Genetic integration of the stress response

T.M. Houslay*[1,2], R.L. Earley[3], S.J. White[1,4], W. Lammers[1], A.J. Grimmer[1], L.M. Travers[1,5], E.L. Johnson[3,6], A.J. Young[1] and A.J. Wilson[1]

* Corresponding author: houslay@gmail.com

[1] Centre for Ecology and Conservation, University of Exeter (Penryn Campus), Cornwall, TR10 9FE, UK.

[2] Current address: Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK.

[3] Department of Biological Sciences, University of Alabama, Biology Building 211-213, Box 870344, Tuscaloosa, AL 35487, USA.

[4] Current address: Department of Ecology and Genetics, Center for Evolutionary Biology, Uppsala University, Uppsala, Sweden.

[5] Current address: School of Biological Sciences, University of East Anglia, Norwich, Norfolk, NR4 7TJ, UK.

[6] Current address: Southern Resesarch, 2000 Ninth Avenue South, Birmingham, AL 35205, USA.

Abstract

The stress response is a product of selection for an integrated suite of behavioural and physiological traits that facilitate coping with acute stressors. As such, genetic variation in the stress response is expected to reflect genetic variation in, and genetic covariation among, its behavioural and physiological components. Such genetic integration among stress response components has yet to be formally demonstrated using multivariate quantitative genetics, despite its profound implications for optimising human and animal health and understanding the responses of wild populations to natural and anthropogenic stressors. Here we use a laboratory population of wild-derived Trinidadian guppies (*Poecilia reticulata*) to determine levels of genetic variation in behavioural and physiological components of the acute stress response, and to establish whether such variation is integrated into a single major axis of genetic (co)variation. First, using a novel method to characterise behavioural components of the stress response from a widely used Open Field Trial paradigm, we find genetic variation in, and genetic covariation among, behavioural parameters that characterise movement patterns under stress. Second, we find a strong genetic component to variation in both the endocrine response to a confinement stressor and the rate at which this response attenuates following repeated exposures to the stressor. Finally, we show that these behavioural and physiological components of the stress response align on a major axis of genetic (co)variation as predicted, suggesting correlational selection in the past has led to genetic integration. This genetic integration could either facilitate or constrain future responses to selection, depending upon the extent to which the direction of selection aligns with this major axis of genetic covariation among stress response traits. This genetic integration also suggests that, while stress-related disease typically arises from physiological stress responses, selection on the genetically correlated behavioural responses could offer a viable non-invasive route to the genetic improvement of health and welfare in captive animal populations.

Introduction

Stress responses are comprised of physiological and behavioural traits that enable individuals to cope with adverse environmental conditions (Romero 2004; Øverli *et al.* 2007; McEwen & Wingfield 2010). Although exposure to such stressors tends to negatively impact organismal performance, populations harbour considerable variation in stress response traits (Korte *et al.* 2005; Koolhaas *et al.* 2007, 2010), such that some individuals may be better at coping with adversity than others. This variation has immediate implications for understanding and predicting susceptibility to stress-related disease in humans and animals (Barton & Iwama 1991; Koolhaas *et al.* 1999; McEwen & Wingfield 2003; Romero 2004; Koolhaas 2008), and for improving animal welfare (e.g., in livestock production; Broom & Johnson 1993; von Borell 1995; Möstl & Palme 2002). Among-individual differences in stress responses are likely underpinned by genetic variation (Koolhaas *et al.* 1999, 2007), enabling a response to selection. This is vital for artificial selection regimes in non-wild populations (Mignon-Grasteau *et al.* 2005). In addition, differences in stress response are expected to be linked directly to fitness variation (Wingfield 2003; Koolhaas 2008), and should therefore be an important target of natural selection. The evolutionary dynamics of the stress response will therefore impact the resilience of wild populations to environmental stressors, whether natural (e.g. predation risk; Clinchy, Sheriff & Zanette 2013) or anthropogenic (Tarlow & Blumstein 2007; Busch & Hayward 2009; Angelier & Wingfield 2012).

Selection – whether natural or artificial – does not act on single traits in isolation, but rather on multivariate phenotypes (Lande & Arnold 1983; Blows 2007). Glucocorticoid (GC) levels are often used as univariate proxies for the physiological stress response (McEwen & Wingfield 2003; Korte *et al.* 2005). However, when coping with acute stressors, an individual's first line of defence against a sudden threat is typically a behavioural one (Moberg 2000). For instance, the well-known 'fight-or-flight' response occurs rapidly, with subsequent GC release then helping to mediate

physiological (and further behavioural) responses to acute stressors (Wingfield *et al.* 1998; Wingfield & Kitaysky 2002). The mechanisms that link GCs with the expression of numerous traits are well established (Sopinka *et al.* 2015; Crossin *et al.* 2016), but if past selection has favoured particular combinations of trait values, then genetic integration of behavioural and physiological stress response traits should also be apparent (McGlothlin & Ketterson 2008; Ketterson, Atwell & McGlothlin 2009; Cox, McGlothlin & Bonier 2016). The premise that the physiological and behavioural components of the stress response will be tightly integrated is pervasive in the literature, as encapsulated in the 'stress coping style' model that predicts an axis of variation from 'reactive' (behavioural immobility coupled with low endocrine reactivity) to 'proactive' (behavioural avoidance and high endocrine reactivity) styles of stress response (Koolhaas *et al.* 1999; Øverli *et al.* 2007). However, with respect to genetic integration, there is a relative paucity of supporting empirical work.

To date, the best evidence for genetic integration of behavioural and physiological stress response traits comes from artificial selection studies on domesticated animal populations. In rainbow trout (*Oncorhynchus mykiss*), successful selection on stress-induced plasma cortisol levels revealed heritable variation in stress physiology (Pottinger and Carrick, 1999), while subsequent comparison of high-responding and low-responding selection lines identified correlated changes in stress-related behaviour (Øverli *et al.* 2001, 2002; Øverli, Winberg & Pottinger 2005; Pottinger & Carrick 2001; Schjolden *et al.* 2005; Ruiz-Gomez *et al.* 2011). However, some results lack consistency and/or appear highly context-dependent: low-responding lines showed more pronounced metabolic stress response under confinement relative to high-responding (Trenzado, Carrick & Pottinger 2003); boldness was not linked to stress-responsiveness under standardised testing procedures (Thomson *et al.* 2011); and behavioural components only of stress coping style were modified by an environmental change (Ruiz-Gomez *et al.* 2008). Artificial selection on stress-related GC levels has also

103 produced correlated stress-related behavioural responses (and/or vice versa) in
104 Japanese quail (*Coturnix japonica*; Hazard et al., 2008b; Hazard et al., 2008a; Jones et al.,
105 1994) and house mice (*Mus musculus domesticus*; Veenema et al., 2003a; Veenema et al.,
106 2003b). In the great tit (*Parus major)*, there is evidence for cortisol levels evolving in
107 response to selection on behavioural 'personality' traits in the great tit (Carere *et al.*
108 2003; Stöwe *et al.* 2010; Baugh *et al.* 2012), and this association has also been shown at
109 the phenotypic level in the wild (Baugh *et al.* 2013).
110 While selection studies provide one useful strategy to test for genetic
111 integration, an alternative approach is to directly estimate the genetic covariance matrix
112 (**G**) among stress-related traits in a population. Selection over long time periods for
113 particular trait combinations is expected to result in tight correlation structure among
114 those traits being evident in G (Ketterson *et al.* 2009). **G** has been widely used to
115 scrutinise genetic integration among sets of traits describing behaviour (Oswald, Singer
116 & Robison 2013), male attractiveness to females (Blows, Chenoweth & Hine 2004; Hine,
117 Chenoweth & Blows 2004; Hunt *et al.* 2007), and (most commonly) morphology
118 (following, e.g., Cheverud 1982). Here, we use a lab-based pedigreed population of wild-
119 derived Trinidadian guppies (*Poecilia reticulata*) to estimate the genetic covariance
120 matrix **G** for behavioural and physiological components of the acute stress response, in
121 order to determine whether (i) these components are genetically integrated into a single
122 major axis of genetic (co)variation, and (ii) the make-up and orientation of this axis
123 conforms with the expectations of the stress coping style model (Koolhaas *et al.* 2010;
124 Boulton *et al.* 2015).
125 The population of guppies studied here is derived from wild individuals sampled
126 from the lower Aripo River, Trinidad in 2008. The colony has subsequently been
127 maintained at high population size with no deliberate inbreeding or selection.
128 Introducing a lone fish to a novel 'open field trial' (OFT) environment is considered a
129 mild stressor for this shoaling species, offering an opportunity to utilise behavioural

130 responses to this treatment as a proxy for their behavioural stress response (Houslay *et*
131 *al.* 2018). Previous work has demonstrated significant additive genetic (co)variance in
132 risk-taking and exploratory behavioural responses in this context (White & Wilson
133 2018; White, Houslay & Wilson 2018), behaviours that also exhibit strong plastic
134 responses to other acute stressors, including simulated predation events by birds and
135 predatory fish (Houslay *et al.* 2018). Using a non-invasive waterborne hormone
136 sampling method, we have also shown that individuals differ in their GC (specifically
137 free circulating cortisol) response to an acute stressor (handling and short term
138 confinement associated with the sampling itself; Houslay *et al.* 2019) and that, on
139 average, this GC response decreases with repeated exposure to the sampling event
140 stressor. However, nothing is known about the genetic basis of variation in these
141 physiological components of the stress response, or of their integration with genetic
142 variation in stress-related behaviours.

143 Here we test for evidence of genetic integration of the behavioural and
144 endocrine components of the stress response. First, we characterise multiple
145 behavioural components of the stress response, by developing a novel phenotyping
146 approach for OFTs, to better distinguish 'flight' behaviour from potentially confounding
147 variation in activity and exploration traits, and by complementing OFTs with additional
148 'emergence trials' (ET) and 'shoaling trials' (ST). Second, we characterise two key
149 physiological components of the stress response by assaying GC levels following
150 exposure to the first and third handling stress treatments in a stress habituation
151 paradigm (see methods). All behavioural and physiological traits were assayed multiple
152 times per individual using fish within a known genetic pedigree structure, enabling us to
153 partition phenotypic (co)variation in the suite of focal traits into its additive genetic,
154 permanent environment (here, among-individual), housing group, and residual
155 components. We predict that there will be a heritable (genetic) component to each trait
156 measured and that the **G** matrix (the pattern of genetic covariation among traits) will

157 contain significant among-trait correlation structure. More specifically, based on the
158 'stress coping style' model (Koolhaas *et al.* 1999) we predict genetic integration of
159 behavioural and physiological traits in **G** such that a major axis of genetic variation is
160 present: at one end, genotypes will be predisposed to a low-activity, exploratory
161 phenotype with lower GC levels, and at the other a 'flighty' phenotype with high GC
162 levels.
163
164 **Results**
165 Our data set includes behavioural observations from 7,637 trials (3,812 OFTs, 1,548 ETs
166 and 1,039 STs) and physiological measures from 1,238 waterborne assays of cortisol
167 (CORT hereafter). These phenotypic data were collected from 1,518 individual fish
168 within a genetic pedigree structure comprised of maternal full-sibships nested within
169 paternal half-sibships (with some connectedness across 5 'generations', as offspring
170 from one round of breeding were used, where available, as parents in the next). The
171 number of individuals phenotyped (OFTs = 1,487, ETs = 806, STs = 532) and the mean
172 number of observations per fish (OFTs = 2.6, ETs = 1.9, STs = 2.0) varied across the
173 behavioural data types. For the 629 fish that were assayed for cortisol (almost all from
174 the final generation), the handling and confinement stressor applied in this assay was
175 performed 3 times (at 48h intervals) for all fish, with the magnitude of the GC response
176 to the stressor being assessed only for the first and last stressor exposures
177 (subsequently $Cortisol_1$ and $Cortisol_3$). Full details of husbandry, phenotyping and
178 analysis are provided in Materials and Methods.
179
180 **Genetic variance in behavioural components of the stress response**
181 We extracted behavioural data from videos of each trial type using video tracking of fish
182 (as described in White, Kells & Wilson 2016; Houslay *et al.* 2018). Time to emerge from
183 the shelter ('*emergence time*') was extracted from ETs and $\log_n$ transformed for analysis,

while *shoaling tendency* was calculated from STs as the time spent in the third of the tank closest to a same-sex shoal (which was visible but physically separated) minus the time spent in the third of the tank farthest from the shoal. Note that both trials are considered to assay the behavioural stress response because the fish are subject to capture and solitary transfer into novel, brightly lit, arenas away from their home tanks and familiar tank mates.

The OFT was used to quantify three traits over a 4m 30s period following release into the tank and 30s acclimation: *track length* (distance swum), *area covered* (as a proportion of the arena floor area), and *time in the middle* (i.e., in the central area of the open field arena away from the tank walls, which is assumed to be perceived as riskier). Though these three OFT traits have previously been found to be repeatable and heritable in this population (White *et al.* 2016, 2018; Houslay *et al.* 2018; White & Wilson 2018), there is not a strong positive association between *track length* and *area covered* (Fig 1A). This is interesting because, if fish moved randomly in the arena, then we would expect *area covered* to increase asymptotically to 100% as *track length* increases. A plausible explanation is that similar *track lengths* can and do arise from two distinct stress-response scenarios: a (putatively) less stressed fish could be exhibiting an exploratory response, leading to relatively high *track length* and *area covered* (fish 1 in Fig 1B), while a (putatively) more stressed fish could be exhibiting a flight response, which typically manifests as rapid swimming around the walls of the arena, leading to a similarly high *track length* but lower *area covered* (Fish 4 in Fig 1B).

To quantitatively discriminate these hypothetical exploratory and flight responses we derived a new trait, '*relative area covered*', using a simple simulation procedure (see Methods). This allowed us to predict the expected *area covered* for a given *track length* under the null model of a 'random swim' within the arena (Fig 1C). *Relative area covered* is then calculated as *observed area covered – expected area covered* (Fig 1D). Thus, higher values of *track length* may reflect an exploratory response when

211 coupled to high values of *relative area covered* and *time in the middle,* but a 'flight'
212 response when coupled to low values of *relative area covered* and *time in the middle*.
213 We used univariate pedigree-based 'animal models' (Kruuk 2004; Kruuk &
214 Hadfield 2007; Wilson *et al.* 2010) to test each trait for additive genetic variation while
215 controlling for additional random effects of social housing group and non-genetic
216 among-individual variance (as well as several fixed effects; see methods for full details).
217 These models confirm the presence of significant additive genetic variation for the
218 derived *relative area covered* trait, as well as for *track length* and *time in the middle* (
219 consistent with previous findings; White & Wilson 2018; White *et al.* 2018) and
220 *emergence time* (Table 1). The heritabilities of these behavioural traits (adjusted for
221 fixed effects; see methods) are low to medium (Table 1) and within the range expected
222 for behavioural traits (Stirling, Réale & Roff 2002). We detected no significant additive
223 genetic variance for *shoaling tendency* (Table 1), despite there being repeatable
224 differences among individuals; R = 0.19 ± 0.04; $\chi^2_{0,1}$ = 20.01, P < 0.001).
225
226 **Genetic variation in physiological components of the stress response**
227 Using a series of nested bivariate animal models, we tested for the presence of additive
228 genetic variation in cortisol levels ($\log_n$ transformed) following stressor exposure
229 (handling and confinement), and for genotype-by-environment interaction (GxE) in
230 cortisol levels across the two samples assayed per fish (i.e. following stressors 1 and 3).
231 In this context, the environment (E) is the time point at which the fish was exposed to
232 the stressor. Any GxE present can therefore be interpreted as genetic variance for
233 habituation to the stressor, given that the average cortisol level was lower following
234 exposure to the third stressor than the first ($\log_n$ transformed mean ± SE; $Cortisol_1$ =
235 8.50 ± 0.05, $Cortisol_3$ = 8.05 ± 0.06, Wald $F_{1,12.9}$ = 120.5, P < 0.001). We first modelled
236 $Cortisol_1$ and $Cortisol_3$ as distinct response variables in a bivariate framework but
237 assuming no GxE (such that we assume $V_{A\text{-}Cortisol1} = V_{A\text{-}Cortisol3}$ and the cross context

238 additive genetic correlation $r_{A\text{-}Cortisol1,Cortisol3} = 1$). This model revealed a significant
239 additive genetic component to variation among individuals in their cortisol levels
240 following stressor exposure ($\chi^2_{0,1} = 6.58$, P = 0.005).

241 Expanding the model to allow GxE (by freely estimating separate genetic
242 variances for $Cortisol_1$ and $Cortisol_3$ as well as the cross-context genetic correlation)
243 provides a significantly better fit to the data ($\chi^2_2 = 9.65$, P = 0.008). This means that
244 GxE is present, or (equivalently) that the magnitude of change in cortisol levels from the
245 first to the third sampling (i.e., the degree of habituation to the stressor) varies among
246 genotypes. The cross-context genetic correlation is strongly positive ($r_{A\text{-}Cortisol1,Cortisol3} \pm SE$
247 $= 0.74 \pm 0.25$), while genetic variance in cortisol levels is greater following exposure to
248 the first stressor than the third ($V_{A\text{-}Cortisol1} = 0.076 \pm 0.028$, $V_{A\text{-}Cortisol3} = 0.047 \pm 0.029$).
249 Both findings are apparent when visualising model predictions (Figure 2): while there is
250 genetic variation in habituation rates (indicated by variation in the slopes of genotypic
251 reaction norms; Figure 2), there is still positive cross-context genetic correlation
252 (indicated by little change in the rank order of individuals across the two contexts;
253 Figure 2). The reduction in genetic variance for cortisol levels between the first and
254 third stressor exposure arises because genotypes with higher than average cortisol
255 levels following the first exposure habituate more rapidly following subsequent
256 exposures (reflected in the model as GxE). In this model we also find that variance in
257 cortisol explained by average differences among groups is similar across contexts
258 ($V_{Group\text{-}Cortisol1} = 0.034 \pm 0.013$, $V_{Group\text{-}Cortisol3} = 0.045 \pm 0.016$), but that residual variance is
259 greater following exposure to the third stressor than the first ($V_{R\text{-}Cortisol1} = 0.166 \pm 0.021$,
260 $V_{R\text{-}Cortisol3} = 0.229 \pm 0.025$). This means that there is no change in variance explained
261 among groups across contexts, but variance within groups is greater after the third
262 stressor relative to the first. Overall, this pattern creates a higher adjusted heritability
263 (the ratio of additive genetic variance to the sum of additive genetic, group and residual

variances after adjusting for fixed effects) for cortisol levels following the first stressor exposure than the third ($h^2_{Cortisol1}$= 0.275 ± 0.093, $h^2_{Cortisol3}$= 0.146 ± 0.088).

Testing for genetic integration and identifying the major axis of genetic variance

Lastly, to test for the hypothesised genetic integration between behavioural and physiological components of the stress response, we built a multivariate animal model to estimate patterns of genetic covariances among these traits (**G**). We excluded *shoaling tendency* given the absence of detectable genetic variance in the univariate model of this trait. We also elected to treat cortisol as a single trait (allowing for a fixed effect of stressor exposure number (1 vs 3) on the mean). Although the analysis described above shows evidence of GxE, the strong positive genetic correlation between $Cortisol_1$ and $Cortisol_3$ justifies combining into a single trait here in order to maximise power to detect overall genetic integration of behaviour and GC expression.

Our final model contained 5 response traits: *relative area covered*, *time in the middle* and *track length*, (log-transformed) *emergence time*, and (log-transformed) *Cortisol* (treated as two repeats of a single trait; see above). Following transformation (where applicable), we standardised all traits to standard deviation units, to assist multivariate model fitting and to prevent eigenvectors (see below) of **G** being dominated by traits with higher variance in observed units. To simplify interpretation of the estimated correlation (and covariance) structure in **G** we multiplied log-transformed *emergence time* by -1, resulting in higher values for all behavioural traits indicating individuals that mounted 'bolder' behavioural responses to stressors. Our estimate of **G** (Table 2) contains significant genetic covariance structure overall (Likelihood Ratio Test of the full model vs. a model in which we did not allow for additive genetic covariance: χ^2_{10} = 36.79, P < 0.001). **G** also contains a number of strong pairwise genetic correlations,

291 both negative and positive, that are statistically significant (based on the bootstrapped
292 95% confidence intervals not crossing zero). Log-transformed *Cortisol* is correlated
293 positively with *track length* at the genetic level, and negatively with *relative area covered*
294 and *time in the middle* (though not all pairwise correlations are individually significant;
295 Figure 3). *Relative area covered* shows significant genetic correlations with both of the
296 other OFT traits: positive with *time in the middle*, and negative with *track length*.
297 Eigen decomposition of **G** provides a more holistic view of the genetic
298 covariance structure and the level of integration among traits. Here the major axis (first
299 principal component, PC1) explains 57.8% of the genetic variance in multivariate
300 phenotype (PC2 = 25.7%; PC3 = 9.3%; PC4 = 5.6%; PC5 = 1.6%). All of the traits except
301 *emergence time* load significantly on this axis (Figure 4). *Relative area covered* and *time*
302 *in the middle* load in one direction, while *track length* and *Cortisol* load in the other
303 direction. This suggests that genotypes at one end of this major axis of genetic
304 (co)variation have more 'exploratory' behavioural responses to stress (i.e., swim shorter
305 distances, spend more time in the central area of the tank, and cover a high area relative
306 to their distance travelled in OFTs) and have weaker physiological responses to stress
307 (i.e., produce lower cortisol levels in response to the stressor). While genotypes at the
308 other end of this axis have more of a 'flight' type of behavioural response to stress (i.e.,
309 swim longer distances, stay closer to the edges of arena, and cover a small area relative
310 to their distance travelled in OFTs) and have stronger physiological responses to stress
311 (i.e., produce higher cortisol levels in response to the stressor).
312

Discussion

In this study we sought to determine whether – and to what extent – there exists genetic variation for, and integration between, behavioural and physiological (endocrine) components of stress response. Our results provide three main novel insights. First, we find that genetic variation underpins individual differences in not only stress-related behaviours (including but not limited to relative area) but also stress physiology. Second, we find genetic covariance structure among these stress response traits – including between behavioural and physiological components. Thirdly, the major axis of genetic covariation conforms to that hypothesised in the stress coping styles model. Overall, by estimating genetic correlation structure among traits we find at least some support for the hypothesis of evolutionary integration between behavioural and endocrine components of the stress response.

The open field trial (OFT) paradigm used here is widely applied to study 'shy-bold' type personality variation in fishes (Toms, Echevarria & Jouandot 2010) and other vertebrates (Carter *et al.* 2013; Perals *et al.* 2017). Furthermore, behavioural differences expected to occur along a 'shy – bold' personality axis align closely with predictions from the 'reactive – proactive' model of stress coping (see Boulton *et al.* 2015). However, whether actual behavioural variation matches expectations of these verbal models is an empirical question. In this guppy population, using the directly observed OFT traits, it does not (White *et al.* 2016; Houslay *et al.* 2018). By identifying the 'null' expected relationship between track length and area covered under a random swim we greatly increase biological interpretability. Specifically, using '*relative area covered*' we are able to demonstrate an axis of repeatable and heritable variation that spans from methodical exploration of the arena by (putatively) unstressed fish through to a 'flight' type response to being placed in the OFT arena. Individuals – and genotypes – predisposed to low relative area also have longer track lengths and spend less time in

340 the middle (i.e., are more thigmotaxic). Given the wide use of OFTs in biomedical
341 research (e.g., Rex *et al.* 1998; von Horsten, Karl & Pabst 2003) as well as in animal
342 behaviour, our approach may have broad applicability for discriminating between
343 exploration and stress/anxiety-related behaviours.

344 Among the non-OFT behaviours assayed, we find less evidence for genetic
345 variation and/or integration. Tendency to shoal varies among individuals but is not
346 detectably heritable. Though not generally considered a stress-response trait *per se*,
347 shoaling is an anti-predator behaviour in guppies (Herbert-Read *et al.* 2017). We had
348 therefore predicted that heightened perception of risk in the open field might be
349 associated with increased shoaling tendency. The absence of detectable genetic variance
350 meant that we could not test this prediction in **G**, though there was no evidence for such
351 a pattern at the level of individual phenotype.

352 By contrast, emergence time is heritable but not significantly correlated with
353 OFT traits in **G** (nor among individuals). While longer emergence times are typically
354 interpreted as reflecting a fear of the open arena (i.e., lack of 'boldness'; see Burns
355 2008), it is notable that the qualitative pattern in **G** in our study actually ran counter to
356 this expectation. Genotypes predisposed to longer emergence times are associated
357 (albeit non-significantly) with higher relative area, lower track length, and more time in
358 the middle during OFT trials. That is, longer emergence times are associated with the
359 bolder, exploratory and (putatively) less stressed end of the stress response continuum.
360 This may indicate that at least some individuals do not perceive the shelter area as safer
361 than the open arena, a possibility also suggested by the fact that some individual
362 guppies decrease (rather than increase) shelter use following simulated predation
363 events (Houslay *et al.* 2018).

364 We also find evidence of significant additive genetic variance in a key
365 physiological component of the stress response: waterborne cortisol concentrations
366 following exposure of the fish to a handling stressor. Our findings suggest that

367 previously detected differences among individuals in cortisol response to a stressor
368 (Houslay *et al.* 2019) are primarily attributable to genetic effects, with the estimated
369 heritability ($h^2 = 0.26$) being >75% of the individual–level repeatability for log(Cortisol).
370 Moreover, by adopting a reaction norm approach to modelling stress physiology, as
371 recently advocated by ourselves and others (e.g., Fürtbauer *et al.* 2015; Hau & Goymann
372 2015; Taff & Vitousek 2016), we detect GxE reflecting genetic differences in the extent of
373 habituation to the stressor over repeated exposures. This result is potentially important
374 since poor habituation of the hypothalamic-pituitary-adrenal/interrenal (HPA/I)
375 response to repeated or ongoing stressors can lead to well documented health problems
376 in human and animal populations (Segerstrom & Miller 2004; Koolhaas 2008; Romero,
377 Dickens & Cyr 2009; Mason 2010). Heritable variation in habituation to stressors raises
378 the possibility of developing targeted selection strategies to improve welfare in captive
379 populations (e.g., Frankham *et al.* 1986; Muir & Craig 1995; Oltenacu & Algers 2009).
380 These findings also highlight the possibility that habituation could be important for
381 adaptation in the wild, as many agents of natural selection can be viewed as
382 environmental stressors (Bijlsma & Loeschcke 2005).
383 Our findings also highlight that there is greater additive genetic variance (and
384 heritability) for cortisol levels following the first exposure to the stressor than following
385 the third. This pattern, which occurs because genotypes that produce the highest
386 cortisol response at first exposure also show the most marked habituation, is consistent
387 with the idea of cryptic genetic variance (Paaby & Rockman 2014) being 'released' by
388 exposure to novel, and so potentially stressful, environments (Ledón-Rettig, Pfennig &
389 Crespi 2010; Ledón-Rettig *et al.* 2014; Berger *et al.* 2011). All else being equal, it also
390 means that selection on cortisol levels following stressor exposure should induce a
391 stronger evolutionary response in naïve relative to habituated fish. However, the strong
392 positive cross-environment correlation means that the ranking of genotypes is
393 consistent across repeated stressor exposures. It also means that we would expect a

394 positively correlated response of habituation to any selection on (average) GC response
395 and *vice versa*.
396 Our estimate of **G** shows genetic integration between behavioural and endocrine
397 components of the stress response: genotypes with more exploratory behavioural
398 phenotypes in the OFT also produce lower levels of cortisol following the handling and
399 confinement stressor. This genetic integration of behaviour and physiology is consistent
400 with the idea that correlational selection in the past has led to the coevolution of these
401 stress response components. Covariance structure in **G** will also modify, and potentially
402 constrain, future evolutionary responses to contemporary selection (whether natural or
403 artificial). Here we have no direct knowledge of selection acting in the wild but note that
404 we do expect acute stress responses in natural population to be broadly adaptive. In
405 contrast, it is clear that prolonged, chronic activation of stress response pathways
406 (notably the HPA(I) axis) frequently jeopardises health and survival in captive animal
407 populations (Huether 1996; Boonstra 2013). Thus, it could follow that more stress-
408 responsive genotypes are disadvantaged in captivity, if responsiveness to acute
409 stressors positively predicts susceptibility to chronic stress. However, even in this
410 scenario it does not follow that high stress-responsiveness is disadvantageous in the
411 wild. Rather, since natural selection should purge alleles that are universally
412 detrimental, it seems more plausible that genetic variation along the major axis
413 described here is maintained by some form of selective trade-off (as widely
414 hypothesised for maintenance of personality variation; e.g., Stamps 2007; Wolf *et al.*
415 2007; Réale *et al.* 2010).
416
417 **Conclusions and future directions**
418 Here we find evidence for genetic variation in – and integration of – behavioural
419 and physiological (endocrine) components of the stress response. The structure of **G**
420 holds with the widely used 'reactive – proactive' model of stress coping style variation

421 (Koolhaas *et al.* 2007), but is more consistent with a continuous axis of variation in
422 acute stress responsiveness. Interestingly, genotypes that are more responsive (in the
423 sense of displaying flight-type behaviour and higher cortisol levels) also exhibit faster
424 habituation of GC physiology when subject to repeated stressors.

425 Our results suggest that correlational selection in the past has likely shaped the
426 multivariate stress response, and that continued evolution of stress-related behaviour
427 will have consequences for glucocorticoid physiology and *vice versa*. Determining
428 contemporary selection on the stress response, and testing the possibility that genetic
429 variation is maintained by fitness trade-offs, is thus an obvious – if empirically
430 challenging – next step. In a more applied context, integration of behavioural and
431 endocrine stress-response components at the genetic level has potential utility for
432 genetic improvement of captive populations. Specifically, it may be possible to identify
433 non-invasive, high throughput, behavioural biomarkers and target them in selection
434 schemes to reduce chronic activation of the HPA/I axis and its attendant deleterious
435 effects. The efficacy of this approach will necessarily depend on (i) the presence of
436 standing genetic variance for health and welfare under chronic stress, and (ii) strong
437 genetic correlation structure between target traits and behavioural markers of acute
438 stress-response.

Table 1: Estimated variance components, along with adjusted heritability, for each trait as estimated in a univariate model (± standard error). Chi-square test statistics and p-values are provided for the pedigree term, testing for the presence of significant additive genetic variance (V_a).

Trait	V_a	V_{pe}	V_{group}	$V_{residual}$	h^2	$\chi^2_{0,1}$	P
Relative area covered	26.35 ± 9.37	72.42 ± 9.28	33.33 ± 6.86	205.22 ± 5.90	0.08 ± 0.03	20.7	<0.001
Time in the middle	588.48 ± 139.61	554.58 ± 109.57	203.29 ± 53.41	2070.06 ± 60.08	0.17 ± 0.04	53.7	<0.001
Track length	26832.64 ± 5925.25	32204.05 ± 4868.88	9956.4 ± 2626.41	93921.54 ± 2711.28	0.16 ± 0.03	86.3	<0.001
Shoaling tendency	0 ± 0	2457.36 ± 570.96	708.87 ± 316.30	9900.95 ± 622.10	0 ± 0	0	0.5
log(Emergence time)	0.12 ± 0.05	0.06 ± 0.06	0.05 ± 0.02	1.07 ± 0.05	0.09 ± 0.04	23.2	<0.001
log(Cortisol)	0.07 ± 0.02	0.02 ± 0.02	0.01 ± 0.01	0.15 ± 0.01	0.26 ± 0.08	22.0	<0.001

Table 2: Additive genetic covariance-correlation matrix (G) from the full multivariate animal model. Genetic variances provided on the shaded diagonal, with genetic covariances below and genetic correlations above. 95% confidence intervals in parentheses are estimated from 5000 bootstrapped replicates. Where the confidence intervals for any estimate do not cross zero the estimate is considered statistically significant (at the 0.05 alpha level) and are shown in bold.

	Relative area covered	*Time in the middle*	*Track length*	-log(*Emergence time*)	log(Cortisol)
Relative area covered	**0.074 (0.029,0.122)**	**0.761 (0.549,0.955)**	**-0.506 (-0.758,-0.184)**	-0.503 (-1.394,0.256)	-0.414 (-1.035,0.225)
Time in the middle	**0.075 (0.031,0.124)**	**0.130 (0.062,0.191)**	**-0.554 (-0.774,-0.295)**	-0.117 (-0.791,0.531)	**-0.686 (-1.165,-0.220)**
Track length	**-0.048 (-0.086,-0.008)**	**-0.070 (-0.116,-0.022)**	**0.121 (0.067,0.171)**	0.559 (-0.026,1.256)	0.279 (-0.238,0.823)
-log(Emergence time)	-0.038 (-0.083,0.014)	-0.012 (-0.070,0.040)	**0.055 (0.001,0.106)**	**0.079 (0.011,0.149)**	-0.177 (-0.910,0.560)
log(Cortisol)	-0.038 (-0.091,0.011)	**-0.082 (-0.138,-0.021)**	0.032 (-0.026,0.085)	-0.017 (-0.073,0.038)	**0.111 (0.036,0.191)**

Figure 1: The lack of a strong positive relationship between observed *tracklength* and *area covered* (panel 1), is initially puzzling given expected autocorrelation and that both are used as positive indicators of exploratory (or 'bold') behaviour. Inset examples of OFT tracks from 4 individuals (panel 2) shed light on this. Fish A and B appear to be exploring the tank, while C and D are engaging in a stereotypical 'flight' behaviour characterised by strong thigmotaxis (remaining close to tank walls) and/or rapid movement along tank walls. As a consequence, individuals B and C have similar *area covered* during the OFT as a consequence, but very different *track lengths*. We simulated random movements to define an expected null relationship between *area covered* and *track length* (panel 3; dashed red line shows the third order polynomial model fit; see Appendix A). The polynomial regression was then used to predict the expected area covered under random movement for each trial's observed *track length*, and the '*relative area covered*' was calculated as the observation minus this prediction. Panel 4 shows the resultant *relative area covered* plotted against *track length* for all trials (dashed red line at *relative area covered* = 0, shows where individuals of any *track length* are expected to lie if they move randomly with respect to direction). From this it is apparent that fish A and B have high *relative area covered*, while C and D do not.

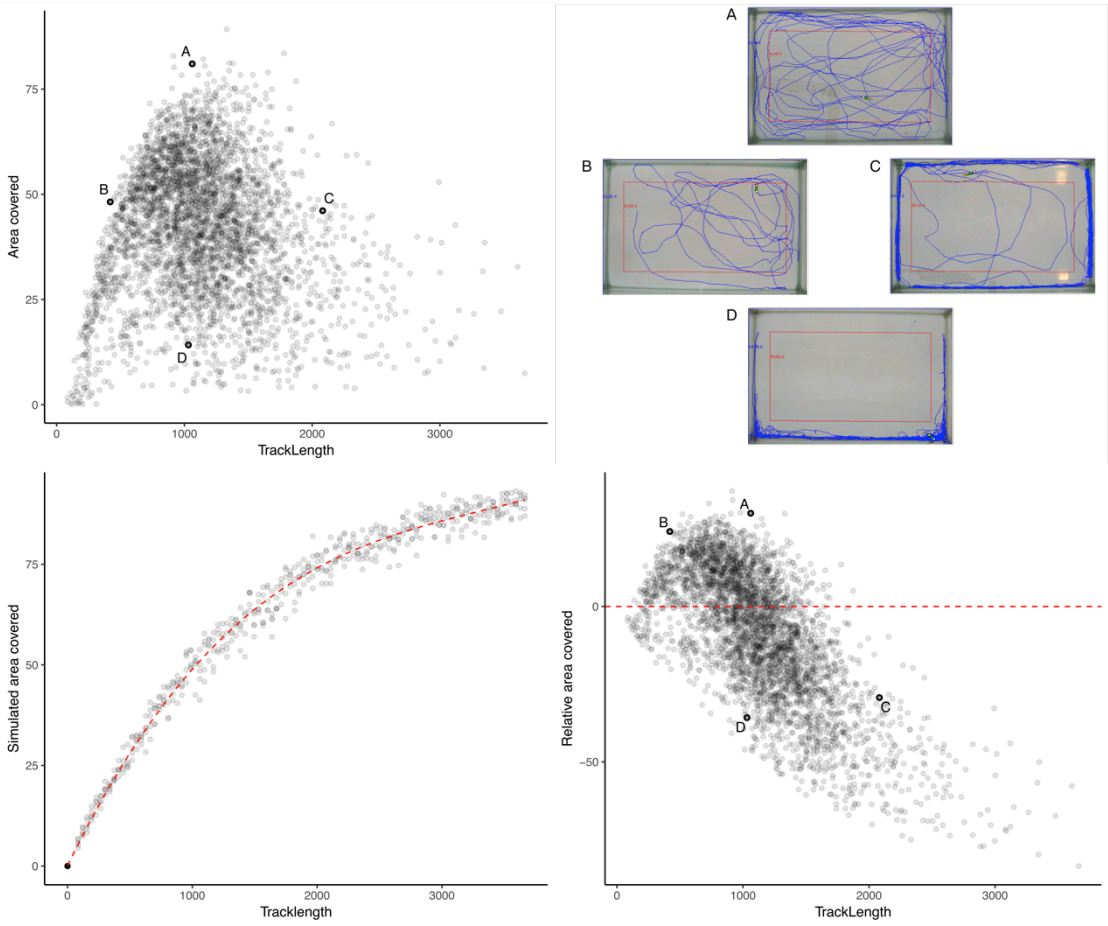

A
B
C
D
Area covered
TrackLength
Simulated area covered
Tracklength
Relative area covered
TrackLength

Figure 2: Guppies habituate to the waterborne sampling procedure, as shown by a decline in log-transformed cortisol levels following stressor exposure declining from their first stressor exposure to their third. Black circles and associated bars denote predicted population means (± standard error) from mixed model analysis. Reaction norms (grey lines) depict the predicted genetic deviations for each individual from the average pattern. GxE is evidenced by the slight reduction in genetic variance over the repeated sampling events (i.e., that the reaction norms (therefore) are not parallel; see main text).

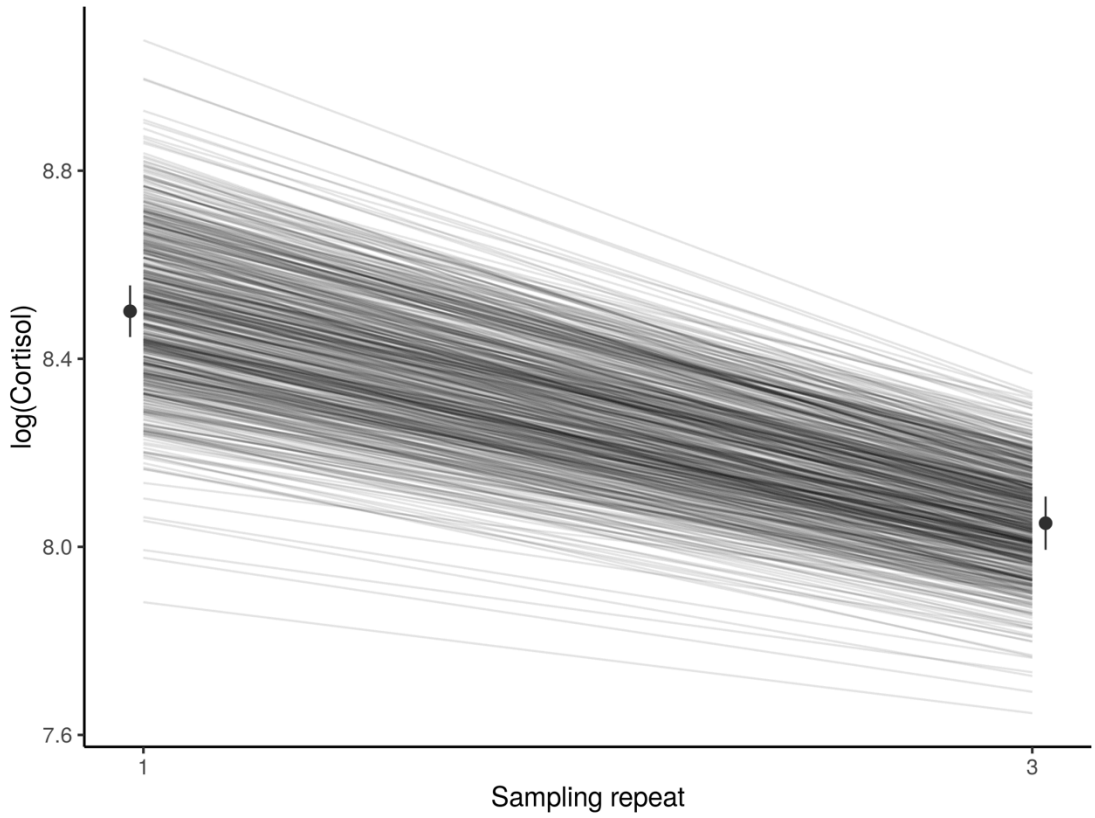
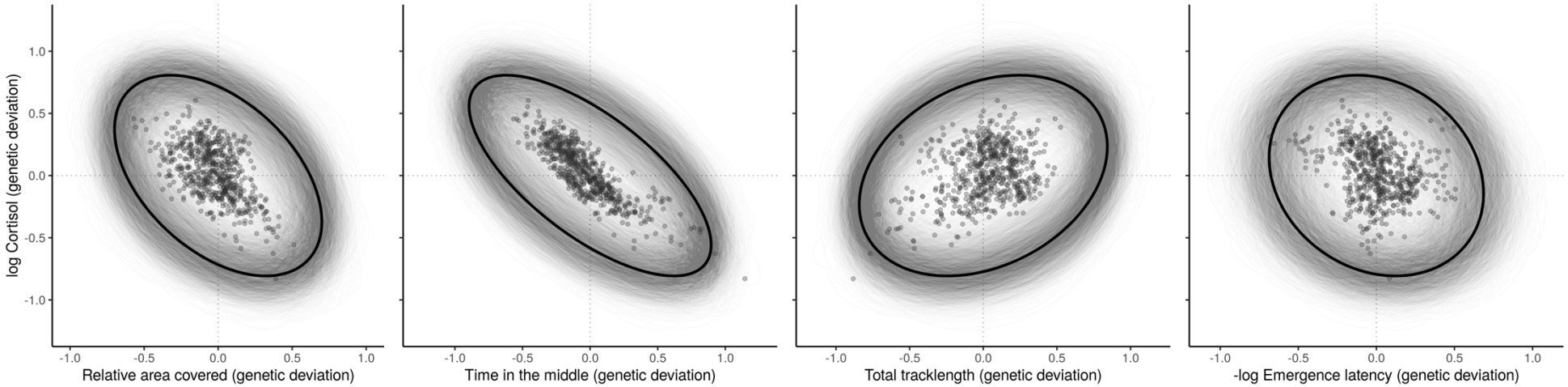
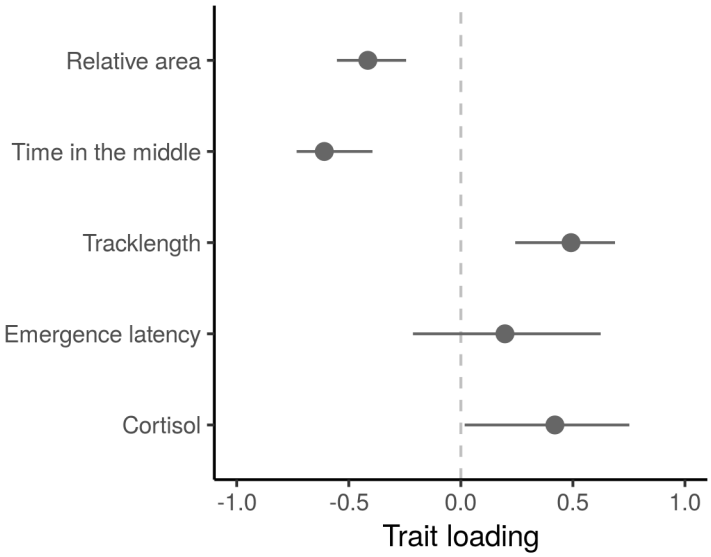


Figure 1: The additive genetic relationship between log-transformed cortisol (y-axis) and four behaviours (a, *relative area covered*; b, *time in the middle*; c, *track length*; d, *-log emergence time*). Points show (predicted) bivariate genetic deviations from the population means, plotted for those individuals in the pedigree with cortisol data. In each case the black ellipse depicts the shape of the relationship as estimated from **G**, with 5000 bootstrapped replicates in grey showing the associated uncertainty.



489 Figure 2: Trait loadings from the first eigen vector (principal component) of **G**. This axis explains
490 57.8% of the genetic (co)variation found in the focal behavioural and physiological components of the
491 stress response in our guppy population. Points show trait loadings from the first eigen vector of our
492 estimate of G, with bars representing 95% confidence intervals on each loading (calculated from 5000
493 bootstrapped replicates of the model).



494

495 **Materials and methods**

496

497 *Husbandry and breeding*

498 We used fish taken from our captive population housed at the University of Exeter's Penryn campus,
499 which is descended from wild fish collected in 2008 from the lower Aripo River in Trinidad. This
500 population has been maintained at a population size of several thousand, and has undergone no
501 deliberate selection or inbreeding. All fish are fed to satiation twice daily (0800 – 1000h and again at
502 1600 – 1800h) using commercial flake food and live *Artemia nauplii*. Water temperature is maintained
503 at 23-24°C in well-aerated closed system tank stacks that undergo 25% water changes each week and
504 with weekly tests for ammonia, nitrate and nitrite levels. Lighting is kept at a 12:12 light/dark cycle.
505 The experiment described in this study was carried out in accordance with the UK Animals (Scientific
506 Procedures) Act 1986 under licence from the Home Office (UK), and with local ethical approval from
507 the University of Exeter.

508 To create our pedigreed sub-population, female fish were sampled haphazardly from the stock
509 tanks and kept in female-only groups for 3 months. Isolation from male contact minimised the chance
510 of females carrying viable sperm from previous matings. For the first generation of offspring, we used
511 a group breeding design (as detailed in White and Wilson, 2018); briefly, females were tagged under
512 anaesthetic (buffered MS222 solution) using visible implant elastomer (VIE) to allow individual
513 identification. We then assigned groups of 4 females to 1 male in 15L breeding tanks (18.5cm x 37cm x
514 22cm), and inspected females daily for high gravidity (swollen abdomens and enlarged 'gravid spots').
515 Heavily gravid females were then isolated in 2.8L brood tanks to give birth (and were returned to the
516 breeding tanks either after producing a brood or two weeks of isolation). Any offspring produced in
517 the breeding tanks were excluded from the experiment as maternal identity could not be positively
518 identified. For the following generations, after 3 months of isolation from males we moved females
519 into individual 2.8L tanks, with 1 male then circulated among 3 females. Males were moved between
520 females every 5-8 days. In this way, females did not have to be moved to brood tanks, and any
521 offspring could be assigned to mothers definitively. In this setup, offspring were moved to a separate
522 brood tank on the day of birth. Note that as the gestation period for guppies is approximately 1 month,

523 any brood produced by a female less than one month after exposure to their designated male was
524 recorded in the pedigree as having unknown paternity.
525 Within 24h of a female producing a brood we recorded her weight (g) and brood size. We kept
526 juvenile fish in full-sib family groups in 2.8L tanks before moving them to 15L 'growth' tanks at an
527 average age of 56 days. At an average age of 133 days (range 59-268) we tagged individuals and
528 placed them into mixed family groups of 16-20 adults (with an even mix of males and females), kept in
529 15L tanks. Note that variation in tagging age arose largely because groups were necessarily
530 established sequentially as sufficient individuals from multiple families reached a large enough size
531 that we deemed the procedure to be safe. Each adult group comprised a mix of fish from different
532 families, reducing the potential for common environment effects to upwardly bias our genetic
533 parameter estimation.
534
535 *Overview of behavioural phenotyping*
536 Behavioural phenotyping commenced at least one week after tagging. In all trials, we filmed
537 movement behaviour of individual fish using a Sunkwang video camera equipped with a 6-60mm
538 manual focus lens suspended over the tank. We used the tracking software Viewer II (BiObserve) to
539 extract behavioural data from each recording (detailed below). The tank was lit from below using a
540 light box, and screened with a cardboard casing to prevent external visual disturbance. After each
541 behavioural trial, the individual tested was weighed and then moved to a temporary 'holding tank'.
542 Once a full group (as described above) had been tested, all were moved from the holding tank back to
543 their home tank. We replaced the water in the testing and holding tanks between groups to reduce the
544 build-up of hormones or other chemicals. The first offspring generation experienced 4 repeat open
545 field trials (OFTs) over a 2-week period, with at least 48h between trials. Subsequent generations
546 experienced 4 repeat behavioural trials, alternating 2 OFTs with 2 emergence trials (ETs). For the final
547 2 generations, we extended the OFTs by including a shoaling trial (ST) at the end of each OFT.
548
549 *Open field trials (OFT)* followed the methodology described by White et al (2016). Briefly, we assessed
550 individual behaviour in a 20cm x 30cm tank, filled to a depth of 5cm with room-temperature water

551 from the main supply. We caught fish individually from their home tank, examined them quickly for
552 identification tags, then placed them immediately into the centre of the OFT tank. After allowing 30s
553 for acclimation, we filmed behaviour for 4m30s. Behaviours characterised from the tracking software
554 were *track length* (the total distance the fish moved during the trial; cm), *area covered* (the percentage
555 of 1cm x 1cm grid squares through which the fish moved during the trial; %), and *time in middle* (time
556 spent in a rectangular inner zone which was defined as being the same size as an outer area; seconds).
557
558 *Shoaling trials (ST)* were appended to a subset of OFTs, by positioning a small tank containing 10 stock
559 fish (of same sex as the test subject) next to one end of the OFT tank but with visual cues blocked by a
560 cardboard divider. At the end of the normal OFT, we removed this divider slowly, allowing the focal
561 animal to have visual contact with the shoal. We began recording the shoaling trial 30s after removing
562 the divider in order to limit any artefacts of slight disturbance. (Note that we used a further cardboard
563 casing around the shoaling tank to avoid any additional external visual stimulus). We then recorded
564 behaviour of the test fish for an additional 3 minutes. We characterised *shoaling tendency* via the
565 tracking software by subdividing the tank area into 3 equal-sized rectangular areas: one next to the
566 tank holding the group of same-sex fish, one farthest from this group, and the central area. We then
567 calculated *shoaling tendency* as the time spent in the 1/3 area closest to the same-sex group after
568 subtracting the time spent in the 1/3 area farthest away. The decision to use a single-sex shoal aimed
569 to reduce any effects of (potential) mate preference and/or avoidance, but also this necessitated
570 replicate arena setups allowing male and female individuals from each group to be tested in the
571 OFT/ST concurrently. We randomised which tank was used for each sex in each group and recorded
572 this information.
573
574 *Emergence trials (ET)* followed the methodology described by White *et al.* (2016). Briefly, we tested
575 individuals in a 20cm x 40cm tank, filled to a depth of 8cm with room-temperature water from the
576 main supply. A 10cm section of the tank length was walled off creating a shelter area (20cm x10cm),
577 the walls and floor of which were painted black. The focal fish was placed into the shelter area and
578 allowed to acclimate for 30s, at which point we opened a sliding door to allow access to the rest of the

579 tank, which was brightly lit from below and otherwise bare. *Time to emerge* (in seconds) was recorded
580 by the tracking software automatically as the fish exited the shelter area and emerged into the open
581 tank section. Trials were ended either at emergence or at 15 min if the fish had not emerged by that
582 point; in the case of non-emergence, fish were given the maximum value (i.e., 900s).
583
584 *Derivation of 'relative area' from OFT trials*
585 The '*area covered'* variable assayed in the OFT is calculated in BiObserve by dividing the arena (i.e., the
586 total area of the tank as viewed from the camera) into 1cm x 1cm grid squares. The path taken by the
587 fish during observation is then used to determine what proportion of these grid squares the fish
588 entered. However, we sought to derive a measure of '*relative area*' that describes whether a fish covers
589 a large, or small area relative to its observed *tracklength.*
590 To do this we simulated 'random swims' within the arena across the observed range of
591 tracklengths. We first selected 40 OFT results at random from our total data set and extracted the
592 coordinates of the fish in each frame from the raw tracking file, creating a set of x and y movements
593 and their associated distances. As original coordinates were recorded in pixels we used the calibration
594 of the software to convert to cm units. We then use a 'random walk' algorithm to select a movement
595 (i.e., step size and direction) from this observed distribution at random, and calculate the new
596 coordinates. If the movement keeps the 'fish' within the bounds of the 'tank' (i.e., defined as a 20cm x
597 30cm arena), the movement is accepted and coordinates added to a movement matrix; if not, a new
598 movement is drawn from the distribution. If the movement is greater than 1cm in distance, we break
599 the movement into a number of smaller parts to be added to the matrix (such that we capture the
600 coordinates of grid squares through which the 'fish' moved along the way). Once the total distance of
601 the random walk reached or exceeded the *tracklength* set as the simulation objective, the path is
602 terminated and the area covered is calculated by counting the number of unique grid squares in the
603 matrix of coordinates and dividing by the total number possible.
604 After simulating random walks across 500 values of *tracklength* (using a vector of 100 values
605 evenly spaced across the range of true data, repeated 5 times), we modelled (simulated) area covered
606 as a fourth order polynomial function of *tracklength*. Using this regression model (which explained

607 97.8% of the variance in simulated data), we calculated the *relative area* for each actual OFT trial as
608 the observed area covered minus the area covered under a random swim, as predicted from our
609 regression model and the observed *tracklength*.
610
611
612 *Waterborne hormone sampling*
613 On completion of behavioural data collection, individuals entering the endocrine testing program were
614 left undisturbed for a minimum of two weeks. Waterborne hormone sampling was then conducted
615 over a 5-day period that included three handling and confinement stressor exposures with 48h
616 between each. We followed the method described by Houslay et al (2019) to obtain repeated non-
617 invasive GC measures of individuals using holding water samples from the first and third
618 confinements. Note that only two samples per fish were analysed because the financial and time costs
619 of doing three was deemed prohibitive. We nonetheless applied the stressor stimulus three times as
620 our prior study showed this was sufficient to produce a strong habituation response, i.e., a significant
621 decrease in water-borne cortisol over the three sampling periods (Houslay *et al.* 2019)).
622 We collected samples between 1200 – 1400h to control for diel fluctuations in GC levels. For
623 each sample, we netted an entire group from their home tank quickly using a large net, transferring
624 them to 2 holding tanks (containing water from the home tank supply) for moving to an adjacent quiet
625 room (performed within 20s of the net first hitting the water). We then transferred fish to individual
626 Pyrex beakers containing 300ml of clean water from the main supply (which serves the main housing
627 units), which has been warmed to the appropriate temperature (mean = 24.1°C, range 23-24.9°C).
628 Beakers were placed within cardboard 'chambers' to prevent fish from seeing each other or
629 experiencing outside disturbance. One fish was transferred every 30s, alternating across holding
630 tanks, such that all fish were in their beakers within 10min of the initial netting. After 60 mins in the
631 beaker, each fish was removed by pouring its sample through a clean net into a second beaker, with
632 the fish then quickly checked to confirm ID and returned to the holding tank until the entire group
633 could be returned to its home tank.

634 We immediately filtered each water sample using Grade 1 filter paper (Whatman), then passed them
635 slowly through solid phase C18 extraction columns (Sep-Pak C18, 3cc, Waters) via high-purity tubing
636 (Tygon 2474, Saint Gobain) under vacuum pressure (Earley *et al.* 2006). Columns were primed
637 beforehand with 2 x 2ml HPLC-grade methanol followed by 2 x 2ml distilled water, and were washed
638 afterwards with a further 2 x 2ml distilled water to purge salts. We then covered both ends of each
639 column with film (Parafilm M, Bemis) and stored them at -20C for future analysis. We washed all
640 beakers, tubes and funnels with 99% ethanol and rinsed them with distilled water prior to each
641 sampling procedure. The remainder of the endocrine assay procedure, involved elution, resuspension,
642 separation and quantification of free cortisol by enzyme immunoassay (EIA) using Cayman Chemicals,
643 Inc EIA kits). Detailed methods are exactly as described by Houslay et al (2019), and so not repeated
644 here (note that here we assayed the free fraction of cortisol only). To validate the cortisol kits, we
645 examined whether the kit standard curve was parallel to a serial dilution curve derived from pooled
646 guppy water-borne hormone extract. 20µl was taken from each of the male samples and pooled; 20µl
647 was taken from each of the female samples and combined into a separate pool. 400µl of the pools was
648 serially diluted from 1:1 to 1:128 and these samples were assayed alongside the kit standard curve on
649 two occasions (June and December 2017, marking the start and finish of sample processing). All
650 dilution curves were parallel to the standard curve (slope comparison test, Zar 1996, p.355; June,
651 male: t_{12} = 0.029, P = 0.97; June, female: : t_{12} = 0.343, P = 0.74; December, male: : t_{12} = 0.119, P = 0.91;
652 December, female: : t_{12} = 0.224, P = 0.83). The serial dilution curves also identified 1:32 as an
653 appropriate dilution to ensure that all samples fell on the linear phase of the standard curve. A total of
654 37, 96-well plates were used and pooled sample was included at the beginning and end of each plate.
655 Intra-assay coefficients of variation ranged from 0.12-19.83% with a median of 3.08%; the inter-assay
656 coefficient of variation was 19.22%.
657
658 **Statistical methods**
659
660 All data handling and analysis was performed in R version 3.4.1 (R Core Team 2017). We used the
661 'tidyverse' set of packages for data handling and visualisation (Wickham 2017), and ASreml-R 3.0

662 (Butler 2009) for fitting linear mixed effects models (as described in full below). We also used 'nadiv'
663 for pedigree preparation and to estimate (approximate) standard errors on linear functions of
664 variance components as estimated from the mixed models (Wolak 2012). All models fitted assumed
665 (multivariate) Gaussian error structures, and we visually assessed residuals to verify this was
666 reasonable (after data transformation in some cases). To test for significance of among individual
667 and/or genetic (co)variance components, we fitted nested models with different random effects
668 structures and compared them using likelihood ratio tests (LRTs). We calculated $\chi^2_{n\mathrm{DF}}$ as twice the
669 difference in model log likelihoods, with the number of degrees of freedom (n) equivalent to the
670 number of additional parameters in the more complex model. When testing a single random effect
671 (variance component), we assumed the difference to be asymptotically distributed as an equal mix of
672 χ^2_0 and χ^2_1 (denoted $\chi^2_{0,1}$; Self and Liang, 1987; Visscher, 2006).
673
674 For each OFT and ST behaviour in turn (*relative area, time in middle, track length, shoaling tendency,*
675 and *emergence time*), we used the random effects specification to partition phenotypic variation (V_p,
676 conditional on fixed effects as described below) into the effects of additive genetics (V_a), permanent
677 environment defined as the non-(additive) genetic component of among-individual differences, V_pe),
678 and housing group (V_group), as well as residual variation (V_residual). We natural log-transformed
679 *emergence time* prior to analysis to meet assumptions of residual normality and homoscedasticity. For
680 all behavioural traits, we included fixed effects of assay *repeat*, the *order within each group* in which
681 the fish was trialled (mean-centred continuous predictor), *temperature* (mean-centred and scaled to
682 standard deviation units), *time* (in minutes from midnight, mean-centred and scaled to standard
683 deviation units), *age* (mean-centred and scaled to standard deviation units), *sex*, and the *generation*
684 from the breeding population. For *shoaling tendency* only, we incorporated an additional fixed effect of
685 *setup* (as detailed above). We tested the significance of genetic variance for each behaviour by LRT
686 comparison of the corresponding full model to one in which the (additive) genetic random effect was
687 excluded.
688

689 Cortisol data were also natural log-transformed for analysis. We formulated a bivariate model to test
690 for both additive genetic variation and genotype-by-environment interaction (GxE) in cortisol levels
691 across the two 'contexts' (i.e. samples retained for each individual at first and third confinement,
692 denoted $Cortisol_1$, $Cortisol_3$). Random effects were first used to partition phenotypic (co)variance
693 (conditional on fixed effects) into among-group and residual components. Fixed effects included the
694 context-specific means, and overall effects of the *order* in which the fish was caught from each group
695 for assay (mean-centred continuous predictor), *temperature* (mean-centred and scaled to standard
696 deviation units), *time of day* (mean-centred and scaled to standard deviation units), *age* (mean-centred
697 and scaled to standard deviation units), and *sex*. In addition, we included fixed covariates of *body mass*
698 (mean-centred and scaled to standard deviation units) and a *sex* by *body mass* interaction (see Houslay
699 *et al.* 2019 for rationale of controlling for body size effects on waterborne hormone levels in this way).
700 Note that modelled in this way each individual is sampled only once for each context-specific cortisol
701 trait so no random effect of individual identity is included. To test for additive genetic variation (V_a)
702 we compared this first bivariate model to a second formulation that also included the (additive)
703 genetic merit, but under the assumption that this is invariant with context within an individual (such
704 that V_{a1}=V_{a3} and $r_{a1,3}$=1 and there is no GxE). We then test for the GxE by comparing the second model
705 to a third in which we allow GxE (i.e., the context-specific genetic variances are free to differ and the
706 cross-context genetic correlation can be <+1).
707
708 Lastly, we built a multivariate animal model to estimate **G** and to test the hypothesised genetic
709 integration among behavioural and physiological stress components. We retained only response traits
710 that harboured significant V_a as shown in univariate models, and so the final model comprised
711 response traits *relative area, time in middle, track length, emergence time* (log transformed), and
712 *Cortisol* (log transformed). We multiplied (transformed) *emergence time* by -1 to simplify
713 interpretation of estimated correlation structures (i.e., higher values for all behavioural traits then
714 represent nominally 'bolder' behaviours). We also scaled all (transformed) response variables to
715 standard deviation units. This was to facilitate model fitting, and also prevent scale effects
716 complicating interpretation of eigenvectors of **G**. Fixed and random effects were fitted on each trait as

specified for the univariate models. Note that one exception to this is that we elected to treat *Cortisol* as a single repeated-measures trait here (with two repeats, one per context) such that a permanent environment effect was now included.

We specified additive genetic genetic (**G**), permanent environment (**PE**), group (**GROUP**), and residual (**R**) covariance structures as unstructured matrices to be estimated. Note that **R** partitions observation-level covariances (as opposed to individual-level in **PE**) that are not definable or statistically identifiable if traits are not measured at the same time (i.e., all covariances relating to *emergence time* or *Cortisol*). Where this was the case we constrained specific covariance terms in **R** to equal zero. We tested for overall additive genetic covariance among the traits by comparing this model against a reduced one in which **G** was specified as a diagonal matrix (i.e., additive variances are estimated but covariances are assumed to equal zero). To aid the interpretation of covariance terms contained in **G,** we calculated the corresponding genetic correlations r_a from the full model. For any pair of traits (x,y), $r_{a(x,y)} = COV_{a(x,y)} / (\sqrt{(V_{a(x)})} \times \sqrt{(V_{a(y)})})$. We also subjected our estimate of **G** to eigen decomposition to determine the proportion of additive genetic variation captured by each principal component and assess whether a single major axis of variation could indeed explain most of the genetic variance in the multivariate phenotype (consistent with a simple proactive-reactive coping style model). We estimated uncertainty on the trait loadings associated with each principal component (eigenvector) using a parametric bootstrap approach as described by Boulton et al (2014).

For visualisation of bivariate relationships at the additive genetic level, we used the R package 'ellipse' (Murdoch & Chow 2018) to determine the coordinates of an ellipse representing the approximate 95% confidence region of deviations based on the point estimate of **G**. We repeated this procedure for the corresponding regions defined from 5000 bootstrapped values of **G** (i.e., to indicate uncertainty arising from estimation of the genetic covariance structure itself).

744 **REFERENCES**

745

746 Angelier, F. & Wingfield, J.C. (2012) Importance of the glucocorticoid stress response in a changing
747 world: Theory, hypotheses and perspectives. *General and Comparative Endocrinology*, **190**, 118–
748 128.

749 Barton, B.A. & Iwama, G.K. (1991) Physiological changes in fish from stress in aquaculture with
750 emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*, **1**, 3–26.

751 Baugh, A.T., van Oers, K., Naguib, M. & Hau, M. (2013) Initial reactivity and magnitude of the acute
752 stress response associated with personality in wild great tits (Parus major). *General and*
753 *Comparative Endocrinology*, **189**, 96–104.

754 Baugh, A.T., Schaper, S. V., Hau, M., Cockrem, J.F., de Goede, P. & Oers, K. van. (2012) Corticosterone
755 responses differ between lines of great tits (Parus major) selected for divergent personalities.
756 *General and Comparative Endocrinology*, **175**, 488–494.

757 Berger, D., Bauerfeind, S.S., Blanckenhorn, W.U. & Schäfer, M.A. (2011) HIGH TEMPERATURES REVEAL
758 CRYPTIC GENETIC VARIATION IN A POLYMORPHIC FEMALE SPERM STORAGE ORGAN.
759 *Evolution*, **65**, 2830–2842.

760 Bijlsma, R. & Loeschcke, V. (2005) Environmental stress, adaptation and evolution: an overview.
761 *Journal of evolutionary biology*, **18**, 744–9.

762 Blows, M.W. (2007) A tale of two matrices: multivariate approaches in evolutionary biology. *Journal of*
763 *evolutionary biology*, **20**, 1–8.

764 Blows, M.W., Chenoweth, S.F. & Hine, E. (2004) Orientation of the genetic variance-covariance matrix
765 and the fitness surface for multiple male sexually selected traits. *The American naturalist*, **163**,
766 329–340.

767 Boonstra, R. (2013) Reality as the leading cause of stress: Rethinking the impact of chronic stress in
768 nature. *Functional Ecology*, **27**, 11–23.

769 von Borell, E. (1995) Neuroendocrine integration of stress and significance of stress for the
770 performance of farm animals. *Applied Animal Behaviour Science*, **44**, 219–227.

771 Boulton, K., Couto, E., Grimmer, A.J., Earley, R.L., Canario, A.V.M., Wilson, A.J. & Walling, C.A. (2015)

How integrated are behavioral and endocrine stress response traits? A repeated measures approach to testing the stress-coping style model. *Ecology and Evolution*, **5**, 618–633.

Boulton, K., Grimmer, A.J., Rosenthal, G.G., Walling, C.A. & Wilson, A.J. (2014) How stable are personalities? A multivariate view of behavioural variation over long and short timescales in the sheepshead swordtail, Xiphophorus birchmanni. *Behavioral Ecology and Sociobiology*, **68**, 791–803.

Broom, D.M. & Johnson, K.G. (1993) Assessing welfare: short-term responses. *Stress and Animal Welfare*, pp. 87–110. Springer Netherlands, Dordrecht.

Burns, J.G. (2008) The validity of three tests of temperament in guppies (Poecilia reticulata). *Journal of comparative psychology*, **122**, 344–356.

Busch, D.S. & Hayward, L.S. (2009) Stress in a conservation context: A discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biological Conservation*, **142**, 2844–2853.

Butler, D. (2009) asreml: asreml() fits the linear mixed model.

Carere, C., Groothuis, T.G.G., Möstl, E., Daan, S. & Koolhaas, J.M. (2003) Fecal corticosteroids in a territorial bird selected for different personalities: Daily rhythm and the response to social stress. *Hormones and Behavior*, **43**, 540–548.

Carter, A.J., Feeney, W.E., Marshall, H.H., Cowlishaw, G. & Heinsohn, R. (2013) Animal personality: What are behavioural ecologists measuring? *Biological Reviews*, **88**, 465–475.

Cheverud, J.M. (1982) Phenotypic, Genetic, and Environmental Morphological Integration in the Cranium. *Evolution*, **36**, 499.

Clinchy, M., Sheriff, M.J. & Zanette, L.Y. (2013) Predator-induced stress and the ecology of fear. *Functional Ecology*, **27**, 56–65.

Cox, R.M., McGlothlin, J.W. & Bonier, F. (2016) Hormones as Mediators of Phenotypic and Genetic Integration: An Evolutionary Genetics Approach. *Integrative and Comparative Biology*, **56**, 126–137.

Crossin, G.T., Love, O.P., Cooke, S.J. & Williams, T.D. (2016) Glucocorticoid manipulations in free-living animals: Considerations of dose delivery, life-history context and reproductive state. *Functional*

Ecology, **30**, 116–125.

Earley, R.L., Edwards, J.T., Aseem, O., Felton, K., Blumer, L.S., Karom, M. & Grober, M.S. (2006) Social interactions tune aggression and stress responsiveness in a territorial cichlid fish (Archocentrus nigrofasciatus). *Physiology and Behavior*, **88**, 353–363.

Frankham, R., Hemmer, H., Ryder, O.A., Cothran, E.G., Soulé, M.E., Murray, N.D. & Snyder, M. (1986) Selection in captive populations. *Zoo Biology*, **5**, 127–138.

Fürtbauer, I., Pond, A., Heistermann, M. & King, A.J. (2015) Personality, plasticity and predation: linking endocrine and behavioural reaction norms in stickleback fish. *Functional Ecology*, n/a-n/a.

Hau, M. & Goymann, W. (2015) Endocrine mechanisms, behavioral phenotypes and plasticity: known relationships and open questions. *Frontiers in Zoology*, **12**, S7.

Hazard, D., Couty, M., Richard, S. & Guémené, D. (2008a) Intensity and duration of corticosterone response to stressful situations in Japanese quail divergently selected for tonic immobility. *General and Comparative Endocrinology*, **155**, 288–297.

Hazard, D., Leclaire, S., Couty, M. & Guémené, D. (2008b) Genetic differences in coping strategies in response to prolonged and repeated restraint in Japanese quail divergently selected for long or short tonic immobility. *Hormones and Behavior*, **54**, 645–653.

Herbert-Read, J.E., Rosén, E., Szorkovszky, A., Ioannou, C.C., Rogell, B., Perna, A., Ramnarine, I.W., Kotrschal, A., Kolm, N., Krause, J. & Sumpter, D.J.T. (2017) How predation shapes the social interaction rules of shoaling fish. *Proceedings of the Royal Society B: Biological Sciences*, **284**, 20171126.

Hine, E., Chenoweth, S.F. & Blows, M.W. (2004) Multivariate quantitative genetics and the lek paradox: genetic variance in male sexually selected traits of Drosophila serrata under field conditions. *Evolution*, **58**, 2754–62.

von Horsten, S., Karl, T. & Pabst, R. (2003) Behavioral phenotyping of mice in pharmacological and toxicological research. *Experimental and Toxicologic Pathology*, **55**, 69–83.

Houslay, T.M., Earley, R.L., Young, A.J. & Wilson, A.J. (2019) Habituation and individual variation in the endocrine stress response in the Trinidadian guppy (Poecilia reticulata). *General and*

828 *Comparative Endocrinology*, **270**, 113–122.

829 Houslay, T.M., Vierbuchen, M., Grimmer, A.J., Young, A.J. & Wilson, A.J. (2018) Testing the stability of
830 behavioural coping style across stress contexts in the Trinidadian guppy. *Functional Ecology*, **32**,
831 424–438.

832 Huether, G. (1996) The central adaptation syndrome: Psychosocial stress as a trigger for adaptive
833 modification of brain structure and brain function. *Progress in Neurobiology*, **48**, 569–612.

834 Hunt, J., Blows, M.W., Zajitschek, F., Jennions, M.D. & Brooks, R.C. (2007) Reconciling strong stabilizing
835 selection with the maintenance of genetic variation in a natural population of black field crickets
836 (Teleogryllus commodus). *Genetics*, **177**, 875–80.

837 Jones, R.B., Satterlee, D.G. & Ryder, F.H. (1994) Fear of Humans in Japanese-Quail Selected for Low or
838 High Adrenocortical-Response. *Physiology & Behavior*, **56**, 379–383.

839 Ketterson, E.D., Atwell, J.W. & McGlothlin, J.W. (2009) Phenotypic integration and independence:
840 Hormones, performance, and response to environmental change. *Integrative and Comparative*
841 *Biology*, **49**, 365–379.

842 Koolhaas, J.M. (2008) Coping style and immunity in animals: Making sense of individual variation.
843 *Brain, Behavior, and Immunity*, **22**, 662–667.

844 Koolhaas, J.M., de Boer, S.F., Buwalda, B. & Van Reenen, K. (2007) Individual variation in coping with
845 stress: A multidimensional approach of ultimate and proximate mechanisms. *Brain, Behavior and*
846 *Evolution*, **70**, 218–226.

847 Koolhaas, J.M., de Boer, S.F., Coppens, C.M. & Buwalda, B. (2010) Neuroendocrinology of coping styles:
848 Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology*, **31**,
849 307–321.

850 Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C.,
851 Ruis, M.A.W. & Blokhuis, H.J. (1999) Coping styles in animals: Current status in behavior and
852 stress- physiology. *Neuroscience and Biobehavioral Reviews*, **23**, 925–935.

853 Korte, S.M., Koolhaas, J.M., Wingfield, J.C. & McEwen, B.S. (2005) The Darwinian concept of stress:
854 Benefits of allostasis and costs of allostatic load and the trade-offs in health and disease.
855 *Neuroscience and Biobehavioral Reviews*, **29**, 3–38.

856 Kruuk, L.E.B. (2004) Estimating genetic parameters in natural populations using the "animal model."
857 *Philosophical Transactions of the Royal Society B: Biological Sciences*, **359**, 873–890.
858 Kruuk, L.E.B. & Hadfield, J.D. (2007) How to separate genetic and environmental causes of similarity
859 between relatives. *Journal of Evolutionary Biology*, **20**, 1890–1903.
860 Lande, R. & Arnold, S.J. (1983) The measurement of selection on correlated characters. *Evolution*, **37**,
861 1210–1226.
862 Ledón-Rettig, C.C., Pfennig, D.W., Chunco, A.J. & Dworkin, I. (2014) Cryptic genetic variation in natural
863 populations: a predictive framework. *Integrative and comparative biology*, **54**, 783–793.
864 Ledón-Rettig, C.C., Pfennig, D.W. & Crespi, E.J. (2010) Diet and hormonal manipulation reveal cryptic
865 genetic variation: Implications for the evolution of novel feeding strategies. *Proceedings of the*
866 *Royal Society B: Biological Sciences*, **277**, 3569–3578.
867 Mason, G.J. (2010) Species differences in responses to captivity: Stress, welfare and the comparative
868 method. *Trends in Ecology and Evolution*, **25**, 713–721.
869 McEwen, B.S. & Wingfield, J.C. (2003) The concept of allostasis in biology and biomedicine. *Hormones*
870 *and Behavior*, **43**, 2–15.
871 McEwen, B.S. & Wingfield, J.C. (2010) What is in a name? Integrating homeostasis, allostasis and stress.
872 *Hormones and Behavior*, **57**, 105–111.
873 McGlothlin, J.W. & Ketterson, E.D. (2008) Hormone-mediated suites as adaptations and evolutionary
874 constraints. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*,
875 **363**, 1611–1620.
876 Mignon-Grasteau, S., Boissy, A., Bouix, J., Faure, J.M., Fisher, A.D., Hinch, G.N., Jensen, P., Le Neindre, P.,
877 Mormède, P., Prunet, P., Vandeputte, M. & Beaumont, C. (2005) Genetics of adaptation and
878 domestication in livestock. *Livestock Production Science*, **93**, 3–14.
879 Moberg, G.P. (2000) Biological response to stress: implications for animal welfare. *The biology of*
880 *animal stress: basic principles and implications for animal welfare*, pp. 1–21.
881 Möstl, E. & Palme, R. (2002) Hormones as indicator of stress. *Domestic animal and endocrinology*, **23**,
882 67–74.
883 Muir, W.M. & Craig, J. V. (1998) Improving animal well-being through genetic selection. *Poultry Science*,

884 **77**, 1781–1788.
885 Murdoch, D. & Chow, E.D. (2018) ellipse: Functions for Drawing Ellipses and Ellipse-like Confidence
886 Regions.
887 Oltenacu, P.A. & Algers, B. (2009) Selection for Increased Production and the Welfare of Dairy Cows:
888 Are New Breeding Goals Needed? *AMBIO: A Journal of the Human Environment*, **34**, 311–315.
889 Oswald, M.E., Singer, M. & Robison, B.D. (2013) The Quantitative Genetic Architecture of the Bold-Shy
890 Continuum in Zebrafish, Danio rerio. *PLoS ONE*, **8**, 1–10.
891 Øverli, Ø., Pottinger, T.G., Carrick, T.R., Overli, E. & Winberg, S. (2002) Differences in behaviour
892 between rainbow trout selected for high- and low-stress responsiveness. *The Journal of*
893 *experimental biology*, **205**, 391–5.
894 Øverli, Ø., Pottinger, T.G., Carrick, T.R., Øverli, E. & Winberg, S. (2001) Brain monoaminergic activity in
895 rainbow trout selected for high and low stress responsiveness. *Brain, Behavior and Evolution*, **57**,
896 214–224.
897 Øverli, Ø., Sørensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W., Summers, C.H. & Nilsson, G.E. (2007)
898 Evolutionary background for stress-coping styles: Relationships between physiological,
899 behavioral, and cognitive traits in non-mammalian vertebrates. *Neuroscience and Biobehavioral*
900 *Reviews*, **31**, 396–412.
901 Øverli, Ø., Winberg, S. & Pottinger, T.G. (2005) Behavioral and Neuroendocrine Correlates of Selection
902 for Stress Responsiveness in Rainbow Trout—a Review. *Integrative and Comparative Biology*, **45**,
903 463–474.
904 Paaby, A.B. & Rockman, M. V. (2014) Cryptic genetic variation: Evolution's hidden substrate. *Nature*
905 *Reviews Genetics*, **15**, 247–258.
906 Perals, D., Griffin, A.S., Bartomeus, I. & Sol, D. (2017) Revisiting the open-field test: what does it really
907 tell us about animal personality? *Animal Behaviour*, **123**, 69–79.
908 Pottinger, T.G. & Carrick, T.R. (1999) Modification of the plasma cortisol response to stress in rainbow
909 trout by selective breeding. *General and Comparative Endocrinology*, **116**, 122–132.
910 Pottinger, T.G. & Carrick, T.R. (2001) Stress responsiveness affects dominant-subordinate
911 relationships in rainbow trout. *Hormones and Behavior*, **40**, 419–427.

R Core Team. (2017) R: A language and environment for statistical computing.

Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V. & Montiglio, P.-O. (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **365**, 4051–4063.

Rex, A., Voigt, J.P., Voits, M. & Fink, H. (1998) Pharmacological evaluation of a modified open-field test sensitive to anxiolytic drugs. *Pharmacology Biochemistry and Behavior*, **59**, 677–683.

Romero, L.M. (2004) Physiological stress in ecology: Lessons from biomedical research. *Trends in Ecology and Evolution*, **19**, 249–255.

Romero, L.M., Dickens, M.J. & Cyr, N.E. (2009) The reactive scope model - A new model integrating homeostasis, allostasis, and stress. *Hormones and Behavior*, **55**, 375–389.

Ruiz-Gomez, M. de L., Huntingford, F.A., Øverli, Ø., Thörnqvist, P.-O. & Höglund, E. (2011) Response to environmental change in rainbow trout selected for divergent stress coping styles. *Physiology & Behavior*, **102**, 317–322.

Ruiz-Gomez, M. de L., Kittilsen, S., Höglund, E., Huntingford, F.A., Sørensen, C., Pottinger, T.G., Bakken, M., Winberg, S., Korzan, W.J. & Øverli, Ø. (2008) Behavioral plasticity in rainbow trout (Oncorhynchus mykiss) with divergent coping styles: When doves become hawks. *Hormones and Behavior*, **54**, 534–538.

Schjolden, J., Backström, T., Pulman, K.G.T., Pottinger, T.G. & Winberg, S. (2005) Divergence in behavioural responses to stress in two strains of rainbow trout (Oncorhynchus mykiss) with contrasting stress responsiveness. *Hormones and Behavior*, **48**, 537–544.

Segerstrom, S.C. & Miller, G.E. (2004) Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychological bulletin*, **130**, 601–30.

Self, S.G. & Liang, K. (1987) Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. *J. Am. Stat. Assoc.*, **82**, 605–610.

Sopinka, N.M., Patterson, L.D., Redfern, J.C., Pleizier, N.K., Belanger, C.B., Midwood, J.D., Crossin, G.T. & Cooke, S.J. (2015) Manipulating glucocorticoids in wild animals: Basic and applied perspectives. *Conservation Physiology*, **3**, 1–16.

Stamps, J.A. (2007) Growth-mortality tradeoffs and "personality traits" in animals. *Ecology Letters*, **10**,

940 355–363.
941 Stirling, D.G., Réale, D. & Roff, D.A. (2002) Selection, structure and the heritability of behaviour. *Journal*
942 *of Evolutionary Biology*, **15**, 277–289.
943 Stöwe, M., Rosivall, B., Drent, P.J. & Möstl, E. (2010) Selection for fast and slow exploration affects
944 baseline and stress-induced corticosterone excretion in Great tit nestlings, Parus major.
945 *Hormones and Behavior*, **58**, 864–871.
946 Taff, C.C. & Vitousek, M.N. (2016) Endocrine Flexibility: Optimizing Phenotypes in a Dynamic World?
947 *Trends in Ecology and Evolution*, **31**, 476–488.
948 Tarlow, E.M. & Blumstein, D.T. (2007) Evaluating methods to quantify anthropogenic stressors on wild
949 animals. *Applied Animal Behaviour Science*, **102**, 429–451.
950 Thomson, J.S., Watts, P.C., Pottinger, T.G. & Sneddon, L.U. (2011) Physiological and genetic correlates of
951 boldness: Characterising the mechanisms of behavioural variation in rainbow trout,
952 Oncorhynchus mykiss. *Hormones and Behavior*, **59**, 67–74.
953 Toms, C.N., Echevarria, D.J. & Jouandot, D.J. (2010) A Methodology Review of Personality-Related
954 Studies in Fish: Focus on the Shy-Bold Axis of Behaviour. *Berkeley Planning Journal*, **26**, 12.
955 Trenzado, C.E., Carrick, T.R. & Pottinger, T.G. (2003) Divergence of endocrine and metabolic responses
956 to stress in two rainbow trout lines selected for differing cortisol responsiveness to stress.
957 *General and Comparative Endocrinology*, **133**, 332–340.
958 Veenema, A.H., Meijer, O.C., de Kloet, E.R. & Koolhaas, J.M. (2003a) Genetic selection for coping style
959 predicts stressor susceptibility. *Journal of Neuroendocrinology*, **15**, 256–267.
960 Veenema, A.H., Meijer, O.C., De Kloet, E.R., Koolhaas, J.M. & Bohus, B.G. (2003b) Differences in basal and
961 stress-induced HPA regulation of wild house mice selected for high and low aggression.
962 *Hormones and Behavior*, **43**, 197–204.
963 Visscher, P.M. (2006) A Note on the Asymptotic Distribution of Likelihood Ratio Tests to Test Variance
964 Components. *Twin Research and Human Genetics*, **9**, 490–495.
965 White, S.J., Houslay, T.M. & Wilson, A.J. (2018) Evolutionary genetics of personality in the Trinidadian
966 guppy II: sexual dimorphism and genotype-by-sex interactions. *Heredity*.
967 White, S.J., Kells, T.J. & Wilson, A.J. (2016) Metabolism, personality and pace of life in the Trinidadian

968 guppy, Poecilia reticulata. *Behaviour*, **153**, 1517–1543.
969 White, S.J. & Wilson, A.J. (2018) Evolutionary genetics of personality in the Trinidadian guppy I:
970 maternal and additive genetic effects across ontogeny. *Heredity*.
971 Wickham, H. (2017) tidyverse: Easily Install and Load "Tidyverse" Packages.
972 Wilson, A.J., Réale, D., Clements, M.N., Morrissey, M.B., Postma, E., Walling, C.A., Kruuk, L.E.B. & Nussey,
973 D.H. (2010) An ecologist's guide to the animal model. *The Journal of animal ecology*, **79**, 13–26.
974 Wingfield, J.C. (2003) Control of behavioural strategies for capricious environments. *Animal Behaviour*,
975 **66**, 807–816.
976 Wingfield, J.C. & Kitaysky, A.S. (2002) Endocrine responses to unpredictable environmental events:
977 Stress or anti-stress hormones? *Integr Comp Biol*, **42**, 600–609.
978 Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M. & Richardson, R.D.
979 (1998) Ecological Bases of Hormone-Behavior Interactions: The "Emergency Life History Stage."
980 *American Zoologist*, **38**, 191–206.
981 Wolak, M.E. (2012) Nadiv: An R package to create relatedness matrices for estimating non-additive
982 genetic variances in animal models. *Methods in Ecology and Evolution*, **3**, 792–796.
983 Wolf, M., van Doorn, G.S., Leimar, O. & Weissing, F.J. (2007) Life-history trade-offs favour the evolution
984 of animal personalities. *Nature*, **447**, 581–4.
985 Zar, J.H. (1996) *Biostatistical Analysis*. Prentice-Hall International, Inc., London.
986