Stress activity is not predictive of coping style in North American red squirrels

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Keywords: coping styles, glucocorticoids, North American red squirrels, behavioral syndromes

Acknowledgments

We thank Agnes MacDonald and her family for long-term access to her trapline, and the Champagne and Aishihik First Nations for allowing us to conduct our work within their traditional territory. We thank Adi Boon, Amanda Kelley, and Ryan W. Taylor for collecting much of the behavioral data and all the volunteers, field assistants, and graduate students for their assistance in data collection. This work was supported by American Society of Mammalogists to SEW; University of Michigan to SEW and BD; National Science Foundation (IOS-1749627) to BD; and Natural Sciences and Engineering Research Council to SB, AGM, JL, and RB. This is publication XX of the Kluane Red Squirrel Project.

Abstract

Individuals vary in their behavioral and physiological responses to environmental changes. These behavioral responses are often described as 'coping styles' along a proactive-reactive continuum. Studies in laboratory populations often, but not always, find that behavioral responses and physiological responses to stressors covary, where more proactive (more aggressive and active) individuals have a lower physiological stress response, specifically as measured by hypothalamic-pituitary-adrenal (HPA) axis activity. These studies support the possibility of hormonal pleiotropy underlying the presentation of behaviors that make up the proactive-reactive phenotype. However, recent research in wild populations is equivocal, with some studies reporting the same pattern as found in many controlled laboratory studies, whereas others do not. We tested the hypothesis that physiological and behavioral stress responses are correlated in wild adult North American red squirrels (*Tamiasciurus hudsonicus*). We used fecal cortisol metabolites (FCMs) as a non-invasive, integrated estimate of circulating glucocorticoids for our measurement of HPA axis activity. We found that FCM concentrations were not correlated with three measures of behavioral coping styles (activity, aggression, and docility) among individuals. This does not support the hypothesis that hormonal pleiotropy underlies a proactive-reactive continuum of coping styles. Instead, our results support the "two-tier" hypothesis that behavioral and physiological stress responses are independent and uncorrelated traits among individuals in wild populations that experience naturally varying environments rather than controlled environments. If also found in other studies, this may alter our predictions about the evolutionary consequences of behavioral and endocrine coping styles in free-living animals.

Significance Statement

Individuals vary in how they respond to stressors through behavior and physiology, but we find the two responses are independent in wild animals. Many laboratory studies find links between the behavioral and physiological stress responses, however studies conducted with wild populations are less conclusive. In wild North American red squirrels, independence between the physiological response and behavioral response may allow adaptive responses to a changing environment without pleiotropic constraint.

Introduction

Organisms can respond to fluctuating environmental challenges and aversive stimuli both through behavioral responses and physiological stress responses. Laboratory studies often find these responses to be associated with one another (but see Steimer and Driscoll 2003; Koolhaas et al. 2007). In behavioral ecology and behavioral neuroscience, 'coping styles' have been recognized as one method of categorizing behavioral reactions to environmental challenges and stressors. Coping styles refer to a consistent set of behavioral responses to a stressor (Gosling 2001; Réale et al. 2007; Koolhaas et al. 2010; Stamps and Groothuis 2010). Furthermore, the suite of behaviors that make up an individual's coping style is theorized to be mediated by hormones that exert pleiotropic actions (Koolhaas et al. 1999; McGlothlin and Ketterson 2008).

This unidimensional model has been repeatedly supported by studies describing how the hypothalamic-pituitary-adrenal (HPA) axis mediates coping styles (Koolhaas et al. 1999). Many of these studies have used selected lines, or have been done under controlled conditions in the laboratory. The conclusion from this model is that the behavioral stress response and physiological stress response run along the same axis. This hypothesis suggests a unidimensional

73 response along a proactive-reactive continuum, where 'proactive' individuals are highly
74 aggressive, highly active, and exhibit lower HPA axis reactivity and activity compared to
75 'reactive' individuals (Koolhaas et al. 1999; Cockrem 2007; Carere et al. 2010). The vast
76 majority of these studies have been conducted using laboratory animals or wild animals selected
77 for specific behavioral phenotypes, producing individuals at the extremes of this behavioral
78 continuum. For example, in wild Great Tits (*Parus major*) lines selected for divergent
79 personality types show the predicted unidimensional relationship between behavioral and stress
80 responses in that more proactive birds exhibited lower HPA axis reactivity in response to capture
81 and restraint (Baugh et al. 2012), and lower baseline corticosterone metabolites (Stöwe et al.
82 2010).

83 As more empirical studies are testing these models, the results from studies in the wild
84 have been equivocal. Whereas there is some support for the unidimensional model in wild
85 animals (see Table 1), recent studies that have used this coping style paradigm to test the
86 relationship between behavior and HPA axis reactivity or activity in free-living animals have
87 found that the proactive-reactive continuum is not predictive of the physiological stress response
88 (Garamszegi et al. 2012; Ferrari et al. 2013; Dosmann et al. 2015). For example, though
89 laboratory selection line results are consistent with predictions of the unidimensional model,
90 when testing Great Tits in the laboratory with natural, non-selected variation in exploratory
91 behavior, the relationship no longer holds (Baugh et al. 2012).

92 Discrepancies in the lab between recent observed relationships (Koolhaas et al. 2007) and
93 the simple unidimensional model (Koolhaas et al. 1999), have recently led to the development of
94 a 'two-tier' coping style model. This two-tier model proposes that individuals in a population can
95 vary independently in both behavioral responses and physiological responses to environmental
96 challenges (Koolhaas et al. 2010). This model of coping styles reframed the original model to

97 establish behavioral coping strategies on a continuum independent of physiological coping
98 strategies (Koolhaas et al. 2010). The distinction between the unidimensional and two-tier coping
99 style models is significant in assessing the ecological and evolutionary consequences of variation
100 in response to stressors. If the phenotypic correlation between behavioral and physiological
101 stress responses (assumed by the unidimensional coping styles model) reflects an underlying
102 correlation mediated by the effects of a hormone, this may present a limitation in the ability of
103 populations to adapt to changing environmental stressors (Sih et al. 2004; Dantzer and Swanson
104 2017). Alternatively, if the two-tier model of coping style is supported, and there are two
105 separate axes for behavioral and physiological stress responses, this suggests the potential for
106 each trait to be an independent target of selection, potentially facilitating rapid adaptation to new
107 environmental challenges (McGlothlin and Ketterson 2008; Ketterson et al. 2009). Exploring
108 how coping styles relate to the physiological stress response in wild populations allows us to test
109 across the entire spectrum of naturally occurring individual variation in behavioral coping styles,
110 thus informing our perspective on how these mechanisms function in wild populations (Réale et
111 al. 2007; Ferrari et al. 2013).

112 We investigated the relationship between three behavioral traits and one measure of HPA
113 axis activity (concentrations of fecal cortisol metabolites, FCM) in a natural population of North
114 American red squirrels (*Tamiasciurus hudsonicus*, hereafter, 'red squirrels'). Previous studies in
115 this species showed that there was a repeatable, correlated suite of behavioral traits, specifically
116 aggression, activity, and docility, across the adulthood of an individual (Boon et al. 2007; Taylor
117 et al. 2012). These suites of behavioral traits can also be placed along the proactive-reactive
118 continuum as coping styles, with the more active, aggressive, and less docile individuals at the
119 proactive end of the continuum. Differences in coping styles in red squirrels have clear
120 environment-dependent fitness correlates (Boon et al. 2007, 2008; Taylor et al. 2014), and

121 variation in heritable coping styles among individuals (Taylor et al. 2012) in this population may
122 be maintained through fluctuating selection caused by changing environmental conditions
123 (Taylor et al. 2014).

124 We used fecal samples as a non-invasive proxy for HPA axis activity and reactivity,
125 which is unaffected by trapping-induced stress (Dantzer et al. 2010). In red squirrels, FCM is
126 representative of the circulating plasma cortisol over the past ~12 hours, with a 10.9 ± 2.3 hours
127 lag time to peak excretion following experimental administration of cortisol (Dantzer et al.
128 2010). Influences of the circadian rhythm on circulating cortisol are not detected in fecal samples
129 collected throughout the day (Dantzer et al. 2010). Additionally, glucocorticoid concentrations in
130 fecal samples have been shown to be representative of HPA activity and reactivity (Sheriff et al.
131 2011; Palme 2019).

132 It is important to note that glucocorticoids are metabolic hormones and only one
133 mediator of the reactive physiological stress response of an individual (Romero et al. 2009).
134 However evidence for both the unidimensional and two-tier models of coping styles specifically
135 connect glucocorticoids with the behavioral response (Table 1), in addition to catecholamines
136 (reviewed in Koolhaas et al. 1999, 2010). While this is not a perfect measure of the overall
137 physiological stress response of an individual, glucocorticoids are an important physiological
138 mediator of the multifaceted stress response (Sapolsky et al. 2000; Romero et al. 2009).
139 Glucocorticoids are secreted to mobilize energy in response to a stressor in the environment, but
140 also exert pleiotropic effects (Sapolsky et al. 2000). For example, fluctuating baseline
141 glucocorticoids act as a mediator of future reproductive investment in European Starlings
142 (*Sturnus vulgaris*) by preparing individuals for energetically expensive reproductive seasons
143 (Love et al. 2014).

144 To test the unidimensional and two-tier models of the overall stress response, we
145 measured FCM as a non-invasive marker of HPA axis activity and the behavior of individuals
146 using three behavioral assays (open-field trial, mirror-image stimulation trial, and handling
147 docility assay) to measure coping style. We then compared the FCM concentrations to the
148 behavioral coping style of individual squirrels. A relationship between FCMs and behavioral
149 coping style across individuals would support the unidimensional model, whereas a lack of
150 relationship would support the two-tier model.
151

152 **Methods**

153 *Study species*

154 North American red squirrels are a sexually monomorphic species of arboreal squirrels
155 (Boutin and Larsen 1993). Females and males are both territorial of their food-cache (located on
156 the center of their territory) year-round (Dantzer et al. 2012; Siracusa et al. 2017). Red squirrels
157 in the region of our study rely on seeds produced by white spruce (*Picea glauca*) trees as their
158 primary food source (Fletcher et al. 2010). Squirrel population density is closely associated with
159 mast seeding of the white spruce, or episodes of booms and busts in food availability (McAdam
160 and Boutin 2003; Fletcher et al. 2010; Dantzer et al., 2013). Red squirrels have one litter per
161 year, with the exception of mast years when autumn spruce seed is superabundant (Boutin et al.
162 2006; McAdam et al. 2007).

163 Our study was conducted as a part of the Kluane Red Squirrel Project, a long-term study
164 of wild population of red squirrels within Champagne and Aishihik First Nation traditional
165 territory along the Alaska Highway in the southwest Yukon, Canada (61°N, 138°W). Each
166 squirrel was tagged with a unique set of alphanumeric stamped ear tags (National Band and Tag
167 Company, Newport, KY, USA). At each live-trapping (Tomahawk Live Trap, Tomahawk, WI,

168 USA) event, body mass and reproductive status of the squirrel were recorded. Female
169 reproductive status was determined through changes in body mass, by nipple condition, and by
170 abdominal palpations of developing fetuses in females. Male reproductive status was determined
171 by palpating for the presence of testicles either in the scrotum (breeding) or abdomen (non-
172 breeding). A more detailed description of the population and general methods can be found in
173 McAdam et al. (2007).

174 The local population of red squirrels was broken down into three study populations in
175 different locations: two were control populations (referred to hereafter as 'control grids') and one
176 was provided with supplemental food between 2004 and 2017, such that squirrel density was
177 increased (Dantzer et al., 2013; hereafter referred to as 'high-density grid'). Squirrels on the
178 high-density grid were provided with 1 kg of peanut butter (no sugar or salt added)
179 approximately every six weeks from October to May (Dantzer et al. 2012). We included these
180 squirrels to increase our sample size, and included study grid type as a covariate in all models to
181 control for variation between the grids. Additionally, high conspecific competition is a
182 significant environmental factor influencing the physiological stress response of red squirrels
183 (Dantzer et al. 2013) and so was important to include as a covariate in our statistical models
184 (Table 2). All work was conducted under the animal ethics approvals from Michigan State
185 University (AUF#04/08-046-00) and University of Guelph (AUP#09R006).

186

187 *Behavioral trials*

188 Squirrels were subjected to two behavioral trials to measure 'activity' and 'aggression':
189 an open-field (OF) trial, and a mirror image stimulation (MIS) trial (Boon et al. 2007; Taylor et
190 al. 2012). These behavioral trials were conducted for other projects and were not evenly
191 distributed across years. We performed trials in 2005, 2008, 2009, 2010, and 2012 (see Table 2

192 for a breakdown of sample sizes). During these years, additional trials were conducted on
193 squirrels in this population for multiple studies (see Boon et al. 2007; Boon et al. 2008; Kelley et
194 al. 2015; Taylor et al. 2012, 2014), but for the purposes of this analyses, we only included trials
195 for which we also had FCM concentration data for that individual. All squirrels were mature
196 adults (>1 year old) at the time of the trial.

197 To measure an individual's coping style, we conducted OF and MIS trials during the
198 same trapping event, with the OF trial completed first followed by the MIS trial. Squirrels
199 habituate to these tests (Archer 1973; Boon et al. 2008; Martin and Réale 2008), but the behavior
200 of individual squirrels over time is known to be repeatable (Boon et al. 2007, 2008; Taylor et al.
201 2014). For simplicity, we thus used only the results of each individual's first test as a measure of
202 its activity and aggression. We transferred focal squirrels from a live trap into the arena using a
203 canvas handling bag. The same portable testing arena was used for both trials, and consisted of a
204 60 x 80 x 50 cm white corrugated plastic box with a clear acrylic lid (Taylor 2012). Four blind
205 holes made with black PVC caps in the bottom of arena allowed the squirrel to explore possible
206 'escape routes'. We exposed a 45 x 30 cm mirror fixed to one end of the arena after the OF trial
207 to begin the MIS trial. A digital video camera recorded behavior in the arena. We performed all
208 behavioral trials on the territory of the focal individual. Between trials, we cleaned the arena
209 using 70% isopropyl alcohol.

210 To quantify behavior from the videos, we used manual scoring methods with an ethogram
211 developed and used in previous red squirrel studies (Boon et al. 2007, 2008; Taylor et al. 2012,
212 2014; Kelley et al. 2015; Supplementary Material Table S1). Because these videos were
213 collected and scored across multiple years, observers used different software programs
214 depending on what program was available the year they were scored. Trials conducted in 2005
215 were scored using The Observer Video-Pro 5.0 (Noldus Information Technology, Wageningen,

216 The Netherlands). Trials conducted from 2008-2010 were scored using JWatcher Video 1.0
217 (Blumstein and Daniel 2007). Trials conducted in 2012 were scored using Cowlog software
218 (Hänninen and Pastell 2009). Regardless of the software used, the ethogram and the overall
219 method of scoring the videos remained consistent. Because this is a manual process and the
220 software simply records keystrokes indicating behaviors observed, it is not likely that the
221 software used impacted the score. Furthermore, a previous study using some of our dataset
222 showed high inter-observer reliability for the behavioral measures we recorded from these videos
223 (Taylor et al., 2012), so it again seems unlikely that the software used would influence the
224 behavioral data we extracted from the videos. During the OF trial, we recorded the mutually
225 exclusive behaviors of time spent walking, sniffing, chewing, rearing, grooming, and being still.
226 Additionally, we recorded the number of jumps and head-dips in the false holes. During the MIS
227 trial, we recorded the amount of time spent in the third of the arena closest to the mirror, and the
228 amount of time spent in the third of the arena farthest from the mirror. We also recorded the
229 number of aggressive contacts with the mirror (attacks), the latency until the first attack, and the
230 latency until the first approach towards the mirror. A detailed description of the video scoring
231 methods can be found in Boon et al. (2008). Following Taylor et al. (2012), behaviors with an
232 inter-observer reliability of greater than 0.7 were used in analyses (see Supplementary Material
233 Table S1 and S2 for a list of behaviors used in the analyses).
234 As an additional behavioral measurement, we also measured 'docility' of individual
235 squirrels. In 2008 and 2009, docility measurements were collected on many squirrels for other
236 studies (see Boon et al. 2007; Taylor et al. 2012), but for the purposes of this study, we focused
237 only on trials that were conducted during a trapping event where fecal samples were also
238 collected and subsequently analyzed (n = 168 trapping events). We quantified docility as the
239 squirrel's response to handling (for examples in other species, see Carere and Oers 2004; Martin

240 and Réale 2008; Montiglio et al. 2012). We transferred squirrels from the trap into a canvas
241 handling bag and placed the squirrel on a flat surface. We measured docility during handling by
242 counting the number of seconds out of 30 seconds in which the squirrel was not struggling. A
243 squirrel that spent most of the time immobile during the test was considered docile, a trait
244 previously demonstrated to be repeatable (Boon et al. 2007; Taylor et al., 2012) and heritable
245 (Taylor et al., 2012) in this population. This test was conducted an average of 8 (min = 1, max =
246 42) times on 79 individual squirrels caught between 2008 and 2009. Docility scores were z-
247 scored for analyses. See Table 2 for detailed sample sizes.
248
249 *Fecal cortisol metabolites*
250 From 2005 to 2014, we opportunistically collected a total of 703 fecal samples during
251 routine trapping of squirrels with peanut butter for measurement of FCM concentrations
252 corresponding to individuals with behavioral data (see Dantzer et al. 2010). Fecal samples were
253 collected from under live-traps within two hours of trapping and placed in 1.5 mL vials stored in
254 a -20 °C freezer within five hours of collection. Urine contaminated feces were excluded. All
255 fecal samples were lyophilized for 14-16 h before being pulverized in liquid nitrogen using a
256 mortar and pestle. Using 0.05 g of dry ground feces, steroid metabolites were extracted by
257 adding 1 mL of 80% methanol and vortexing samples at 1450 RPM for 30 min, and then
258 centrifuging for 15 min at 2500 g (Dantzer et al. 2010; Palme et al. 2013). The resulting
259 supernatant was stored at -20 °C for analysis via glucocorticoid metabolite assay using a 5α-
260 pregnane-3β,11β,21-triol-20-one antibody enzyme immunoassay (EIA; see Touma et al. 2003).
261 A detailed validation and description of steroid extraction and EIA with red squirrel fecal
262 samples can be found in Dantzer et al. (2010).

263 Fecal samples were analyzed across multiple assays and in two different labs (n = 355 at
264 University of Toronto Scarborough and n = 348 at University of Michigan) but using the same
265 protocol. We confirmed that our measures of FCM concentrations were highly repeatable across
266 assays or labs through the following. First, a separate group of fecal samples (n = 128 samples)
267 were analyzed in both labs and the optical density of these samples were closely correlated
268 (Pearson correlation = 0.88). This indicates that the data were comparable, but we also included a
269 covariate in our statistical models for where the data were analyzed (see below). Second, using
270 pooled samples that were run repeatedly on different plates (n = 115), we found that the
271 estimates of optical density for these pool samples were highly repeatable (R = 0.85, 95% CI =
272 0.54-0.93). Finally, using a linear mixed-effects model, we partitioned the variance in the optical
273 density recorded for the pooled samples that were run across these different plates. We found
274 that most of the variance was due to the sample itself (85.1%) with relatively little of it being
275 explained by intra-assay variation as all samples were run in duplicate (4.9%) or by inter-assay
276 variation (9.9%). Together, this indicates that our measures of FCM concentrations should be
277 comparable across assays and across labs. See Table 2 for a representation of how sample sizes
278 were broken down in each dataset.
279
280 *Statistical methods*
281 All statistical analyses were conducted in R version 3.4.3 (R Core Team 2016). For the
282 OF and MIS trials, we used two principal components analyses to reduce the redundancy among
283 behavioral measurements and calculate composite behavioral scores for each trial, as we have
284 done previously in this system (Boon et al. 2007, 2008; Taylor et al. 2012, 2014; Kelley et al.
285 2015). To conduct the principal components analyses with correlation matrices, we used the R
286 package 'ade4' version 1.7-10 (Dray and Dufour 2007). By reducing the multiple behaviors

287 observed down to one metric for each trial, we were able to assess the primary variation among
288 individuals along those axes. All further analyses used the scores calculated from the principal
289 component loadings (Supplementary Material Table S2) for each trial. From the OF trial, we
290 interpreted the first principal component as a measure of overall 'activity', as it has previously
291 been interpreted in this population (Boon et al. 2007, 2008; Taylor et al. 2012, 2014). In our data
292 set, the first component explained 64% of the variation in behavior across OF trials. From the
293 MIS trial, we interpreted the first principal component as a measure of 'aggression', as it has also
294 previously been interpreted (Boon et al. 2007, 2008; Taylor et al. 2012, 2014). In our data set,
295 the first component explained 60% of the variation in behavior across MIS trials.

296 Because we were interested in an estimate of the covariance of FCM concentrations and
297 personality, we used a multivariate framework to conservatively address how the two types of
298 stress responses interact, as explained in Houslay and Wilson (2017). In this study, we were
299 interested in how FCM concentrations and behavioral traits co-varied among individuals. To
300 investigate this in a multivariate framework, we used a Bayesian generalized linear mixed effects
301 multivariate model based on a Markov chain Monte Carlo algorithm with the R package
302 'MCMCglmm' version 2.25 (Hadfield 2010) to assess the relationship between FCM
303 concentrations and behavior. All fecal cortisol metabolite concentration data were ln-transformed
304 to improve normality of residuals.

305 For docility analyses, we used measurements of docility paired with the FCM
306 concentrations of that trapping event. Using a bivariate generalized linear mixed-effects model,
307 we asked whether individuals with higher mean FCM concentrations have higher mean docility
308 scores (among-individual covariance), and whether individual observations of FCM
309 concentration and docility relative to the individual's mean concentrations were correlated
310 (within-individual covariance). Within-individual covariance indicates how the FCM

311 concentrations and docility scores of one individual covary across multiple observations for that
312 individual; in essence if we have multiple unique measurements of FCM concentration and
313 docility from one individual, does the docility score predict FCM concentrations on that day? In
314 contrast, among-individual covariance measures the relationship between FCM concentration
315 and docility across individuals in the population; in other words, does an individual's average
316 docility score predict its average FCM concentration? The bivariate model for docility and FCM
317 concentration included fixed effects of study grid (control or high-density), sex, Julian date of
318 trapping event (continuous), trial number, breeding status (breeding/non-breeding/lactating), and
319 a variable to indicate where the fecal sample was processed (UT Scarborough/UM). Docility
320 measurements were taken across multiple trapping events for a squirrel, therefore we included
321 trial number to control for any variation caused by habituation to the process of being trapped
322 and restrained in the bag (Boon et al., 2007; Taylor et al., 2012). We specified in the model to
323 estimate the fixed effects of Julian date of trapping event and the location of fecal sample
324 processing for only FCM concentration. These fixed effects were included because they have
325 previously been shown to influence FCM concentration in red squirrels (Dantzer et al. 2010,
326 2013), and thus were included to control for variation among these variables. Although the
327 correlation between UT Scarborough and UM samples was high (0.88), we included location of
328 fecal sample processing to account for any minor variation between the locations.
329 Because we only had one measurement of aggression and activity per individual, we were
330 unable to estimate the within-individual covariance between FCM concentration and the
331 activity/aggression of that individual. Thus, the bivariate models for activity and aggression were
332 structured to control for only one individual activity and aggression score per individual, and
333 only estimate among-individual covariance. The models for activity and aggression included the
334 same fixed effects as the model for docility, except trial number was not included. All trials were

335 the first trial in the lifetime of that individual, so there is no variance in novelty of the arena
336 across individuals. Again, we estimated fixed effects of the Julian date of trapping event and
337 location of fecal sample processing for only FCM concentration. With this model structure, we
338 were able to more precisely control for variation in FCM concentration due to reproductive
339 condition and time of year.

340 We fit all bivariate MCMCglmm models with uninformative priors (as in Houslay and
341 Wilson 2017) for 2,100,000 iterations with the first 100,000 discarded, and 1 out of every 1,000
342 of the remaining iterations used for parameter estimations. Credible intervals (95%) around the
343 correlation were based on the MCMC chain iterations. To confirm convergence using a
344 combination of methods, as suggested in a comparative review (Cowles and Carlin 1996), we ran
345 all MCMCglmm models three times for comparison using the Gelman-Rubin statistic (Gelman
346 and Rubin 1992), and we also ran the Geweke diagnostic (Geweke 1992). All models passed
347 both diagnostics for convergence.

348

349 *Data availability*

350 The datasets analyzed during the current study available from the corresponding author on
351 reasonable request.

352

353 **Results**

354 Our results indicate that docility, activity, and aggression did not co-vary with FCM
355 concentrations among individuals. Using a bivariate generalized linear mixed effects model
356 approach, the within-individual covariance indicated that an individual's FCM concentrations
357 did not correlate with docility (r = 0.020, CI = [-0.14, 0.28]). Our models also indicated that
358 among individuals, FCM concentrations did not correlate with docility (r = 0.14, CI = [-0.64,

359 0.83], Figure 1), activity (r = 0.15, CI = [-0.17, 0.47], Figure 2) or aggression (r = 0.29, CI = [-
360 0.098, 0.56], Figure 2) (Table 3). Regardless of the statistical significance of these relationships,
361 the direction of the observed effect was opposite to the predicted relationship between behavior
362 and FCM concentrations. The direction of these correlations suggest more active and more
363 aggressive squirrels may have higher FCM concentrations, but this is not conclusive due to
364 confidence intervals overlapping zero.
365
366 **Discussion**
367 We demonstrated that the behavioral coping style (represented by three behaviors) and
368 one measure of the physiological stress response (FCM concentrations) did not co-vary in a free-
369 ranging mammal. Independent variation between the behavioral and physiological stress
370 responses supports the two-tier model of coping strategies proposed by Koolhaas et al. (2010).
371 This model proposes that within a species, individuals can exhibit a consistent behavioral
372 response anywhere along the proactive-reactive continuum but independent of their
373 physiological stress response, which can range from a low to a high HPA axis activity. Contrary
374 to many studies (Raulo and Dantzer, 2018), proactive, or highly active/aggressive red squirrels
375 did not always exhibit lower HPA axis activity than reactive individuals. In fact, the parameter
376 estimates were in the opposite direction from those predicted by the unidimensional model.
377 Specifically, the unidimensional model predicts that a more active behavioral stress response and
378 HPA axis activity should be negatively correlated and we found that they were instead positively
379 correlated, though again these confidence intervals overlapped zero. Although we did find that
380 the three behavioral measures were phenotypically correlated (see Supplementary Material),
381 physiological stress, as measured by FCM concentration, does not appear to be the pleiotropic
382 mechanism causing this covariation.

383 Previous studies that have found behavior and HPA axis reactivity are linked have used a
384 different statistical framework than our study. Thus, it is possible our results are simply the
385 outcome of using a more conservative statistical test. However, our results were robust across
386 statistical techniques as we also ran the same models using a different statistical technique (linear
387 mixed effects models) that has been used in previous studies (e.g. Lendvai et al. 2011; Montiglio
388 et al. 2012). These results from the linear mixed effects models (Supplementary Material Table
389 S5) and those from the bivariate models (presented above) both support the hypothesis that
390 behavior and physiology are independent in our study.

391 Our study contributes to an emerging trend of a lack of a strong relationship between
392 behavioral and physiological stress responses in wild and laboratory animals (reviewed by Raulo
393 and Dantzer 2018). For example, wild alpine marmots (*Marmota marmota*) exhibit a lack of
394 among-individual correlation between activity and plasma cortisol concentrations, as well as
395 between docility and plasma cortisol (Ferrari et al. 2013). Likewise, docility and exploration
396 were not correlated with a change in plasma cortisol in response to a stressor in plateau pika
397 (*Ochotona curzoniae*: Qu et al. 2018). Additional studies measuring fecal glucocorticoid
398 metabolites demonstrate that HPA axis activity does not correlate with shy-bold behavioral types
399 in wild flycatchers (*Ficedula albicollis*; Garamszegi et al. 2012), or with exploration/activity in
400 Belding's ground squirrels (*Spermophilus beldingi*; Dosmann et al. 2015). In captivity, Holstein
401 Friesian heifer calves (*Bos taurus*) HPA axis reactivity to ACTH is not correlated with their
402 response to novelty (Van Reenen et al. 2005).

403 The unidimensional model posits that both HPA axis activity and reactivity should be
404 lower in proactive animals (Koolhaas et al. 1999). However, it should be noted that
405 measurements of fecal glucocorticoid metabolites in red squirrels may not allow for direct
406 measurement of the reactivity of the HPA axis, which may correlate more strongly with

407 behavioral stress responses compared to basal regulation (Baugh et al. 2012). Although a study
408 on free-ranging eastern chipmunks (*Tamias striatus*) showed evidence supporting covariance of
409 behavioral response and physiological stress response from fecal samples, the study used only
410 one metric of physiological stress (coefficient of variation of fecal glucocorticoid metabolites)
411 per individual (Montiglio et al. 2012). This statistical method was limiting in that it did not
412 consider the uncertainty around each individual's measure of HPA axis activity. Our research
413 expands upon the chipmunk study by using more conservative statistical methods, which were
414 not widely used until recently (Houslay and Wilson 2017), in addition to linear models and
415 multiple behavioral assays to establish coping styles. Using both of these approaches, we showed
416 that the behavioral coping style (comprised of three correlated behaviors) does not covary with
417 one measure of physiological stress. We acknowledge that the studies included in Table 1 are
418 across multiple taxa, behaviors, and HPA axis activity measurements. This likely contributes to
419 the equivocal nature of support for these models in wild animals. Though we focused only on
420 wild animals in our brief review (Table 1), empirical studies using laboratory animals also
421 include variation in measurements. Due to the large variability across studies in measurements of
422 HPA axis activity and behavior, perhaps a less generalized model of the relationship between
423 behavior and physiological stress may be more predictive for future studies than our current
424 models.

425 Our study was conducted using adult red squirrels. In this population, high juvenile
426 mortality results in a high opportunity for selection during the first year of life (McAdam et al.
427 2007). Due to these strong selective pressures, we must consider the possibility that selection
428 may have already eroded the (co)variance of physiological and behavioral stress responses in
429 surviving adults. For example, perhaps juveniles with high covariance of the physiological and
430 behavioral stress response were unable to adaptively respond to environmental conditions,

431 whereas juveniles with low covariance were able to adaptively respond to conditions with the
432 two stress responses decoupled. Additionally, these selective pressures fluctuate across years
433 because red squirrels rely on a masting food source (white spruce) that goes through episodes of
434 booms and busts in production of reproductive cones (McAdam and Boutin 2003; Fletcher et al.
435 2010). Following the masting of spruce trees, squirrel populations increase in density, which may
436 generate density-dependent selection on juvenile traits (Dantzer et al. 2013; Fisher et al. 2017).
437 These fluctuations in selection may maintain genetic variation in behavioral traits in this
438 population (Taylor et al. 2014), and, if a pleiotropic hormone was the mechanism underlying
439 these behavioral correlations, it could limit an adaptive behavioral response to this fluctuating
440 selection if the selective forces on hormone levels and the behavior push in opposite directions
441 (Ketterson and Nolan Jr. 1999; McGlothlin and Ketterson 2008). For example, if a high activity
442 is beneficial in one environmental condition but high HPA axis reactivity is not, a strong
443 correlation between the two traits would constrain an individual's ability to show an adaptive
444 behavioral response to the current environmental conditions. Recent work, however, suggests a
445 hormonal pleiotropic relationship is likely not powerful enough to constrain independent
446 evolution of two traits (Dantzer and Swanson 2017). Alternatively, if the hormone does not show
447 a pleiotropic relationship with behavior and selection for both is working in the same direction,
448 then the absence of a correlation could slow their adaptive response relative to a situation with a
449 positive pleiotropic relationship between the two traits (Ketterson et al. 2009).
450 We also must consider the possibility that different behavioral traits are favored at
451 different life stages. Additional work in this study system has shown wider variation in these
452 behavioral traits among juvenile squirrels, with individuals at both extremes of the proactive-
453 reactive continuum, and individuals regress to the mean as they age (Kelley et al. 2015). This is a
454 potential limitation of our study. Studies using selection lines, and therefore individuals at

455 extremes in behavioral response, may therefore be more appropriate for making predictions
456 about juvenile red squirrels, though perhaps not appropriate for predictions about adults. In both
457 Great Tits and rainbow trout (*Oncorhynchus mykiss*), studies using exploration selection lines
458 have found evidence to support a correlation between behavioral and physiological stress
459 responses (Øverli et al. 2007; Baugh et al. 2012). This suggests the potential to detect a
460 relationship between behavioral and physiological stress responses in juvenile red squirrels,
461 when individuals are more widely dispersed along the proactive-reactive continuum. Future work
462 on the relationship between coping styles and physiological stress responses should investigate
463 the ontogeny of the relationship, and how it may change across life stages.

464 Our study helps establish a foundation to use in exploring the fitness consequences of
465 variability across two axes of the stress response, behavioral and physiological. Building upon
466 this current work, we have an opportunity to explore the mechanisms contributing to each axis of
467 variation independently. For instance, the maternal environment during ontogeny may influence
468 the development of the physiological stress axis, or contribute to the behavioral coping style
469 (reviewed in Meaney 2001). Furthermore, our study provides additional evidence supporting the
470 lack of direct phenotypic correlation between behavioral and physiological stress responses in
471 wild animals exhibiting natural variation in stress responses. Our study, in conjunction with
472 previous studies on these models in wild animals (Table 1), suggests a need for a more
473 generalizable model of the relationship between the behavioral and physiological stress
474 responses, perhaps taking into account the environmental conditions experienced by the species.
475 Further studies in wild animals are needed to explore the mechanisms underlying this variation
476 along the phenotypic landscape of the stress response and the adaptive value of such variation.

477 Studies on the relationship between the behavioral and physiological stress phenotypes in
478 wild animals in variable environments provide insight into the pleiotropic constraints on the

evolutionary paths these populations may take. Our study contributes to a growing body of work in support of the two-tier model of coping styles and physiological stress reactivity and activity in wild and laboratory populations. Specifically, our study demonstrated that the FCM concentration of wild red squirrels is independent of an individual's activity, aggression, and docility. Given that red squirrels in this region experience a fluctuating environment in terms of competitors (Dantzer et al. 2013), food (Boutin et al. 2006), and predators (O'Donoghue et al. 1998; Studd et al. 2014) and also fluctuating selection on behavioral traits (Boon et al., 2007; Taylor et al., 2014), having behavioral and physiological responses that are uncorrelated may be beneficial for adapting to this environmental variability. If similar results are found in other species, the lack of a phenotypic relationship between the behavioral and physiological stress responses could have important evolutionary implications, particularly for those species living in fluctuating environments.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflict of interest: The authors declare that they have no conflict of interest.

498 **References**

499 Archer J (1973) Tests for emotionality in rats and mice: A review. Anim Behav 21:205–235. doi:
500 10.1016/S0003-3472(73)80065-X

501 Baugh AT, Schaper S V., Hau M, et al (2012) Corticosterone responses differ between lines of
502 great tits (*Parus major*) selected for divergent personalities. Gen Comp Endocrinol
503 175:488–494. doi: 10.1016/j.ygcen.2011.12.012

504 Blumstein DT, Daniel JC (2007) Quantifying behavior the JWatcher way. Sinaeur Associates,
505 Inc., Sunderland, MA

506 Boon AK, Reale D, Boutin S (2008) Personality, habitat use, and their consequences for survival
507 in North American red squirrels *Tamiasciurus hudsonicus*. Oikos 117:1321–1328. doi:
508 10.1111/j.2008.0030-1299.16567

509 Boon AK, Réale D, Boutin S (2007) The interaction between personality, offspring fitness and
510 food abundance in North American red squirrels. Ecol Lett 10:1094–1104. doi:
511 10.1111/j.1461-0248.2007.01106.x

512 Boutin S, Larsen KW (1993) Does food availability affect growth and survival of males and
513 females differently in a promiscuous small mammal, *Tamiasciurus hudsonicus*? J Anim
514 Ecol 62:364–370. doi: 10.2307/5367

515 Boutin S, Wauters LA, McAdam AG, et al (2006) Anticipatory reproduction and population
516 growth in seed predators. Science 314:1928–1930. doi: 10.1126/science.1135520

517 Carere C, Caramaschi D, Fawcett TW (2010) Covariation between personalities and individual
518 differences in coping with stress: Converging evidence and hypotheses. Curr Zool 56:728–
519 741

520 Carere C, Oers K Van (2004) Shy and bold great tits (*Parus major*): body temperature and breath
521 rate in response to handling stress. Physiol Behav 82:905–912. doi:

522 10.1016/j.physbeh.2004.07.009
523 Cockrem JF (2007) Stress, corticosterone responses and avian personalities. J Ornithol 148:169–
524 178. doi: 10.1007/s10336-007-0175-8
525 Cowles MK, Carlin BP (1996) Markov chain Monte Carlo convergence diagnostics: A
526 comparative review. J Am Stat Assoc 91:883–904. doi: 10.1080/01621459.1996.10476956
527 Dantzer B, Boutin S, Humphries MM, McAdam AG (2012) Behavioral responses of territorial
528 red squirrels to natural and experimental variation in population density. Behav Ecol
529 Sociobiol 66:865–878. doi: 10.1007/s00265-012-1335-2
530 Dantzer B, McAdam AG, Palme R, et al (2010) Fecal cortisol metabolite levels in free-ranging
531 North American red squirrels: Assay validation and the effects of reproductive condition.
532 Gen Comp Endocrinol 167:279–286. doi: 10.1016/j.ygcen.2010.03.024
533 Dantzer B, Newman AEM, Boonstra R, et al (2013) Density triggers maternal hormones that
534 increase adaptive offspring growth in a wild mammal. Science 340:1215–1217. doi:
535 10.1126/science.1235765
536 Dantzer B, Swanson EM (2017) Does hormonal pleiotropy shape the evolution of performance
537 and life history traits? Integr Comp Biol 57:372–384. doi: 10.1093/icb/icx064
538 Dosmann AJ, Brooks KC, Mateo JM (2015) Within-individual correlations reveal link between a
539 behavioral syndrome, condition, and cortisol in free-ranging Belding's ground squirrels.
540 Ethology 121:125–134. doi: 10.1111/eth.12320
541 Dray S, Dufour A-B (2007) The ade4 package: implementing the duality diagram for ecologists.
542 J Stat Softw 22:. doi: 10.18637/jss.v022.i04
543 Ferrari C, Pasquaretta C, Carere C, et al (2013) Testing for the presence of coping styles in a
544 wild mammal. Anim Behav 85:1385–1396. doi: 10.1016/j.anbehav.2013.03.030
545 Fisher DN, Boutin S, Dantzer B, et al. (2017) Multilevel and sex-specific selection on

competitive traits in North American red squirrels. Evolution 71:1841–1854. doi: 10.1111/evo.13270

Fletcher QE, Boutin S, Lane JE, et al (2010) The functional response of a hoarding seed predator to mast seeding. Ecology 91:2673–2683. doi: 10.1890/09-1816.1

Garamszegi LZ, Rosivall B, Rettenbacher S, et al (2012) Corticosterone, avoidance of novelty, risk-taking and aggression in a wild bird: No evidence for pleiotropic effects. Ethology 118:621–635. doi: 10.1111/j.1439-0310.2012.02049.x

Gelman A, Rubin DB (1992) Inference from iterative simulation using multiple sequences. Stat Sci 7:457–472. doi: 10.1214/ss/1177011136

Geweke J (1992) Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. In: Bernardo J, Berger A, Dawid A, Smith A (eds) Bayesian Statistics 4. Oxford University Press, Oxford, pp 169–193

Gosling SD (2001) From mice to men: what can we learn about personality from animal research? Psychol Bull 127:45–86. doi: 10.1037/0033-2909.127.1.45

Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. J Stat Softw 33:. doi: 10.18637/jss.v033.i02

Hänninen L, Pastell M (2009) CowLog: Open-source software for coding behaviors from digital video. Behav Res Methods 41:472–476. doi: 10.3758/BRM.41.2.472

Houslay TM, Wilson AJ (2017) Avoiding the misuse of BLUP in behavioural ecology. Behav Ecol 28:948–952. doi: 10.1093/beheco/arx023

Kelley AD, Humphries MM, McAdam AG, Boutin S (2015) Changes in wild red squirrel personality across ontogeny: activity and aggression regress towards the mean. Behaviour 152:1291–1306. doi: 10.1163/1568539X-00003279

Ketterson ED, Atwell JW, McGlothlin JW (2009) Phenotypic integration and independence:

570 Hormones, performance, and response to environmental change. Integr Comp Biol 49:365–
571 379. doi: 10.1093/icb/icp057
572 Ketterson ED, Nolan, Jr. V (1999) Adaptation, exaptation, and constraint: A hormonal
573 perspective. Am Nat 154:S4–S25. doi: 10.1086/303280
574 Koolhaas JM, De Boer SF, Buwalda B, Van Reenen K (2007) Individual variation in coping with
575 stress: A multidimensional approach of ultimate and proximate mechanisms. Brain Behav
576 Evol 70:218–226. doi: 10.1159/000105485
577 Koolhaas JM, de Boer SF, Coppens CM, Buwalda B (2010) Neuroendocrinology of coping
578 styles: Towards understanding the biology of individual variation. Front Neuroendocrinol
579 31:307–321. doi: 10.1016/j.yfrne.2010.04.001
580 Koolhaas JM, Korte SM, De Boer SF, et al (1999) Coping styles in animals: Current status in
581 behavior and stress-physiology. Neurosci Biobehav Rev 23:925–935. doi: 10.1016/S0149-
582 7634(99)00026-3
583 Lendvai ÁZ, Bókony V, Chastel O (2011) Coping with novelty and stress in free-living house
584 sparrows. J Exp Biol 214:821–828. doi: 10.1242/jeb.047712
585 Love OP, Madliger CL, Bourgeon S, et al. (2014) Evidence for baseline glucocorticoids as
586 mediators of reproductive investment in a wild bird. Gen Comp Endocrinol 199:65–69. doi:
587 10.1016/j.ygcen.2014.01.001
588 Martin JGA, Réale D (2008) Temperament, risk assessment and habituation to novelty in eastern
589 chipmunks, *Tamias striatus*. Anim Behav 75:309–318. doi: 10.1016/j.anbehav.2007.05.026
590 McAdam AG, Boutin S (2003) Variation in viability selection among cohorts of juvenile red
591 squirrels (*Tamiasciurus hudsonicus*). Evolution 57:1689–1697. doi: 10.1111/j.0014-
592 3820.2003.tb00374.x
593 McAdam AG, Boutin S, Sykes AK, Humphries MM (2007) Life histories of female red squirrels

594 and their contributions to population growth and lifetime fitness. Ecoscience 14:362. doi:
595 10.2980/1195-6860(2007)14[362:LHOFRS]2.0.CO;2
596 McGlothlin JW, Ketterson ED (2008) Hormone-mediated suites as adaptations and evolutionary
597 constraints. Philos Trans R Soc B Biol Sci 363:1611–1620. doi: 10.1098/rstb.2007.0002
598 Meaney MJ (2001) Maternal care, gene expression, and the transmission of individual
599 differences in stress reactivity across generations. Annu Rev Neurosci 24:1161–1192
600 Montiglio P-O, Garant D, Pelletier F, Réale D (2012) Personality differences are related to long-
601 term stress reactivity in a population of wild eastern chipmunks, *Tamias striatus*. Anim
602 Behav 84:1071–1079. doi: 10.1016/j.anbehav.2012.08.010
603 O'Donoghue M, Boutin S, Krebs CJ, et al (1998) Behavioural responses of coyotes and lynx to
604 the snowshoe hare cycle. Oikos 82:169. doi: 10.2307/3546927
605 Øverli Ø, Sørensen C, Pulman KGT, et al (2007) Evolutionary background for stress-coping
606 styles: Relationships between physiological, behavioral, and cognitive traits in non-
607 mammalian vertebrates. Neurosci Biobehav Rev 31:396–412. doi:
608 10.1016/j.neubiorev.2006.10.006
609 Palme R (2019) Non-invasive measurement of glucocorticoids: Advances and problems. Physiol
610 Behav 199:229–243. doi: 10.1016/j.physbeh.2018.11.021
611 Palme R, Touma C, Arias N, et al (2013) Steroid extraction: Get the best out of faecal samples.
612 Wiener Tierärztliche Monatsschrft 100:238–246
613 Qu J, Fletcher QE, Réale D, et al (2018) Independence between coping style and stress reactivity
614 in plateau pika. Physiol Behav 197:1–8. doi: 10.1016/j.physbeh.2018.09.007
615 Raulo A, Dantzer B (2018) Associations between glucocorticoids and sociality across a
616 continuum of vertebrate social behavior. Ecol Evol 1–20. doi: 10.1002/ece3.4059
617 Réale D, Reader SM, Sol D, et al (2007) Integrating animal temperament within ecology and

618 evolution. Biol Rev 82:291–318. doi: 10.1111/j.1469-185X.2007.00010.x
619 Romero LM, Dickens MJ, Cyr NE (2009) The reactive scope model - A new model integrating
620 homeostasis, allostasis, and stress. Horm Behav 55:375–389. doi:
621 10.1016/j.yhbeh.2008.12.009
622 Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress
623 responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr
624 Rev 21:55–89. doi: 10.1210/er.21.1.55
625 Sheriff MJ, Dantzer B, Delehanty B, et al (2011) Measuring stress in wildlife: Techniques for
626 quantifying glucocorticoids. Oecologia 166:869–887. doi: 10.1007/s00442-011-1943-y
627 Sih A, Bell AM, Johnson JC, Ziemba RE (2004) Behavioral syndromes: An integrative
628 overview. Q Rev Biol 79:241–277. doi: 10.1086/422893
629 Siracusa E, Morandini M, Boutin S, et al (2017) Red squirrel territorial vocalizations deter
630 intrusions by conspecific rivals. Behaviour 154:1259–1273. doi: 10.1163/1568539X-
631 00003467
632 Stamps J, Groothuis TGG (2010) The development of animal personality: Relevance, concepts
633 and perspectives. Biol Rev 85:301–325. doi: 10.1111/j.1469-185X.2009.00103.x
634 Steimer T, Driscoll P (2003) Divergent stress responses and coping styles in psychogenetically
635 selected Roman high-(RHA) and low-(RLA) avoidance rats: Behavioural, neuroendocrine
636 and developmental aspects. Stress 6:87–100. doi: 10.1080/1025389031000111320
637 Stöwe M, Rosivall B, Drent PJ, Möstl E (2010) Selection for fast and slow exploration affects
638 baseline and stress-induced corticosterone excretion in Great tit nestlings, Parus major.
639 Horm Behav 58:864–871. doi: 10.1016/j.yhbeh.2010.08.011
640 Studd EK, Boutin S, McAdam AG, et al (2014) Predators, energetics and fitness drive neonatal
641 reproductive failure in red squirrels. J Anim Ecol 84:249–259. doi: 10.1111/1365-

642 2656.12279
643 Taylor RW (2012) Quantitative genetics, selection, mate choice and red squirrel behavior in a
644 fluctuating environment. Michigan State University
645 Taylor RW, Boon AK, Dantzer B, et al. (2012) Low heritabilities, but genetic and maternal
646 correlations between red squirrel behaviours. J Evol Biol 25:614–624. doi: 10.1111/j.1420-
647 9101.2012.02456.x
648 Taylor RW, Boutin S, Humphries MM, McAdam AG (2014) Selection on female behaviour
649 fluctuates with offspring environment. J Evol Biol 27:2308–2321. doi: 10.1111/jeb.12495
650 Touma C, Sachser N, Möstl E, Palme R (2003) Effects of sex and time of day on metabolism and
651 excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol 130:267–278.
652 doi: 10.1016/S0016-6480(02)00620-2
653 Van Reenen CG, O'Connell NE, Van der Werf JTN, et al (2005) Responses of calves to acute
654 stress: Individual consistency and relations between behavioral and physiological measures.
655 Physiol Behav 85:557–570. doi: 10.1016/j.physbeh.2005.06.015
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Figure 1. Stress reactivity and activity, as measured by average FCM concentration, is not predicted by docility in North American red squirrels. Size of the points represents number of fecal samples included for that individual.

Figure 2. Stress reactivity and activity, as measured by average FCM concentration, is not predicted by activity or aggression in North American red squirrels. Activity and aggression are from scores determined by the principal component analyses. Size of the points represents number of fecal samples included for that individual.

Table 1. Review of field studies testing for the covariance in behavioral coping styles and HPA activity

This non-comprehensive table includes studies conducted with natural populations testing the unidimensional (Koolhaas et al. 1999b) and two-tier models (Koolhaas et al. 2010) of the among individual relationship between behavioral coping styles ('Behavioral Trait') and HPA axis activity ('Physiological Measurement'). The first section includes studies that support the main prediction from the unidimensional model that more proactive individuals have lower HPA axis activity. The second section includes studies that are contrary to the main prediction from the unidimensional model that more proactive individuals would have lower HPA axis activity. These studies do show that behavior and HPA activity covary, but the relationship is in the opposite of the direction predicted by the unidimensional model with proactive individuals having lower HPA axis activity. The third section includes studies that support the two-tier model that predicts that behavior and HPA axis activity do not covary in either direction. Correlations or estimates are included if available in the corresponding manuscript. Confidence/credible intervals are included if available; if not, p-values are included when available. If the confidence/credible interval overlapped zero, we interpreted this as the behavior trait measured and the physiological measurement did not covary. Non-true baseline samples were the first sample collected but involved some handling or trapping stress. (n = sample size of individuals, NS = non-significant ($p > 0.05$), FCM = fecal cortisol metabolites, DEX = dexamethasone (synthetic corticosteroid that exerts negative feedback on the HPA axis), ACTH = adrenocorticotropic hormone that increases adrenocortical activity)

Species	Behavioral Trait	Physiological Measurement	n	Correlation or Estimate [CI]
Evidence supporting the predictions of the unidimensional model that behavioral and physiological traits are negatively correlated				
Great Tits (*Parus major*) (Baugh et al. 2017)	Exploration	Blood corticosterone *(ACTH challenge induced)*	85	$R^2 = 0.051$, $p = 0.04$
Great Tits (*Parus major*) (Baugh, van Oers, Naguib, & Hau, 2013)	Exploration	Blood corticosterone *(90-min handling-restraint stress-induced)*	16	b = -0.417, $p = 0.015$
	Exploration	Blood corticosterone *(area under the stress-induced curve)*	16	b = -24.66, $p = 0.007$
Belding's ground squirrels (*Urocitellus belingi*) (Clary et al. 2014)	Vigilance	FCM *(nominal baseline)*	12	b = 2.109 [0.17, 4.05]
Brook charr (*Salvelinus fontinalis*) (Farwell et al. 2014)	Activity	Whole-body cortisol *(baseline and handling/novel object stress-induced samples)*	66	NA*
House Sparrows (*Passer domesticus*) (Lendvai et al. 2011)	Hovering	Blood corticosterone *(30 min handling-restraint stress-induced)*	18	Pearson's r = -0.58, $p = 0.017$
Eastern chipmunks (*Tamias striatus*) (Montiglio et al. 2012)	Exploration	FCM *(coefficient of variation)*	58	b = -13.68 [-27.62, -4.45]
Plateau pika (*Ochotona curzoniae*) (Qu et al. 2018)	Shyness	Blood cortisol *(true baseline)*	292	posterior R = 0.45 [0.09, 0.66]
Evidence in the opposite direction of predictions of the unidimensional model				
Poeciliid fish (*Brachyrhaphis episcopi*) (Archard et al. 2012)	Exploration, Activity	Water-borne cortisol *(open-field stress-induced)*	96	Pearson's r = -0.29, $p = 0.005$
Great Tits (*Parus major*) (Baugh et al. 2013)	Exploration	Blood corticosterone *(true baseline)*	82	b = 0.536, $p = 0.003$
Alpine marmots (*Marmota marmota*) (Costantini et al. 2012)	Exploration, Activity	Blood cortisol *(non-true baseline)*	28	b = 0.54, Fischer *C*-statistic model selection
Graylag Geese (*Anser anser*) (Kralj-Fišer et al. 2009)	Aggression	FCM *(handling stress-induced)*	10	Spearman's r = 0.782, $p = 0.008$
Evidence supporting the predictions of the two-tier model that behavioral and physiological traits are not significantly correlated				

Species	Behavioral Trait	Physiological Measurement	n	Correlation or Estimate [CI]
Great Tits (*Parus major*)	Exploration	Blood corticosterone *(handling-restrain stress-induced)*	85	$R^2 = 0.03$, $p = 0.14$
(Baugh, Davidson, Hau, & van Oers, 2017)	Exploration	Blood corticosterone *(DEX challenge response)*	85	$R^2 = 0.02$, $p = 0.20$
Belding's ground squirrels (*Urocitellus belingi*)	Exploration	FCM *(non-true baseline)*	12	b = -0.76 [-1.12, 0.98]
(Clary et al., 2014)				
Alpine marmots (*Marmota marmota*)	Exploration, Activity	Blood cortisol *(pre-restraint to post-restraint response)*	28	NA**
(Costantini et al., 2012)				
Belding's ground squirrels (*Urocitellus belingi*)	Activity	FCM *(non-true baseline)*	157	posterior R = -0.007, [-0.123, 0.095]
(Dosmann et al. 2015)	Exploration	FCM *(non-true baseline)*	157	posterior R = -0.046, [-0.175, 0.036]
	Docility	FCM *(non-true baseline)*	157	posterior R = 0.016, [-0.077, 0.139]
Alpine marmots (*Marmota marmota*)	Activity	Blood cortisol *(non-true baseline)*	146	posterior R = 0.04, [-0.56, 0.71]
(Ferrari et al. 2013)	Impulsivity	Blood cortisol *(non-true baseline)*	146	posterior R = 0.08, [-0.68, 0.62]
	Docility	Blood cortisol *(non-true baseline)*	146	posterior R = 0.14, [-0.64, 0.63]
Collard Flycatchers (*Ficedula albicollis*)	Novel object avoidance	FCM *(non-true baseline)*	51	Pearson's r = -0.017, [-0.291, 0.260]
(Garamszegi et al. 2012)	Aggression	FCM *(non-true baseline)*	56	Pearson's r = -0.076, [-0.332, 0.191]
	Risk-taking	FCM *(non-true baseline)*	54	Pearson's r = 0.074, [-0.198, 0.335]
Nazca Boobies (*Sula granti*)	Gardening (non-social)	Blood corticosterone *(true baseline)*	222	b = 0.26, [-0.09, 0.61]
(Grace and Anderson 2014)	Shaking (non-social)	Blood corticosterone *(true baseline)*	222	b = -0.06, [-0.25, 0.13]
	Aggression (non-social)	Blood corticosterone *(true baseline)*	222	b = 0.19, [-0.07, 0.45]
	Gardening (non-social)	Blood corticosterone *(maximum value across 4 time points during capture-restraint)*	222	b = -0.30, [-0.98, 0.38]
	Shaking (non-social)	Blood corticosterone *(maximum value across 4 time points during capture-restraint)*	222	b = -0.08, [-0.44, 0.28]
	Aggression (non-social)	Blood corticosterone *(maximum value across 4 time points during capture-restraint)*	222	b = -0.07, [-0.35, 0.21]
	Gardening (non-social)	Blood corticosterone *(area under the curve across 4 time points during capture-restraint)*	222	b = -0.16, [-0.7, 0.38]
	Shaking (non-social)	Blood corticosterone *(area under the curve across 4 time points during capture-restraint)*	222	NA, CI in figure includes 0
	Aggression (non-social)	Blood corticosterone *(area under the curve across 4 time points during capture-restraint)*	222	NA, CI in figure includes 0
	Gardening (social)	Blood corticosterone *(true baseline)*	222	b = 0.20, [0.02, 0.38]
	Shaking (social)	Blood corticosterone *(true baseline)*	222	b = -0.04 [-0.18, 0.1]

Species	Behavioral Trait	Physiological Measurement	n	Correlation or Estimate [CI]
	Aggression (social)	Blood corticosterone *(true baseline)*	222	b = -0.02 [-0.11, 0.07]
	Gardening (social)	Blood corticosterone *(maximum value across 4 time points during capture-restraint)*	222	b = -0.03 [-0.23, 0.17]
	Shaking (social)	Blood corticosterone *(maximum value across 4 time points during capture-restraint)*	222	b = 0.04 [-0.2, 0.28]
	Aggression (social)	Blood corticosterone *(maximum value across 4 time points during capture-restraint)*	222	b = -0.01 [-0.02, 0]
	Gardening (social)	Blood corticosterone *(area under the curve across 4 time points during capture-restraint)*	222	b = 0.02 [-0.15, 0.19]
	Shaking (social)	Blood corticosterone *(area under the curve across 4 time points during capture-restraint)*	222	NA, CI in figure includes 0
	Aggression (social)	Blood corticosterone *(area under the curve across 4 time points during capture-restraint)*	222	NA, CI in figure includes 0
Graylag Geese (*Anser anser*)	Aggression	FCM *(non-true baseline)*	10	Spearman's r = -0.503, NS
(Kralj-Fišer et al. 2009)	Sociability	FCM *(non-true baseline)*	10	Spearman's r = -0.212, NS
	Sociability	FCM *(handling stress-induced)*	10	Spearman's r = -0.127, NS
House Sparrows (*Passer domesticus*)	Exploration	Blood corticosterone *(30 min handling-restraint stress-induced)*	18	Pearson's r = -0.07, *p* = 0.797
(Lendvai et al. 2011)	Shy-bold	Blood corticosterone *(30 min handling-restraint stress-induced)*	18	Pearson's r = -0.04, *p* = 0.866
	Exploration	Blood corticosterone *(true baseline)*	18	Pearson's r = -0.18, *p* = 0.487
	Shy-bold	Blood corticosterone *(true baseline)*	18	Pearson's r = -0.07, *p* = 0.801
	Hovering	Blood corticosterone *(true baseline)*	18	Pearson's r = -0.14, *p* = 0.573
Plateau pika (*Ochotona curzoniae*)	Docility, in cage	Blood cortisol *(true baseline)*	292	posterior R = 0.25 [-0.02, 0.63]
(Qu et al. 2018)	Docility, in bag	Blood cortisol *(true baseline)*	292	posterior R = -0.06 [-0.28, 0.31]
	Exploration	Blood cortisol *(true baseline)*	292	posterior R = -0.16 [-0.52, 0.05]
	Docility, in cage	Blood cortisol *(40 min post-capture change from baseline)*	292	posterior R = 0.22 [-0.06, 0.47]
	Docility, in bag	Blood cortisol *(40 min post-capture change from baseline)*	292	posterior R = 0.16 [-0.07, 0.48]
	Exploration	Blood cortisol *(40 min post-capture change from baseline)*	292	posterior R = 0.10 [-0.42, 0.24]
	Shyness	Blood cortisol *(40 min post-capture change from baseline)*	292	posterior R = -0.23 [-0.28, 0.22]

4

685 * Used AICc model selection approach. All top models included activity as predictor of cortisol.
686 Based on figure in text, the top model indicates more active individuals have less whole-body
687 cortisol.
688 ** Used nested path model selection approach. Based on figure in text, the top model does not
689 include a relationship between change in cortisol and coping style.
690

691 **Table 2. Bivariate model structures and sample sizes.** This table breaks down the sample sizes and the variables (fixed and random
692 effects) considered in each bivariate mixed effects model. Response variables shown are for the bivariate models that tested the
693 association between behavioral traits (docility, activity, or aggression) and a measure of the physiological stress response (fecal
694 cortisol metabolites or FCM). Italics indicate fixed effects estimated for FCM concentration only.

Model	Response Variables	Fixed Effects	Random Effect	Year	# of individuals	# of fecal samples
1	docility, FCM	study grid (control or high-density) sex docility trial number breeding, non-breeding, or lactating *fecal processing lab* *Julian date of trapping event*	squirrel ID			
				2008:	32	36
				2009:	56	132
				total:	**79***	**168**
2	activity, FCM	study grid (control or high-density) sex breeding, non-breeding, or lactating *fecal processing lab* *Julian date of trapping event*	squirrel ID	2005:	2	9
				2008:	27	139
				2009:	15	117
				2010:	1	10
				2012:	28	260
				total:	**72***	**484**
3	aggression, FCM	study grid (control or high-density) sex breeding, non-breeding, or lactating *fecal processing lab* *Julian date of trapping event*	squirrel ID	2005:	2	9
				2008:	27	139
				2009:	15	117
				2010:	1	10
				2012:	28	260
				total:	**72***	**484**

695 *Some squirrels were sampled in multiple years.

696 **Table 3. Multivariate results for relationships between FCM and behaviors**

697 Results from our three bivariate generalized linear mixed-effects model models to examine the
698 relationships between FCM and each of three behaviors individually (activity and aggression
699 models: n = 484 fecal samples; docility model: n = 168 fecal samples). Among-individual
700 variances are listed on the diagonal, covariances below and correlations above (with the lower
701 and upper bounds of 95% CIs in parentheses).

	ln(FCM)	**Docility**
ln(FCM)	0.049 [1.86-8, 0.13]	0.14 [-0.63, 0.83]
Docility	0.012 [-0.074, 0.11]	0.40 [0.15, 0.71]

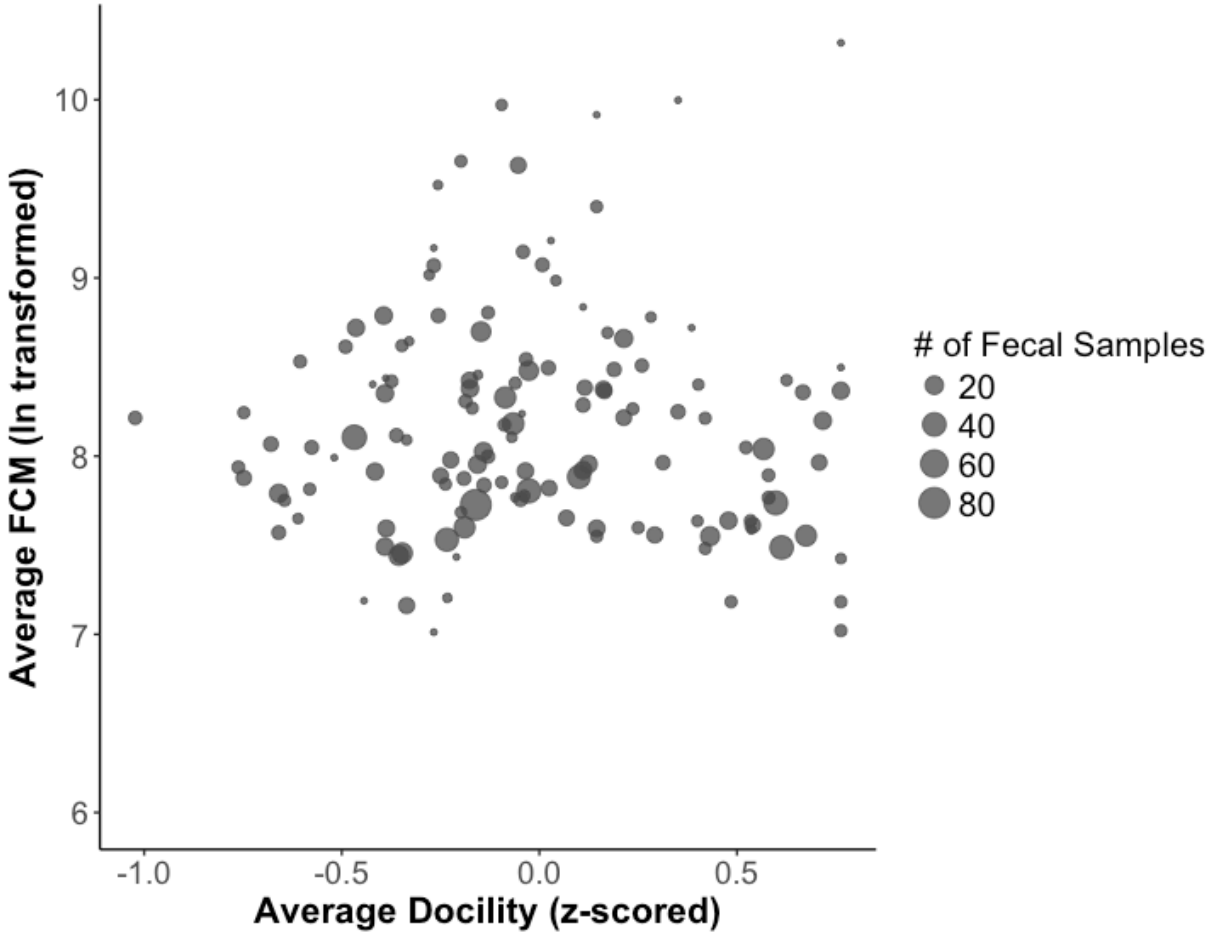
	ln(FCM)	**Activity**
ln(FCM)	0.075 [0.018, 0.14]	0.15 [-0.17, 0.47]
Activity	0.04 [-0.034, 0.11]	0.55 [0.38, 0.73]

	ln(FCM)	**Aggression**
ln(FCM)	0.077 [0.016, 0.14]	0.29 [-0.098, 0.56]
Aggression	0.061 [-0.03, 0.16]	1.00 [0.68, 1.33]

702

703

704 **Figure 1.**



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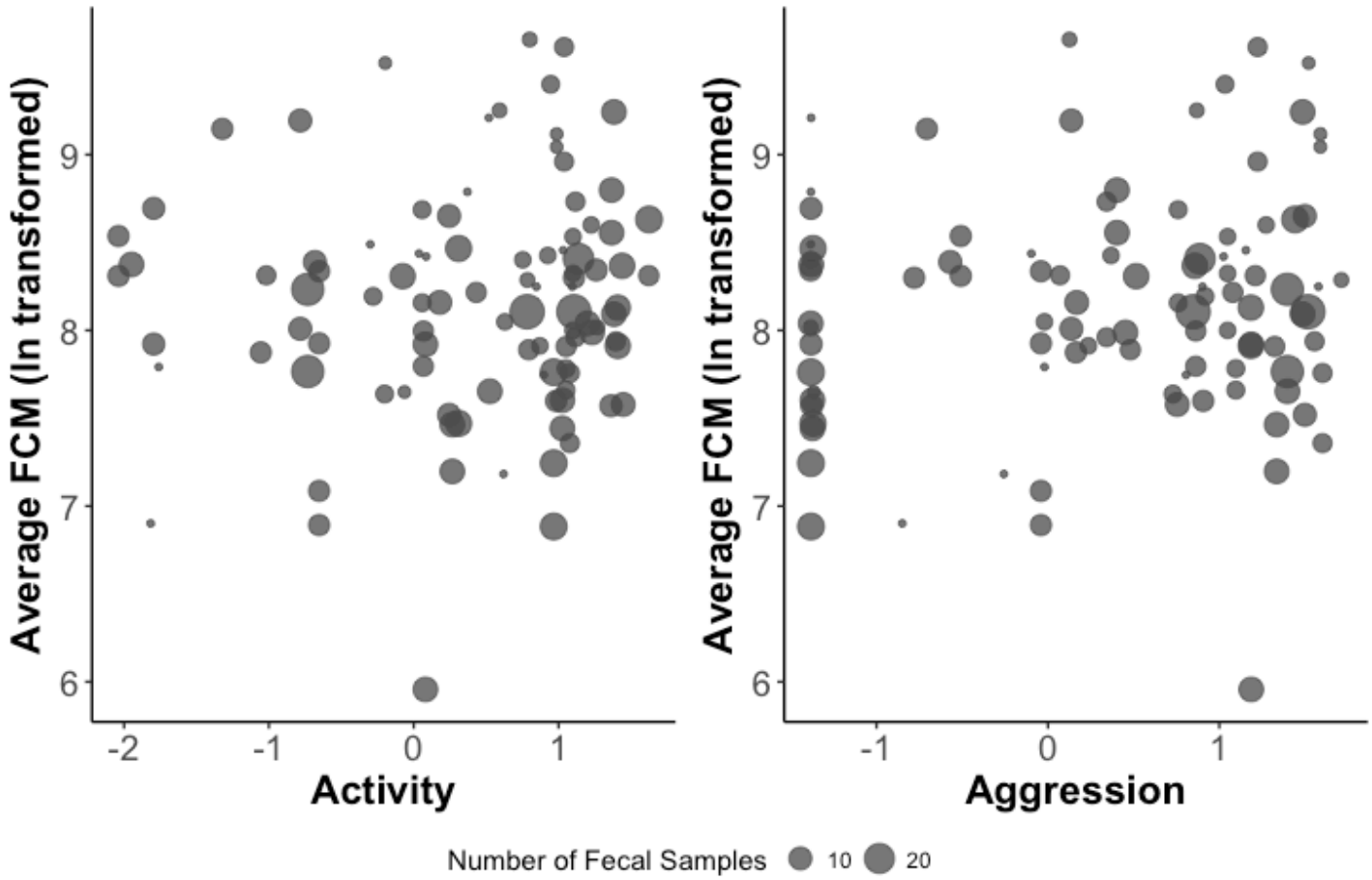
708

709 **Figure 2.**

710



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