RA, EIF2AK2, RSAD2). There
272 was GO enrichment of three categories: "immune response" (p = 0.019), "mitotic cell cycle
273 process" (p=0.029), and "defense response to virus" (p = 0.049) (Table S3).
274
275 *Differential Expression – Pair Type*
276 Pair type had the largest effect on gene expression, with 881 genes DE between offspring
277 from the two different pair types (FDR < 0.10, Table S2). Some known stress response genes
278 were more highly expressed in nestlings in WxT nests, including the glucocorticoid receptor
279 (NR3C1), superoxide dismutase (SOD)1 & SOD2, DEP domain-containing mTOR-interacting
280 protein (DEPTOR), and several ubiquitin-mediated proteolysis pathway genes (e.g. UBE2D3,
281 PSMD3, PSMD6). Additionally, immune system related genes were also expressed more highly
282 in WxT nests, including cytokines (e.g. IL2RA, IL7R), suppressor of cytokine signaling 1
283 (SOCS1), and five putative major histocompatibility complex (MHC) class I loci. No GO
284 categories were significantly enriched, however.
285 We next tested for a morph-specific response to pair type. Within WxT nests, 40 genes
286 were DE (p <0.10) between T and W morph nestlings. Twelve of these genes (30%) are located
287 within the inversion, again reflecting an enrichment of inversion genes among those differentially
288 expressed between morph (χ^2=34.44, df=1, p<0.00001). Only two genes (THSD7B & CFAP44)
289 were DE between morphs within TxW nests, both of which are uniquely DE between morphs in
290 TxW nests. No GO categories were enriched in either comparison.
291
292 *WGCNA – Morph*
293 WGCNA revealed 26 modules, five of which were correlated with morph (Table 1,
294 Figure 1). The light cyan module (183 genes, R^2=0.67, p=5x10^{-5}) and ivory module (72 genes,
295 R^2=-0.66, p=9x10^{-5}) contained genes expressed higher and lower, respectively, in W morph
296 nestlings relative to T morph nestlings. These modules are both enriched for genes located within

This article is protected by copyright. All rights reserved
Author Manuscript

297 the chromosomal inversion (light cyan module = 70/183 (38%) genes, χ^2=266.49, df=1,
298 p<0.00001; ivory module = 40/72 (56%), χ^2=261.60, df=1, p<0.00001) (Figure S5). The hubs of
299 each of these modules are also located in the chromosomal inversion (Table 1, Figure S5).
300 Additionally, the sky blue module (58 genes, R^2=0.53, p=0.003) and dark red module (102 genes,
301 R^2=0.47, p=0.009) (Figure S6) contained genes expressed at higher levels in W morph nestlings
302 and many of these genes overlap with the immune related genes described in the morph DE tests
303 above. The hubs of these networks (e.g. sky blue: EIF2AK2, IFIT5, OASL; dark red: TRAF5)
304 (Table 1) reflect a conserved innate immunity network structure in avian blood (Kernbach et al.,
305 2019) (Figure S6). Lastly, the salmon module (294 genes, R^2=-0.50, p=0.005) contained genes
306 expressed at lower levels in W morph nestlings and did not exhibit any enriched GO categories.
307

308 *WGCNA – Pair Type*

309 We found seven modules correlated with pair type (Table 2, Figure 1). The blue module
310 represented genes that are expressed at higher levels in nestlings from WxT nests (1,142 genes,
311 R^2 = -0.45, p=0.01). This module contained both the largest number of genes and
312 correspondingly, the strongest functional enrichment. Many of these GO enrichments were
313 related to protein function, resulting from the presence of ribosomal genes. Interestingly, several
314 GO categories for metabolism, catabolism, and proteolysis were also enriched, driven by genes
315 encoding ubiquitin-conjugating enzymes and proteasome subunits (e.g. "proteasomal protein
316 catabolic process", p=2.34x10^{-4}; "proteasome-mediated ubiquitin-dependent protein catabolic
317 process", p=5.32x10^{-4}) (Table S4). Many of these (e.g. PSMF1, PSMD3, PSMD6, UBE2D2,
318 UBE2D3, UBE3C) were also DE between offspring of the two pair types (Figure 2). Lastly, the
319 blue module contains one hub gene, NDUFB3 (DD=42) (Figure 2), which is involved in the
320 mitochondrial electron transport chain.

321 The beige and light green modules represented candidate stress response networks. These
322 modules showed contrasting expression patterns in nestlings from WxT nests (Figure 4).
323 Although not significantly enriched for any GO categories, the beige module comprised 335
324 genes that shower greater expression in WxT nests than in TxW nests (R^2=-0.61, p=3x10^{-4}).
325 DEPTOR, which functions as an inhibitor of the mTOR pathway in response to stress (e.g.
326 Desantis et al. 2015), was the single hub in the beige module (DD=39, Figure 3). The beige

Author Manuscript

This article is protected by copyright. All rights reserved

327 module also contained NR3C1, which is activated in response to increased glucocorticoid
328 secretion. Lastly, the light green module (116 genes, R^2=0.60, p=4x10^{-4}) contained genes with
329 low expression in TxW nests relative to WxT nests. There were three hub genes (DD > 28),
330 CDK19, CHD4, and EPG5, each with previously described roles in the stress response (Figure
331 4).
332 For each pair type module, the correlation was stronger for the overall effect of pair type
333 than any individual nest, indicating that one nest did not drive the correlation. This trend was
334 reflected in gene expression plots of hub genes and candidate genes described above (Figure S7).
335 We did not observe modules correlated with pair type that were also correlated with nestling
336 morph or sex, suggesting there is no morph or sex-specific response to a given pair type at the
337 network level.
338
339 **Discussion**
340 By assessing genome-wide transcription in nestlings raised by different WTSP pair types we
341 have identified distinct transcriptomic signatures that suggest nestlings raised by WxT pairs
342 exhibited a stronger stress response relative to nestlings raise by TxW pairs. This is reflected both
343 by differential expression of several genes involved in protein degradation as well as networks of
344 co-expressed genes with stress response hubs. Additionally, we identified morph-specific gene
345 expression driven by innate immunity genes and genes located in the chromosome 2 inversion.
346 As adults, the genes within the inversion strongly influence the WTSP neural transcriptome
347 (Balakrishnan et al. 2014, Zinzow-Kramer et al. 2015). Our results here suggest that as nestlings,
348 parental genotypes and associated behaviors, rather than nestling genotype, have the strongest
349 influence on the nestling transcriptome.
350
351 *Gene expression differences resulting from pair type*
352 We found 881 genes DE between nestlings raised by the two pair types. Many of these
353 genes function in the proteasome or ubiquitin-mediated proteolysis. Cells naturally use the
354 proteasome for degradation of proteins targeted by the ubiquitination process, but genes involved
355 in proteasome formation (e.g. PSMD6, PSMD11) and ubiquitination (e.g. UBE2B) are up-
356 regulated in cells experiencing mild oxidative stress (Aiken et al. 2011, Shang & Taylor 2011,

Author Manuscript

This article is protected by copyright. All rights reserved

357 Livneh et al. 2016) or organisms experiencing abiotic stress (Dhanasiri et al. 2013, Tomalty et al.
358 2015). Thus, increased expression of these genes in nestlings from WxT nests suggests they are
359 responding to oxidative stress. As a result, there may be a cost to having a W morph father and T
360 morph mother at the nestling stage.

361 To complement our differential expression approach, we also constructed co-expression
362 networks with *WGCNA*. Using this approach, we identified 26 modules of co-regulated genes in
363 this dataset (Figure 1), seven of which were significantly correlated with parental pair type. The
364 blue module contains genes that are expressed at higher levels in nestlings in WxT nests. The
365 blue module hub gene was NDUFB3 (Module Membership [MM]=0.938, DD=42) (Figure 2),
366 which encodes a subunit of the mitochondrial membrane respiratory chain. Interestingly, many of
367 the same proteolysis-related genes highlighted in the differential expression results are also
368 present in this module, resulting in the enrichment of several metabolism and stress-related GO
369 categories (Table S4).

370 Two modules, light green and beige, contained stress responsive hub genes. The light
371 green module contains genes that are expressed at lower levels in nestlings in WxT nests, with
372 three hub genes: CDK19, CHD4, and EPG5 (Figure 4). The absence of EPG5 expression (via
373 knockout) and reduction in CHD4 expression (via knockdown) has been associated with
374 increased DNA damage (Zhao et al. 2013, Larsen et al. 2010). Similarly, down-regulation of
375 CDK19 following knockdown is associated with an increased stress response (Audetat et al.
376 2017). Suppression of these genes in these nestlings could be indicative of increased cellular
377 damage. The beige module contains genes whose expression is higher in nestlings from WxT
378 nests and contains one hub gene, DEPTOR, which is an inhibitor of mTOR signaling (Figure 3).
379 The exact role of DEPTOR remains unclear, but up-regulation likely inhibits the mTORC1
380 pathway to reduce endoplasmic reticulum stress, promote cell survival, and avoid apoptosis
381 (Peterson et al. 2009, Desantis et al. 2015, Catena et al. 2016). Thus, nestlings raised in WxT
382 nests may be activating DEPTOR to alleviate the effects of endoplasmic reticulum stress.

383 Higher expression levels for genes in the beige module in these nestlings and the high
384 connectivity of DEPTOR to other co-expressed genes provide further support for a
385 transcriptional stress response in nestlings sampled within WxT nests. The beige module also
386 contains two well-studied stress responsive genes, superoxide dismutase 2 (SOD2) and the

Author Manuscript

This article is protected by copyright. All rights reserved

387 glucocorticoid receptor (NR3C1). SOD2 mitigates the effects of exposure to reactive oxygen
388 species by scavenging free radicals (Zelko et al. 2002). NR3C1 binds glucocorticoids and has
389 primarily been studied in the context of ELS and methylation of an upstream promoter. NRC3C1
390 methylation is often associated with down-regulation of NR3C1 (e.g. McGowan et al. 2009) and
391 impairment of the HPA axis, but up-regulation following methylation has also been observed as
392 part of the stress response (Turner et al. 2006, Bockmühl et al. 2015). The expression pattern
393 observed here directly implicates the HPA axis and suggests these nestlings may be activating
394 SOD2 and NR3C1 to cope with elevated levels of reactive oxygen species and corticosterone,
395 respectively (Wang et al. 2018, Finsterwald & Alberini 2014). However, further work is needed
396 to investigate stress physiology, corticosterone levels, and uncover the epigenetic state of NR3C1
397 in these nestlings and how this may relate to ELS (Banerjee et al. 2011, McCoy et al. 2016,
398 Rubenstein et al. 2016, Quirici et al. 2016, Greggor et al. 2017).
399
400 *How does parental genotype influence offspring gene expression?*
401 In a non-experimental study, we have limited power to make inferences about the
402 mechanism by which parental genotype impacted offspring gene expression. Given the well-
403 studied reproductive biology of WTSPs, however, two mechanisms seem especially likely:
404 hormone-mediated maternal effects and/or differences in parental provisioning and behavior. In
405 weighing the evidence for these two non-mutually exclusive possibilities, we conclude that the
406 difference in parental provisioning is the most plausible explanation for the observed gene
407 expression differences. As described above, WTSP morphs differ in hormone levels. Only
408 oestradiol, however, has so far been shown to differ between adult female morphs during the
409 breeding season and is higher in W morph females during the pre-laying and laying stages
410 (Horton et al. 2014). No baseline differences in any other hormone measured to date
411 (corticosterone, testosterone, DHEA, DHT) have been described during the breeding season
412 (Spinney et al. 2006, Swett & Breuner 2009, Horton & Holberton 2010, Horton et al. 2014).
413 Taken together this suggests that hormone deposition into eggs may not differ dramatically
414 between the morphs. By contrast, there is strong evidence of differences in provisioning among
415 morph types (Knapton & Falls 1983, Kopachna & Falls 1993, Horton & Holberton 2010, Horton
416 et al. 2014). Reduced provisioning by W morph males appears to be stable across populations

This article is protected by copyright. All rights reserved

Author Manuscript

417 resulting in female-biased parental care in WxT nests (Knapton & Falls 1983, Horton et al.
418 2014). Therefore, parental care variation is a likely source of behaviorally mediated maternal or
419 paternal effects (see Crean & Bonduriansky 2014) that could explain the strong signature of
420 stress exposure in the expression data.

421 Previous work revealed no difference in clutch size between pair types (Knapton et al.
422 1984, Formica et al. 2004) and no effect of pair type on nestling mass (Knapton et al. 1984,
423 Tuttle et al. 2017). Also, nestlings did not differ in mass at time of sampling between the TxW
424 and WxT nests used in this study (Smith et al. in review). Increased provisioning by females to
425 compensate for reduced care by males could explain this observation, and this has been observed
426 previously in a separate WTSP population (Knapton & Falls 1983). In this scenario reduced
427 brooding and increased maternal separation could also negatively impact nestling physiology and
428 act as a source of ELS (reviewed in Ledón-Rettig et al. 2013). Somewhat surprisingly, given the
429 gene expression findings described here, a recent study in our study population did not detect
430 differences in reactive oxygen metabolites in plasma of offspring of the two different pair types
431 (Grunst et al. 2019). ROM, however, only provides a limited overview of the stress response and
432 the RNA-seq response we observed could even mitigate long-term consequences of ELS. The
433 results here further highlight the utility of blood RNA-seq as a highly sensitive measure of
434 environmental exposures (Louder et al. 2018).

435 Our study is limited by the fact that we did not perform a cross-fostering experiment. We
436 aimed to mitigate potential environmental confounds by restricting sampling of nestlings to a
437 short time period of nine days and sampling nests of both pair types throughout this period.
438 Certainly, the environment may influence gene expression in our samples, but consistent changes
439 among the samples in the two pair types suggest the role of parents is a significant driver of
440 nestling gene expression, rather than temporal or spatial environmental variation. Although the
441 two pair types are equally abundant in our study population, our study had unbalanced sample
442 sizes between the pair types (21 TxW, 9 WxT). The biased sample size resulted from technical
443 difficulties in RNA extraction, as many of these samples contained very little starting tissue.
444 Future studies should prioritize larger tissue samples for RNA based analyses. Lastly, aspects of
445 male behavior during incubation (e.g. provisioning females) could also influence nestling stress
446 and warrant further targeted behavioral observations of males.

Author Manuscript

This article is protected by copyright. All rights reserved

447

448 *Morph-specific gene expression*

449 We were also interested in morph-specific gene expression and how nestling morph may
450 respond to differences in parental pair type. WTSPs have been studied extensively as adults, but
451 very rarely in other life stages. W morph males and T morph females exhibit earlier reproductive
452 and actuarial senescence, potentially resulting from the high energy expenditure lifestyle of W
453 morph males and biased parental care given by T morph females (Grunst et al. 2018a, Grunst et
454 al. 2018b). There also appears to be annual variation in fitness between the morphs as adults.
455 Following cold, wet winters, W morph males exhibit lower recruitment in the breeding grounds,
456 leading to an overproduction of W morph male nestlings, potentially to stabilize morph
457 frequencies in the population (Tuttle et al. 2017). Thus, morph specific differences may arise in
458 early life. We found 92 genes DE between morphs, including 14 innate immune-related genes
459 and genes located within the inversion (65/92 genes, Table S2). *WGCNA* revealed five modules
460 correlated with morph (Figure 1). These included two innate immunity-related modules with
461 increased expression in W morphs (Dark Red & Sky Blue) and two modules enriched with genes
462 located in the inversion (Ivory = 40/72, Light Cyan = 70/183) (Figures S5, S6). The sky blue
463 module contains nine hub genes and the dark red module contains one hub gene, both of which
464 include well-studied anti-viral genes (e.g. sky blue: OASL, RSAD2; dark red: TRAF5). These
465 genes also form a co-expression module in avian blood following West Nile virus infection
466 (Kernbach et al., 2019). Adult WTSP morphs differ in their ability to clear infection (Boyd et al.
467 2018), so the immune activation here may be indicative of an increased parasite load in W morph
468 nestlings, although further investigation is required. The light cyan module contains genes
469 expressed at higher levels in W morph nestlings and contains eight hub genes, each located in the
470 inversion (Table 1). Three of these, EPM2A, BPNT1, and TAF5L, were also identified as hub
471 genes in brain tissues of adult W morph males (Zinzow-Kramer et al. 2015). These nestlings thus
472 exhibit expression differences in inversion genes prior to any phenotypic or behavioral
473 differences, revealing the importance of the inversion in maintaining morph phenotypes
474 throughout life. Additionally, the conservation of network hub genes in a different tissue and life
475 stage highlights avenues for further investigation into WTSP gene regulation.

Author Manuscript

This article is protected by copyright. All rights reserved

476 Despite broad gene expression differences between the morphs, within pair types morph-
477 specific expression was limited. Nestlings in TxW nests only had two genes DE between morphs.
478 There was a larger effect of morph within WxT nests, where the number of DE genes increased
479 to 40. These genes encompassed a wide range of gene functions without any obvious stress-
480 related candidate genes. Of these 40 genes, 34 are uniquely DE within WxT nests and do not
481 overlap with the overall list of 92 genes DE between morphs using all samples. Interestingly, W
482 morph nestlings in WxT nests expressed glucocorticoid-induced transcript 1 (GLCCI1) at higher
483 levels than T morph nestlings. The function of GLCCI1 remains unclear (Kim et al. 2016), but
484 expression differences between morphs observed here implicates the role of glucocorticoids in
485 response to pair type. This suggests that nestling morphs may respond differently to the parental
486 pair type though larger sample sizes will be needed to explore this further.
487
488 **Conclusions**
489 Using the WTSP, a system with alternative parental care strategies, we show that
490 nestlings in WxT nests (female-biased parental care) have increased expression of stress-related
491 genes, and parental genotypes may act as a source of ELS in the species. Nestling morph also
492 influences transcription, but parental pair type appears to have the greatest effect on their
493 transcriptome. Combined, this supports the parental effects hypothesis (Wade 1998, Schrader et
494 al. 2018), where offspring phenotypes are primarily a result of the nest environment and care
495 received, rather than from offspring genotypes (i.e. T vs. W). Nearly 54% of observed pairs have
496 been WxT (Tuttle et al. 2016). Thus, roughly half of the nestlings in every population will
497 experience female-biased parental care. Our results suggest that these differences in parental pair
498 type have at least short-term consequences on offspring physiology. While we have identified
499 impacts at the level of transcription, an integrative approach assessing nestling WTSP physiology
500 and performing cross-fostering experiments will further elucidate the consequences of variation
501 in parental pair type. Importantly, it remains unclear whether female-biased parental care or
502 differences in maternal effects translate into long-term fitness consequences for offspring. There
503 appears to be a cost associated with parental genotype, as less cooperative reproductive strategy
504 (WxT pairs) accelerates senescence (Grunst et al. 2018a, Grunst et al. 2018b). We show here that
505 this cost is also translated into nestlings within WxT nests via increased stress-related gene

Author Manuscript

This article is protected by copyright. All rights reserved

506 expression. This work sets the stage to further explore morph-specific fitness consequences in
507 nestlings experiencing alternative parental care strategies.
508

509 **Acknowledgements**

510 We acknowledge Lindsay Forrette, Andrea Grunst, and Melissa Grunst for assistance in the field,
511 Sarah Ford for assistance with molecular work, Rachel Wright for WGCNA code, and Cranberry
512 Lake Biological Station. Funding provided by East Carolina University, Indiana State University,
513 The National Science Foundation (grant no. DUE-0934648) and the National Institutes of Health
514 (grant no. 1R01Gm084229 to E.M.T and R.A.G.) and a Sigma Xi Grants in Aid of Research
515 award to DJN. Birds were banded with color bands and a Fish and Wildlife band (Master
516 Banding Permit 22296 to EMT and permit 24105 to RAG). Dr. Alvaro Hernandez and Chris
517 Wright provided guidance and oversight on sequencing carried out at the University of Illinois.
518 All methods were conducted in accordance with legal and ethical standards and were approved
519 by Indiana State University's Institutional Animal Care and Use Committee (protocols 562158-
520 1:ET/RG and 562192-1:ET/RG).
521

522 **References**

523 Aiken, C. T., Kaake, R. M., Wang, X., & Huang, L. (2011). Oxidative Stress-Mediated Regulation of Proteasome
524 Complexes. *Molecular & Cellular Proteomics*, *10*(5). Retrieved from
525 http://www.mcponline.org/content/10/5/R110.006924.abstract

526 Alyamani, R. A. S., & Murgatroyd, C. (2018). Epigenetic Programming by Early-Life Stress. In *Progress in*
527 *Molecular Biology and Translational Science* (Vol. 157, pp. 133–150). doi: 10.1016/bs.pmbts.2018.01.004

528 Anastasiadi, D., Esteve-Codina, A., & Piferrer, F. (2018). Consistent inverse correlation between DNA methylation
529 of the first intron and gene expression across tissues and species. *Epigenetics & Chromatin*, *11*(1). doi:
530 10.1186/s13072-018-0205-1

531 Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq-A Python framework to work with high-throughput sequencing
532 data. *Bioinformatics*, *31*(2), 166–169. doi: 10.1093/bioinformatics/btu638

533 Audetat, K. A., Galbraith, M. D., Odell, A. T., Lee, T., Pandey, A., Espinosa, J. M., … Taatjes, D. J. (2017). A
534 kinase-independent role for CDK19 in p53 response. *Molecular and Cellular Biology*. *37*(13), e00626-16
535 Retrieved from http://mcb.asm.org/content/early/2017/04/12/MCB.00626-16.abstract

536 Balakrishnan, C. N., Mukai, M., Gonser, R. A., Wingfield, J. C., London, S. E., Tuttle, E. M., & Clayton, D. F.
537 (2014). Brain transcriptome sequencing and assembly of three songbird model systems for the study of
538 social behavior. *PeerJ*, *2*, e396–e396. doi: 10.7717/peerj.396

Author Manuscript

This article is protected by copyright. All rights reserved

539 Banerjee, S. B., Arterbery, A. S., Fergus, D. J., & Adkins-Regan, E. (2011). Deprivation of maternal care has long-
540 lasting consequences for the hypothalamic-pituitary-adrenal axis of zebra finches. *Proceedings of the Royal*
541 *Society B: Biological Sciences*, *279*(1729), 759–766. doi: 10.1098/rspb.2011.1265
542 Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate - a practical and powerful approach to
543 multiple testing. *J R Stat Soc B Method*, *57*(1), 289-300.
544 Berger, S. L. (2007). The complex language of chromatin regulation during transcription. *Nature*, *447*(7143), 407–
545 412. doi: 10.1038/nature05915
546 Bockmühl, Y., Patchev, A. V., Madejska, A., Hoffmann, A., Sousa, J. C., Sousa, N., … Spengler, D. (2015).
547 Methylation at the CpG island shore region upregulates Nr3c1 promoter activity after early-life stress.
548 *Epigenetics*, *10*(3), 247–257. doi: 10.1080/15592294.2015.1017199
549 Bolton, J. L., Molet, J., Ivy, A., & Baram, T. Z. (2017). New insights into early-life stress and behavioral outcomes.
550 *Current Opinion in Behavioral Sciences*, *14*, 133–139. doi: 10.1016/j.cobeha.2016.12.012
551 Boyd, R. J., Kelly, T. R., MacDougall-Shackleton, S. A., & MacDougall-Shackleton, E. A. (2018). Alternative
552 reproductive strategies in white-throated sparrows are associated with differences in parasite load following
553 experimental infection. *Biology letters*, *14*(7), 20180194.
554 Catena, V., Bruno, T., De Nicola, F., Goeman, F., Pallocca, M., Iezzi, S., … Fanciulli, M. (2016). Deptor
555 transcriptionally regulates endoplasmic reticulum homeostasis in multiple myeloma cells. *Oncotarget*,
556 *7*(43), 70546–70558. doi: 10.18632/oncotarget.12060
557 Cingolani, P., Patel, V., Coon, M., Nguyen, T., Land, S., Ruden, D., & Lu, X. (2012). Using *Drosophila*
558 *melanogaster* as a Model for Genotoxic Chemical Mutational Studies with a New Program, SnpSift.
559 *Frontiers in Genetics*, *3*, 35–35.
560 Cottrell, E. C., & Secki, J. R. (2009). Prenatal stress, glucocorticoids and the programming of adult disease.
561 *Frontiers in Behavioral Neuroscience*, *3,19*. doi: 10.3389/neuro.08.019.2009
562 Crean, A. J., & Bonduriansky, R. (2014). What is a paternal effect? *Trends in Ecology & Evolution*, *29*(10), 554–
563 559. doi: 10.1016/j.tree.2014.07.009
564 Crespi, E. J., Williams, T. D., Jessop, T. S., & Delehanty, B. (2012). Life history and the ecology of stress: how do
565 glucocorticoid hormones influence life-history variation in animals? *Functional Ecology*, *27*(1), 93–106.
566 doi: 10.1111/1365-2435.12009
567 Desantis, A., Bruno, T., Catena, V., De Nicola, F., Goeman, F., Iezzi, S., … Fanciulli, M. (2015). Che-1-induced
568 inhibition of mTOR pathway enables stress-induced autophagy. *The EMBO Journal*, *34*(9), 1214–1230.
569 Dhanasiri, A. K. S., Fernandes, J. M. O., & Kiron, V. (2013). Liver Transcriptome Changes in Zebrafish during
570 Acclimation to Transport-Associated Stress. *PLOS ONE*, *8*(6), e65028–e65028.
571 Diaz-Real, J., Kim, S.-Y., & Velando, A. (2017). Plumage colour and the expression of stress-related genes in gull
572 chicks. *Journal of Avian Biology*, *48*(9), 1216–1225. doi: 10.1111/jav.01460
573 Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., … Gingeras, T. R. (2013). STAR: ultrafast
574 universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15–21.

This article is protected by copyright. All rights reserved

Author Manuscript

575 Eden, E., Lipson, D., Yogev, S., & Yakhini, Z. (2007). Discovering Motifs in Ranked Lists of DNA Sequences.
576 *PLOS Computational Biology*, *3*(3), e39–e39.
577 Eden, E., Navon, R., Steinfeld, I., Lipson, D., & Yakhini, Z. (2009). GOrilla: a tool for discovery and visualization
578 of enriched GO terms in ranked gene lists. *BMC Bioinformatics*, *10*(1), 48–48. doi: 10.1186/1471-2105-10-
579 48
580 Finsterwald, C., & Alberini, C. M. (2014). Stress and glucocorticoid receptor-dependent mechanisms in long-term
581 memory: From adaptive responses to psychopathologies. *Neurobiology of Learning and Memory*, *112*, 17–
582 29. doi: 10.1016/j.nlm.2013.09.017
583 Formica, V. A., Gonser, R. A., Ramsay, S., & Tuttle, E. M. (2004). Spatial dynamics of alternative reproductive
584 strategies: the role of neighbors. *Ecology*, *85*(4), 1125-1136.
585 Gluckman, P. D., Hanson, M. A., Cooper, C., & Thornburg, K. L. (2008). Effect of In Utero and Early-Life
586 Conditions on Adult Health and Disease. *New England Journal of Medicine*, *359*(1), 61–73. doi:
587 10.1056/NEJMra0708473
588 Gott, A. J. (2018). Effects of early-life adversity on the adult phenotype in European starlings. Doctoral dissertation,
589 Newcastle University.
590 Greggor, A. L., Spencer, K. A., Clayton, N. S., & Thornton, A. (2017). Wild jackdaws' reproductive success and
591 their offspring's stress hormones are connected to provisioning rate and brood size, not to parental
592 neophobia. *General and Comparative Endocrinology*, *243*, 70–77. doi: 10.1016/j.ygcen.2016.11.006
593 Griebel, I. A., Fairhurst, G. D., Marchant, T. A., & Clark, R. G. (2019). Effects of parental and nest-site
594 characteristics on nestling quality in the Tree Swallow (*Tachycineta bicolor*). *Canadian Journal of Zoology*,
595 *97*(1), 63–71. doi: 10.1139/cjz-2018-0109
596 Griffiths, R., Double, M. C., Orr, K., & Dawson, R. J. G. (1998). A DNA test to sex most birds. *Molecular Ecology*,
597 *7*(8), 1071–1075. doi: 10.1046/j.1365-294x.1998.00389.x
598 Grunst, A. S., Grunst, M. L., Formica, V. A., Korody, M. L., Betuel, A. M., Barcelo-Serra, M., … Tuttle, E. M.
599 (2018a). Morph-Specific Patterns of Reproductive Senescence: Connections to Discrete Reproductive
600 Strategies. *The American Naturalist*, *191*(6), 744–755. doi: 10.1086/697377
601 Grunst, A. S., Grunst, M. L., Gonser, R. A., & Tuttle, E. M. (2019). Developmental stress and telomere dynamics in
602 a genetically polymorphic species. *Journal of Evolutionary Biology, 32(2), 134-143.* doi:
603 10.1111/jeb.13400
604 Grunst, M. L., Grunst, A. S., Formica, V. A., Korody, M. L., Betuel, A. M., Barcelo-Serra, M., … Tuttle, E. M.
605 (2018b). Actuarial senescence in a dimorphic bird: different rates of ageing in morphs with discrete
606 reproductive strategies. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1892), 20182053. doi:
607 10.1098/rspb.2018.2053
608 Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma
609 and depression: Insights from HPA axis studies in humans. *Psychoneuroendocrinology*, *33*(6), 693–710.
610 doi: 10.1016/j.psyneuen.2008.03.008

Author Manuscript

This article is protected by copyright. All rights reserved

611 Horton, B. M., & Holberton, R. L. (2010). Morph-Specific Variation in Baseline Corticosterone and the
612 Adrenocortical Response in Breeding White-Throated Sparrows (*Zonotrichia albicollis*). *The Auk*, *127*(3),
613 540–548. doi: 10.1525/auk.2010.09096
614 Horton, B. M., Moore, I. T., & Maney, D. L. (2014). New insights into the hormonal and behavioural correlates
615 of polymorphism in white-throated sparrows, *Zonotrichia albicollis*. *Animal Behaviour*, *93*, 207–219. doi:
616 10.1016/j.anbehav.2014.04.015
617 Horvath, S. (2011). *Weighted network analysis: application in genomics and systems biology*. New York, NY:
618 Springer.
619 Hu, Z., Chang, Y.-C., Wang, Y., Huang, C.-L., Liu, Y., Tian, F., … DeLisi, C. (2013). VisANT 4.0: Integrative
620 network platform to connect genes, drugs, diseases and therapies. *Nucleic Acids Research*, *41*(W1), W225–
621 W231.
622 Jacquin, L., Blottière, L., Haussy, C., Perret, S., & Gasparini, J. (2012). Prenatal and postnatal parental effects on
623 immunity and growth in 'lactating' pigeons: Prenatal and postnatal parental effects. *Functional Ecology*,
624 *26*(4), 866–875. doi: 10.1111/j.1365-2435.2012.01988.x
625 Kernbach, M.E., Newhouse, D.J., Miller, J.M., Hall, R.J., Gibbons, J., Oberstaller, J., Selechnik, D., Jiang, R.,
626 Unnasch, T.R., Balakrishnan, C.N., & L.B. Martin. Light pollution increases West Nile virus competence of
627 a ubiquitous passerine reservoir species. *Proceedings of the Royal Society B: Biological Sciences*, *286*. doi:
628 10.1098/rspb.2019.1051
629 Kim, S.-H., Kim, H.-J., & Kim, C.-W. (2016). GLCCI1 is a novel component associated with the PI3K signaling
630 pathway in podocyte foot processes. *Experimental & Molecular Medicine*, *48*(5), e233–e233. doi:
631 10.1038/emm.2016.28
632 Kinnally, E. L., Feinberg, C., Kim, D., Ferguson, K., Leibel, R., Coplan, J. D., & John Mann, J. (2011). DNA
633 methylation as a risk factor in the effects of early life stress. *Brain, Behavior, and Immunity*, *25*(8), 1548–
634 1553. doi: 10.1016/j.bbi.2011.05.001
635 Knapton, R. W., Cartar, R. V., & Falls, J. B. (1984). A comparison of breeding ecology and reproductive success
636 between morphs of the white-throated sparrow. *The Wilson Bulletin*, 60-71.
637 Knapton, R. W., & Falls, J. B. (1983). Differences in parental contribution among pair types in the polymorphic
638 White-throated Sparrow. *Canadian Journal of Zoology*, *61*(6), 1288–1292. doi: 10.1139/z83-173
639 Kopachena, J. G., & Falls, J. B. (1993). Aggressive Performance as a Behavioral Correlate of Plumage
640 Polymorphism in the White-Throated Sparrow (*Zonotrichia albicollis*). *Behaviour*, *124*(3/4), 249–266.
641 Krause, E. T., Honarmand, M., Wetzel, J., & Naguib, M. (2009). Early Fasting Is Long Lasting: Differences in Early
642 Nutritional Conditions Reappear under Stressful Conditions in Adult Female Zebra Finches. *PLoS ONE*,
643 *4*(3), e5015. doi: 10.1371/journal.pone.0005015
644 Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC*
645 *Bioinformatics*, *9*(1), 559–559. doi: 10.1186/1471-2105-9-559

This article is protected by copyright. All rights reserved

Author Manuscript

646 Larsen, D. H., Poinsignon, C., Gudjonsson, T., Dinant, C., Payne, M. R., Hari, F. J., … Lukas, J. (2010). The
647 chromatin-remodeling factor CHD4 coordinates signaling and repair after DNA damage. *The Journal of*
648 *Cell Biology*, *190*(5), 731–740.
649 Ledón-Rettig, C. C., Richards, C. L., & Martin, L. B. (2013). Epigenetics for behavioral ecologists. *Behavioral*
650 *Ecology*, *24*(2), 311–324. doi: 10.1093/beheco/ars145
651 Lewis, C. R., & Olive, M. F. (2014). Early-life stress interactions with the epigenome: potential mechanisms driving
652 vulnerability toward psychiatric illness. *Behavioural Pharmacology*, 1. doi:
653 10.1097/FBP.0000000000000057
654 Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population
655 genetical parameter estimation from sequencing data. *Bioinformatics*, *27*(21), 2987–2993.
656 Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *ArXiv:1303.3997*
657 *[q-Bio]*. Retrieved from http://arxiv.org/abs/1303.3997
658 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., … Subgroup, 1000 Genome Project Data
659 Processing. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–
660 2079.
661 Lima, S. L. (2009). Predators and the breeding bird: behavioral and reproductive flexibility under the risk of
662 predation. *Biological Reviews*, *84*(3), 485–513. doi: 10.1111/j.1469-185X.2009.00085.x
663 Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., … Meaney, M. J. (1997). Maternal Care,
664 Hippocampal Glucocorticoid Receptors, and Hypothalamic-Pituitary-Adrenal Responses to Stress. *Science*,
665 *277*(5332), 1659–1662.
666 Livneh, I., Cohen-Kaplan, V., Cohen-Rosenzweig, C., Avni, N., & Ciechanover, A. (2016). The life cycle of the 26S
667 proteasome: from birth, through regulation and function, and onto its death. *Cell Research*, *26*, 869–869.
668 Lloyd, J. D., & Martin, T. E. (2004). Nest-site preference and maternal effects on offspring growth. *Behavioral*
669 *Ecology*, *15*(5), 816–823. doi: 10.1093/beheco/arh085
670 Longmire, J. L., Gee, G. F., Hardekopf, C. L., & Mark, G. A. (1992). Establishing paternity in whooping cranes
671 (*Grus americana*) by DNA analysis. *The Auk*, *109*(3), 522-529.
672 Louder, M. I. M., Hauber, M. E., & Balakrishnan, C. N. (2018). Early social experience alters transcriptomic
673 responses to species-specific song stimuli in female songbirds. *Behavioural Brain Research*, *347*, 69–76.
674 doi: 10.1016/j.bbr.2018.02.034
675 Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data
676 with DESeq2. *Genome Biology*, *15*(12), 550–550. doi: 10.1186/s13059-014-0550-8
677 Lowdon, R. F., Jang, H. S., & Wang, T. (2016). Evolution of Epigenetic Regulation in Vertebrate Genomes. *Trends*
678 *in Genetics*, *32*(5), 269–283. doi: 10.1016/j.tig.2016.03.001
679 Lowther, J. K. (1961). Polymorphism in the white-throated sparrow, *Zonotrichia albicollis* (Gmelin). *Canadian*
680 *Journal of Zoology*, *39*(3), 281–292. doi: 10.1139/z61-031

Author Manuscript

This article is protected by copyright. All rights reserved

681 Lowther, J. K. (1962). *Colour and Behavioural Polymorphism in the White-throated Sparrow, Zonotrichia Albicollis (Gmelin)*. Doctoral dissertation, University of Toronto.

683 Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445. doi: 10.1038/nrn2639

685 Marco, E. M., Echeverry-Alzate, V., López-Moreno, J. A., Giné, E., Peñasco, S., & Viveros, M. P. (2014). Consequences of early life stress on the expression of endocannabinoid-related genes in the rat brain. *Behavioural Pharmacology*, *25*(5–6), 547–556. doi: 10.1097/FBP.0000000000000068

688 Marini, F., & Binder, H. (2016). Development of Applications for Interactive and Reproducible Research: a Case Study. *Genomics and Computational Biology*, *3*(1), 39. doi: 10.18547/gcb.2017.vol3.iss1.e39

690 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10–10. doi: 10.14806/ej.17.1.200

692 McCoy, C. R., Rana, S., Stringfellow, S. A., Day, J. J., Wyss, J. M., Clinton, S. M., & Kerman, I. A. (2016). Neonatal maternal separation stress elicits lasting DNA methylation changes in the hippocampus of stress-reactive Wistar Kyoto rats. *European Journal of Neuroscience*, *44*(10), 2829–2845. doi: 10.1111/ejn.13404

695 McEwen, B. S. (2007). Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. *Physiological Reviews*, *87*(3), 873–904. doi: 10.1152/physrev.00041.2006

697 McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonté, B., Szyf, M., … Meaney, M. J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience*, *12*, 342–342.

700 Meaney, M. J. (2001). Maternal Care, Gene Expression, and the Transmission of Individual Differences in Stress Reactivity Across Generations. *Annual Review of Neuroscience*, *24*(1), 1161–1192. doi: 10.1146/annurev.neuro.24.1.1161

703 Michopoulos, V., Maney, D. L., Morehouse, C. B., & Thomas, J. W. (2007). A genotyping assay to determine plumage morph in the white-throated sparrow (*Zonotrichia albicollis*). *The Auk*, *124*(4), 1330. doi:10.1642/0004-8038(2007)124[1330:AGATDP]2.0.CO;2

706 Millstein, R. A., & Holmes, A. (2007). Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. *Neuroscience & Biobehavioral Reviews*, *31*(1), 3–17. doi: 10.1016/j.neubiorev.2006.05.003

709 Mock, D. W., & Parker, G. A. (1997). *The evolution of sibling rivalry*. Oxford ; New York: Oxford University Press.

710 Moghadam, H. K., Johnsen, H., Robinson, N., Andersen, Ø., H. Jørgensen, E., Johnsen, H. K., … Tveiten, H. (2017). Impacts of Early Life Stress on the Methylome and Transcriptome of Atlantic Salmon. *Scientific Reports*, *7*(1). doi: 10.1038/s41598-017-05222-2

713 Monaghan, P. (2014). Organismal stress, telomeres and life histories. *Journal of Experimental Biology*, *217*(1), 57–66. doi: 10.1242/jeb.090043

715 Mousseau, T. A., & Fox, C. W. (Eds.). (1998). *Maternal effects as adaptations*. New York: Oxford University Press.

Author Manuscript

This article is protected by copyright. All rights reserved

716 Murgatroyd, C., Patchev, A. V., Wu, Y., Micale, V., Bockmühl, Y., Fischer, D., … Spengler, D. (2009). Dynamic
717 DNA methylation programs persistent adverse effects of early-life stress. *Nature Neuroscience*, *12*(12),
718 1559–1566. doi: 10.1038/nn.2436
719 Noguera, J. C., Kim, S.-Y., & Velando, A. (2017). Family-transmitted stress in a wild bird. *Proceedings of the*
720 *National Academy of Sciences*, *114*(26), 6794-6799. Retrieved from
721 http://www.pnas.org/content/early/2017/06/06/1706164114.abstract
722 Nord, A., & Nilsson, J.-Å. (2011). Incubation Temperature Affects Growth and Energy Metabolism in Blue Tit
723 Nestlings. *The American Naturalist*, *178*(5), 639–651. doi: 10.1086/662172
724 Pértille, F., Brantsæter, M., Nordgreen, J., Coutinho, L. L., Janczak, A. M., Jensen, P., & Guerrero-Bosagna, C.
725 (2017). DNA methylation profiles in red blood cells of adult hens correlate with their rearing conditions.
726 *The Journal of Experimental Biology*, *220*(19), 3579–3587. doi: 10.1242/jeb.157891
727 Peterson, T. R., Laplante, M., Thoreen, C. C., Sancak, Y., Kang, S. A., Kuehl, W. M., … Sabatini, D. M. (2009).
728 DEPTOR Is an mTOR Inhibitor Frequently Overexpressed in Multiple Myeloma Cells and Required for
729 Their Survival. *Cell*, *137*(5), 873–886. doi: 10.1016/j.cell.2009.03.046
730 Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features.
731 *Bioinformatics*, *26*(6), 841–842.
732 Quirici, V., Guerrero, C. J., Krause, J. S., Wingfield, J. C., & Vásquez, R. A. (2016). The relationship of telomere
733 length to baseline corticosterone levels in nestlings of an altricial passerine bird in natural populations.
734 *Frontiers in Zoology*, *13*(1). doi: 10.1186/s12983-016-0133-5
735 R Core Team (2013). R: A language and environment for statistical computing.
736 Reshetnikov, V. V., Studenikina, A. A., Ryabushkina, J. A., Merkulova, T. I., & Bondar, N. P. (2018). The impact of
737 early-life stress on the expression of HPA-associated genes in the adult murine brain. *Behaviour*, *155*(2–3),
738 181–203. doi: 10.1163/1568539X-00003482
739 Romanov, M. N., Tuttle, E. M., Houck, M. L., Modi, W. S., Chemnick, L. G., Korody, M. L., … Ryder, O. A.
740 (2009). The value of avian genomics to the conservation of wildlife. *BMC Genomics*, *10*(Suppl 2), S10. doi:
741 10.1186/1471-2164-10-S2-S10
742 Royle, N. J., Smiseth, P. T., & Kölliker, M. (Eds.). (2012). *The evolution of parental care*. Oxford University Press.
743 Rubenstein, D. R., Skolnik, H., Berrio, A., Champagne, F. A., Phelps, S., & Solomon, J. (2016). Sex-specific fitness
744 effects of unpredictable early life conditions are associated with DNA methylation in the avian
745 glucocorticoid receptor. *Molecular Ecology*, *25*(8), 1714–1728. doi: 10.1111/mec.13483
746 Schrader, M., Jarrett, B. J. M., & Kilner, R. M. (2018). Parental care and sibling competition independently increase
747 phenotypic variation among burying beetle siblings. *Evolution*, *72(11), 2546-2552*. doi: 10.1111/evo.13607
748 Schwander, T., Libbrecht, R., & Keller, L. (2014). Supergenes and Complex Phenotypes. *Current Biology*, *24*(7),
749 R288–R294. doi: 10.1016/j.cub.2014.01.056
750 Shang, F., & Taylor, A. (2011). Ubiquitin–proteasome pathway and cellular responses to oxidative stress. *Free*
751 *Radical Biology and Medicine*, *51*(1), 5–16. doi: 10.1016/j.freeradbiomed.2011.03.031

Author Manuscript

This article is protected by copyright. All rights reserved

752 Sheldon, E. L., Schrey, A. W., Ragsdale, A. K., & Griffith, S. C. (2018). Brood size influences patterns of DNA
753 methylation in wild Zebra Finches (*Taeniopygia guttata*). *The Auk*, *135*(4), 1113–1122. doi:
754 10.1642/AUK-18-61.1
755 Silberman, D. M., Acosta, G. B., & Zorrilla Zubilete, M. A. (2016). Long-term effects of early life stress exposure:
756 Role of epigenetic mechanisms. *Pharmacological Research*, *109*, 64–73. doi: 10.1016/j.phrs.2015.12.033
757 Smith, J.N., Newhouse, D.J., Balakrishnan, C.N., Tuttle, E.M.,& Gonser, R.A. Differential morph gene expression in
758 chromosome three of the white-throated sparrow (*Zonotrichia albicollis*). *In review*.
759 Spencer, K. A. (2017). Developmental stress and social phenotypes: integrating neuroendocrine, behavioural and
760 evolutionary perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
761 *372*(1727). Retrieved from http://rstb.royalsocietypublishing.org/content/372/1727/20160242.abstract
762 Spencer, K. A., Evans, N. P., & Monaghan, P. (2009). Postnatal Stress in Birds: A Novel Model of Glucocorticoid
763 Programming of the Hypothalamic-Pituitary-Adrenal Axis. *Endocrinology*, *150*(4), 1931–1934. doi:
764 10.1210/en.2008-1471
765 Spinney, L. H., Bentley, G. E., & Hau, M. (2006). Endocrine correlates of alternative phenotypes in the white-
766 throated sparrow (*Zonotrichia albicollis*). *Hormones and Behavior*, *50*(5), 762–771. doi:
767 10.1016/j.yhbeh.2006.06.034
768 Swett, M. B., & Breuner, C. W. (2009). Plasma Testosterone Correlates with Morph Type across Breeding Substages
769 in Male White-Throated Sparrows. *Physiological and Biochemical Zoology*, *82*(5), 572–579. doi:
770 10.1086/605392
771 Szyf, M. (2009). The early life environment and the epigenome. *Biochimica et Biophysica Acta (BBA) - General*
772 *Subjects*, *1790*(9), 878–885. doi: 10.1016/j.bbagen.2009.01.009
773 Szyf, M., Weaver, I., & Meaney, M. (2007). Maternal care, the epigenome and phenotypic differences in behavior.
774 *Reproductive Toxicology*, *24*(1), 9–19. doi: 10.1016/j.reprotox.2007.05.001
775 Taylor, S., & Campagna, L. (2016). Avian supergenes. *Science*, *351*(6272), 446–447. doi: 10.1126/science.aae0389
776 Thomas, J. W., Cáceres, M., Lowman, J. J., Morehouse, C. B., Short, M. E., Baldwin, E. L., … Martin, C. L. (2008).
777 The Chromosomal Polymorphism Linked to Variation in Social Behavior in the White-Throated Sparrow
778 (*Zonotrichia albicollis*) Is a Complex Rearrangement and Suppressor of Recombination. *Genetics*, *179*(3),
779 1455 LP – 1468.
780 Thorneycroft, H. B. (1966). Chromosomal Polymorphism in the White-Throated Sparrow, *Zonotrichia albicollis*
781 (Gmelin). *Science*, *154*(3756), 1571-1572.
782 Thorneycroft, H. B. (1975). A cytogenetic study of the white-throated sparrow, Zonotrichia albicollis (Gmelin).
783 *Evolution*, *29*(4), 611–621. doi:10.1111/j.1558-5646.1975.tb00855.x
784 Tomalty, K. M. H., Meek, M. H., Stephens, M. R., Rincón, G., Fangue, N. A., May, B. P., & Baerwald, M. R.
785 (2015). Transcriptional Response to Acute Thermal Exposure in Juvenile Chinook Salmon Determined by
786 RNAseq. *G3: Genes|Genomes|Genetics*, *5*(7), 1335 LP – 1349.

Author Manuscript

This article is protected by copyright. All rights reserved

787 Trivers, R.L. (1972). Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (B.
788 Campbell, ed), pp. 136–179. Aldine, Chicago, IL.
789 Turner, J. D., Schote, A. B., Macedo, J. A., Pelascini, L. P. L., & Muller, C. P. (2006). Tissue specific glucocorticoid
790 receptor expression, a role for alternative first exon usage? *Biochemical Pharmacology*, *72*(11), 1529–1537.
791 doi: 10.1016/j.bcp.2006.07.005
792 Tuttle, E. (2003). Alternative reproductive strategies in the white-throated sparrow: behavioral and genetic evidence.
793 *Behavioral Ecology*, *14*(3), 425–432.
794 Tuttle, E. M., Grunst, A. S., Grunst, M. L., Korody, M. L., Betuel, A. M., Barcelo-Serra, M., … Gonser, R. A.
795 (2017). Climatically driven changes in population composition and offspring sex-morph ratio in a
796 polymorphic species. *Ecosphere*, *8*(4), e01762–e01762. doi: 10.1002/ecs2.1762
797 Tuttle, E. M., Bergland, A. O., Korody, M. L., Brewer, M. S., Newhouse, D. J., Minx, P., … Balakrishnan, C. N.
798 (2016). Divergence and Functional Degradation of a Sex Chromosome-like Supergene. *Current Biology*,
799 *26*(3), 344–350. doi: 10.1016/j.cub.2015.11.069
800 van Oers, K., Kohn, G. M., Hinde, C. A., & Naguib, M. (2015). Parental food provisioning is related to nestling
801 stress response in wild great tit nestlings: implications for the development of personality. *Frontiers in*
802 *Zoology*, *12*(Suppl 1), S10. doi: 10.1186/1742-9994-12-S1-S10
803 Wade, M. J. (1998). The evolutionary genetics of maternal effects Pp. 5–21 *in* Mousseau T. A & Fox C. W.,
804 eds. Maternal effects as adaptations. Oxford Univ. Press, New York.
805 Wang, Y., Branicky, R., Noë, A., & Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ROS
806 damage and regulating ROS signaling. *The Journal of Cell Biology*, *217*(6), 1915–1928. doi:
807 10.1083/jcb.201708007
808 Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., … Meaney, M. J.
809 (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, *7*, 847–847.
810 Weaver, I. C. G., Meaney, M. J., & Szyf, M. (2006). Maternal care effects on the hippocampal transcriptome and
811 anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proceedings of the National*
812 *Academy of Sciences of the United States of America*, *103*(9), 3480 LP – 3485.
813 Wolf, J. B., & Wade, M. J. (2009). What are maternal effects (and what are they not)? *Philosophical Transactions of*
814 *the Royal Society B: Biological Sciences*, *364*(1520), 1107–1115. doi: 10.1098/rstb.2008.0238
815 Zelko, I. N., Mariani, T. J., & Folz, R. J. (2002). Superoxide dismutase multigene family: a comparison of the CuZn-
816 SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free*
817 *Radical Biology and Medicine*, *33*(3), 337–349. doi: 10.1016/S0891-5849(02)00905-X
818 Zhang, B., & Horvath, S. (2005). A General Framework for Weighted Gene Co-Expression Network Analysis.
819 *Statistical Applications in Genetics and Molecular Biology*, *4*. doi: 10.2202/1544-6115.1128
820 Zhao, H., Zhao, Y. G., Wang, X., Xu, L., Miao, L., Feng, D., … Zhang, H. (2013). Mice deficient in *Epg5*exhibit
821 selective neuronal vulnerability to degeneration. *The Journal of Cell Biology*, *200*(6), 731 LP – 741.

Author Manuscript

This article is protected by copyright. All rights reserved

822 Zinzow-Kramer, W. M., Horton, B. M., McKee, C. D., Michaud, J. M., Tharp, G. K., Thomas, J. W., … Maney, D.
823 L. (2015). Genes located in a chromosomal inversion are correlated with territorial song in white-throated
824 sparrows. *Genes, Brain and Behavior*, *14*(8), 641–654. doi: 10.1111/gbb.12252
825

826 **Data Accessibility**

827 The 32 RNAseq libraries used in this study are deposited in the NCBI SRA under
828 Bioproject PRJNA546611. Count files and R code are located in this project's GitHub page:
829 https://github.com/danielnewhouse/wtsp
830

831 **Author Contributions**

832 DJN designed and performed research, analyzed the data, and wrote the paper. MBS
833 performed research, contributed samples, and reviewed drafts of the paper. EMT designed and
834 performed research and contributed samples. RAG designed and performed research, contributed
835 samples, and reviewed drafts of the paper. CNB designed and performed research, contributed
836 reagents, and reviewed drafts of the paper.

837 **Tables and Figures**

838 Table 1. WGCNA modules correlated with morph, strength of correlation (R^2), p-value, hub
839 gene(s) of module, and the degree distribution of hub gene(s).

Module	R^2	p-value	Hub genes	DD of hub gene(s)
Dark Red	0.47	0.009	TRAF5	32
Ivory	-0.66	$9x10^{-5}$	GOPC, HDAC2, HINT3, TAF5L, TRMT61B, MARC2	>29
Light Cyan	0.67	$5x10^{-5}$	BPNT1, EPM2A, LOC102066536 (GST-like), MAN1A1, MEI4, RNASET2, SLC18B1, TTC32	>27
Salmon	-0.5	0.005	NSL1	39
Sky Blue	0.53	0.003	DTX3L, EIF2AK2, IFIT5, LOC102064521 (OASL),	>22

Author Manuscript

This article is protected by copyright. All rights reserved

			LOC102065196 (IFI27L2), PARP9, PARP14, RSAD2, ZNFX1	

840

841 Table 2. WGCNA modules correlated with pair type, strength of correlation (R^2), p-value, hub
842 gene(s) of module, and the degree distribution of hub gene(s).

Module	**R^2**	**p-value**	**Hub genes**	**DD of hub gene(s)**
Beige	-0.61	$3x10^{-4}$	DEPTOR	39
Blue	-0.45	0.01	NDUFB3	42
Cyan	0.46	0.01	HELZ	36
Dark Orange	0.7	$1x10^{-5}$	NCOA6	45
Light Green	0.6	$4x10^{-4}$	CDK19, CHD4, EPG5	>28
Orange	-0.45	0.01	ZFX	31
Pink	0.37	0.04	LOC102060916 (C12orf4)	19

843

844

845 Figure 1. WGCNA module-trait correlation matrix. Each box contains the R^2 correlation value
846 followed by p-value in parentheses of a given trait with the module. Correlation values range
847 from -1 to 1, with orange colors representing positive correlation and blue colors representing
848 negative correlation.

849

850 Figure 2. (A) Network of blue module, highlighting hub gene NDUFB3, along with normalized
851 expression plots of (B) NDUFB3, (C) PSMD6, and (D) UBE2D3. NDUFB3 is a hub of the blue
852 module, while PSMD6 and UBE2D3 represent candidate ubiquitin-mediated proteolysis genes.
853 TxW represents samples from nests sired by a T male and a W female. WxT represents samples
854 from nests sired by a W male and a T female. Each circle represents a gene and diamonds
855 represent hub genes described in Table 2.

856

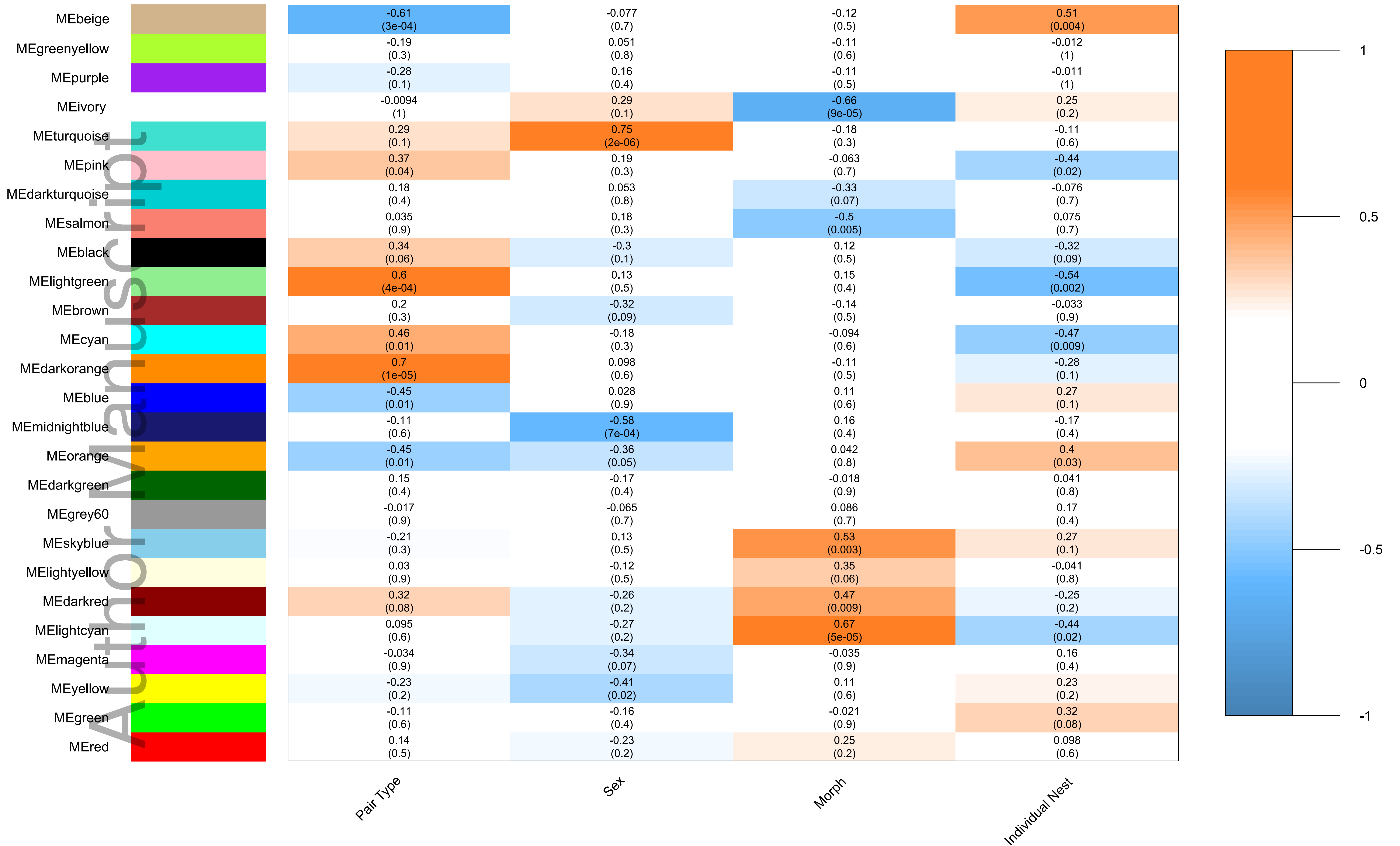
Author Manuscript

This article is protected by copyright. All rights reserved

857 Figure 3. (A) Network of beige module, highlighting hub gene DEPTOR, along with normalized
858 expression plots of (B) DEPTOR, (C) SOD2 and (D) NR3C1. DEPTOR is a hub of the beige
859 module, while SOD2 and NR3C1 represent candidate stress responsive genes. TxW represents
860 samples from nests sired by a T male and a W female. WxT represents samples from nests sired
861 by a W male and a T female.
862
863 Figure 4. (A) Network of light green module and normalized expression plots of hub genes (B)
864 CDK19, (C) CHD4, and (D) EPG5. TxW represents samples from nests sired by a T male and a
865 W female. WxT represent samples from nests sired by a W male and a T female. Each circle
866 represents a gene and diamonds represent hub genes described in Table 2.

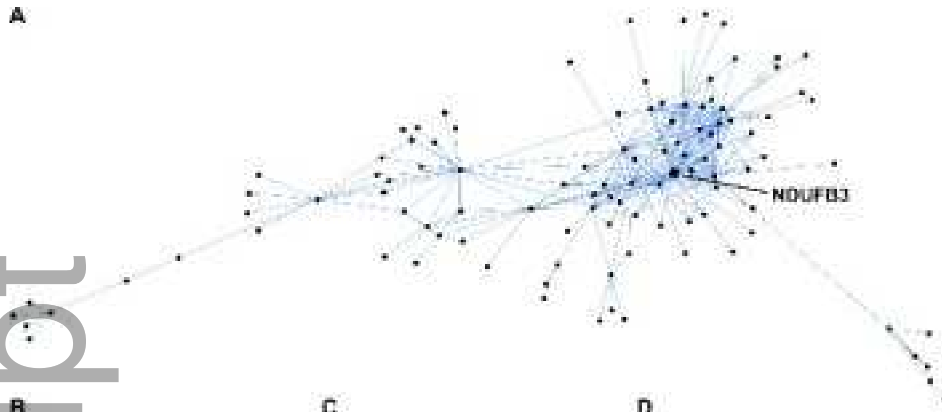
Author Manuscript

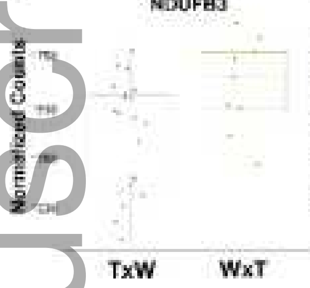
This article is protected by copyright. All rights reserved

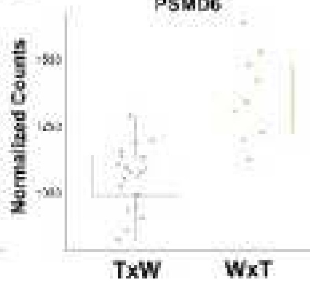


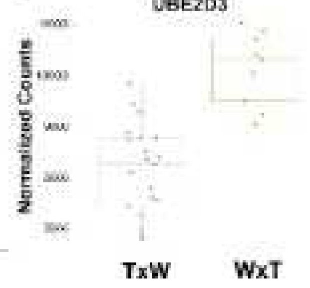
Author Manuscript

This article is protected by copyright. All rights reserved







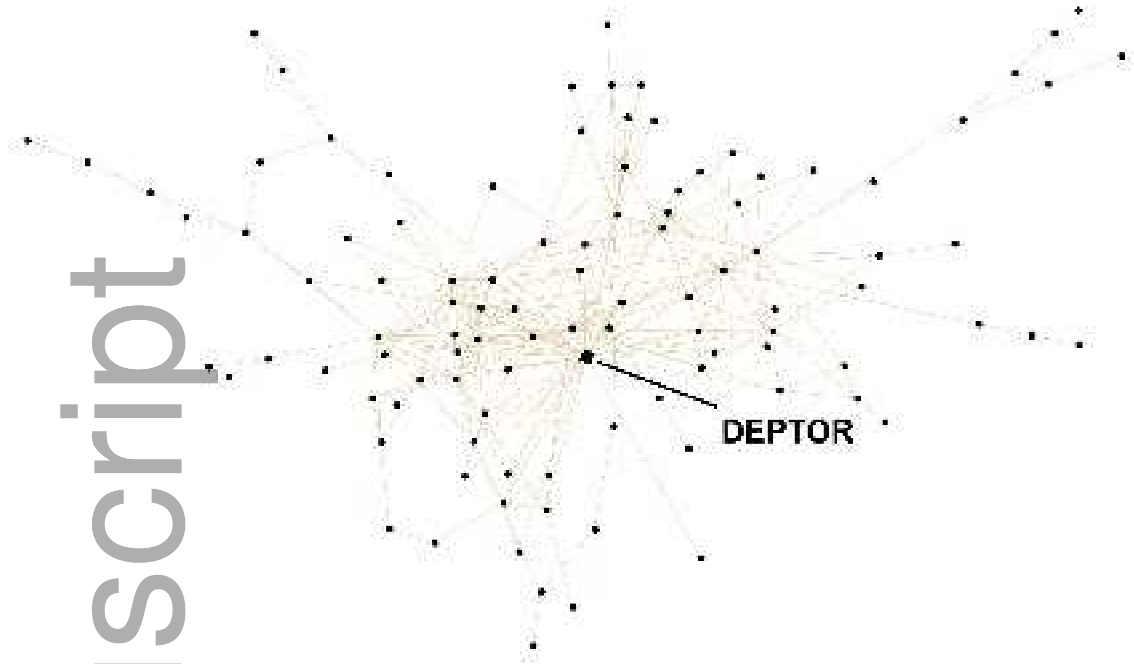


mec_15205_f2.png

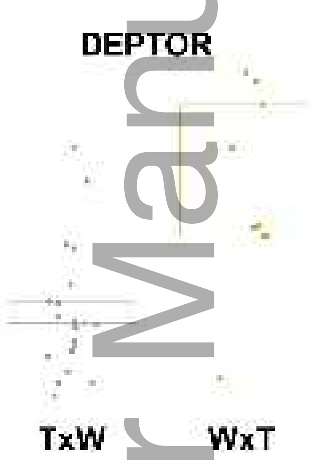
Author Manuscript

This article is protected by copyright. All rights reserved

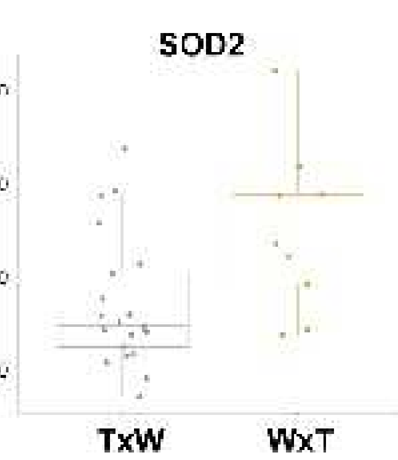
A



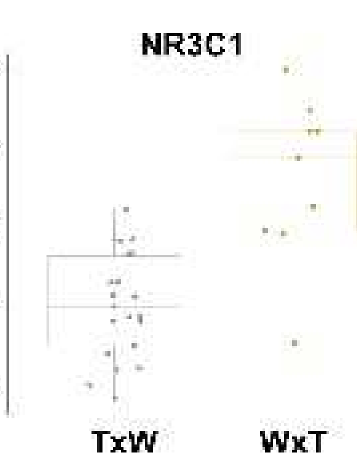
B



C



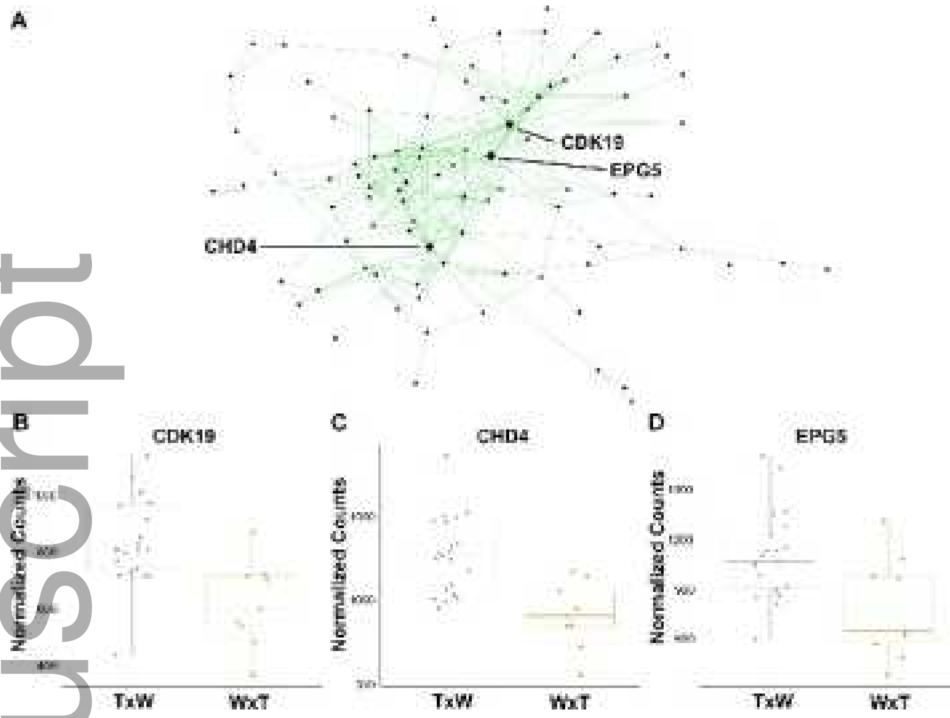
D



mec_15205_f3.png

Author Manuscript

This article is protected by copyright. All rights reserved



mec_15205_f4.png

Author Manuscript

This article is protected by copyright. All rights reserved