Behavioral syndromes shape evolutionary trajectories via conserved genetic architecture.

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Abstract

Behaviors are often correlated within broader syndromes, creating the potential for evolution in one behavior to drive evolutionary changes in other behaviors. Despite demonstrations that behavioral syndromes are common across taxa, whether this potential for evolutionary effects is realized has not yet been demonstrated. Here we show that populations of field crickets (*Gryllus integer*) exhibit a genetically conserved behavioral syndrome structure despite differences in average behaviors. We found that the distribution of genetic variation and genetic covariance among behavioral traits was consistent with genes and cellular mechanisms underpinning behavioral syndromes rather than correlated selection. Moreover, divergence among populations' average behaviors was constrained by the genetically conserved behavioral syndrome. Our results demonstrate that a conserved genetic architecture linking behaviors has shaped the evolutionary trajectories of populations in disparate environments—illustrating an important way by which behavioral syndromes result in shared evolutionary fates.

Introduction

14 Behavior is frequently assumed to have been shaped by selection (Grafen 1984) and thus
15 populations are expected to differ in a range of behaviors based on local selective
16 pressures. This implies that behaviors are able to evolve independently, an assumption
17 increasingly challenged by the ubiquity of behavioral syndromes—correlations among
18 behaviors (Table 1; Sih et al. 2004a, Sih et al. 2004b)—which have been documented across
19 taxonomic groups (Garamszegi et al. 2012, 2013) and are comprised of both genetic and
20 environmental contributions (Dochtermann 2011, Dingemanse and Dochtermann 2014,
21 Dochtermann et al. 2015).

22 Given the contribution of genetic correlations to behavioral syndromes, these
23 syndromes have the potential to constrain the ability of populations to diverge and respond
24 to local selective pressures (Dochtermann and Dingemanse 2013). Specifically, based on
25 quantitative genetic theory, if syndromes stem from pleiotropic effects—wherein a single
26 gene affects multiple behaviors—populations will be constrained to diverge along shared
27 evolutionary pathways. Further, this divergence is predicted to occur in the direction in
28 trait space that contains the most variation (Figure 1; Schluter 1996). As a result, if
29 syndromes have a constraining effect on evolution, the pattern of correlations among traits
30 will be conserved among populations (Figure 1). Alternatively, if genetic correlations
31 underpinning syndromes are the result of selection historically favoring particular trait
32 combinations (i.e. selection-induced linkage disequilibrium (Roff 1997, Saltz et al. 2017)),
33 the divergence of populations will be generally unconstrained as these genetic correlations

are expected to rapidly break down when selection changes (Roff 1997, Conner 2002, Saltz et al. 2017).

These two quantitative genetic explanations for behavioral syndromes have explicit analogs in the behavioral literature: whether syndromes emerge from pleiotropy, tight genetic linkage, or other shared physiological and cellular effects has been termed the "constraints hypothesis," as opposed to selection-induced linkage disequilibrium, which has been termed the "adaptive hypothesis" (Figure 1, *sensu* Bell (2005)). While some studies have compared phenotypic or among-individual correlations across populations (e.g. Dingemanse et al. 2007, Pruitt et al. 2010, Dochtermann et al. 2012, Royauté et al. 2014, Michelangeli et al. 2018), only population comparisons of behavioral syndromes at the additive genetic level allow for properly testing these competing hypotheses. Unfortunately, data at the additive genetic level has been restricted to a single comparison of two populations (Bell 2005). Consequently, the overall role of behavioral syndromes in shaping population divergence is an important gap in our knowledge as support for the constraints versus adaptive hypotheses remains insufficiently tested.

Knowing whether behavioral syndromes emerge from genetic constraints or selection induced linkage disequilibrium is important because these mechanisms differentially affect evolutionary outcomes (Saltz et al. 2017). These potential effects are broad, from altering responses to environmental changes to speciation dynamics. For example, in *Anolis* lizards, constraints imposed by genetic correlations on morphological traits have shaped divergence and what phenotypes can be expressed during adaptive radiations (McGlothlin et al. 2018). Behavioral syndromes may have an even greater

56 constraining effect: Dochtermann and Dingemanse (2013) reported that the average
57 magnitude of genetic correlations between behaviors was sufficient to constrain
58 evolutionary responses to a greater degree than do correlations between life-history or
59 morphological traits. Unfortunately, this conclusion was based on data that could not
60 distinguish between the constraints and adaptive hypotheses. If indeed the constraints
61 hypothesis underpins behavioral syndromes then syndromes will reduce the rate of
62 adaptation and reduce the rate at which populations and species diverge. Evaluating
63 evidence for the constraints and adaptive hypotheses is therefore necessary in order to
64 understand whether behavioral syndromes are important in behavioral evolution.

65 Here we evaluated predictions of the adaptive and constraints hypotheses (Figure
66 1) and tested whether behavioral syndromes have diverged at the genetic level among
67 populations of the field cricket, *Gryllus integer*. Specifically, according to the constraints
68 hypothesis we predicted that genetic variation would be expressed in a consistent manner
69 among populations and genetic correlations would be maintained across generations.
70 Specific predictions are more difficult to make for the adaptive hypothesis without
71 knowledge of local selective pressures. This hypothesis can, however, be assessed
72 indirectly because behavioral divergence is expected to not be constrained and correlations
73 are expected to rapidly degrade. We evaluated these predictions via estimation and
74 comparisons of behavioral genetic (co)variance matrices, i.e. **G** (Table 1), estimated for
75 multiple populations of *G. integer* (Figure 2).

Methods

76 <u>Cricket collection</u>

77 *G. integer* is a particularly appropriate species to evaluate the constraints and adaptive
78 hypotheses for behavioral syndromes as the species exhibits population differences in
79 ecologically relevant behaviors that can be assayed in the laboratory (e.g. Hedrick and
80 Kortet 2006, Kortet and Hedrick 2007, Niemela et al. 2012a). *G. integer* can also readily be
81 bred in the lab according to quantitative genetic designs making it an ideal model for our
82 questions of interest (Hedrick 1988).

83 We collected adult female crickets from four populations throughout the
84 southwestern and western US: Socorro, NM; Las Cruces, NM; Aguila, AZ; and Dunnigan, CA
85 (Figure 2) during the summer of 2017. Crickets from these locations are formally
86 recognized as members of *Gryllus integer* but additional splitting is currently being
87 considered (D. Weissman, personal communication). These populations are also
88 geographically distant from each other (Figure 2) and vary in predator and parasitoid
89 abundance (Hedrick and Kortet 2006).

90 Around 50 females on average were collected from each population (Table S1) and
91 taken to animal housing facilities at North Dakota State University. Females were housed
92 individually in 0.71 L containers and provided with ad libitum food (Purina Chick Starter)
93 and water (water was provided in glass vials capped with cotton). Each cricket was also
94 provided with a small piece of cardboard egg carton for shelter. The cricket housing room
95 was maintained on a 12:12 dark:light cycle reversed such that the room was dark during
96 daytime hours. The housing room was kept at ~27C.

97 <u>Breeding design</u>

98 Females collected from the field (generation P) were allowed to oviposit in water vials
99 while in their containers. Offspring of these females were designated generation F_0 as sires
100 were unknown: mating occurred prior to capture and multiple mating is common in the
101 genus (Simmons 1986). F_0 offspring hatched in their dams' containers and were then
102 moved to individual housing prior to maturation. We assayed the behavior of 387 F_0
103 individuals (see below) upon maturation (Table S1). After behavioral trials, F_0 individuals
104 were assigned to breeding pairs such that individual males were mated to multiple
105 randomly assigned females from the same population but different dams according to a
106 standard full-sib, half-sib breeding design (Lynch and Walsh 1998). Matings were
107 conducted as follows: females were moved from their normal housing containers to a
108 larger container (34.6 × 21 × 12.4 cm) along with their food dish, water vial, and egg carton
109 shelter. After the female had been transferred, the assigned male was likewise moved to
110 the large container, also with its food dish, water vial, and egg carton. The male and female
111 remained in these containers for 24 hours to allow sufficient time for courtship and
112 multiple mating. After 24 hours the male and female crickets were returned to their
113 original containers. If males were to be mated with additional females, they were allowed a
114 minimum break of 24 hours before repeating the above procedure. These F_0 females were
115 subsequently allowed to oviposit into water vials within their containers. Resulting F_1
116 offspring were moved to individual housing prior to maturation and had their behaviors
117 assayed upon maturation. After behavioral assays, F_1 individuals were likewise paired with
118 F_1 individuals of the same population but different sires in the same manner as above and
119 resulting F_2 offspring were moved to individual housing and had their behavior measured
120 upon maturation. This resulted in the behavioral testing of 395 F_1 individuals and 163 F_2

121 individuals (Table S1). Across the three generations this represented behavioral testing of
122 946 individual crickets.

123 Behavioral testing

124 All behavioral tests followed standard procedures previously validated in the literature for
125 Gryllid crickets (Kortet and Hedrick 2007, Kortet et al. 2007, Hedrick and Kortet 2012,
126 Niemela et al. 2012b, Royauté et al. 2015, Royauté and Dochtermann 2017, Royauté et al.
127 2019). These assays encompass how individuals vary in risk-taking behavior (Kortet et al.
128 2007), exploratory propensity (Royauté et al. 2015, Royauté and Dochtermann 2017,
129 Royauté et al. 2019), and response to predation threat (Royauté and Dochtermann 2017,
130 Royauté et al. 2019). Based on the known relatedness among individuals we then
131 estimated **G**—the matrix of additive genetic behavioral variances and covariances—for
132 each population. Below, we describe these behavioral assays and their ecological relevance.

133 - Latency to emerge from shelter -

134 Gryllid crickets, including *G. integer*, use small burrows and natural cracks for refuge from
135 predators and to which they retreat when under threat. As a result, latency to emerge from
136 shelter after disturbance can be considered a proxy for risk-taking behavior or "boldness"
137 (Kortet et al. 2007). Here, we conducted latency tests wherein individuals were transferred
138 from their home containers to small artificial burrows (40 cm^3) placed within a 34.6 × 21
139 cm arena. These artificial burrows were capped so that individuals could not immediately
140 emerge. Crickets were forced to remain in the artificial burrow for two minutes after which
141 the cap was removed. Crickets were then allowed six minutes and thirty seconds to emerge
142 from the artificial burrow. During this test we recorded how long it took for an individual

143 to emerge (in seconds). Individuals that did not emerge were given a maximum latency of
144 390 seconds.

145 - Open field exploratory behavior -

146 Open field tests are a classic behavioral assay across taxa (Walsh and Cummins 1976)
147 which measure the exploratory propensity of individuals (Réale et al. 2007), including
148 crickets (Royauté et al. 2015, Royauté and Dochtermann 2017, Royauté et al. 2019).
149 Individuals that move through more of the arena are considered more thorough explorers
150 (Réale et al. 2007). Here we used open field tests to measure activity and exploratory
151 propensity in a 30 × 30 cm plexiglass arena. Individuals were introduced into the arena
152 under a small container and allowed to rest for 30 seconds after introduction. At the end of
153 this 30 seconds, the container was removed and the cricket was allowed to explore the
154 arena for 3 minutes and 40 seconds. The arena was cleaned with isopropyl alcohol between
155 trials to remove any chemosensory cues from the arena. We used Ethovision XT to record
156 the total distance the individual moved during the trial (cm), the number of unique zones of
157 the arena an individual visited during the trial, and the variance in velocity of individuals.
158 This latter measure indicates whether an individual's speed of exploration was constant
159 (low velocity variance) or whether individuals had frequent activity bursts punctuated by
160 long bouts of inactivity (high velocity variance).

161 - Response to cues of predator presence -

162 How individuals respond to cues of predator presence often varies within and among
163 populations and is likely to covary with fitness (Herman and Valone 2000). Crickets
164 respond to chemical cues of predator presence by either freezing or increasing activity

depending on whether confronted by predator cues of sit-and-wait or active predators (Storm and Lima 2008, Binz et al. 2014). Here we used a behavioral assay to measure response to cues of predator presence also previously used with another Gryllid species (Royauté and Dochtermann 2017, Royauté et al. 2019). Specifically, individuals were introduced into a 15 cm diameter circular arena (7.5 cm height), the floor of which was covered with dry filter paper that had been soaked with diluted excreta from leopard geckos (*Eublepharis macularius*). Crickets respond to exposure to leopard gecko cues by increasing activity (Royauté and Dochtermann 2017, Royauté et al. 2019). All leopard geckos were fed a diet of *G. integer* with occasional diet supplementation of mealworms (i.e. larval *Tenebrio molitor*) and the related decorated cricket (*Gryllodes sigillatus*). Crickets were introduced to a portion of the arena without predator cue under a small shelter. After a 30 second rest period, the shelter was removed and the individual allowed to freely move throughout the arena for 3 minutes and 40 seconds. We then used Ethovision XT to record the total distance an individual moved during the trial (cm). Total distance moved during the predator cue trial, the latency to first movement (in seconds), and the variance in velocity were used in subsequent analyses.

<u>Statistical analyses</u>

- **G** matrix estimation -

We used multi-response mixed effect animal models (Kruuk 2004) implemented using the `MCMCglmm` package in R (Hadfield 2010) to estimate genetic variances and covariances (i.e. the **G** matrix). We included the effects of temperature, day and time of testing in the behavioral arena room along with sex, life-stage and mass of the individual as fixed effects.

We used the individual relatedness matrix (based on the known pedigree) as a random effect. Traits for which variances and covariances were estimated were: (i) the latency that an individual emerged from the shelter during the trial (modeled as censored Gaussian), (ii) the distance moved during the open field trial (Gaussian), (iii) the number of unique zones an individual visited during the open field trial (Poisson), (iv) the log-transformed variance in velocity during the open field trial (Gaussian), (v) the square-root transformed distance an individual moved during the predator cue response trial (Gaussian), (vi) the latency to initiate movement in the antipredator response trial (Poisson) and (vii) the log-transformed variance in velocity during the antipredator response trial (Gaussian). The inclusion of dam ID as a random effect did not improve model fit, indicating negligible or non-existent maternal effects and was not included in final model runs. Multi-response models were fit individually by population with each population's variances and covariances estimated from the posterior of an MCMC chain of 4.8×10^6 iterations, with an 800,000 burn-in period and a thinning interval of 4,000. A prior that was minimally informative for both variances and covariances was used. All variances and covariances were estimated at the additive genetic level and on the latent scale (Table S4).

- Evaluating the constraints and adaptive hypotheses -

The location, orientation, and distribution of genetic variation in multivariate space—in our case seven dimensional space—can differ among groups in a variety of complex ways (Phillips and Arnold 1999, Blows et al. 2004, Walsh 2007, Roff et al. 2012, Aguirre et al. 2014). We therefore used a suite of statistical approaches to compare the orientation of

genetic variation and evaluated agreement among multiple statistical approaches in support for either the constraints or adaptive hypothesis.

To determine whether behavioral syndrome structure at the additive genetic level was shared among populations we used two approaches:

(i) testing whether populations exhibited shared subspaces (dimensions) of **G** based on Krzanowski's common subspace analysis (Aguirre et al. 2014);

(ii) comparing alignment of dominant eigenvectors among populations (i.e. $\mathbf{g}_{\mathbf{max}}$, Table 1 (Schluter 1996))

Krzanowski's common subspace analysis determines whether genetic variation is expressed in the same dimensions and direction across groups (Aguirre et al. 2014). This can be thought of as analogous to asking whether the populations shared principal components (Phillips and Arnold 1999). In two dimensions, this is similar to the directions the ellipses point in Figure 1, with shared subspaces when the ellipses point in the same direction. For our data there were seven possible dimensions of overlap but some dimensions may not possess substantive variation (Table S4). Following Aguirre et al. (2014) we therefore considered those subspaces that additively contained greater than 90% of the genetic variation, which were then summarized in matrix form (**H**)(Aguirre et al. 2014). Here, this included three possible shared subspaces ($\mathbf{h}_1$, $\mathbf{h}_2$, and $\mathbf{h}_3$; Table 1) and we tested whether these subspaces were shared among populations to a degree greater than expected by chance. Under the constraints hypothesis we expect genetic variation to be expressed in the same dimensional direction among populations, manifested as shared genetic subspaces. A lack of shared subspaces would therefore contradict the predictions of

230 the constraints hypothesis. However, shared subspaces may also be observed under the
231 adaptive hypothesis if selective pressures are the same across populations.

232 Because evolutionary trajectories are biased by the dimensional direction (vector)
233 in which most genetic variation is expressed, we also compared this vector, i.e. $\mathbf{g_{max}}$
234 (Schluter 1996), among populations. This approach is similar to Krzanowski's common
235 subspace analysis but focuses on the dimension in which most variation is expressed. Here
236 we calculated the vector correlation between the $\mathbf{g_{max}}$s of each population and, via
237 randomization testing, determined whether these correlations significantly differed from 0.

238 Even if genetic covariances constrain the path of evolutionary change, average
239 behaviors can change. Therefore, we estimated "**D**" (Table 1)—a matrix that describes the
240 phenotypic divergence amongst populations and was here estimated as the (co)variance of
241 species means (Schluter 1996, McGlothlin et al. 2018). The eigenvectors (**d**, Table 1) of this
242 matrix describe the direction in multivariate space in which most divergence has occurred.
243 If behavioral syndromes have constrained the evolution of populations, we would expect
244 the direction in which most phenotypic divergence in average behavior has occurred ($\mathbf{d_1}$)
245 to be correlated with shared subspaces identified by Krzanowski's common subspace
246 analysis (McGlothlin et al. 2018). Here we calculated the vector correlation between $\mathbf{d_1}$ and
247 $\mathbf{h_{1:3}}$. Non-zero correlations between $\mathbf{d_1}$ and any shared subspace would be consistent with
248 the constraints hypothesis.

249 As with average behaviors, genetic variances across dimensions may differ among
250 populations even while populations share an overall conserved genetic behavioral
251 syndrome. We can therefore ask whether genetic variances have been constrained by a
252 conserved behavioral syndrome. To do so we used methods described by Hine et al. (2009)

253 to calculate what are known as genetic covariance eigentensors (**E**, Table 1). This approach
254 starts by calculating the variances and covariances of **G** matrices across populations.
255 Resulting matrices can subsequently be subjected to further eigen analysis, producing
256 eigentensor matrices (**E**) and eigenvectors (**e**, Table 1) to describe in which dimension the
257 **G**s of populations differ the most. If a conserved genetic behavioral syndrome, consistent
258 with the constraints hypothesis, has affected the divergence of **G**s among populations we
259 would expect correlations between the eigenvectors of eigentensors (**e**) and any shared
260 subspaces ($\mathbf{h}_{1:3}$). Put another way, if populations share genetic variation in the same
261 dimension in which most of the genetic divergence occurred, this would be evidence that
262 syndromes channel behavioral evolution along a line of least resistance.

263 For the above approaches we followed the recommendations of Aguirre et al. (2014)
264 in that all tests were based on the full MCMC posterior distributions and null distributions
265 for population comparisons were based on randomizations of breeding values. To compare
266 whether eigenvectors were significantly aligned, we also generated a random distribution
267 of vector correlations following McGlothlin et al. (2018). The critical values of vector
268 correlations based on this distribution were 0.93 ($P < 0.001$), 0.85 ($P < 0.01$), 0.71 ($P <$
269 0.05) and 0.62 ($P < 0.1$). To assess the significance of eigenvalues of **H** and **E** against
270 random expectations, we calculated the largest posterior quantiles for which these
271 distributions did not overlap (Figures S2 and S3 respectively). This threshold serves as a
272 Bayesian probability in favor of the observed distribution being generated by patterns
273 other than chance (hereafter, P_{mcmc}). Because there are no clear-cut rules for interpreting
274 these Bayesian probabilities, we provide the following scale to indicate how we interpreted
275 support for inferences: $P_{mcmc} < 0.7$: poor evidence of difference compared to random

276 expectations; $P_{mcmc} > 0.8$: moderate evidence of difference compared to random
277 expectations; $P_{mcmc} > 0.9$ strong evidence of difference compared to random expectations;
278 $P_{mcmc} > 0.95$: very strong evidence of difference compared to random expectations. Other
279 reported probabilities (hereafter P) were interpreted according to standard criteria.
280 To further assess support for the adaptive and constraints hypotheses we also
281 compared genetic correlations across generations. Genetic correlations due to selection-
282 induced linkage disequilibrium are expected to decline across generations with random
283 mating. Specifically, Conner (2002) argued that with random mating and in the absence of
284 physical linkage, the magnitude of genetic correlations should halve every generation.
285 Because of our breeding design we were able to opportunistically and separately estimate
286 phenotypic and genetic correlations among behaviors by generation. Here, while mating
287 was restricted to be within populations, mating was random with regard to behavior and
288 we would therefore expect both genetic correlations (r_A) and phenotypic correlations (r_P)
289 to decrease during the duration of the experiment under the adaptive hypothesis. We
290 therefore generated expected genetic and phenotypic average absolute correlations under
291 these assumptions (Appendix S1) and compared the observed estimates to these
292 expectations. According to the constraints hypothesis we would expect correlations to
293 remain stable across generations while under the adaptive hypothesis we would expect
294 them to degrade toward a correlation of zero.
295 Finally, based on the estimated **G** matrices for each population, we calculated
296 "autonomy" ($\bar{a}$, Table 1) throughout multivariate space following Hansen and Houle
297 (2008). Autonomy provides an estimate of the "fraction of genetic variation that is
298 independent of potentially constraining characters"(Hansen and Houle 2008). Put another

299 way, autonomy estimates the degree to which genetic variation is free to respond to
300 selection (max $\bar{a}$ = 1) versus constrained by covariance (min $\bar{a}$ = 0). We did not have
301 predictions as to values for autonomy under either the constraints or adaptive hypotheses.
302 Instead, these values indicate the potential for future evolutionary constraints for each
303 population.

304 *Results*

305 Behavioral syndromes were genetically conserved among populations. Based on
306 Krzanowski's common subspace analysis (**H**, Table 1; Aguirre et al. (2014)), the behavioral
307 syndrome of *G. integer* was characterized by three dimensions of genetic covariance ($\mathbf{h}_{1\text{-}3}$,
308 Table 1). These dimensions, and thus the overall syndrome, were shared among
309 populations, as indicated by all Bayesian probabilities, p_{mcmc}, being < 0.65 (Fig. S1). p_{mcmc}
310 values that are closer to 1 for this test would indicate departure from random expectations
311 and would therefore support a lack of shared syndrome structure among populations. The
312 shared behavioral syndrome was comprised of: *i*) genetic covariation between shelter
313 emergence time and predator cue responsiveness ($\mathbf{h}_1$, Table 2); *ii*) a genetic boldness-
314 activity syndrome in which active individuals were more prone to ignore predator cues and
315 were quicker to exit from their shelter ($\mathbf{h}_2$, Table 2); and *iii*) genetic covariance between
316 activity and shelter emergence ($\mathbf{h}_3$, Table 2). Each of these three axes explained around
317 one-third of the observed genetic variance (Table 2).

318 Following the demonstration of genetic conservation of behavioral syndromes, we
319 determined whether genetic variation was primarily expressed in the same direction in
320 multivariate space across populations ($\mathbf{g}_{\mathbf{max}}$ alignment; Table 1). Put another way, given the

321 general conservation of behavioral syndrome structure at the genetic level, did populations
322 express most genetic variation in the same combinations of traits? Indeed, the $\mathbf{g}_{\mathbf{max}}$s of the
323 Aguila and Dunnigan and Socorro and Dunnigan populations were strongly correlated with
324 each other (vector correlation $r > 0.7$, $p < 0.05$) (Figure 2). Moreover, the $\mathbf{g}_{\mathbf{max}}$s of each
325 population were aligned with the shared axes (Figure 2). This alignment demonstrates that
326 the genetically conserved behavioral syndrome captured the genetic variation expressed in
327 each population and confirmed that the orientation of genetic variation in multivariate
328 space was conserved among the populations.

329 Despite the genetic conservation of behavioral syndrome structure, populations did
330 exhibit some divergence. Specifically, the populations have diverged in their multivariate
331 behavioral averages (i.e. "**D,**" Figure 3, Table 1 and S2) and in the magnitude of genetic
332 variation present in each population (Figure S3). Importantly, however, the direction of
333 divergence in both means and variances was aligned with the shared behavioral syndrome
334 (e.g. $r_{d1,h3} = 0.85$, $p < 0.01$; $r_{h1,e11} = 0.92$, $p < 0.01$; Table S2). This alignment demonstrates
335 that divergence has been constrained by the shared structure of behavioral syndromes.

336 Behavioral syndromes emerging from either the adaptive or constraints hypotheses
337 are expected to respond differently to random mating. Specifically, under the adaptive
338 hypothesis, genetic correlations are expected to erode by 50% every generation. Because
339 we mated individuals at random, we were able to compare the observed average genetic
340 and phenotypic correlations (r_A and r_P) with their expected values under the adaptive
341 hypothesis (see Appendix S1 for details). Contrary to the expectations of the adaptive
342 hypothesis, but as predicted according to the constraints hypothesis, average genetic and

343 phenotypic correlations remained stable over the course of three successive laboratory
344 generations (posterior mean and 95 % credible intervals; $r_{A\ Observed}$ F_1 = 0.36 [0.23; 0.52], $r_{A\ Observed}$ F_2 = 0.38 [0.23; 0.53], Figure 4).

346 Finally, for each population, we calculated autonomy (Table 1), which estimates the
347 degree of constraint on evolutionary outcomes imposed by the genetic architecture
348 connecting traits. Autonomy varies between 0 and 1, with higher values indicating greater
349 potential for independent evolution. For *Gryllus integer*, autonomy varied between 0.47 and
350 0.61 (DUN: $\bar{a}$ = 0.48 [0.31; 0.68], SOC: $\bar{a}$ = 0.47 [0.32; 0.67], AG: $\bar{a}$ = 0.60 [0.44; 0.78], LC: $\bar{a}$ =
351 0.61 [0.43; 0.76], all populations combined: $\bar{a}$ = 0.57 [0.43; 0.70]). This suggests that the
352 constraining effect of behavioral syndromes is likely to persist over future generations.

353 *Discussion*

354 Three key results demonstrate conservation of behavioral syndromes at the genetic
355 level despite differences among populations in average behavior, providing strong support
356 for the constraints hypothesis. This support for the constraints hypothesis is unexpected
357 given that a previous study with stickleback (Bell 2005) found that two populations
358 differed in the magnitude of heritabilities and genetic correlations between two
359 behaviors—albeit with overlapping confidence intervals—providing support in that case
360 for the adaptive hypothesis. This conservation of behavioral syndrome structure has also
361 had the effect of channeling population divergence. Our results therefore suggest that
362 studying a broader suite of behavioral traits may reveal evolutionary constraints not
363 apparent from pair-wise correlations.

364 Our first major result supporting the constraints hypothesis was that the genetic
365 variation among the four populations was shared along three dimensions. These
366 dimensions describe the genetic structure of the species' behavioral syndromes and their
367 being shared demonstrates that the orientation of genetic variation was conserved among
368 populations. The overall behavioral syndrome consisted of a boldness-activity dimension
369 ($\mathbf{h}_2$, Table 2) frequently described in the literature. This dimension genetically links
370 activity, exploration and risk-prone behaviors. This dimension has been described at the
371 phenotypic level (Wilson and Godin 2009, Bókony et al. 2012) but demonstrations at the
372 genetic level are rare (see Bell (Bell 2005) for one example). The other conserved
373 dimension ($\mathbf{h}_1$ and $\mathbf{h}_3$) represent potential trade-offs between risk management strategies,
374 in which individuals either compensate for risk during foraging by being less prone to
375 resume activity when threatened ($\mathbf{h}_3$, Table 2), or take risks in one context (not moving
376 away from a predator cue) while avoiding risk in another (taking longer to emerge from
377 shelter) ($\mathbf{h}_1$, Table 2). Alternatively, dimension $\mathbf{h}_1$ might indicate that individuals with long
378 latencies are less active. As a result, these individuals may encounter fewer predator cues
379 resulting in weaker antipredator responses.

380 Our second major result supporting the constraints hypothesis was that the $\mathbf{g}_{max}$s of
381 the Aguila and Dunnigan and Socorro and Dunnigan populations were strongly correlated
382 and all $\mathbf{g}_{max}$s were aligned with the shared behavioral syndrome. This validates that
383 behavioral syndrome structure is shared among the populations and that the behavioral
384 syndrome captures the majority of observed genetic variation. Schluter (1996)
385 demonstrated that morphological divergence among several pairs of populations and
386 species of vertebrates is constrained by $\mathbf{g}_{max}$. Specifically, evolutionary divergence was

387 greatest when populations and species shared a common $\mathbf{g}_{max}$ and there was directional
388 selection for morphological trait combinations in this same direction in phenotypic space
389 (Schluter 1996). We found that $\mathbf{g}_{max}$ was conserved and that divergence in both average
390 behavior and genetic (co)variance among the four populations was aligned with $\mathbf{g}_{max}$.. This
391 demonstrates that behavioral syndromes affect population divergence in a manner similar
392 to that observed for morphology.

393 Our third result in support of the constraints hypothesis stems from the prediction
394 that, under the adaptive hypothesis, genetic correlations are expected to decrease by about
395 50% each generation due to the effects of recombination (Conner 2002). This prediction
396 assumes an absence of genetic linkage and random mating (Appendix S1). However,
397 genetic linkage sufficiently strong to resist recombination is also consistent with the
398 constraints hypothesis—see, for example, the effects of supergenes (Purcell et al. 2014,
399 Küpper et al. 2016)—and so we consider this assumption appropriate. In contrast to this
400 prediction of declining correlations, we found that the average genetic correlation did not
401 change across generations (Figure 4). Similarly, phenotypic correlations did not decrease
402 according to predictions (Figure 4). Because we were not able to study replicate lines
403 under random mating, this finding is not conclusive on its own. Instead the result is one
404 additional line of evidence consistent with the constraints hypothesis and in contradiction
405 of the adaptive hypothesis.

406 Importantly, the first two results—shared dimensions of genetic variation and
407 correlated $\mathbf{g}_{max}$s—could also be observed under the adaptive hypothesis if the selective
408 pressures each of the populations experienced were the same. We consider this unlikely for

409 three reasons. First, the degree of geographic separation among populations was extensive,
410 totaling more than 1500 km in some cases (Figure 2). This degree of geographic separation
411 makes it unlikely that the populations experienced the exact same selective regime.
412 Moreover, climate (Table S5) as well as predation and parasitism regimes are highly
413 variable among the populations (Hedrick and Kortet 2006). Second, if similarity in
414 selection regimes was the driving force behind these converging patterns of genetic
415 covariance, we would expect the geographically closest populations to have the greatest
416 similarity in $\mathbf{g}_{max}$. This was not the case and, in fact, $\mathbf{g}_{max}$ was most similar among
417 populations that were geographically most separated (Figure 2). Finally, our third main
418 result directly contradicts the adaptive hypothesis: if trait correlations, like those of
419 behavioral syndromes, arise due to the adaptive hypothesis and therefore selection-
420 induced linkage disequilibrium, they are expected to rapidly degrade under random mating
421 (Roff 1997, Conner 2002). In direct contradiction to this expectation we observed that
422 correlations did not decrease across generations (Figure 4). Put another way, our first two
423 results—which showed that the multivariate composition of behavioral syndromes was
424 shared among populations—are consistent with the predictions of the constraints
425 hypothesis. Next, our third result—the maintenance of behavioral correlations despite
426 random mating—demonstrates the failure of predictions made by the adaptive hypothesis.

427 Our results indicate that the conserved genetic architecture of behavioral
428 syndromes leads to populations having quantitatively constrained evolutionary trajectories
429 (Houle 2001) and that these syndromes have limited population divergence. This
430 quantitative constraint and resulting limitation on divergence is also expected to persist
431 into the future due to the behavioral syndrome structure imposed by each population's **G**

matrix. Based on these **G** matrices, we found similar degrees of autonomy (Hansen and Houle 2008) among populations ranging from 0.47-0.61, a stronger constraint than observed for life-history or morphological traits (Dochtermann and Dingemanse 2013). These autonomies indicate that behaviors will rarely evolve independently and the observed genetic behavioral syndrome will affect future evolution.

Despite the conservation of behavioral syndrome structure at the genetic level across populations, *Gryllus integer* populations did exhibit divergence in both mean behaviors and magnitudes of genetic variation present in each population. Both the divergence in means and variances was strongly aligned with the shape of the shared behavioral syndrome, demonstrating that the syndrome itself was channeling the evolutionary divergence of the populations (Table S2). The divergence in means was most strongly related to differences in latency and distance moved in both the open-field and antipredator assays (Table 2). Specifically, the Dunnigan, CA and Aguila, AZ populations exhibited the greatest differences in average behaviors (Figure 4). Similarly, the divergence in magnitude of genetic variation was driven by the three easternmost populations having less genetic variation than the Dunnigan, CA population (Figure S1). Whether this represents a loss of variation due to selection, stochastic effects on the three eastern populations, or the accumulation of variation for the western population is not currently clear.

Throughout we have referred to the adaptive and constraints hypothesis as competing hypotheses for the expression of behavioral syndromes. However, two caveats to this framing exist: First, according to a Tinbergian framework (Tinbergen 1963), these

454 hypotheses are not addressing questions at the same level. Within the framework of
455 Tinbergen's four questions, the constraints hypothesis is a proximate causation question
456 and reduces to a question about pleiotropy (or other molecular mechanisms) versus
457 linkage and is agnostic as to selection. In contrast, the adaptive hypothesis is an ultimate
458 question of function and would be assessed by determining the alignment between **G** and
459 selection gradients (e.g. following methods described by Berdal and Dochtermann 2019).
460 This framing does not, however, carry-over to the quantitative genetic literature wherein
461 selection-induced linkage disequilibrium and molecular mechanisms such as pleiotropy are
462 considered competing explanations (e.g. Roff 1997). The difference in perspective is partly
463 due to the history of the fields but is also because the quantitative genetic framework
464 recognizes the necessary role of continuing correlated selection in maintaining covariances
465 stemming from linkage disequilibrium. As a second caveat it is important to note that the
466 hypotheses are not strictly mutually exclusive (Conner et al. 2011, Saltz et al. 2017). It is
467 possible that some portion of an estimated genetic covariance might stem from pleiotropy
468 while some other portion stems from linkage disequilibrium (the two could even cancel
469 each other out). Nonetheless, our results consistently supported the predictions of the
470 constraints hypothesis across several lines of evidence.

471 The surprising degree of shared genetic variation in behavioral syndrome reported
472 here suggests an unrecognized and important role for behavioral syndromes in the
473 evolution of populations. Behaviors such as those measured here—exploratory behaviors
474 and responses to predation threat—are frequently assumed to have been under selection
475 and their responses to selection have been assumed to be unconstrained. In contrast, we
476 have shown that the genetic contribution to behavioral expression is highly conserved, that

477 populations share evolutionary fates, and that conserved behavioral variation may be a
478 driver of population divergence and perhaps even speciation.

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624

Table 1. Terms and symbol definitions.

Term or Symbol	Definition
Behavioral syndrome	Among-individual correlations of behavior. For example, individuals that are on average more aggressive also, on average, show a higher degree of exploratory propensity.
$\mathbf{G}$	Additive genetic covariance matrix.
$\mathbf{g}_{\mathbf{max}}$	The dominant eigenvector of $\mathbf{G}$, describes the dimension in multivariate trait space with the highest additive genetic variance.
$\mathbf{D}$	Among-population divergence matrix describing patterns of population divergence in average phenotype.
$\mathbf{d}_i$	Eigenvectors of $\mathbf{D}$, $\mathbf{d}_1$ represent the dimension capturing most of the divergence in average phenotype among populations.
$\mathbf{H}$	Common subspaces of genetic variation for all four populations; describes the trait combinations that share the most genetic variation among populations.
$\mathbf{h}_i$	Eigenvectors of $\mathbf{H}$, $\mathbf{h}_1$ is analogous to $\mathbf{g}_{\mathbf{max}}$ and describe the major axis of shared genetic variance among populations.
$\mathbf{E}_i$	Eigentensors describing subspaces for which $\mathbf{G}$ varies among population.
$\mathbf{e}_{ij}$	j^{th} eigenvector of the ith eigentensor, describes the trait combinations for which genetic divergence has occurred among populations.
r	Correlation among eigenvectors, values close to 0 indicate independence of eigenvectors, values close to 1 indicate alignment of eigenvectors.
$\bar{a}$	Autonomy of $\mathbf{G}$, indicates the proportion of genetic variation unconstrained by covariance among traits. Values closer to 0 indicate stronger evolutionary constraints and values closer to 1 indicate complete autonomy of genetic variation, meaning that each trait can evolve independently in response to future selection.

Table 2. Eigenvectors of phenotypic divergence (**d**), conserved genetic variation (**h**) and divergence in **G** (**e**). Traits legend: Latency = latency to exit from the shelter, OF.Dist = distance travelled in the open-field test, UZ = number of unique zones explore in the open-field arena, OF.Var.Velo = variance in velocity in the open-field test, AP.Dist = distance travelled in the antipredator response test, AP.Lat.Mov = latency to initiate movement in the antipredator response test, AP.Var.Velo = variance in velocity in the antipredator response test.

						E1 (53 %)	**E**2 (31 %)	
Traits	$\mathbf{d}_1$	$\mathbf{d}_2$	$\mathbf{h}_1$	$\mathbf{h}_2$	$\mathbf{h}_3$	$\mathbf{e}_{11}$	$\mathbf{e}_{21}$	$\mathbf{e}_{22}$
Latency	**-0.32**	**0.90**	**-0.31**	**0.86**	**0.47**	**0.63**	0.08	**0.77**
OF.Dist	**-0.80**	**-0.34**	0.18	**-0.37**	**0.88**	-0.13	**-0.79**	**0.42**
UZ	-0.02	-0.06	0.02	-0.02	0.06	-0.01	-0.08	0.06
OF.Var.Velo	-0.09	-0.06	0.02	-0.02	0.07	-0.02	-0.10	0.06
AP.Dist	**-0.48**	-0.04	**0.93**	**0.35**	0.02	**-0.74**	**-0.59**	**-0.45**
AP.Lat.Mov	-0.05	0.24	-0.10	-0.01	0.02	0.16	0.07	0.15
AP.Var.Velo	-0.07	-0.03	0.05	0.02	0.01	-0.05	-0.05	-0.02
% Variance explained	58.2	31.4	33.1	33.0	32.9	97.4	69.5	30.4

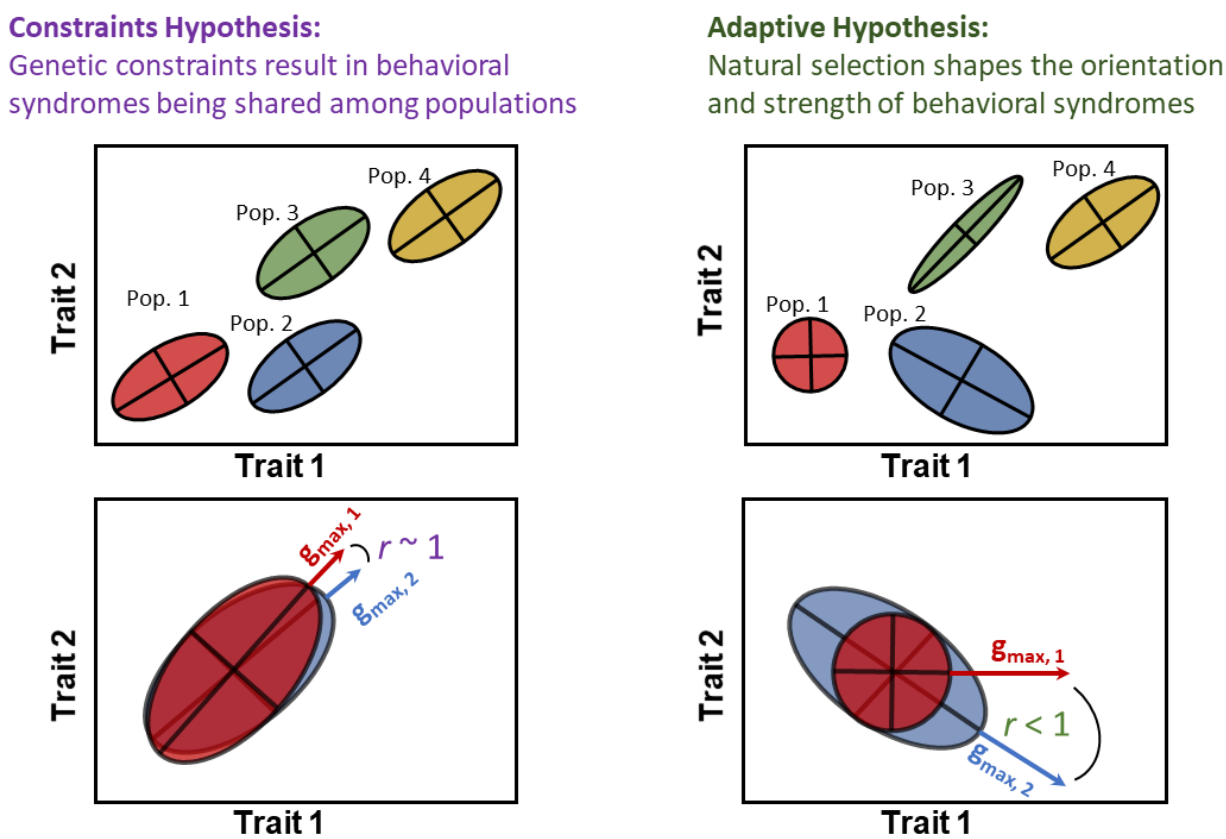

Figure 1. Two contrasting hypotheses can explain the presence of genetic correlations among behavioral traits (i.e. behavioral syndromes): Genetic constraints arising from pleiotropy and shared molecular mechanisms should lead to the expression of the same behavioral syndrome (top left panel). As a result, the vector correlations between major axes of genetic variation ($\mathbf{g}_{\mathbf{max}}$) are predicted to be approaching 1 (bottom left panel). Alternatively, selection-induced linkage disequilibrium should lead to differing orientation and strength of behavioral syndromes when selective pressures differ among populations (top right panel). The vector correlation between $\mathbf{g}_{\mathbf{max}}$s should therefore be below 1 (bottom right panel).

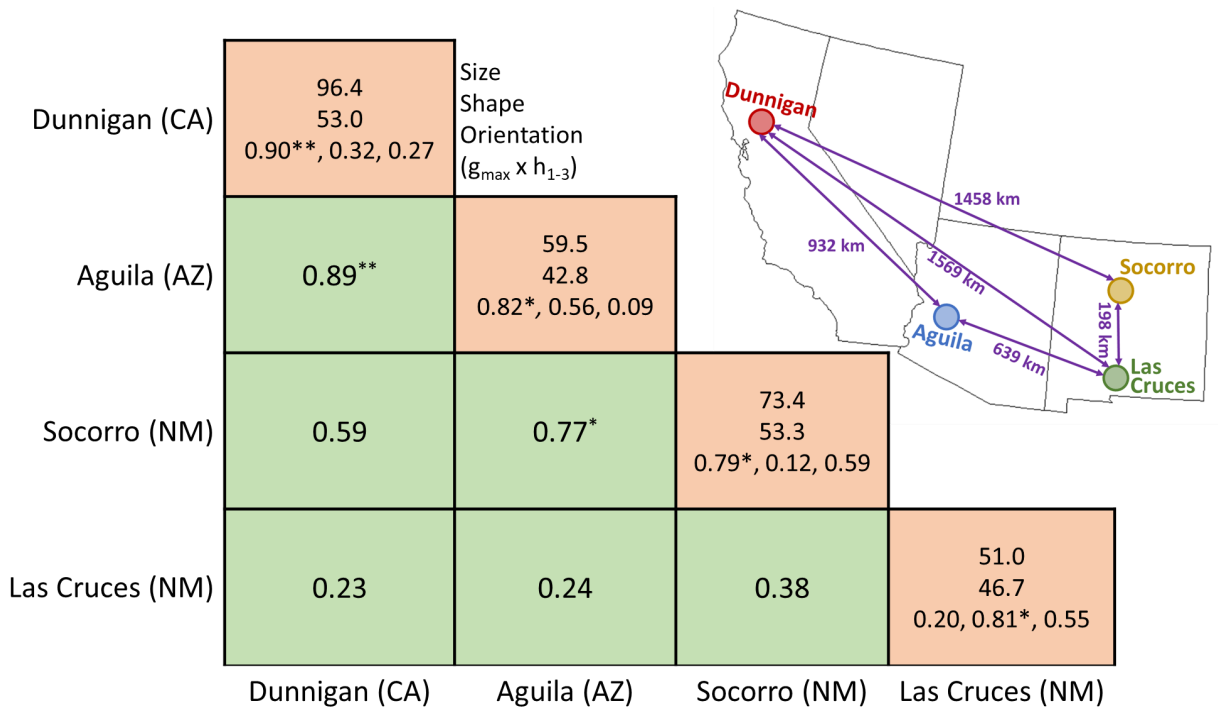

Figure 2. The genetic architecture of behavioral syndromes is shared in four isolated populations of *Gryllus integer*. Values along the diagonal (peach shading) describe the multivariate structure of behavioral variation: first row the size (total genetic variance), second the shape (percent of variance explained by the major axis of genetic variation, $\mathbf{g}_{\mathbf{max}}$), and, third, orientation (vector correlation between $\mathbf{g}_{\mathbf{max}}$ and conserved genetic subspaces $\mathbf{h}_{1\text{-}3}$). Off-diagonal elements represent the correlation between the $\mathbf{g}_{\mathbf{max}}$ of each population (top row) and the probability that alignment differed from 0: ** P < 0.01, * P < 0.05.

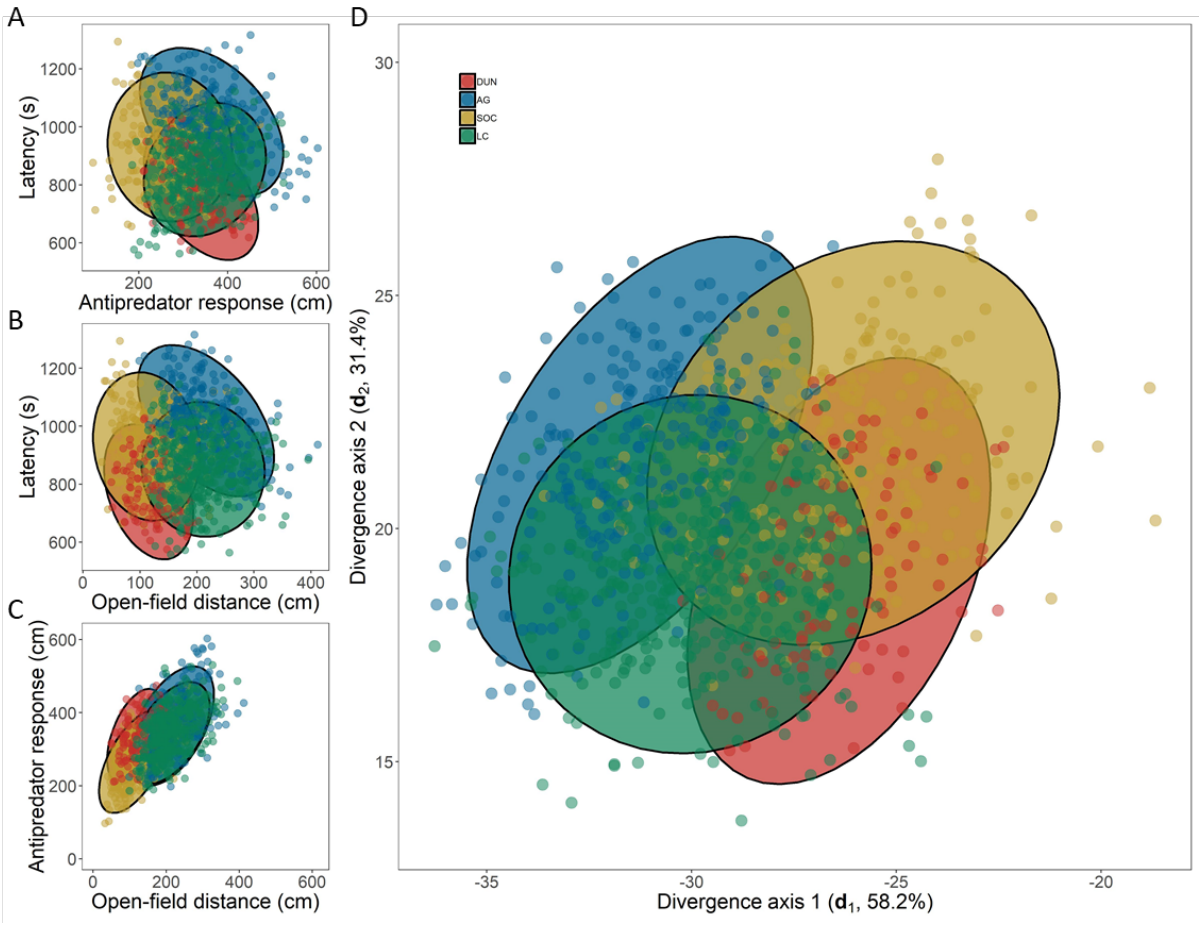

Figure 3. Evolutionary divergence in the structure of behavioral syndromes occurs along shared axes of genetic variation. A-C) Correlations between pairs of traits that exhibit the greatest variation in divergence (Table S2). Points represent breeding values for each individual within a population centered around the population mean for that trait. >50% of divergence was in latency to emerge from shelter by antipredator response activity D) Population-specific divergence in average behaviors. Population-specific **G** matrices were visualized by transforming estimated breeding values for each trait based on the divergence among populations. Ellipses represent the 95% confidence ellipses for each population centered at the multivariate species mean (DUN: Dunnigan CA, AG: Aguila AZ, SOC: Socorro NM, LC: Las Cruces NM).

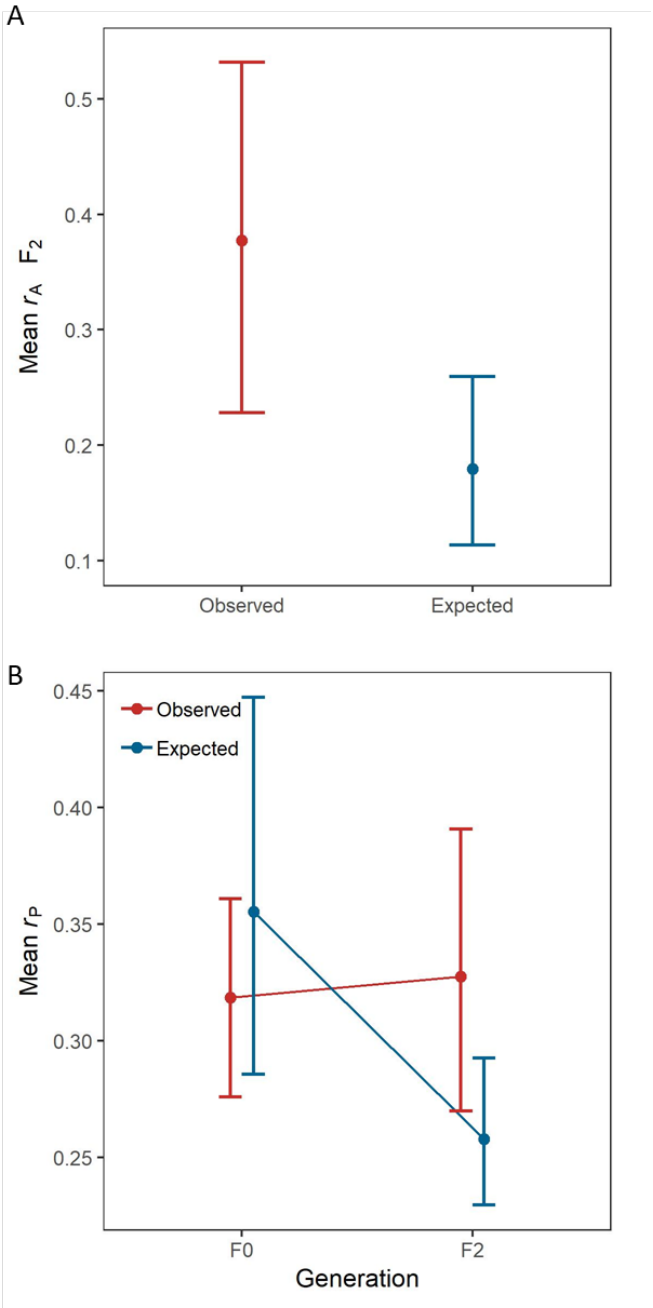

Figure 4. A) Additive genetic (r_A) and B) phenotypic correlations (r_P) remained stable over the course of three successive generations compared to theoretical expectations based on selection-induced linkage disequilibrium and random mating ($r_{A\ observed}$ = 0.38, $r_{A\ expected}$ = 0.18, P_{mcmc} for difference from expectations under selection-induced linkage disequilibrium > 0.85; $r_{P\ observed}$ F_0 = 0.32, $r_{P\ observed}$ F_2 = 0.33, $r_{P\ expected}$ F_0 = 0.35, $r_{P\ expected}$ F_2 = 0.26, P_{mcmc} F_2 > 0.80). Error bars correspond to 95% credibility intervals around the posterior mean.