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Testing the behavioral origins of novelty: did increased aggression lead to scale-eating in pupfishes?

Michelle E. St. John¹, Joseph A. McGirr¹, Christopher H. Martin^{1*}

¹*Department of Biology, University of North Carolina at Chapel Hill, NC, USA*

*Correspondence: Christopher H Martin. Department of Biology, University of North Carolina at Chapel Hill, 120 South Rd., Chapel Hill, NC, 27599, United States

Email: chmartin@unc.edu

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23 **Abstract**

24 How novelty evolves is still largely unknown. Environmental changes are often assumed to
25 precede novelty; however, behavioral shifts may also play a role. Here, we examine whether a
26 shift in aggression explains the origin of a novel scale-eating pupfish species (*Cyprinodon*
27 *desquamator*) within an adaptive radiation on San Salvador Island, Bahamas. We compared
28 aggression using behavioral and gene expression data across three sympatric species in the San
29 Salvador radiation (generalist, snail-eating specialist, and scale-eating specialist), and
30 additionally measured behavioral aggression in an outgroup generalist from North Carolina.
31 Surprisingly, we found increased behavioral aggression and differential expression of
32 aggression-related genes in both the scale-eating and snail-eating species. Furthermore, male
33 scale-eaters and female snail-eaters showed the highest levels of aggression compared to other
34 groups. Differential gene expression in each specialist during larval development also suggested
35 sex-mediated differences in male-male aggression and maternal care. Ultimately, our data
36 indicate that aggression is not unique to scale-eating specialists. Instead, selection may increase
37 aggression in other contexts such as niche specialization, mate competition, or selection on other
38 ecologically relevant traits, including jaw size. Indeed, some adaptive variants associated with
39 oral jaw size in the San Salvador radiation occur in genetic pathways with pleiotropic effects on
40 aggression.

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47 **Introduction**

48 The origins of evolutionary novelty are still poorly understood. For example, both novel
49 behavior and morphology play a role in the evolution of novel resource use, but their relative
50 importance and order in which they evolve are still unknown. Changes in behavior may precede
51 the evolution of novel morphologies, as they can expose organisms to novel environments and
52 selective pressures (Huey et al. 2003). Investigations of novelty, however, overwhelmingly
53 ignore this possibility (although see: Huey et al. 2003; Losos et al. 2004; Duckworth 2006) .
54 Instead, previous studies have focused on novel adaptive morphologies or on how environmental
55 changes expose organisms to new selective pressures (Liem 1973; Barton and Partridge 2000;
56 Janovetz 2005a; Hulsey et al. 2008). One reason behavior has been overlooked as an origin for
57 novelty is because it is still unclear whether it drives or inhibits evolution, as behavior is often
58 extremely plastic. However, in order to determine if novelty has a behavioral origin we must
59 first understand its variation within and among taxa.

60 One outstanding example of novelty is lepidophagy (scale-eating) in fishes. Scale-eating
61 has been documented in five freshwater and seven saltwater families of fishes and has
62 independently evolved at least 19 times (Sazima 1983; Janovetz 2005b; Martin and Feinstein
63 2014; Kolmann et al. 2018). Scale-eating includes both novel morphologies and novel behaviors.
64 For example, some scale-eaters have premaxillary external teeth for scraping scales
65 (Novakowski et al. 2004), some use aggressive mimicry to secure their prey (Boileau et al.
66 2015), others sneak scales from the surface of fish that they are cleaning (Losey 1979), and still
67 others use ambush tactics to obtain scales (Nshombo et al. 1985). Even though scale-eating is an
68 outstanding example of the convergent evolution of novel trophic ecology across disparate

69 environments and taxa and displays a wide variety of morphologies and behaviors, its origins
70 have yet to be explored.

71 There are currently only three hypothesized behavioral origins for scale-eating. The
72 algae-grazer hypothesis predicts that scale-eating arises from the incidental ingestion of scales
73 during algae scraping (Fryer et al. 1955; Greenwood 1965; Sazima 1983). Many scale-eaters are
74 closely related to algae-grazers. For example, many rock-dwelling Malawi cichlids are algae-
75 scrapers (Greenwood 1965; Fryer and Iles 1972; Ribbink et al. 1983), but the radiation also
76 includes two sister species of scale-eaters (*Corematodus shiranus* and *Corematodus taeniatus*)
77 and a second independent origin of scale-eating in *Genyochromis mento* (Trewavas 1947;
78 Greenwood 1965). Similarly, the extinct Lake Victorian scale-eater *Haplochromis welcommei*
79 was nested within rock-dwelling algae scrapers (Greenwood 1965). This hypothesis, however,
80 does not address why algae-grazing fish would seek food on the surface of other fish
81 (Greenwood 1965). The second hypothesis, termed the cleaner hypothesis, tries to address this
82 gap by arguing that scale-eating arose from the incidental ingestion of scales during the
83 consumption of ectoparasites from the surface of other fishes (Greenwood 1965; Sazima 1983).
84 One line of evidence supporting this hypothesis is that cleaner fish sometimes eat scales. For
85 example, the Hawaiian cleaner wrasse (*Labroides phthirophagus*) and two species of juvenile
86 sea chub (*Hermosilla azurea* and *Girella nigricans*) consume both ectoparasites and scales
87 (Demartini and Coyer 1981; Sazima 1983; Losey 1972). However, most scale-eating fishes are
88 not known to forage on ectoparasites, nor are they closely related to fish that do. In fact, the
89 closest examples of this are the false cleaner fishes (*Aspidontus taeniatus* and *Plagiotremus*
90 *rhinorhynchs*) who aggressively mimic cleaner wrasse (*Labroides dimidiatus*) in order to
91 consume scales. Despite their morphological similarities, however, these fish are not closely

92 related (Hundt et al. 2014). Finally, the aggression hypothesis predicts that scale-eating evolved
93 due to the accidental ingestion of scales during inter- or intra-species aggression (Sazima 1983).
94 This hypothesis is supported by the fact that two characid species of scale-eaters (*Probolodus*
95 *heterostomus* and *Exodon paradoxus*) are closely related to the aggressive *Astyanax tetras*
96 (Sazima 1983; Kolmann et al. 2018); a similar argument can be made for the scale-eating
97 piranha (*Cataprin mento*) (Janovetz 2005).

98 The scale-eating pupfish, *Cyprinodon desquamator*, is an excellent species for
99 investigating the origins of scale-eating because it is, by far, the youngest scale-eating specialist.
100 The species is nested within a sympatric adaptive radiation of pupfishes endemic to the
101 hypersaline lakes of San Salvador island, Bahamas (Martin and Wainwright 2011, 2013a). In
102 addition to the scale-eating pupfish, this radiation also includes a widespread generalist (*C.*
103 *variegatus*) and an endemic snail-eating specialist (*C. brontotheroides*). Other generalist pupfish
104 lineages are also distributed across the Caribbean and western Atlantic Ocean. Phylogenetic
105 evidence indicates that scale-eater's most recent ancestor was a generalist feeder (Martin 2016;
106 Richards and Martin 2017). Furthermore, geological evidence suggests that the hypersaline
107 lakes of San Salvador island—and thus the radiation containing the scale-eater—is less than 10
108 thousand years old (Hagey and Mylroie 1995; Martin and Wainwright 2013a,b).

109 We investigated the possible behavioral origins of novelty by examining whether a shift
110 in aggression led to the evolution of scale-eating in pupfish. We compared measures of
111 aggression using both behavioral and gene expression data between scale-eaters and closely
112 related species within their radiation (a sympatric generalist species and a snail-eating species
113 from San Salvador Island) as well as an additional outgroup (a generalist species from North
114 Carolina). We predicted high levels of aggression in scale-eaters, intermediate levels in the San

115 Salvador generalist and snail-eating pupfish, and low levels of aggression in the North Carolina
116 (NC) outgroup generalist. Surprisingly, we found that male scale-eaters and female snail-eaters
117 displayed increased levels of aggression, while other groups displayed relatively low levels of
118 aggression. This suggests that aggression alone cannot explain the origins of scale-eating in
119 pupfish. We also identified promising candidate genes that are differentially expressed between
120 species associated with differences in aggression and linking behavior, morphology, and the
121 evolution of novel ecology.

122

123 **Methods**

124 *Sampling*

125 Generalist, snail-eating, and scale-eating pupfish were collected by seine from Crescent Pond,
126 Great Lake, Little Lake, Osprey Lake, and Oyster Pond of San Salvador Island, Bahamas in July,
127 2016. In June 2017, generalist pupfish were collected by seine from the Cape Fear river drainage
128 (Fort Fisher) on the coast of North Carolina. Fishes were housed in 40 – 80 liter tanks in mixed-
129 sex groups at 5-10 ppt salinity in temperatures ranging from 23°C-30°C. Fish were fed a diet of
130 frozen blood worms, frozen mysis shrimp, or commercial pellet food daily.

131

132 *Aggression assay*

133 We quantified levels of aggression for each pupfish species and sex using mirror tests (Vøllestad
134 and Quinn 2003; Francis 2010). To control for individual size and motivation, we incited
135 aggression using a compact mirror (10 cm X 14 cm) placed in a 2-liter trial tank (25 cm X 16 cm
136 X 17 cm). We randomly chose adult fish and isolated each one in 2-liter tanks that contained a

137 single bottom synthetic yarn mop for cover and opaque barriers between adjacent tanks. We gave
138 the fish at least 12 hours to acclimate to their new environment before performing an assay.
139 During a 5-minute focal observation period, we measured three metrics as a proxy for
140 aggression: latency to approach mirror image, latency to attack mirror image, and total number
141 of attacks toward the mirror image. A trial began as soon as the mirror was securely lowered into
142 the tank. We measured latency to approach as the time elapsed before an individual approached
143 the mirror to within one-body length. Similarly, we measured latency to attack as the time
144 elapsed before an individual attacked their mirror image for the first time. Finally, we counted
145 the total number of attacks an individual performed during the entirety of the trial. We also
146 measured the standard length of each fish after the trial.

147
148 *Statistical analyses*
149

150 We used time-to-event analyses to determine if species and sex were associated with 1) latency
151 to approach their mirror image and 2) latency to attack their mirror image. For the latency to
152 approach metric (time in seconds) and the latency to attack metric (time in seconds) we used a
153 mixed-effects Cox proportional hazards model (coxme package; Therneau 2012) in R (R
154 Development Core Team 2016). These models allow right censored data, i.e. individuals who did
155 not approach or attack their mirror image were not excluded and contributed to Kaplan-Meier
156 estimates and time-to-event curves (Rich et al. 2010). For both the latency to approach model
157 and the latency to attack model we included species, sex, and their interaction as fixed effects
158 and lake population as a random effect. We compared these models to equivalent models that
159 also included size (log scale) as a covariate using AICc (Burnham and Anderson 2002; stats
160 package; R Development Core Team 2016) . For the latency to approach model, size was non-
161 significant ($P = 0.36$) and we removed it from the model. For the latency to attack model,

162 however, size was a significant covariate and retained in the final model. We used the likelihood
163 ratio test to determine if species, sex, or their interaction were associated with latency to
164 approach or attack the mirror image. Additionally, we used a Cox proportional hazards model
165 without mixed effects to plot the resulting time-to-event curves and made pairwise comparisons
166 between curves using log-rank tests (Survival Package; survminer package; Therneau 2015).

167 We analyzed the total number of attacks using a generalized linear mixed model
168 (GLMM) with a negative binomial distribution for this response variable. We modeled species,
169 sex, and their interaction as fixed effects, and population as a random effect. We compared this
170 model to a model including size (log scale) as a continuous covariate using AICc, but accounting
171 for size did not substantially increase the likelihood of the model ($\Delta\text{AICc} = 0.96$). We used a
172 Wald chi-square test (type II) to determine if species, sex, or their interaction significantly
173 affected the total number of attacks performed and used Tukey's HSD to make direct
174 comparisons between groups.

175

176 *Identifying candidate genes affecting differences in aggression between species*

177 We searched a previously published dataset of 15 San Salvador pupfish transcriptomes to
178 identify candidate genes underlying behavioral differences in aggression among all three species
179 (Mcgirr and Martin 2018). This previous study did not analyze gene expression pathways
180 annotated for effects on behavior. Briefly, purebred F₁ and F₂ offspring from the three-species
181 found on San Salvador island were raised in a common garden laboratory environment. Larvae
182 were euthanized in an overdose of MS-222 at 8-10 days post fertilization (dpf) and were
183 immediately preserved in RNAlater (Ambion, Inc.) and stored at -20 C after 24 hours at 4 C.
184 Total mRNA was extracted from 6 generalists, 6 snail-eaters, and 3 scale-eaters (RNeasy kits,

185 Qiagen). Stranded sequencing on an Illumina HiSeq 4000 at the High Throughput Genomic
186 Sequencing Facility at UNC Chapel Hill resulted in 363 million raw reads that were aligned to
187 the *Cyprinodon variegatus* reference genome (NCBI, *C. variegatus* Annotation Release 100,
188 total sequence length =1,035,184,475; number of scaffold=9,259, scaffold N50, =835,301; contig
189 N50=20,803; Lencer et al. 2017). Aligned reads were mapped to annotated features using STAR
190 (v. 2.5(33)), with an average read depth of 309x per individual and read counts were generated
191 across annotated features using the featureCounts function from the Rsubread package (Liao et
192 al. 2013). DEseq2 (Love et al. 2014, v. 3.5) was used to normalize counts and identify: 1) 1,014
193 differentially expressed genes between snail-eaters vs generalists and 2) 5,982 differentially
194 expressed genes between scale-eaters vs generalists (McGirr and Martin 2018).

195 We identified one-way best hit zebrafish orthologs for genes differentially expressed
196 between 1) snail-eaters vs generalists (n=722) and 2) scale-eaters vs generalists (n=3,966) using
197 BlastP (V. 2.6; E-value <1). We compared this list of orthologs to gene ontologies describing
198 aggressive behavior (GO: 0002118), inter-male aggressive behavior (GO: 0002121), maternal
199 aggressive behavior (GO:0002125), maternal care behavior (GO: 0042711), and territorial
200 aggressive behavior (GO: 0002124; AmiGo; Carbon et al. 2009; Ashburner et al. 2000;
201 The Gene Ontology Consortium 2017). We also searched gene ontologies for three hormone
202 pathways commonly associated with aggression (the vasopressin pathway, the androgen
203 pathway, and the estradiol pathway).

204

205 **Results**

206 ***Behavioral aggression***

207 Male scale-eaters and female snail-eaters showed increased levels of aggression compared to
208 other groups. Scale-eaters (at the species level) approached their mirror image significantly more
209 than NC generalists (Table 1A; Fig. 1A; log-rank test, $P = .038$). Additionally, male scale-eaters
210 attacked their mirror image significantly more than male San Salvador generalists (Table 1B;
211 Fig. 1B; log-rank test, $P = 0.032$). Lastly, male scale-eaters were the only group to exhibit
212 increased aggression compared to their female counterparts. More male scale-eaters attacked
213 their mirror image than did female scale-eaters (Table 1B; Fig. 1B: log-rank test, $P = 0.003$), and
214 they performed significantly more total attacks (Table 1C; Fig. 2; Tukey HSD, $P = 0.0003$).

215 Female snail-eaters showed a similar pattern of increased aggression. Snail-eaters (at the
216 species level) approached their mirror image significantly more than NC generalists (Table 1A;
217 Fig. 1A; log-rank test, $P = .038$). Significantly more female snail-eaters attacked their mirror
218 image than all other female groups (Table 1B; Fig. 1B; log-rank test, NC generalist $P = 0.032$,
219 San Salvador generalist $P = 0.0027$, San Salvador scale-eater $P = 0.0081$). Finally, female snail-
220 eaters performed significantly more attacks than female San Salvador scale-eaters and generalists
221 (Table 1C; Fig. 2; Tukey HSD, generalist $P = 0.0032$, scale-eater $P = 0.0006$).

222

223 ***Gene Expression***

224 We searched genes that were differentially expressed between scale-eaters *vs* generalists or
225 between snail-eaters *vs* generalists for gene ontologies describing aggressive behavior, inter-male
226 aggressive behavior, maternal aggressive behavior, maternal care behavior, the vasopressin
227 hormone pathway, and the androgen hormone pathway (Table 2). Despite over one thousand
228 differentially expressed genes at this developmental stage, only five genes were associated with
229 these aggression-related ontologies in the snail-eater *vs* generalist comparison (Table 2A). Scale-

230 eaters also exhibited differential expression of genes associated with inter-male aggression and
231 vasopressin when compared to their generalist sister species (Table 2B). Interestingly, one of
232 these genes (*gnaq*) contains a fixed variant in scale-eaters, which is known to function in
233 craniofacial development and shows signs of a hard selective sweep (McGirr and Martin 2017;
234 McGirr JA unpublished data). None of these ontologies were significantly over-represented in
235 either species comparison, which were instead enriched for cranial skeleton, metabolism, and
236 pigmentation terms (McGirr and Martin 2018).

237

238 **Discussion**

239 The origins of novelty have overwhelmingly been examined from a morphological perspective,
240 often ignoring behavior's potential role. However, shifts in behavior may also be a viable origin
241 for novelty. Unfortunately, only a few previous studies have directly investigated this possibility.
242 The origin of novelty in the Pacific field cricket (*Teleogryllus oceanicus*)—which exhibits a
243 novel silent morph—is one of the few examples of evolutionary novelty with a behavioral origin
244 (Zuk et al. 2006; Tinghitella and Zuk 2009; Bailey et al. 2010). Increased brain size in birds has
245 also been linked to behavioral shifts and novelty. Birds that display innovative feeding behaviors
246 have larger brains and are more successful at invading novel environments (Nicolakakis and
247 Lefebvre 2000; Sol and Lefebvre 2000; Overington et al. 2009). Likewise, the role of behavior in
248 evolutionary novelty has also been explored in western bluebirds (*Sialia Mexicana*; Duckworth
249 2006) and *Anolis* lizards (Losos et al. 2004, 2006). Despite the growing empirical evidence of
250 behavior's role in evolutionary innovation, a consensus has not yet been reached on whether
251 behavior ultimately drives or inhibits novelty. Furthermore, studies that investigate behavioral
252 origins of novelty rarely do so using both behavioral and genetic approaches. However, by

253 leveraging our gene expression data, we gained some mechanistic insight into the divergent
254 origins of increased behavioral aggression in each specialist species.

255 We tested whether increased aggression contributed to the origin of scale-eating in a
256 species of Caribbean pupfish using both behavioral and gene expression data. The aggression
257 hypothesis predicts that scale-eating arose due to increased inter- and intra- specific aggression
258 (Sazima 1983). Contrary to these predictions, both snail-eaters and scale-eaters showed increased
259 levels of aggression. Our gene expression data supported these findings, as both scale-eaters and
260 snail-eaters showed differential expression of genes involved in several aggression-related
261 pathways during larval development. Additionally, both scale-eaters and snail-eaters displayed
262 surprising differences in aggression between the sexes. While male scale-eaters showed
263 increased levels of aggression, female scale-eaters showed extremely low levels of aggression.
264 Conversely, female snail-eaters showed increased levels of aggression compared to females of
265 other species. These results suggest that the aggression hypothesis alone cannot explain the
266 evolution of scale-eating. Instead, selection may have favored increased levels of aggression in
267 other contexts, such as mate competition or trophic specialization in general. Increased levels of
268 aggression could have also arisen indirectly due to selection for other behaviors or traits,
269 including several genes involved in both aggression and craniofacial morphology (e.g. *gnaq*).

270 One caveat is that there is still discussion whether mirror tests accurately predict levels of
271 aggression in the field. Balzarini et al. (2014) argue that, while mirror tests are a valid method of
272 measuring aggression in some species, they are inappropriate for others. For example, some
273 species use lateral displays of aggression which primarily occur head to tail—a maneuver that is
274 impossible with a mirror image. Additional studies also indicate that mirror tests may not
275 accurately predict aggressive display frequency, duration, or orientation (Elwood et al. 2014;

276 Arnott et al. 2016). It is possible that our method of measuring aggression may have
277 underestimated aggression for scale-eating pupfish. This may be particularly true for female
278 scale-eaters. Our study primarily measured direct displays of aggression (i.e. attacks), however,
279 females often display aggression indirectly (Rosvall 2011; Stockley and Campbell 2013). Our
280 methods of measuring aggression, therefore, may have missed increased levels of aggression in
281 female scale-eaters while still detecting them in males.

282 A second caveat is that we compared differential gene expression in an early larval
283 developmental stage, 8-10 dpf, long before sexual maturity in this species. Thus, we are not
284 comparing adult differences in gene expression between the sexes in each species. Instead, by
285 examining early larval stages our gene expression analyses provide insight into species-specific
286 differences in aggression-related genetic pathways established during an early developmental
287 timepoint. This has the advantage of defining structural developmental differences in each
288 species, rather than transient differences in transcription between adult male and female fish
289 sensitive to dominance status, reproductive state, and mood. Furthermore, we found surprising
290 congruence between our behavioral and transcriptomic data supporting the conclusions of
291 increased aggression in both San Salvador specialists due to different aggression-related genetic
292 pathways.

293

294 *New hypotheses for varying levels of aggression between pupfish species*

295 If increased levels of aggression are not associated with scale-eating, then what explains this
296 variation between species? One possibility is that selection may have directly favored increased
297 aggression in the context of dietary specialization. Aggression may be positively correlated with
298 traits associated with specialization (Genner et al. 1999; Peiman and Robinson 2010; Blowes et

299 al. 2013), suggesting that specialists should show increased levels of aggression compared to
300 generalists. Existing evidence supports this as increased levels of aggression have been
301 documented in specialist butterfly fish (*Chaetodontids*; Blowes et al. 2013), specialist striped
302 surfperch (*Embiotoca lateralis*; Holbrook and Schmitt 1992), and have even been observed in
303 game-theoretic simulation models (Chubaty et al. 2014). A second possibility is that increased
304 aggression may be associated with colonizing a novel niche. Aggression is tightly correlated with
305 boldness in a phenomenon termed the aggressiveness-boldness syndrome (Sih et al. 2004). Many
306 studies have shown that increased boldness in species such as cane toads, mosquitofish, and
307 Trinidadian killifish leads to increased dispersal into novel habitats or niches (Fraser et al. 2001;
308 Rehage and Sih 2004; Gruber et al. 2017). This relationship indicates that increased aggression
309 may be an incidental effect of selection for increased boldness and occupation of a novel niche.
310 Our data supports either scenario, as we observed increased aggression in both San Salvador
311 specialists. Neither of these hypotheses, however, explain the variation in aggression between
312 sexes.

313

314 ***Aggression and mating system***

315 Increased aggression may be favored in the context of courtship or mate competition. It is well
316 documented across multiple taxa that the sex with the higher potential reproductive rate should
317 have increased levels of aggression as they must compete more heavily for access to mates
318 (Clutton-Brock and Parker 1992; Andersson 1994; Jennions and Petrie 2007). Normally, males
319 have higher potential reproductive rates since mating is energetically cheap for them (Trivers
320 1972). Scale-eaters, and *Cyprinodon* pupfishes in general, seem to adhere to this standard as they
321 mate in a lekking system and do not provide parental care (Gumm 2012). Male scale-eaters may

322 be more aggressive to compete for mates. We found some support for this in our gene expression
323 data. Specifically, we found differential expression in the *rac2* and *ube3a* genes between scale-
324 eaters *vs* generalists. The *rac2* gene is associated with the visualization of visible light,
325 metabolism, and behavior (Elsaesser et al. 2010; Goergen et al. 2014). Mutations in the *rac2*
326 gene affect both male aggression and courtship in *Drosophila* (Goergen et al. 2014). Differential
327 expression of *ube3a* has also been linked to male aggression. Interestingly, the *ube3a* gene is
328 responsible for producing Ubiquitin-protein ligase E3A, an enzyme that aids in the degradation
329 of proteins, which may be adaptive for the protein-rich diet of scale-eaters which exhibit
330 substantial differential expression of metabolism-related genes (McGirr and Martin 2018).
331 Differential expression of *ube3a* has also been linked to variation in levels of aggression in male
332 rats (Kurian et al. 2007; Stoppel 2014). However, snail-eater and generalist pupfish also adhere
333 to a lekking mating system, although there may be quantitative differences in male competition
334 and degree of lekking among species and lake populations (CHM pers. obs.).

335 The increased female aggression of snail-eaters may also be explained by mating system.
336 Although snail-eaters have been observed mating in the lekking system, not much is known
337 about how their courting behaviors differ from generalists. It is possible that increased levels of
338 female aggression are part of the species' courting ritual. Alternatively, female aggression may
339 have increased incidentally due to selection for decreased maternal care. Our gene expression
340 data indicates that snail-eaters show increased levels of expression for the *nr2e1* gene compared
341 to generalists. This gene (nuclear receptor subfamily 2, group E, member 1) produces a receptor
342 which has been linked to abnormal brain and eye development, as well as increased aggression
343 and lack of maternal care (Young et al. 2002; Abrahams et al. 2005). It is possible that selection
344 for differing levels of maternal care in snail-eaters, compared to generalists or scale-eaters, also

345 incidentally increased levels of aggression for females. For example, one closely related
346 outgroup to *Cyprinodon*, *Jordanella floridae*, exhibits paternal care of eggs through pectoral
347 fanning (St Mary et al. 2004).

348

349 ***Increased aggression due to indirect selection***

350 Alternatively, aggression may have increased via selection on other traits. For example, melanin
351 production and aggression are physiologically linked via the melano-cortin system (Cone 2005;
352 Price et al. 2008). This association has been documented across a wide array of vertebrates and
353 suggests that selection for increased melanin pigmentation in other contexts (e.g. mate choice or
354 camouflage) may incidentally increase aggression (Mcgraw et al. 2003; Ducrest et al. 2008;
355 Price et al. 2008). Indeed, territorial male scale-eating pupfish exhibit jet black breeding
356 coloration, unique among cyprinodontiform fishes, and territorial snail-eating pupfish exhibit the
357 lightest breeding coloration of any *Cyprinodon* species (Martin and Wainwright 2013a).
358 Similarly, selection for morphological traits may also indirectly increase aggression. We found
359 differential gene expression between scale-eater vs generalist pupfish in the *gnaq* gene, which is
360 annotated for maternal care (Table 2B). *Gnaq* is one of four Gq class α -subunits and aids in
361 phospholipase C- β – receptor coupling (Offermanns et al. 1998). Silencing this gene produces
362 severe craniofacial defects in mice, especially in the mandible (Offermanns et al. 1998). *C.*
363 *desquamator* show extreme craniofacial features, including enlarged oral jaws that may be
364 beneficial for scale-eating. Thus, it is intriguing that selection for increased jaw size may have
365 indirectly selected for increased aggression in this species. Given the enlarged oral jaws of most
366 scale-eating species, this may be a general mechanism indirectly contributing to increased
367 aggression in scale-eaters depending on how frequently this genetic pathway is modified.

368 In conclusion, our data suggest that the aggression hypothesis is not a sufficient
369 explanation for the origin of scale-eating in pupfish. Instead, increased aggression in both
370 specialists indicates that aggression may function in dietary specialization or occupation of a
371 novel niche. Alternatively, increased aggression may be an incidental effect of selection on other
372 ecological or sexual traits. Specifically, the aggression-boldness syndrome, the melanocortin
373 system, selection for increased oral jaw size, or metabolic adaptations for increased intake of
374 protein could all have indirectly increased aggression. Future studies should investigate whether
375 aggression is adaptive for scale- and snail-eating in pupfish.

376

377

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385

386 **Author contributions**

387 MES and CHM conceptualized the project, MES and JAM collected data and performed
388 analyses, MES wrote the manuscript, and all authors revised the manuscript.

389

390 **Data accessibility**

391 All behavioral datasets from this study will be deposited in the Dryad Digital Repository.
392 Transcriptomic raw sequence reads are deposited at the NCBI BioProject database (Title:
393 Craniofacial divergence in Caribbean Pupfishes. Accession: PRJNA391309).

394

395

396 **References**

397

398 Abrahams, B. S., M. C. H. Kwok, E. Trinh, S. Budaghzadeh, S. M. Hossain, and E. M. Simpson.

399 2005. Pathological aggression in "fierce" mice corrected by human nuclear
400 receptor 2E1. *J. Neurosci.* 25:6263–70. Society for Neuroscience.

401 Andersson, M. B. 1994. Sexual selection. *Monogr. Behav. Ecol.* 5347:599.

402 Arnott, G., E. Beattie, and R. W. Elwood. 2016. To breathe or fight? Siamese fighting fish differ
403 when facing a real opponent or mirror image. *Behav. Processes* 129:11–17.

404 Ashburner, M., C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K.

405 Dolinski, S. S. Dwight, J. T. Eppig, M. A. Harris, D. P. Hill, L. Issel-Tarver, A. Kasarskis,
406 S. Lewis, J. C. Matese, J. E. Richardson, M. Ringwald, G. M. Rubin, and G. Sherlock.

407 2000. Gene Ontology: tool for the unification of biology. *Nat. Genet.* 25:25–29.

408 Bailey, N. W., B. Gray, and M. Zuk. 2010. Acoustic Experience Shapes Alternative Mating

409 Tactics and Reproductive Investment in Male Field Crickets. *Curr. Biol.* 20:845–849.

410 Balzarini, V., M. Taborsky, S. Wanner, F. Koch, and J. G. Frommen. 2014. Mirror, mirror on the

411 wall: the predictive value of mirror tests for measuring aggression in fish. *Behav. Ecol.*

412 *Sociobiol.* 68:871–878.

413 Barton, N., and L. Partridge. 2000. Limits to natural selection.

- 414 Blowes, S. A., M. S. Pratchett, and S. R. Connolly. 2013. Heterospecific Aggression and
415 Dominance in a Guild of Coral-Feeding Fishes: The Roles of Dietary Ecology and
416 Phylogeny. *Am. Nat.* 182:157–168.
- 417 Boileau, N., F. Cortesi, B. Egger, M. Muschick, A. Indermaur, A. Theis, H. H. Büscher, and W.
418 Salzburger. 2015. A complex mode of aggressive mimicry in a scale-eating cichlid fish.
419 *Biol. Lett.* 11:20150521. The Royal Society.
- 420 Burnham, K. P., and D. R. Anderson. 2002. *Model Selection and Multimodel Inference: A*
421 *Practical Information-Theoretic Approach* (2nd ed).
- 422 Carbon, S., A. Ireland, C. J. Mungall, S. Shu, B. Marshall, and S. Lewis. 2009. AmiGO: online
423 access to ontology and annotation data. *Bioinforma. Appl. NOTE* 25:288–28910.
- 424 Chubaty, A. M., B. O. Ma, R. W. Stein, D. R. Gillespie, L. M. Henry, C. Phelan, E. Palsson, F.
425 W. Simon, and B. D. Roitberg. 2014. On the evolution of omnivory in a community
426 context. *Ecol. Evol.* 4:251–265.
- 427 Clutton-Brock, T. H., and G. A. Parker. 1992. Potential Reproductive Rates and the Operation of
428 Sexual Selection. *Q. Rev. Biol.* 67:437–456.
- 429 Cone, R. D. 2005. Anatomy and regulation of the central melanocortin system.
- 430 Demartini, E. E., and J. A. Coyer. 1981. Cleaning and Scale-Eating in Juveniles of the Kyphosid
431 Fishes, *Hermosilla azurea* and *Girella nigricans*. *Copeia* 785–789.
- 432 Duckworth, R. A. 2006. Aggressive behaviour affects selection on morphology by influencing
433 settlement patterns in a passerine bird. *Proc. R. Soc. B Biol. Sci.* 273:1789–1795. The Royal
434 Society.
- 435 Ducrest, A. L., L. Keller, and A. Roulin. 2008. Pleiotropy in the melanocortin system, coloration
436 and behavioural syndromes.

- 437 Elsaesser, R., D. Kalra, R. Li, and C. Montell. 2010. Light-induced translocation of *Drosophila*
438 visual Arrestin2 depends on Rac2. *Proc. Natl. Acad. Sci. U. S. A.* 107:4740–5. National
439 Academy of Sciences.
- 440 Elwood, R. W., V. Stoilova, A. McDonnell, R. L. Earley, and G. Arnott. 2014. Do mirrors reflect
441 reality in agonistic encounters? A test of mutual cooperation in displays. *Anim. Behav.*
442 97:63–67. Elsevier Ltd.
- 443 Francis, R. C. 2010. Temperament in a Fish: A Longitudinal Study of the Development of
444 Individual Differences in Aggression and Social Rank in the Midas Cichlid. *Ethology*
445 86:311–325.
- 446 Fraser, D. F., J. F. Gilliam, M. J. Daley, A. N. Le, and G. T. Skalski. 2001. Explaining
447 Leptokurtic Movement Distributions: Intrapopulation Variation in Boldness and
448 Exploration. *Am. Nat.* 158:124–135.
- 449 Fryer, G., P. H. Greenwood, and E. Trewavas. 1955. Scale-eating Habits of African Cichlid
450 Fishes. *Nature* 175:1089–1090.
- 451 Fryer, G., and T. D. Iles. 1972. cichlid fishes of the great lakes of Africa. Oliver & Boyd.
- 452 Genner, M. J., G. F. Turner, and S. J. Hawkins. 1999. Resource control by territorial male cichlid
453 fish in Lake Malawi. *J. Anim. Ecol.* 68:522–529. Blackwell Science Ltd.
- 454 Goergen, P., A. Kasagiannis, H. B. Schiöth, and M. J. Williams. 2014. The *Drosophila* Small
455 GTPase Rac2 is Required for Normal Feeding and Mating Behaviour. *Behav. Genet.*
456 44:155–164.
- 457 Greenwood, P. H. 1965. Two new species of *Haplochromis* (Pisces, Cichlidae) from Lake
458 Victoria. *J. Nat. Hist. Ser.* 13 8:303–318.
- 459 Gruber, J., G. Brown, M. J. Whiting, and R. Shine. 2017. Geographic divergence in dispersal-

- 460 related behaviour in cane toads from range-front versus range-core populations in Australia.
461 Behav. Ecol. Sociobiol. 71:38. Springer Berlin Heidelberg.
- 462 Gumm, J. M. 2012. Sex recognition of female-like sneaker males in the Comanche Springs
463 pupfish, *Cyprinodon elegans*. Anim. Behav. 83:1421–1426.
- 464 Hagey, F. M., and J. E. Myroie. 1995. Pleistocene lake and lagoon deposits, San Salvador
465 Island, Bahamas. Pp. 77–90 in Special Paper 300: Terrestrial and shallow marine geology of
466 the Bahamas and Bermuda.
- 467 Holbrook, S. J., and R. J. Schmitt. 1992. Causes and Consequences of Dietary Specialization in
468 Surfperches □: Patch Choice and Intraspecific Competition. Ecology 73:402–412.
- 469 Huey, R. B., P. E. Hertz, and B. Sinervo. 2003. Behavioral Drive versus Behavioral Inertia in
470 Evolution: A Null Model Approach. Am. Nat. 161:357–366.
- 471 Hulsey, C. D., R. J. Roberts, A. S. P. Lin, R. Guldborg, and J. T. Streebman. 2008. Convergence
472 in a mechanically complex phenotype: Detecting structural adaptations for crushing in
473 cichlid fish. Evolution (N. Y). 62:1587–1599.
- 474 Hundt, P. J., S. P. Iglésias, A. S. Hoey, and A. M. Simons. 2014. A multilocus molecular
475 phylogeny of combtooth blennies (Percomorpha: Blennioidei: Blenniidae): Multiple
476 invasions of intertidal habitats. Mol. Phylogenet. Evol. 70:47–56. Academic Press.
- 477 Janovetz, J. 2005a. Functional morphology of feeding in the scale-eating specialist *Catoprion*
478 *mento*. J. Exp. Biol. 208:4757–4768.
- 479 Janovetz, J. 2005b. Functional morphology of feeding in the scale-eating specialist *Catoprion*
480 *mento*. J. Exp. Biol. 208:4757–4768.
- 481 Jennions, M. D., and M. Petrie. 2007. Variation in mate choice and mating preferences: a review
482 of causes and consequences. Biol. Rev. 72:283–327.

- 483 Kolmann, M. A., J. M. Huie, K. Evans, and A. P. Summers. 2018. Specialized specialists and the
484 narrow niche fallacy: a tale of scale-feeding fishes. , doi: 10.1098/rsos.171581.
- 485 Kurian, J. R., R. M. Forbes-Lorman, and A. P. Auger. 2007. Sex difference in *Mecp2* expression
486 during a critical period of rat brain development. *Epigenetics* 2:173–178.
- 487 Lencer, E. S., W. C. Warren, R. Harrison, and A. R. McCune. 2017. The *Cyprinodon variegatus*
488 genome reveals gene expression changes underlying differences in skull morphology among
489 closely related species. *BMC Genomics* 18:424.
- 490 Liao, Y., G. K. Smyth, and W. Shi. 2013. The Subread aligner: Fast, accurate and scalable read
491 mapping by seed-and-vote. *Nucleic Acids Res.* 41.
- 492 Liem, K. F. 1973. Evolutionary Strategies and Morphological Innovations: Cichlid Pharyngeal
493 Jaws. *Syst. Zool.* 22:425.
- 494 Losey, G. S. 1979. Fish cleaning symbiosis: proximate causes of host behavior. *Anim. Behav.*
495 27:669–685.
- 496 Losey, G. S. 1972. The ecological importance of cleaning symbiosis. *Copeia* 820–833.
- 497 Losos, J. B., T. W. Schoener, R. B. Langerhans, and D. A. Spiller. 2006. Rapid temporal reversal
498 in predator-driven natural selection. *Science* (80-.). 314:1111.
- 499 Losos, J. B., T. W. Schoener, and D. A. Spiller. 2004. Predator-induced behaviour shifts and
500 natural selection in field-experimental lizard populations. *Nature* 432:505–508. Nature
501 Publishing Group.
- 502 Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion
503 for RNA-seq data with DESeq2. *Genome Biol.* 15:550. BioMed Central.
- 504 Martin, C. H. 2016. The cryptic origins of evolutionary novelty: 1 , 000-fold- faster trophic
505 diversification rates without increased ecological opportunity or hybrid swarm. 1–51.

- 506 Martin, C. H., and L. C. Feinstein. 2014. Novel trophic niches drive variable progress towards
507 ecological speciation within an adaptive radiation of pupfishes. *Mol. Ecol.* 23:1846–1862.
- 508 Martin, C. H., and P. C. Wainwright. 2013a. A Remarkable Species Flock of Cyprinodon
509 Pupfishes Endemic to San Salvador Island, Bahamas. *Bull. Peabody Museum Nat. Hist.*
510 54:231–241.
- 511 Martin, C. H., and P. C. Wainwright. 2013b. On the Measurement of Ecological Novelty: Scale-
512 Eating Pupfish Are Separated by 168 my from Other Scale-Eating Fishes. *PLoS One*
513 8:e71164.
- 514 Martin, C. H., and P. C. Wainwright. 2011. Trophic novelty is linked to exceptional rates of
515 morphological diversification in two adaptive radiations of cyprinodon pupfish. *Evolution*
516 (N. Y). 65:2197–2212.
- 517 McGirr, J. A., and C. H. Martin. 2018. Parallel evolution of gene expression between trophic
518 specialists despite divergent genotypes and morphologies. *Evol. Lett.*, doi: 10.1002/evl3.41.
- 519 McGraw, K. J., J. Dale, and E. A. Mackillop. 2003. Social Environment during Molt and the
520 Expression of Melanin-Based Plumage Pigmentation in Male House Sparrows (*Passer*
521 *domesticus*). *Source Behav. Ecol. Sociobiol. Behav Ecol Sociobiol* 53.
- 522 Nicolakakis, N., and L. Lefebvre. 2000. Forebrain size and innovation rate in European birds:
523 Feeding, nesting and confounding variables. *Behaviour* 137:1415–1429. Brill.
- 524 Novakowski, G. C., R. Fugi, and N. S. Hahn. 2004. Diet and dental development of three species
525 of *Roeboides* (Characiformes: Characidae). *Neotrop. Ichthyol.* 2:157–162. Sociedade
526 Brasileira de Ictiologia.
- 527 Nshombo, M., Y. Yanagisawa, and M. Nagoshi. 1985. Scale-Eating in *Perissodus microlepis*
528 (*Cichlidae*) and of Its Food Habits with Growth. *Japanese J. Ichthyol.* 32:66–73.

- 529 Offermanns, S., L. P. Zhao, A. Gohla, I. Sarosi, M. I. Simon, and T. M. Wilkie. 1998. Embryonic
530 cardiomyocyte hypoplasia and craniofacial defects in $G\alpha(q)/G\alpha11$ -mutant mice. *EMBO J.*
531 17:4304–4312.
- 532 Overington, S. E., J. Morand-Ferron, N. J. Boogert, and L. Lefebvre. 2009. Technical
533 innovations drive the relationship between innovativeness and residual brain size in birds.
534 *Anim. Behav.* 78:1001–1010.
- 535 Peiman, K. S., and B. W. Robinson. 2010. Ecology and Evolution of Resource-Related
536 Heterospecific Aggression. *Q. Rev. Biol.* 85:133–158. The University of Chicago Press .
- 537 Price, A., C. Weadick, J. Shim, and F. Helen Rodd. 2008. Pigments, Patterns, and Fish Behavior.
538 *Zebrafish* 5:297–307.
- 539 R Development Core Team. 2016. R: A Language and Environment for Statistical Computing. R
540 Found. Stat. Comput. Vienna Austria 0:{ISBN} 3-900051-07-0.
- 541 Rehage, J. S., and A. Sih. 2004. Dispersal behavior, boldness, and the link to invasiveness: A
542 comparison of four gambusia species. *Biol. Invasions* 6:379–391.
- 543 Ribbink, A. J., B. A. Marsh, A. C. Marsh, A. C. Ribbink, and B. J. Sharp. 1983. A preliminary
544 survey of the cichlid fishes of rocky habitats in Lake Malawi. *South African J. Zool.*
545 18:149–310. Routledge.
- 546 Rich, J. T., J. G. Neely, R. C. Paniello, C. C. J. Voelker, B. Nussenbaum, and E. W. Wang. 2010.
547 A practical guide to understanding Kaplan-Meier curves. *Otolaryngol. Neck Surg.* 143:331–
548 336.
- 549 Richards, E., and C. Martin. 2017. Adaptive introgression from distant Caribbean islands
550 contributed to the diversification of a microendemic radiation of trophic specialist
551 pupfishes. *PLOS Genet.*, doi: 10.1101/115055.

- 552 Rosvall, K. A. 2011. Intrasexual competition in females: Evidence for sexual selection? *Behav.*
553 *Ecol.* 22:1131–1140.
- 554 Sazima, I. 1983. Scale-eating in characoids and other fishes. *Environ. Biol. Fishes* 9:87–101.
- 555 Sih, A., A. M. Bell, J. C. Johnson, and R. E. Ziemba. 2004. Behavioral Syndromes: An
556 Integrative Overview. *Q. Rev. Biol.* 79:241–277.
- 557 Sol, D., and L. Lefebvre. 2000. Behavioural flexibility predicts invasion success in birds
558 introduced to New Zealand. *Oikos* 90:599–605.
- 559 St Mary, C. M., E. Gordon, and R. E. Hale. 2004. Environmental effects on egg development and
560 hatching success in *Jordanella floridae*, a species with parental care. *J. Fish Biol.* 65:760–
561 768. Blackwell Science Ltd.
- 562 Stockley, P., and A. Campbell. 2013. Female competition and aggression: interdisciplinary
563 perspectives. *Philos. Trans. R. Soc. B Biol. Sci.* 368:20130073–20130073.
- 564 Stoppel, D. C. 2014. Social Behavior and Gene Expression Disturbances in Mouse Models of
565 Angelman Syndrome and Idic15 Autism. Harvard University.
- 566 The Gene Ontology Consortium. 2017. Expansion of the Gene Ontology knowledgebase and
567 resources. *Nucleic Acids Res.* 45:D331–D338.
- 568 Therneau, T. 2012. A Package for Survival Analysis in S. R package version.
- 569 Therneau, T. M. 2015. A Package for Survival Analysis in S.
- 570 Tinghitella, R. M., and M. Zuk. 2009. Asymmetric mating preferences accommodated the rapid
571 evolutionary loss of a sexual signal. *Evolution (N. Y.)*. 63:2087–2098. Wiley/Blackwell.
- 572 Trewavas, E. 1947. An Example of “Mimicry” in Fishes. *Nature* 160:120–120.
- 573 Trivers, R. L. 1972. Parental investment and sexual selection.
- 574 Vøllestad, L. A., and T. P. Quinn. 2003. Trade-off between growth rate and aggression in

575 juvenile coho salmon, *Oncorhynchus kisutch*. *Anim. Behav.* 66:561–568.

576 Young, K. A., M. L. Berry, C. L. Mahaffey, J. R. Saionz, N. L. Hawes, B. Chang, Q. Y. Zheng,
577 R. S. Smith, R. T. Bronson, R. J. Nelson, and E. M. Simpson. 2002. Fierce: A new mouse
578 deletion of *Nr2e1*; violent behaviour and ocular abnormalities are background-dependent.
579 *Behav. Brain Res.* 132:145–158.

580 Zuk, M., E. Bastiaans, T. Langkilde, and E. Swanger. 2014. The role of behaviour in the
581 establishment of novel traits. *Anim. Behav.* 92:333–344. Elsevier Ltd.

582 Zuk, M., J. T. Rotenberry, and R. M. Tinghitella. 2006. Silent night: adaptive disappearance of a
583 sexual signal in a parasitized population of field crickets. *Biol. Lett.* 2:521–524. The Royal
584 Society.

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597 **Table 1.** Results of likelihood ratio test for: a) the latency to approach mirror image (mixed-
 598 effect Cox proportional hazards model); b) the latency to attack mirror image (mixed-effect Cox
 599 proportional hazards model); and c) the total number of attacks (generalized linear mixed
 600 model). Significant results are indicated in bold.

model	response variable	predictor	df	χ^2	P
a) proportional hazards	Latency to Attack Mirror Image	Species	3	9.78	0.02
		Sex	1	0.3	0.58
		Species:Sex	3	7.4	0.06
b) proportional hazards	Latency to Attack Mirror Image	Species	3	11.21	0.01
		Sex	1	4.29	0.04
		log SL	1	4.8	0.03
		Species:Sex	3	11.12	0.01
c) GLMM	Total Number of Attacks	Species	3	7.08	0.07
		Sex	1	7.58	0.01
		Species:Sex	3	20.91	0.0001

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608 **Table 2.** List of all differentially expressed genes in aggression-related pathways between *a)*
 609 *sail-eaters vs generalists* and *b) scale-eaters vs generalists*. 3-6 individuals of each species
 610 sampled at 8-10 dpf.

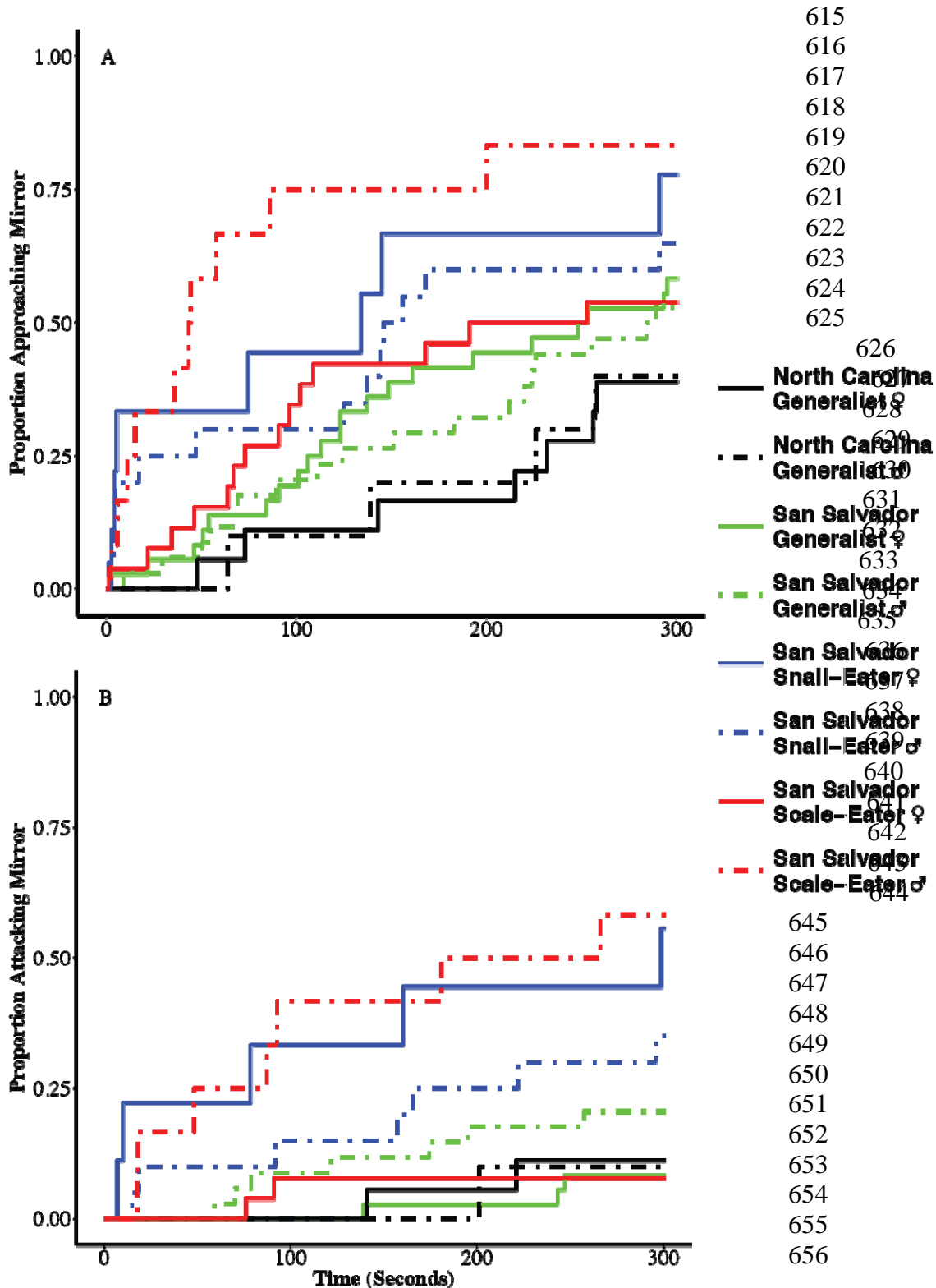
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species comparison	gene	log fold change	GO pathway
<i>a) Snail-Eater vs Generalist</i>			
	<i>nr2e1</i>	1.44	Aggression
	<i>heyl</i>	-0.83	Androgen
	<i>rhoa</i>	-0.2	Androgen
	<i>smarca4</i>	0.53	Androgen
	<i>crebrf</i>	-0.7	Maternal Care
<i>b) Scale-Eater vs Generalist</i>			
	<i>crhbp</i>	-1.14	Aggression/Maternal Care/ Maternal Aggression Aggression/Inter-Male
	<i>fkbp14</i>	1.24	Aggression
	<i>penk</i>	1.12	Aggression
	<i>rac2</i>	-0.9	Aggression
	<i>arid1a</i>	0.46	Androgen
	<i>ddx5</i>	0.5	Androgen
	<i>fkbp4</i>	0.62	Androgen
	<i>foxp1</i>	-0.91	Androgen
	<i>heyl</i>	-1.03	Androgen
	<i>igf1</i>	1.22	Androgen
	<i>med12</i>	-0.98	Androgen
	<i>med14</i>	0.74	Androgen
	<i>med16</i>	1.24	Androgen
	<i>rhoa</i>	-0.46	Androgen
	<i>smarca4</i>	0.72	Androgen
	<i>taf1</i>	0.4	Androgen
	<i>ube3a</i>	-0.96	Androgen
	<i>crebrf</i>	-1.41	Maternal Care

<i>dbh</i>	2.02	Maternal Care
<i>gnaq</i>	-1.53	Maternal Care
<i>cul5</i>	0.58	Vasopressin

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613 **Figure 1.** Time-to-event curves for *a*) the latency to approach mirror image (Cox proportional
 614 hazards model) and *b*) the latency to attack mirror image (Cox proportional hazards model).



657 **Figure 2.** Box plots illustrating the total number of attacks performed by each species and sex (n
658 = 165 total individuals tested). Squares represent the total number of attacks for individual
659 females, while triangles represent the total number of attacks for individual males.

