Behavioral syndromes shape evolutionary trajectories via conserved genetic architecture.

Raphael Royauté [a]
Ann Hedrick [b]
Ned A. Dochtermann [a, c]

[a] Department of Biological Sciences; North Dakota State University
[b] Department of Neurobiology, Physiology, and Behavior; University of California, Davis
[c] corresponding author: ned.dochtermann@gmail.com

Abstract

Correlations among traits can affect how populations evolve, even to the point of completely preventing populations from responding to selection. Consistent individual differences in behavior (i.e. animal personality) are often correlated within what are known as behavioral syndromes but the potential of animal personality and behavioral syndromes to affect evolutionary outcomes is unknown. Here we show that geographically isolated populations of field crickets (*Gryllus integer*) exhibit a genetically conserved syndrome structure and that the degree of genetic constraint was consistent among populations. Moreover, divergence among populations was constrained by this genetically conserved behavioral syndrome. Our results demonstrate that a conserved genetic architecture shaped the evolutionary trajectories of populations in disparate environments.

Introduction

Behavior is frequently assumed to have been shaped by selection[1] and thus populations are expected to differ in a range of behaviors based on local selective pressures. This implies that behaviors are able to evolve independently, an assumption increasingly challenged by the ubiquity of behavioral syndromes—correlations among behaviors[2] (Table 1)—which have been documented across taxonomic groups[3,4] and are comprised of both genetic and environmental contributions[5,6].

Given the contribution of genetic correlations to behavioral syndromes[5,7], these syndromes have the potential to constrain the ability of populations to diverge and respond to local selective pressures[8]. Specifically, based on quantitative genetic theory, if syndromes stem from pleiotropic effects—wherein a single gene affects multiple behaviors—populations will be constrained to diverge along shared evolutionary pathways. Further, this divergence is predicted to occur in the direction in trait space that contains the most variation[9] (Figure 1). As a result, if syndromes have a constraining effect on evolution, the pattern of correlations among traits will be conserved among populations (Figure 1). Alternatively, if genetic correlations underpinning syndromes are the result of selection historically favoring particular trait combinations (i.e. selection-induced linkage disequilibrium[10]), the divergence of populations will be relatively unconstrained, as these genetic correlations are expected to rapidly break down when selection changes[11].

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, see also[12]). Knowing whether the constraints or adaptive hypothesis drives the emergence of behavioral syndromes is of importance because these hypotheses differentially affect evolutionary outcomes ranging from responses to environmental changes to speciation dynamics. For example, in *Anolis* lizards, constraints imposed by morphological genetic correlations have shaped divergence and the phenotypes possible during adaptive radiations[13]. Whether behavioral syndromes have similar effects is an open question. While some studies have compared phenotypic or among-individual correlations across populations[14-18], 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[12]. Consequently, the overall importance of behavioral syndromes in shaping population divergence remains an important gap in our knowledge as the prevalence of the constraints versus adaptive hypotheses remains insufficiently tested.

Here we critically evaluated predictions of the adaptive and constraints hypotheses (Figure 1) and tested whether behavioral syndromes have diverged at the genetic level among populations of the field cricket, *Gryllus integer*. We tested these predictions via comparisons of behavioral genetic (co)variance matrices, i.e. **G** (Table 1), estimated for multiple populations of *G. integer* (Figure 2). We collected crickets from four populations, one in California (USA), one in Arizona (USA) and two in New Mexico (USA; Figure 2, Table S1) during the summer of 2017. These populations are geographically distant from each

other (Figure 2) and are known to vary in predator and parasitoid abundance[19]. Collected females (F_P) were individually housed under standard conditions and allowed to oviposit into a cotton substrate (see Methods and Table S1). Offspring (F_0) were then reared to maturity, after which known matings of males and females within populations were conducted[20], producing a subsequent generation of crickets (F_1) of known parentage. This was repeated for a second generation (F_2). We measured seven behaviors of F_0, F_1, and F_2 crickets (965 individuals in total, Table S1) in three ecologically relevant behavioral assays: latency to emerge from shelter, activity/exploration of a novel area, and locomotive response to the cues of predator presence (see Methods). These assays encompass how individuals vary in risk-taking behavior[21], exploratory propensity[22-24], and response to predation threat[22,23]. Based on the known relatedness among individuals we estimated **G** for each population.

Results

Behavioral syndromes were genetically conserved among populations. Based on Krzanowski's common subspace analysis[25] (**H**, Table 1), the behavioral syndrome of *G. integer* was characterized by three axes of genetic covariance ($\mathbf{h}_{1\text{-}3}$, Table 1). These axes, and thus the overall syndrome, were shared among populations, as indicated by all Bayesian probabilities, p_{mcmc}, being < 0.65 (Fig. S1). p_{mcmc} values closer to 1 indicate departure from random expectations and would therefore support a lack of shared syndrome structure among populations (see Methods for details). The shared behavioral syndrome was comprised of: *i*) genetic covariation between shelter emergence time, and predator cue responsiveness ($\mathbf{h}_1$, Table 2); *ii*) a genetic boldness-activity syndrome in

which active individuals were more prone to ignore predator cues and were quicker to exit from their shelter ($\mathbf{h}_2$, Table 2); and *iii*) genetic covariance between activity and shelter emergence ($\mathbf{h}_3$, Table 2).

Following the demonstration of genetic conservation of behavioral syndromes, we determined whether genetic variation was primarily expressed in the same direction in multivariate space across populations ($\mathbf{g}_{\mathbf{max}}$ [9] alignment; Table 1). Indeed, the $\mathbf{g}_{\mathbf{max}}$s of the Aguila and Dunnigan and Socorro and Dunnigan populations were strongly correlated with each other (vector correlation $r > 0.7$, $p < 0.05$) (Figure 2). Moreover, the $\mathbf{g}_{\mathbf{max}}$s of each population were aligned with the shared axes (Figure 2). This alignment demonstrated that the genetically conserved behavioral syndrome properly captured the genetic variation expressed in each population, and confirmed that the orientation of genetic variation in multivariate space was conserved among the populations.

Behavioral syndromes emerging from either the adaptive or constraints hypotheses are expected to respond differently to relaxed selection. Specifically, under the adaptive hypothesis, genetic correlations are expected to erode by 50% every generation. Because we mated individuals at random, we were able to compare the observed average genetic and phenotypic correlations (r_A and r_P) with their expected values under the adaptive hypothesis (see Appendix S1 for details). Contrary to the expectations of the adaptive hypothesis but as predicted according to the constraints hypothesis, average genetic and phenotypic correlations remained highly stable over the course of three successive laboratory generations (posterior mean and 95 % credible intervals; $r_{A\ \mathrm{Observed}}\ F_1 = 0.36$ [0.23; 0.52], $r_{A\ \mathrm{Observed}}\ F_2 = 0.38$ [0.23; 0.53], Figure 3).

Despite the genetic conservation of behavioral syndrome structure and the maintenance of genetic correlations, populations did exhibit some divergence. Specifically, the populations have diverged in their multivariate behavioral averages (i.e. "**d,**" Figure 4, Table 1 and S2) and in the magnitude of genetic variation present in each population (Figure S3). Importantly, however, the direction of divergence in both means and variances was aligned with the shared behavioral syndrome (Table S2). This alignment demonstrates that divergence has been constrained by the shared structure of behavioral syndromes. 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 or stochastic effects on the three eastern populations or the accumulation of variation for the western population is not currently clear.

Finally, for each population, we calculated autonomy[26] (Table 1), which estimates the degree of constraint on evolutionary outcomes imposed by the genetic architecture connecting traits. Autonomy varies between 0 and 1, with higher values indicating greater potential for independent evolution. For *Gryllus integer* autonomy varied between 0.47 and 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: $\bar{a}$ = 0.61 [0.43; 0.76], all populations combined: $\bar{a}$ = 0.57 [0.43; 0.70]). This suggests that the constraining effect of behavioral syndromes is likely to persist over future generations.

Discussion

Three key results demonstrate conservation of behavioral syndromes at the genetic level despite differences among populations in average behavior, providing strong support

for the constraints hypothesis. This support for the constraints hypothesis is unexpected given that a previous study with stickleback[12] found that two populations differed in the magnitude of heritabilities and genetic correlations between behaviors—albeit with overlapping confidence intervals—providing support in that case for the adaptive hypothesis. This conservation of behavioral syndrome structure has also had the effect of channeling population divergence. Our results therefore suggest that studying a broader array of behavioral traits reveals evolutionary constraints not apparent from pair-wise correlations.

Our first major result supporting the constraints hypothesis was that the four populations shared three axes in multivariate space. These axes describe the genetic structure of the species' behavioral syndromes and that they are shared demonstrates that the orientation of genetic variation was conserved among the populations. The overall behavioral syndrome consisted of a boldness-activity axis ($\mathbf{h}_2$, Table 2) frequently described in the literature. This axis genetically links activity, exploration and risk-prone behaviors. This axis has been described at the phenotypic level[27,28] but demonstrations at the genetic level are rare (see Bell[12] for one example). The other conserved axes ($\mathbf{h}_1$ and $\mathbf{h}_3$) represent potential trade-offs between risk management strategies, in which individuals either compensate for risk during foraging by being less prone to resume activity when threatened ($\mathbf{h}_3$, Table 2), or take risks in one context (not moving away from a predator cue) while avoiding risk in another (taking longer to emerge from shelter) ($\mathbf{h}_1$, Table 2). Alternatively, axis $\mathbf{h}_1$ might indicate that individuals with long latencies are less active. As a result, these individuals may encounter fewer predator cues resulting in weaker antipredator responses.

147 Second, the $\mathbf{g}_{max}$s of the Aguila and Dunnigan and Socorro and Dunnigan populations
148 were strongly correlated and all $\mathbf{g}_{max}$s were aligned with the shared behavioral syndrome.
149 This validates that behavioral syndrome structure is shared among the populations and
150 that the behavioral syndrome captures the majority of observed genetic variation.
151 Schluter[9] demonstrated that morphological divergence among several pairs of populations
152 and species of vertebrates is constrained by $\mathbf{g}_{max}$. Specifically, evolutionary divergence was
153 greatest when populations and species shared a common $\mathbf{g}_{max}$ and there was directional
154 selection for morphological trait combinations in this same direction in phenotypic space[9].
155 We found that $\mathbf{g}_{max}$ was conserved and that divergence in both average behavior and
156 genetic (co)variance among the four populations was aligned with $\mathbf{g}_{max}$.. This demonstrates
157 that behavioral syndromes affect population divergence in a manner similar to that
158 observed for morphology.

159 Our third result in support of the constraints hypothesis stems from the prediction
160 that, under the adaptive hypothesis, genetic correlations are expected to decrease by about
161 50% each generation due to the effects of recombination[11]. This prediction assumes an
162 absence of genetic linkage and random mating (Appendix S1). Genetic linkage sufficiently
163 strong to resist recombination is consistent with the constraints hypothesis—see, for
164 example, the effects of supergenes[29,30]—and so we consider this first assumption
165 appropriate. In contrast to this prediction, we found that the average genetic correlation
166 did not change between generations (Figure 3). Similarly, phenotypic correlations did not
167 decrease according to predictions (Figure 3). This lack of change in correlations is
168 consistent with the constraints hypothesis and directly in opposition to the adaptive
169 hypothesis.

Importantly, the first two results—shared subspaces and correlated $\mathbf{g}_{max}$s—could also be observed under the adaptive hypothesis if the selective pressures each of the populations experienced were the same. We consider this unlikely for three reasons. First, the degree of geographic separation among populations was extensive, totaling more than 1500 km in some cases (Figure 2). This degree of geographic separation makes it unlikely that the populations experienced the exact same selective regime. Moreover, climate (Table S5) as well as predation and parasitism regimes are highly variable among the populations[19]. Second, if similarity in selection regimes was the driving force behind these converging patterns of genetic covariance, we would expect the geographically closest populations to have the greatest similarity in $\mathbf{g}_{max}$. This was not the case and, in fact, $\mathbf{g}_{max}$ was most similar among populations that were geographically most separated (Figure 2). Finally, our third main result directly contradicts the adaptive hypothesis: if trait correlations, like those of behavioral syndromes, arise due to the adaptive hypothesis and therefore selection-induced linkage disequilibrium, they are expected to rapidly degrade under random mating[10,11]. In direct contradiction to this expectation we observed that correlations did not decrease across generations (Figure 3). Put another way, our first two results—which showed that the multivariate composition of behavioral syndromes was shared among populations—are consistent with the predictions of the constraints hypothesis. Next, our third result—the maintenance of behavioral correlations despite random mating—demonstrates the failure of predictions made by the adaptive hypothesis.

Our results indicate that the conserved genetic architecture of behavioral syndromes leads to populations having quantitatively constrained[31] evolutionary trajectories and that these syndromes have limited population divergence. This

quantitative constraint and resulting limitation on divergence is also expected to persist 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[26] among populations ranging from 0.47-0.61, a stronger constraint than observed for life-history or morphological traits[8]. These autonomies demonstrate an inability of behaviors to independently evolve.

The surprising degree of shared genetic variation in behavioral syndrome reported here suggests an unrecognized and important role for behavioral syndromes in the evolution of populations. Behaviors such as those measured here—exploratory behaviors and responses to predation threat—are frequently assumed to have been under selection and their responses to selection have been assumed to be unconstrained. In contrast, we show that the genetic contribution to behavioral expression is highly conserved, that populations share evolutionary fates, and that conserved behavioral variation may be a primary driver of population divergence and perhaps even speciation.

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Author Contributions

N.A.D. and A.H. conceived the project and supervised the gathering of data, R.R analyzed the data, and N.A.D., A.H., and R.R. wrote the manuscript.

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335 **Table 1.** Terms and symbol definitions.

Term or Symbol	Definition
Animal personality	The general phenomenon that individuals differ consistently from each other in their behavior.
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.
G	Additive genetic covariance matrix.
$\mathbf{g}_{max}$	The dominant eigenvector of **G**, describes the dimension in multivariate trait space with the highest additive genetic variance.
D	Among-population divergence matrix describing patterns of population divergence in average phenotype.
$\mathbf{d}_i$	Eigenvectors of **D**, $\mathbf{d}_1$ represent the dimension capturing most of the divergence in average phenotype among populations.
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 **H**, $\mathbf{h}_1$ is analogous to $\mathbf{g}_{max}$ and describe the major axis of shared genetic variance among populations.
$\mathbf{E}_i$	Eigentensors describing subspaces for which **G** varies among population.
$\mathbf{e}_{ij}$	j^{th} eigenvector of the i^{th} 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 **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

Figures.

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 isolated populations to express 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) represent describe the multivariate structure of behavioral variation: first 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 (green shading) represent the correlation between the $\mathbf{g}_{\mathbf{max}}$ of each population (top row) and the probability that alignment differed from 1: ** P < 0.01, * P < 0.05.

Figure 3. 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.

Figure 4. 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).

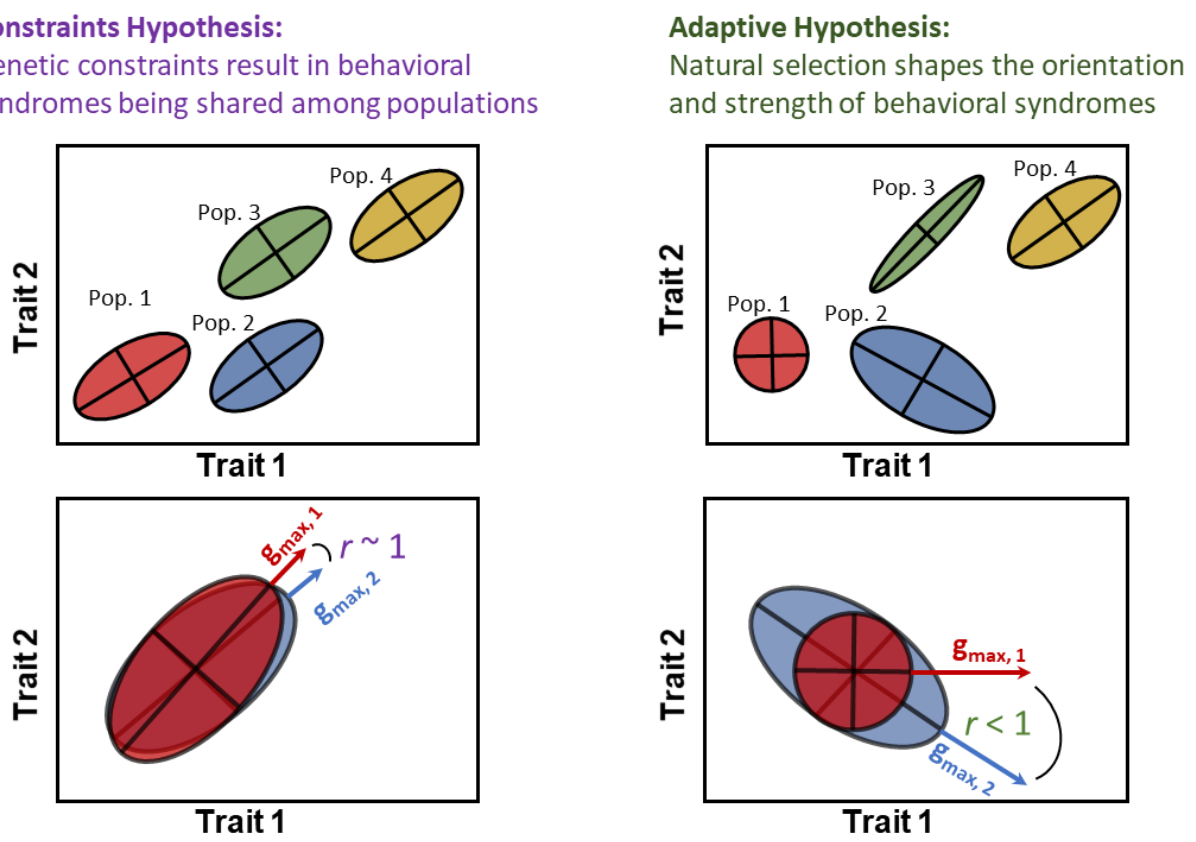


Figure 1. Two contrasting hypotheses can explain the presence of genetic correlations among behavioral traits (i.e. behavioral syndromes): Genetic constraints arising from pleiotropy and shared molecular mechanisms should lead isolated populations to express the same behavioral syndrome (top left panel). As a result, the vector correlations between major axes of genetic variation ($\mathbf{g}_{max}$) 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 $\mathbf{g}_{max}$s should therefore be below 1 (bottom right panel).

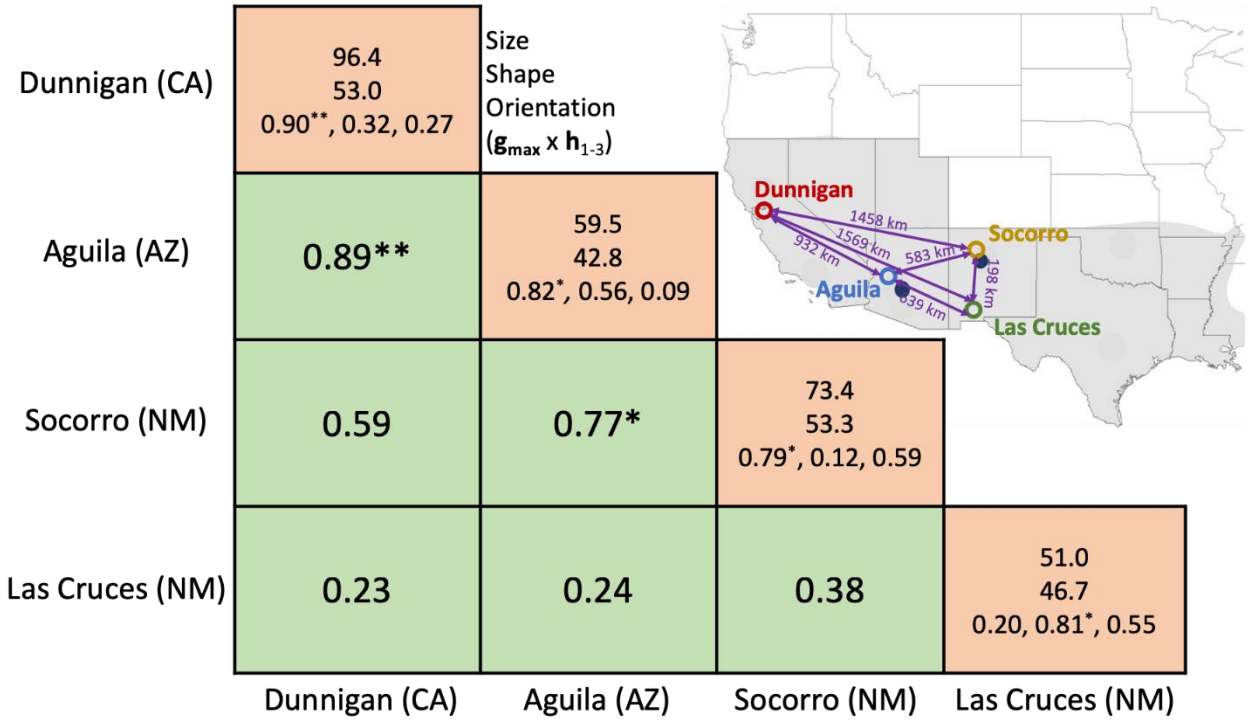


Figure 2. The genetic architecture of behavioral syndromes is shared in four isolated populations of *Gryllus integer*. Values along the diagonal represent (peach shading) describe the multivariate structure of behavioral variation: first the size (total genetic variance), second the shape (percent of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

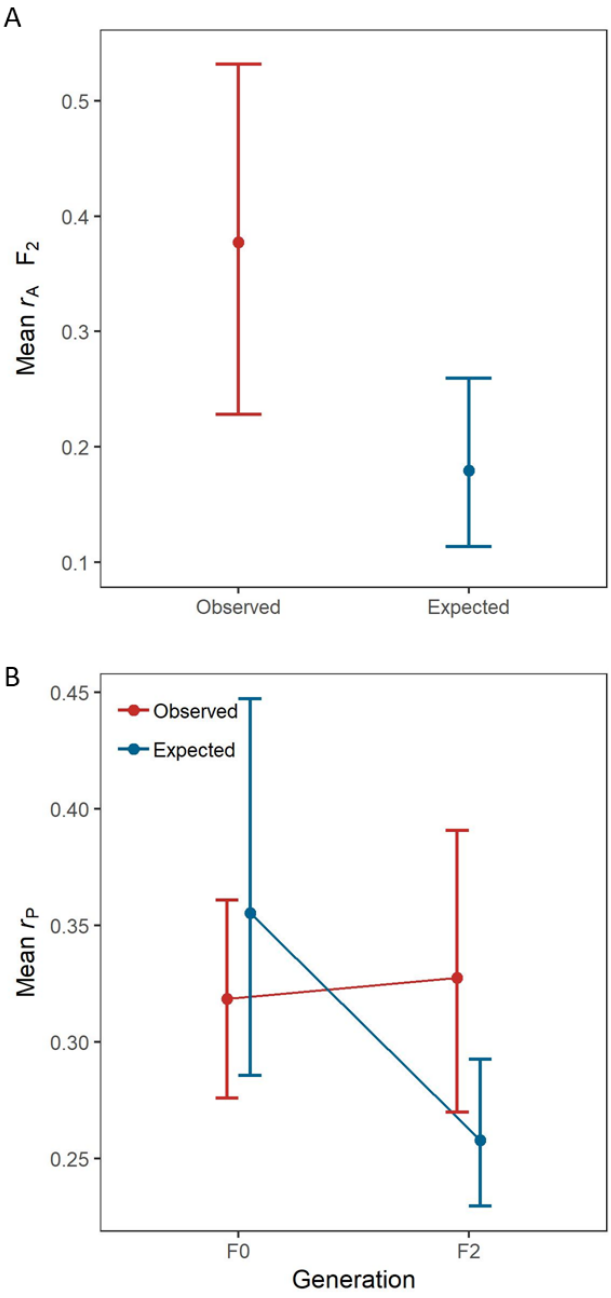
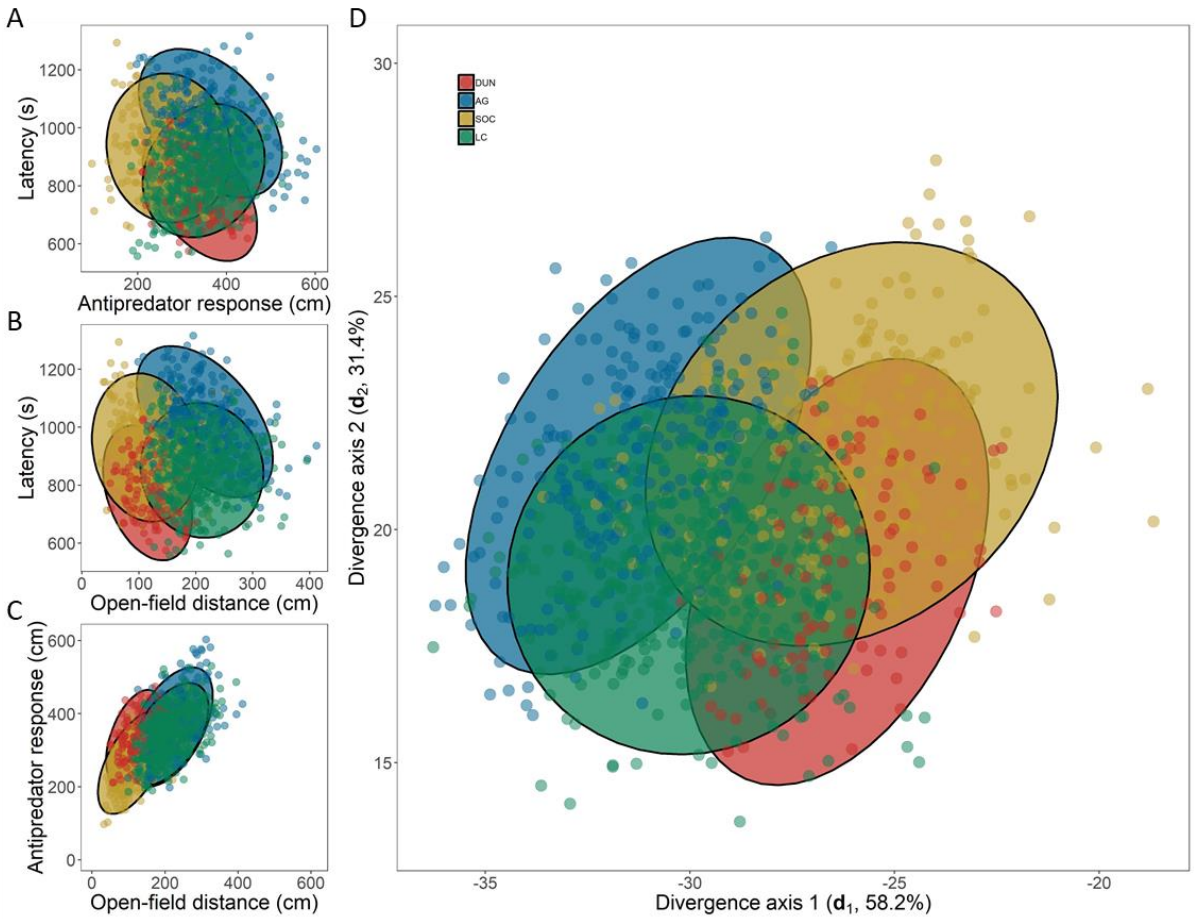


Figure 3. A) Additive genetic (r_A) and B) phenotypic correlations (r_P) remained stable over the course of three successive generations compared to theoretical expectations based on selection-induced linkage disequilibrium and random mating ($r_{A\ observed}$ = 0.38, $r_{A\ expected}$ = 0.18, P_{mcmc} 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.



370 **Figure 4.** Evolutionary divergence in the structure of behavioral syndromes occurs along
371 shared axes of genetic variation. A-C) Correlations between pairs of traits that exhibit the
372 greatest variation in divergence (Table S2). Points represent breeding values for each
373 individual within a population centered around the population mean for that trait. >50% of
374 divergence was in latency to emerge from shelter by antipredator response activity D)
375 Population-specific divergence in average behaviors. Population-specific **G** matrices were
376 visualized by transforming estimated breeding values for each trait based on the
377 divergence among populations. Ellipses represent the 95% confidence ellipses for each
378 population centered at the multivariate species mean (DUN: Dunnigan CA, AG: Aguila AZ,
379 SOC: Socorro NM, LC: Las Cruces NM).

Methods

Cricket collection

We collected adult female crickets from four populations throughout the southwest and western US: Socorro, NM; Las Cruces, NM; Aguila, AZ; and Dunnigan, CA (Figure 1) during the summer of 2017. Crickets from these locations are all formally recognized as members of *Gryllus integer* but additional splitting out of subspecies or different species based on population genetic structure is currently being considered (Weissman, personal communication). Around 50 females on average were collected from each population (Table S1) and taken to animal housing facilities at North Dakota State University. Females were housed individually in 0.71 L containers and provided with ad libitum food (Purina Chick Starter) and water (water was provided in glass vials capped with cotton). Each cricket was also provided with a small piece of cardboard egg carton for shelter. The cricket housing room was maintained on a 12:12 dark:light cycle reversed such that the room was dark during daytime hours. The housing room was kept at ~27C.

Breeding design

Females collected from the field (generation P) were assumed to have mated prior to capture (with the possibility of multiple mating, as is common in the genus[32]) and were allowed to oviposit in water vials while in their containers. Offspring of these females (termed generation F_0 as sires were unknown) hatched in their dams' containers and were moved to individual housing prior to maturation. We assayed the behavior of 387 F_0 individuals (see below) upon maturation (Table S1). After behavioral trials, F_0 individuals were assigned to breeding pairs such that individual males were mated to multiple

402 randomly assigned females from the same population but different dams according to a
403 standard full-sib, half-sib breeding design[20]. Matings were conducted as follows: females
404 were moved from their normal housing containers to a larger container (34.6 × 21 × 12.4
405 cm) along with their food dish, water vial, and egg carton shelter. After the female had been
406 transferred, the assigned male was likewise moved to the large container, also with its food
407 dish, water vial, and egg carton. The male and female remained in these containers for 24
408 hours to allow sufficient time for courtship and multiple mating. After 24 hours the male
409 and female crickets were returned to their original containers. If males were to be mated
410 with additional females, they were allowed a minimum of 24 hours before repeating the
411 above procedure. These F_0 females were subsequently allowed to oviposit into water vials
412 within their containers. Resulting F_1 offspring were moved to individual housing prior to
413 maturation and had their behaviors assayed upon maturation. After behavioral assays, F_1
414 individuals were likewise paired with F_1 individuals of the same population but different
415 sires in the same manner and resulting F_2 offspring moved to individual housing and had
416 their behavior measured upon maturation. This resulted in the behavioral testing of 395 F_1
417 individuals and 163 F_2 individuals (Table S1). Across the three generations this
418 represented behavioral testing of 946 individual crickets.

419 **Behavioral testing**

420 All behavioral tests followed standard procedures previously validated in the literature for
421 Gryllid crickets[21-24,33-35]. Below, we briefly describe these tests and their ecological
422 relevance.

423 *Latency to emerge from shelter*

Gryllid crickets, including *G. integer*, use small burrows and natural cracks for refugee from predators and to which they retreat when under threat. As a result, latency to emerge from shelter after disturbance can be considered a proxy for risk-taking behavior or "boldness"[21]. Here, we conducted latency tests wherein individuals were transferred from their home containers to small artificial burrows (40 cm^3) placed within a 34.6 × 21 cm arena. These artificial burrows were capped so that individuals could not immediately emerge. Crickets were forced to remain in the artificial burrow for two minutes after which the cap was removed. Crickets were then allowed six minutes and thirty seconds to emerge from the artificial burrow. During this test we recorded how long it took for an individual emerge (in seconds). Individuals that did not emerge were given a maximum latency of 390 seconds.

Open field exploratory behavior

Open field tests are a classic behavioral assay across taxa[36] which measure the exploratory propensity of individuals[37], including crickets[22-24]. Individuals that move through more of the arena are considered more thorough explorers[37]. Here we used open field tests to measure activity and exploratory propensity in a 30 × 30 cm plexiglass arena. Individuals were introduced into the arena under a small container and allowed to rest for 30 seconds after introduction. At the end of this 30 seconds, the container was removed and the cricket was allowed to explore the arena for 3 minutes and 40 seconds. The arena was cleaned with isopropyl alcohol between trials to remove any chemosensory cues from the arena. We used Ethovision XT to record the total distance the individual moved during the trial (cm), the number of unique zones of the arena an individual visited during the trial, and the

446 variance in velocity of individuals $(cm/s)^2$. This latter measure indicates whether an
447 individual's speed of exploration was constant (low velocity variance) or whether
448 individuals had frequent activity bursts punctuated by long bouts of inactivity (high
449 velocity variance).

450 *Response to cues of predator presence*

451 How individuals respond to cues of predator presence often varies within and among
452 populations and is likely to covary with fitness[15,38]. Crickets respond to chemical cues of
453 predator presence by either freezing or increasing activity depending on whether
454 confronted by predator cues of sit-and-wait or active predators[39,40]. Here we used a
455 behavioral assay to measure response to cues of predator presence previously validated
456 with another Gryllid species[22,23]. Specifically, individuals were introduced into a 15 cm
457 diameter circular arena (7.5 cm height), the floor of which was covered with dry filter
458 paper that had been soaked with diluted excreta from leopard geckos (*Eublepharis*
459 *macularius*). All leopard geckos were fed a diet of *G. integer* with occasional diet
460 supplementation of mealworms (i.e. larval *Tenebrio molitor*) and the related decorated
461 cricket (*Gryllodes sigillattus*). Crickets were introduced to a portion of the arena without
462 predator cue under a small shelter. After a 30s rest period, the shelter was removed and
463 the individual allowed to freely move throughout the arena for 3 minutes and 40 seconds.
464 We then used Ethovision XT to record the total distance an individual moved during the
465 trial (cm). Total distance moved during the predator cue trial, the latency to first movement
466 (in seconds), and the variance in velocity were used in subsequent analyses.

467 **Statistical analyses**

468 **G** *matrix estimation*

469 We used multi-response mixed effect animal models[41] implemented using the MCMCglmm
470 package in R[42] to estimate genetic variances and covariances. We included the effects of
471 temperature of the behavioral arena room, sex of the individual, and mass of the individual
472 as fixed effects and the individual relatedness matrix (based on the known pedigree) as a
473 random effect. Traits for which variances and covariances were estimated were: (i) the
474 latency that an individual emerged from the shelter during the trial (censored Gaussian),
475 (ii) the distance moved during the open field trial (Gaussian), (iii) the number of unique
476 zones an individual visited during the open field trial (Poisson), (iv) the log-transformed
477 variance in velocity during the open field trial (Gaussian), (v) the square-root transformed
478 distance an individual moved during the predator cue response trial (Gaussian), (vi) the
479 latency to initiate movement in the antipredator response trial (Poisson) and (vii) the log-
480 transformed variance in velocity during the antipredator response trial (Gaussian). Multi-
481 response models were fit individually by population with each population's variances and
482 covariances estimated from the posterior of an MCMC chain of 4.8×10^6 iterations, with an
483 800,000 burn-in period and a thinning interval of 4,000. A prior that was minimally
484 informative for both variances and covariances was used. All variances and covariances
485 were estimated at the additive genetic level and on the latent scale (Table S4).

486 *Population comparisons of* **G**

487 To compare behavioral syndrome structure at the additive genetic level we:

488 (i) compared alignment of dominant eigenvectors among populations (i.e. $\mathbf{g}_{max}$,
489 Table S5[9]);

(ii) tested whether populations exhibited shared subspaces of **G** [25];

(iii) tested how populations differed in their variances and covariances (i.e. genetic covariance tensor analysis[25]).

We followed the recommendations of Aguirre et al. (2014) in that all tests were based on the full MCMC posterior distributions, null distributions for population comparisons were based on randomizations of breeding values, and vector correlations estimated against null expectations of 1 (see also[43]). Specifically, to compare whether eigenvectors were significantly aligned, we generated a random distribution of vector correlations following [13]. The critical values of this distribution were 0.93 ($P < 0.001$), 0.85 ($P < 0.01$), 0.71 ($P < 0.05$) and 0.62 ($P < 0.1$). To assess the significance of eigenvalues of **H** and **E** against random expectations, we calculated the largest posterior quantiles for which these distributions did not overlap (Figures S2 and S3 respectively). This threshold serves as a Bayesian probability in favor of the observed distribution being generated by patterns other than chance (hereafter, P_{mcmc}). We interpret these probabilities on the following scale: $P_{mcmc} < 0.7$: poor evidence of difference compared to random expectations; $P_{mcmc} > 0.8$: moderate evidence of difference compared to random expectations; $P_{mcmc} > 0.9$ strong evidence of difference compared to random expectations; Pmcmc > 0.95: very strong evidence of difference compared to random expectations.

Based on the estimated **G** matrices for each population, we also calculated "autonomy" ($\bar{a}$) throughout multivariate space following Hansen and Houle [26]. Autonomy provides an estimate of the "fraction of genetic variation that is independent of potentially constraining characters"[26]. Put another way, autonomy estimates the degree to which

512 genetic variation is free to respond to selection (max $\bar{a}$ = 1) versus constrained by

513 covariance (min $\bar{a}$ = 0).