Animal personality shapes evolutionary trajectories via conserved genetic architecture.

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Abstract

Consistent individual differences in behavior, i.e. animal personality, are often correlated within broader syndromes[1] that can cause evolution in one trait to drive correlated changes in other behaviors via genetic correlations[2]. Despite abundant demonstration that these behavioral syndromes are common across taxa[3,4], it has not been clear whether syndromes arise due to genetic and cellular mechanisms or because particular combinations of behavioral responses are favored. 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 covariance among behavioral traits was consistent with genes and cellular mechanisms underpinning behavioral syndromes rather than historic correlated selection. Moreover, divergence among populations' average behaviors was constrained by the genetically conserved behavioral syndrome. By constraining the divergence of populations, animal personality may therefore also affect the ability of species to behaviorally diverge and therefore speciation. This suggests important ways in which labile traits—traits that are highly plastic, like behavior, physiology, and life-history traits—might affect evolutionary outcomes.

Behavior is frequently assumed to have been shaped by selection[5] and thus populations are expected to differ in a range of behaviors based on local selective pressures. This perspective implies that behaviors are able to evolve independently, an assumption increasingly challenged by the ubiquity of behavioral syndromes—correlations among behaviors[6]—which have been documented across taxonomic groups[3,4] and are comprised of both genetic and environmental contributions[2,7].

Given the contribution of genetic correlations to behavioral syndromes[7,8], syndromes have the potential to constrain the ability of populations to diverge and respond to local selective pressures[9]. Specifically, based on quantitative genetic theory, if syndromes reflect pleiotropic effects—where 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[10] (Figure 1). Consequently, 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[11]), the divergence of populations will be relatively unconstrained as these genetic correlations are expected to rapidly break down when selection changes[12].

These two quantitative genetic explanations for behavioral syndromes have explicit analogs in the behavioral literature: whether syndromes emerge from pleiotropy or other mechanistic effects is termed the “constraints hypothesis,” as opposed to selection-induced linkage disequilibrium, which is termed the “adaptive hypothesis” (Figure 1, see also[13]).

Knowing whether the constraints or adaptive hypothesis drives the emergence of behavioral syndromes is of critical importance. 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[14]. Whether behavioral syndromes have similar effects is an open question. Unfortunately, while some studies have compared phenotypic or among-individual correlations across populations[15-19], population comparison of behavioral syndromes at the additive genetic level has been restricted to a single pairwise comparison of two populations[13]. Consequently, the generality of the constraints versus adaptive hypotheses is unclear and the general effect of behavioral syndromes on population divergence is unknown.

Here we tested whether behavioral syndromes have diverged at the genetic level among populations of the field cricket, *Gryllus integer*. We critically evaluated predictions of the adaptive and constraints hypotheses (Figure 1) via comparisons of behavioral genetic (co)variance matrices, i.e. **G**, 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. 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 population 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

64 individuals in total, Table S1) in three ecologically relevant behavioral assays: latency to
65 emerge from shelter, activity/exploration of a novel area, and locomotive response to cues
66 of predator presence (see Methods). Based on the known relatedness among individuals
67 we estimated **G** for each population. We then compared the four populations to determine
68 whether the differences among **G**s were consistent with expectations of either the adaptive
69 or constraints hypotheses based on the distribution of genetic variation in multivariate
70 trait space[10,14,21-23] and based on whether correlations degraded across generations as
71 expected under the adaptive hypothesis[12].

72 As predicted under the constraints hypothesis, the populations exhibited conserved
73 behavioral syndrome structure. This conclusion is supported by three primary lines of
74 evidence: First, and most importantly, the four populations shared three subspaces in
75 multivariate space that explained 99% of the observed genetic variation (Table S2; all
76 Bayesian probabilities < 0.6 for differences). These subspaces were primarily characterized
77 by variation in latencies to emerge from shelter, distance traveled during exploration, and
78 in response to cues of predator presence (Table S2). These subspaces describe the
79 structure of the species' behavioral syndrome and their being shared demonstrates that the
80 orientation of genetic variation was conserved among the populations. Second, we found
81 that the $\mathbf{g}_{max}$'s (the dimension in which most behavioral variation was expressed[10]) of the
82 Aguila and Dunnigan and Socorro and Aguila populations were strongly correlated (Figure
83 2). This, again, demonstrates that behavioral syndrome structure is shared among the
84 populations. Third, under the adaptive hypothesis, genetic correlations are expected to
85 decrease by about 50% each generation with a corresponding decrease in phenotypic
86 correlations (Appendix S1); these correlations were, instead, constant (Figure 3).

Despite this conservation of behavioral syndrome structure, populations did exhibit some degree of divergence. Importantly, however, this divergence was constrained by the structure of behavioral syndromes (Table S3). Specifically, the populations have diverged in their multivariate averages (Figure 4, Table S2) and in the magnitude of genetic variation present (Figure S3), but this divergence aligned with the shared behavioral syndrome (i.e. shared subspaces) of the populations (Table S3). 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 for the three eastern populations or the accumulation of variation for the western population is not currently clear.

We thus have three key results demonstrating conservation of behavioral syndromes at the genetic level despite differences among populations in average behavior. This conservation of behavioral syndrome structure has had the effect of constraining population divergence. Jointly these results provide strong support for the constraints hypothesis for the expression of behavioral syndromes. However, the first two results—shared subspaces and correlated $\mathbf{g}_{max}$'s—could also result under the adaptive hypothesis if the selective pressures each of the populations experienced were the same. We consider this unlikely because of the degree of geographic separation among populations (Figure 2) and because $\mathbf{g}_{max}$ was most similar among populations that were geographically most separated (Figure 2). Moreover, 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[11,12]. In direct contradiction of 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.

In contrast to our findings, a previous study with stickleback[13] found that two populations differed in the magnitude of heritabilities and genetic correlations between behaviors, albeit with overlapping confidence intervals. This earlier result is suggestive of behavioral syndromes emerging from correlated selection but predates the development of methods for the comparison of the full multivariate structure among populations as done here. Unfortunately, examination and comparison of pairwise genetic correlations can be misleading in regard to evolutionary constraints. For example, while two traits may be uncorrelated, each may be strongly correlated to others and differ in the magnitude of genetic variation available, thereby generating constraints not apparent from correlations. Instead, it is the overall multivariate structure of variation that shapes evolutionary responses and leads to quantitative constraints on evolution[24,25]—constraints that we have evaluated for behavioral syndromes for the first time here.

Behaviors like 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. Our results suggest an unrecognized and important role for behaviors like these and for behavioral syndromes in

the evolution of populations. That behavioral syndromes are important for trait divergence among populations implies that they may also affect speciation patterns. While correlations among morphological traits have been implicated in constraining speciation[14,26,27] and sexually selected behaviors have been argued to play a role in pre-zygotic reproductive isolation in allopatry[28], the potential for behaviors like those examined here to influence speciation has not been critically evaluated. Moreover, genetic correlations are common across trait types[2,29,30] and, given that genetic variance is rarely limited[31], these correlations should be expected to quantitatively constrain divergence and responses to selection even if they do not act as absolute constraints. Because behaviors exhibit heritabilities similar to those observed for life-history and physiological traits[29,32,33], our results also suggest that correlations among labile traits—traits that are highly plastic—may actually play a role in shaping differences among populations and species. Our findings suggest that a more diverse range of traits and behaviors may have shaped population divergence and speciation patterns than previously considered.

Acknowledgements

We thank Monica Berdal, Kathelyn Cannon, Jeremy Dalos, Sarah Felde, Brady Klock, Ishan Joshi, Hannah Lambert, Jenna LaCoursiere and Alondra Neunsinger for assistance in conducting behavioral trials and in rearing and care of the crickets and Martori Farms, David Lightfoot, Scott Bundy, Nico Franz, Sangmi Lee, Cameron Jones, Kenny Chapin, Ti Eriksson, Meranda Feagins, Charlotte Mulloney, Melody Martinez, Allyson Richins, Mauriel Rodriguez, Helen Vessels and David Wikman for assistance in collecting the crickets. We thank J. Pruitt, J. Bowsher, and members of the Gillam and Dochtermann labs at NDSU for important comments on earlier versions of this paper. This work was supported by US NSF IOS grants 1557951and 1558069 to N.A.D. and A.H. respectively.

156 *Author Contributions*

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

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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 ($\mathbf{g}_{max}$) 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 (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, $\mathbf{g}_{max}$), and, third, orientation (vector correlation between $\mathbf{g}_{max}$ and conserved genetic subspaces $\mathbf{h}_{1\text{-}3}$). Off-diagonal elements (green shading) 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: P_{mcmc} for difference from expectations under selection-induced linkage disequilibrium > 0.85; r_P: 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

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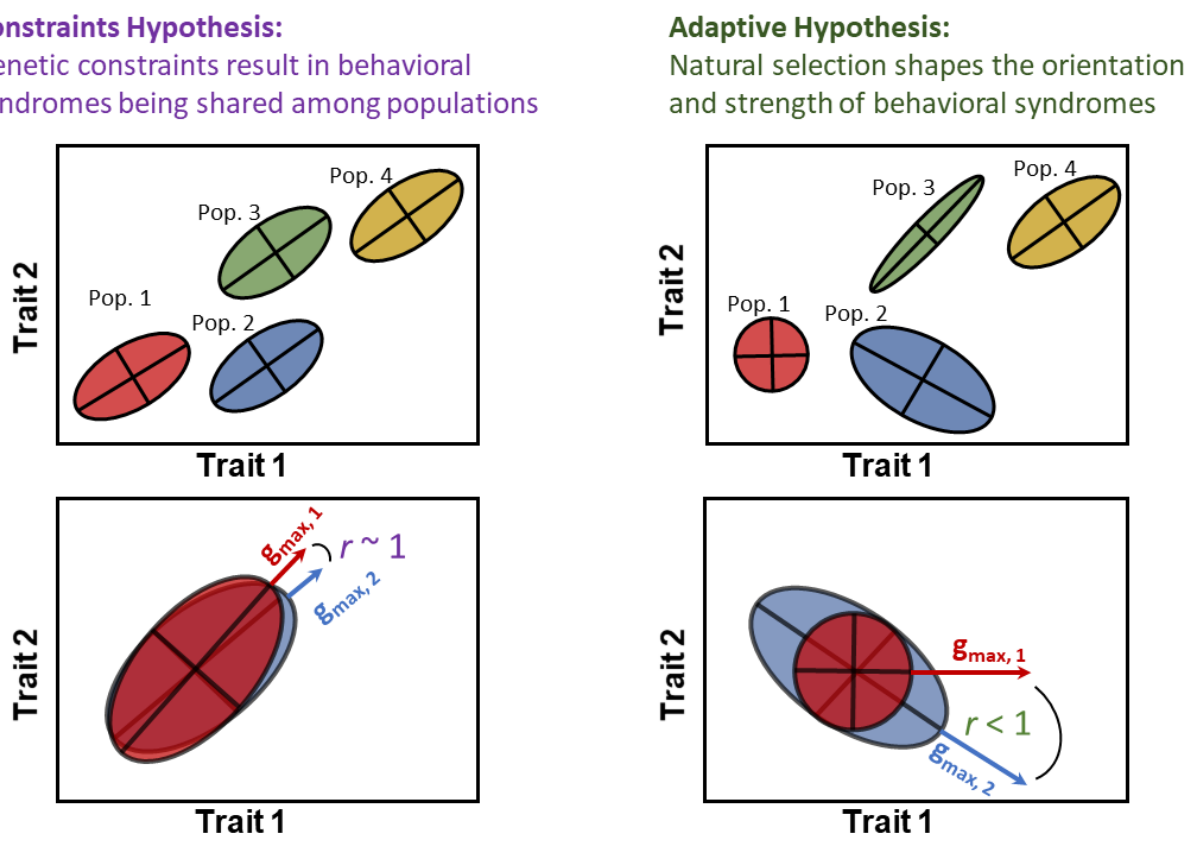

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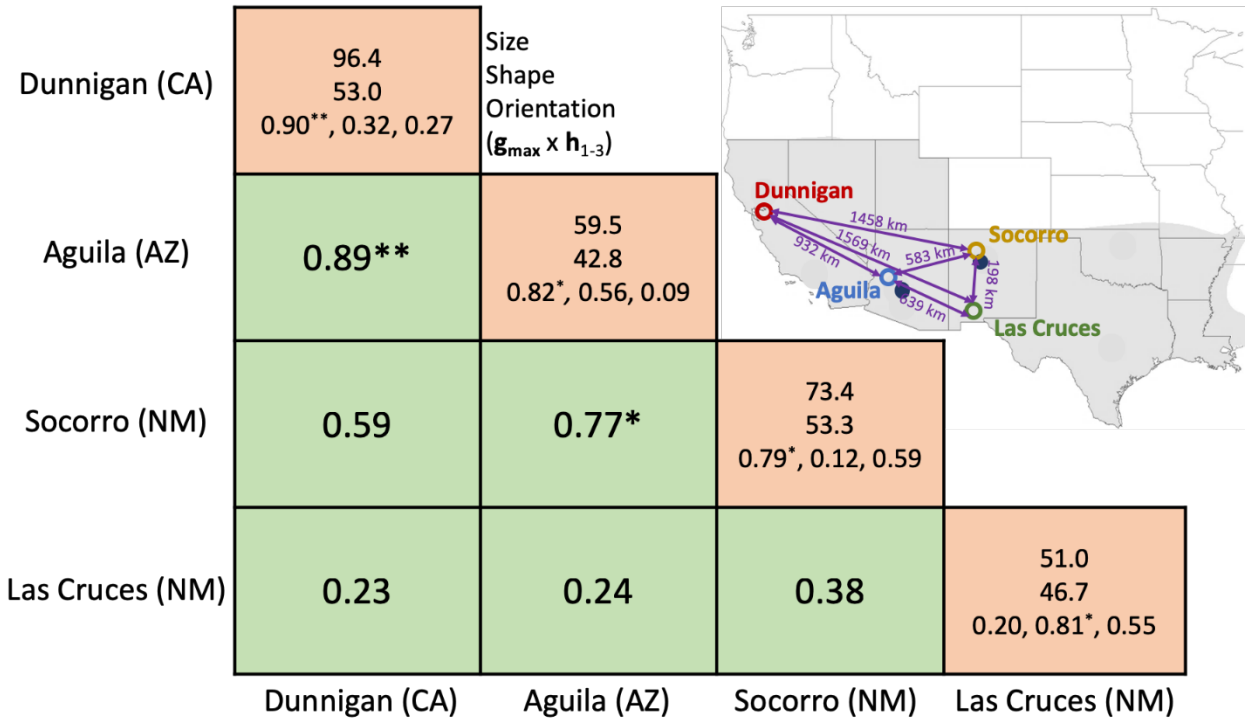

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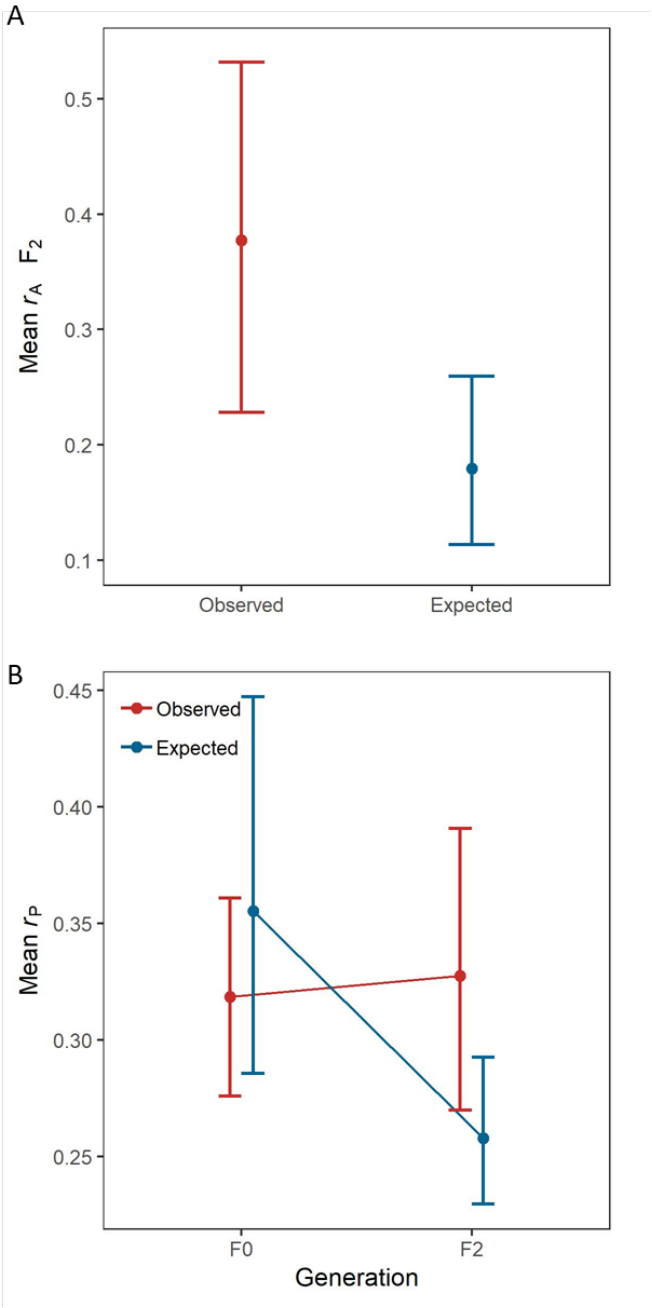

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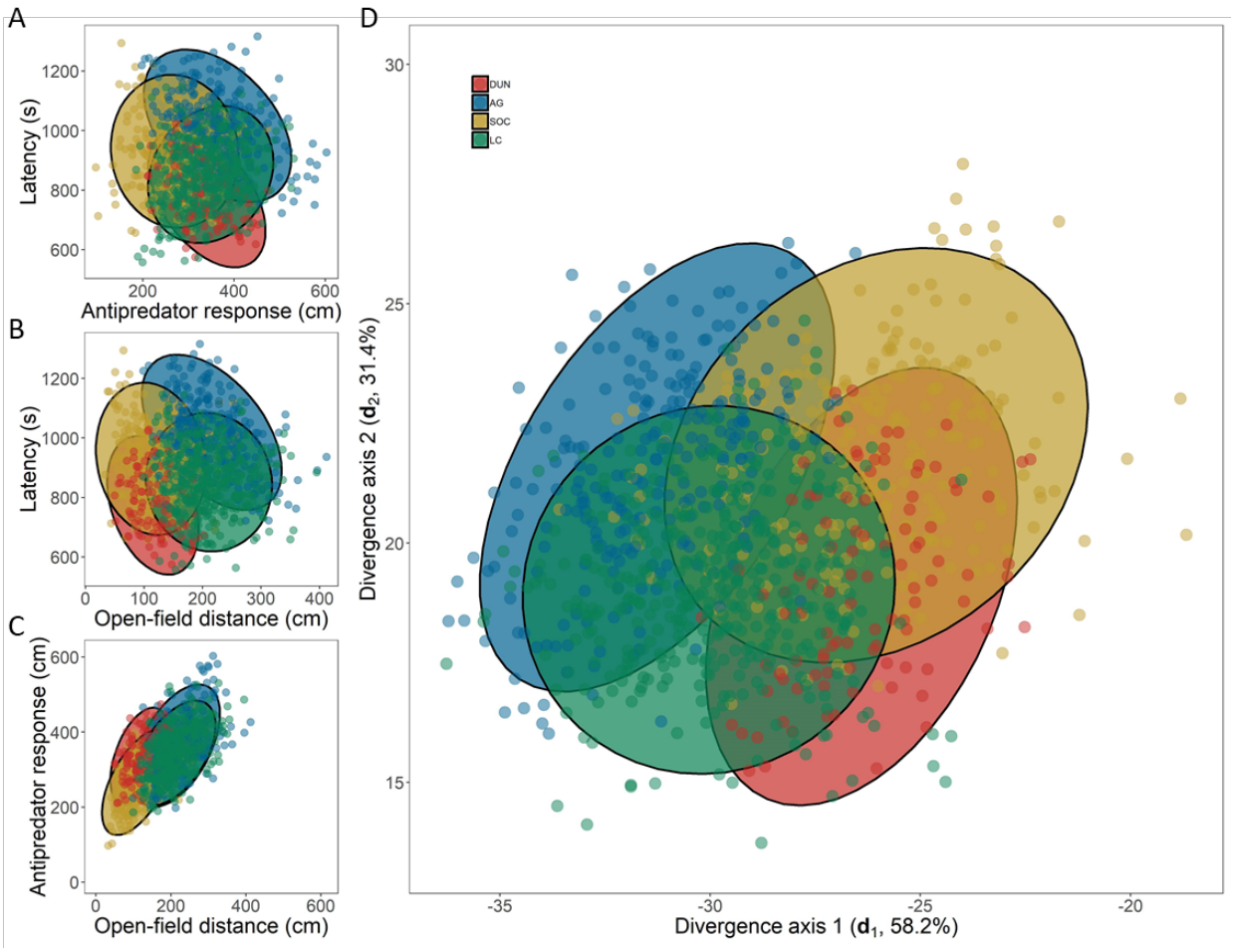

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Methods

Cricket collection

We collected adult female crickets from four populations throughout the southwest and western US: Socorro, NM; Las Cruces, NM; Aguilla, AZ; and Dunnigan, CA (Figure 1) during the summer of 2017. Crickets from these locations are formally recognized as all members of *Gryllus integer* but additional splitting out of subspecies or different species based on population genetic structure is currently being considered (Weissman, personal communications). 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[34]) 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 dam's container 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

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

Behavioral testing

All behavioral tests followed standard procedures previously validated in the literature for Gryllid crickets[35-41]. Below, we briefly describe these tests and their ecological relevance.

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"[37]. 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[42] which measure the exploratory propensity of individuals[43], including for crickets[39-41]. Individuals that move through more of the arena are considered more thorough explorers[43]. 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. From this test 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 variance in velocity of individuals $(cm/s)^2$. This latter measure indicates whether an individual's speed of exploration was constant (low velocity variance) or whether individuals had frequent activity bursts punctuated by long bouts of inactivity (high velocity variance).

Response to cues of predator presence

How individuals respond to cues of predator presence often varies within and among populations and is likely to covary with fitness[16,44]. Crickets 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[45,46]. Here we used a behavioral assay to measure response to cues of predator presence previously validated with another Gryllid species[39,40]. Specifically, individuals were introduced into a 15 cm diameter circular arena (7.5 cm height) the floor of which is covered with dry filter paper that had been soaked with diluted excreta from leopard geckos (*Eublepharis macularius*). 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 sigillattus*). Crickets were introduced to a portion of the arena without predator cue under a small shelter. After a 30s 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.

Statistical analyses

409 **G** *matrix estimation*

410 We used multi-response mixed effect animal models[47] implemented using the MCMCglmm
411 package in R[48] to estimate genetic variances and covariances. We included the effects of
412 temperature of the behavioral arena room, sex of the individual, and mass of the individual
413 as fixed-effects and the individual relatedness matrix (based on the known pedigree) as a
414 random effect. Traits for which variances and covariances were estimated were: (i) the
415 latency that an individual emerged from the shelter during the trial (censored gaussian),
416 (ii) the distance moved during the open field trial (gaussian), (iii) the number of unique
417 zones an individual visited during the open field trial (Poisson), (iv) the log-transformed
418 variance in velocity during the open field trial (gaussian), (v) the square-root transformed
419 distance an individual moved during the predator cue response trial (gaussian), (vi) the
420 latency to initiate movement in the antipredator response trial (Poisson) and (vii) the log-
421 transformed variance in velocity during the antipredator response trial (gaussian). Multi-
422 response models were fit individually by population with each population's variances and
423 covariances estimated from the posterior of an MCMC chain of 4.8×10^6 iterations, with an
424 800,000 burn-in period and a thinning interval of 4,000. A prior that was minimally
425 informative for both variances and covariances was used. All variances and covariances
426 were estimated at the additive genetic level and on the latent scale (Table S4).

427 *Population comparisons of* **G**

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

429 (i) compared alignment of dominant eigenvectors among populations (i.e. $\mathbf{g}_{max}$,
430 Table S5[10]);

(ii) tested whether populations exhibited shared subspaces of **G** [21];

(iii) tested how populations differed in their variances and covariances (i.e. genetic covariance tensor analysis[21]).

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 angles estimated against null expectations of 0 (see also[49]). Specifically, to compare whether eigenvectors were significantly aligned, we generated a random distribution of vector correlations following [14]. 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.

Supplementary Materials

Appendix S1. Comparing observed patterns of phenotypic correlations with expectations based on SILD and random mating.

Table S1. Sample sizes by population and generation.

Table S2. Eigenvectors of phenotypic divergence (**d**), conserved genetic variation (**h**) and divergence in **G** (**e**).

Table S3. Vector correlation (*r*) between major axes of phenotypic divergence (**d**), subspaces of conserved genetic variation (**h**) and subspaces representing genetic divergence (**e**).

Table S4. Genetic variances and covariances of four populations of field crickets.

Table S5. Eigen decomposition of **G** for each population.

Figure S1. Population coordinates and genetic variance along the leading eigentensors capturing most of the variation in divergence (**E**1 and **E**2).

Figure S2. Eigenvalues of **H** for a comparison of the first three eigenvectors of each **G** matrix compared against random expectations.

Figure S3. Eigenvalues of the first three positive eigentensors of **S**.

Appendix S1. Comparing observed patterns of phenotypic correlations with expectations based on SILD and random mating.

Genetic correlations are expected to decline every new generation when they arise from selection-induced linkage disequilibrium (SILD). Conner [12] showed that in absence of both selection and linkage, the magnitude of genetic correlations under a SILD model and random mating should halve with every new generation. In contrast, genetic correlations caused by pleiotropic effects are expected to remain stable even after multiple generations of random mating. From this we expect the additive genetic correlation in the F_2 generation to be half as large as that of the F_1 generation:

$$(1)\ r_{A,\,F_2} = \frac{1}{2} r_{A,\,F_1}$$

To test this hypothesis, we estimated the observed genetic covariance based on multivariate animal models including the data of the F_0 and F_1 and F_1 and F_2 generations respectively. These models were specified similarly to the multivariate models described in the main text, but population origin was added as a fixed effect to control for population differences in average trait expression. We transformed the genetic covariance matrix to a correlation matrix and calculated the observed average correlation coefficients, $r_{A\ observed}$, for every generation. We then compared, $r_{A\ observed}$ to the expected average correlation shown in equation (1). Since our multivariate models were specified within a Bayesian framework, we performed these operations on every slices of the posterior estimates to obtain 95 % credible intervals of observed and expected estimates and base our inference on the overlap between the two 95 % credible intervals.

Because phenotypic correlations result from the combined influence of genetic and environmental influences, it is also possible to compare the expected decline in phenotypic

correlations under SILD and random mating with the phenotypic correlations observed in every generation. The expected relationship between the phenotypic covariance in generation F_0, F_1 and F_2 can therefore be expressed by the following sets of equations:

$$(2)\ \mathbf{P}_{\mathbf{F_0}} = \mathbf{2}\ \mathbf{G}_{\mathbf{F_1}} + \mathbf{E}$$

$$(3)\ \mathbf{P}_{\mathbf{F_1}} = \mathbf{G}_{\mathbf{F_1}} + \mathbf{E}$$

$$(4)\ \mathbf{P}_{\mathbf{F_2}} = \frac{\mathbf{1}}{\mathbf{2}}\mathbf{G}_{\mathbf{F_1}} + \mathbf{E}$$

Where $\mathbf{P}_{\mathbf{F_x}}$, and $\mathbf{G}_{\mathbf{F_x}}$ represent the phenotypic and genetic covariance matrices for generation F_x respectively and **E** represents the overall environmental covariance matrix estimated across all generations. Note that these formulas only apply to the covariance among traits (i.e. the off-diagonal elements) while the genetic variances (i.e. the diagonal elements) are kept constant across generations. $\mathbf{G}_{\mathbf{F_1}}$ was used as our reference here because this generation had the largest sample size and thus had the most influence on the estimation of **G**. We estimated $\mathbf{G}_{\mathbf{F_1}}$ from the animal model used to estimate equation (1) and we estimated **E** using a model containing the data from all populations for every generations.

We then compared these expected covariances to the observed phenotypic covariance estimated for each generation separately by fitting multivariate models excluding pedigree information so that only the phenotypic covariance was estimated. As above, we converted each covariance matrix to a correlation matrix and compared the observed average correlation coefficient $r_{P\ observed}$, with the average correlation coefficient $r_{P\ expected}$ for every generation.

510 **Table S1.** Number of sires, dams and phenotyped offspring for each population and
511 generation. The F_0 population represents the first population established in the laboratory
512 after collection of gravid females in the field. N indicates the number field collected females
513 for the F_P generation.

514

Population and generation	No. of sires	No. of dams	No. offspring in pedigree	No. phenotyped offspring
Dunnigan (CA) (N = 38)				
F_0	0	8	30	30
F_1	6	8	36	36
F_2	6	8	35	35
Total	12	24	101	101
Aguila (AZ) (N = 71)				
F_0	0	32	144	143
F_1	16	21	92	92
F_2	2	2	16	16
Total	18	55	252	251
Socorro (NM) (N = 65)				
F_0	0	19	112	112
F_1	10	20	126	125
F_2	4	4	8	8
Total	14	43	246	245
Las Cruces (NM) (N = 38)				
F_0	0	13	105	102
F_1	20	47	157	142
F_2	13	16	104	104
Total	33	76	366	349

515

Table S2. 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

Table S3. Vector correlation (r) between major axes of phenotypic divergence ($\mathbf{d}_1$, $\mathbf{d}_2$), subspaces of conserved genetic variation ($\mathbf{h}_1$, $\mathbf{h}_2$, $\mathbf{h}_3$) and subspaces representing genetic divergence ($\mathbf{e}_{11}$, $\mathbf{e}_{21}$, $\mathbf{e}_{22}$). Probability of significant alignment: *** P < 0.001, ** P < 0.01, * P < 0.05. Italics indicate significant alignment at P < 0.1.

	$\mathbf{d}_1$	$\mathbf{d}_2$	$\mathbf{h}_1$	$\mathbf{h}_2$	$\mathbf{h}_3$
$\mathbf{h}_1$	0.49	0.40			
$\mathbf{h}_2$	0.15	0.89**			
$\mathbf{h}_3$	0.85**	0.06			
$\mathbf{e}_{11}$	0.25	*0.69*	0.92**	0.33	0.18
$\mathbf{e}_{21}$	0.90**	0.40	0.72*	0.16	*0.67*
$\mathbf{e}_{22}$	0.38	0.60	0.59	0.34	0.72*

Table S4. Genetic variances (shaded diagonal elements) and covariances (bottom off-diagonal elements) of four populations of field crickets sampled in Dunnigan (CA), Aguila (AZ), Socorro (NM) and Las Cruces (NM). The probability of excluding zero (Pmcmc) are indicated on the top diagonal. Bold values indicate Pmcmc > 0.95, bold and italics indicate Pmcmc > 0.90 and italics indicate Pmcmc > 0.80.

	Latency	OF.Dist	UZ	OF.Var.Velo	AP.Dist	AP.Lat.Mov	AP.Var.Velo
Dunnigan (CA)							
Latency	33.66	0.58	0.66	0.61	*0.88*	**0.95**	0.77
OF.Dist	1.89	21.58	**0.95**	**0.99**	0.57	0.54	0.64
UZ	0.62	**1.96**	0.69	**0.99**	0.55	0.73	0.67
OF.Var.Velo	0.38	**2.18**	**0.34**	0.43	0.57	0.63	0.70
AP.Dist	*-14.73*	2.78	0.27	0.43	36.36	***0.94***	**0.99**
AP.Lat.Mov	**5.20**	0.02	0.27	0.10	***-5.44***	3.43	0.66
AP.Var.Velo	-0.90	0.41	0.08	0.08	***2.09***	-0.15	0.28
Aguila (AZ)							
Latency	20.67	0.60	0.57	0.54	0.76	0.68	0.61
OF.Dist	-2.01	18.48	**0.99**	**0.99**	0.69	0.79	0.60
UZ	-0.17	**1.48**	0.37	**0.99**	0.54	0.70	0.47
OF.Var.Velo	-0.13	**1.52**	**0.17**	0.30	0.61	*0.80*	0.65
AP.Dist	-4.28	2.80	0.10	0.21	17.89	*0.83*	**0.99**
AP.Lat.Mov	0.91	1.13	0.09	*0.15*	*-1.27*	1.61	0.69
AP.Var.Velo	-0.19	0.15	0.00	0.03	**0.91**	-0.06	0.16
Socorro (NM)							
Latency	20.57	0.54	0.65	0.54	0.51	0.55	0.46
OF.Dist	-1.91	26.26	**0.99**	**1.00**	**0.96**	***0.90***	***0.91***
UZ	-0.48	**2.16**	0.46	**1.00**	***0.91***	*0.89*	*0.82*
OF.Var.Velo	-0.23	**2.78**	**0.29**	0.41	***0.94***	***0.92***	***0.92***
AP.Dist	-0.74	**12.83**	***1.24***	***1.58***	24.39	**0.99**	**0.99**
AP.Lat.Mov	0.24	***-1.68***	*-0.20*	***-0.23***	**-2.54**	1.09	0.76
AP.Var.Velo	0.07	***0.97***	*0.08*	***0.13***	**1.68**	-0.09	0.22
Las Cruces (NM)							
Latency	22.00	0.55	0.64	0.68	0.74	0.69	0.77
OF.Dist	0.43	12.46	**0.99**	**1.00**	***0.92***	*0.83*	*0.84*
UZ	-0.17	**0.65**	0.21	**0.99**	*0.86*	*0.84*	0.77
OF.Var.Velo	0.26	**1.02**	**0.09**	0.18	*0.87*	0.76	***0.94***
AP.Dist	3.16	***4.90***	*0.41*	*0.54*	14.70	**0.97**	**1.00**
AP.Lat.Mov	-0.59	*-0.92*	*-0.10*	-0.09	**-1.67**	1.30	0.72
AP.Var.Velo	0.34	*0.38*	0.03	***0.07***	**0.90**	-0.06	0.13

Table S5. Eigen decomposition of **G** for each population. Bold values indicate loadings > 0.25 to help interpretation.

	$\mathbf{g}_{max}$	$\mathbf{g}_2$	$\mathbf{g}_3$	$\mathbf{g}_4$	$\mathbf{g}_5$	$\mathbf{g}_6$	$\mathbf{g}_7$	$\mathbf{g}_{max}$	$\mathbf{g}_2$	$\mathbf{g}_3$	$\mathbf{g}_4$	$\mathbf{g}_5$	$\mathbf{g}_6$	$\mathbf{g}_7$
	Dunnigan (CA)							Aguila (AZ)						
λ	51.11	24.59	17.64	2.28	0.52	0.17	0.13	25.47	17.59	14.52	1.38	0.27	0.16	0.11
% Variance	53.00	25.50	18.29	2.37	0.54	0.17	0.14	42.81	29.56	24.40	2.32	0.46	0.26	0.18
Latency	**-0.66**	**-0.47**	**0.57**	-0.11	0.00	0.01	-0.01	**0.70**	**-0.53**	**0.48**	-0.04	0.00	0.00	0.00
OF.Dist	0.03	**-0.78**	**-0.61**	-0.02	-0.12	-0.05	-0.04	**-0.44**	**-0.84**	**-0.29**	-0.08	0.11	0.03	-0.01
UZ	0.00	-0.08	-0.04	0.12	**0.92**	**-0.33**	-0.15	-0.03	-0.07	-0.03	-0.02	**-0.88**	**0.42**	-0.20
OF.Var.Velo	0.00	-0.09	-0.05	0.05	**0.34**	**0.72**	**0.59**	-0.03	-0.07	-0.02	0.04	**-0.46**	**-0.79**	**0.40**
AP.Dist	**0.73**	**-0.40**	**0.54**	0.10	-0.03	-0.04	0.05	**-0.57**	0.00	**0.82**	0.08	-0.01	0.03	0.04
AP.Lat.Mov	-0.16	-0.01	0.00	**0.98**	-0.14	-0.05	0.05	0.04	-0.09	-0.07	**0.99**	0.01	0.04	-0.02
AP.Var.Velo	0.04	-0.03	0.02	0.08	0.07	**0.60**	**-0.79**	-0.03	0.00	0.04	0.00	0.00	**-0.45**	**-0.89**
	Socorro (NM)							Las Cruces (NM)						
λ	39.13	20.44	12.52	0.83	0.29	0.11	0.08	23.81	17.36	8.39	1.09	0.19	0.10	0.06
% Variance	53.31	27.85	17.06	1.13	0.39	0.14	0.11	46.69	34.05	16.46	2.13	0.37	0.19	0.11
Latency	0.10	**0.99**	-0.10	0.01	-0.02	0.01	0.00	**0.86**	**0.49**	-0.13	-0.01	0.01	0.02	0.00
OF.Dist	**-0.72**	**0.01**	**-0.68**	-0.03	0.10	0.05	0.05	0.23	**-0.61**	**-0.75**	-0.03	-0.07	0.04	0.03
UZ	-0.06	-0.01	-0.04	0.08	**-0.95**	0.19	0.20	0.01	-0.04	-0.03	0.04	**0.91**	**0.37**	0.18
OF.Var.Velo	-0.08	0.00	-0.06	0.04	**-0.26**	**-0.63**	**-0.72**	0.03	-0.05	-0.06	-0.01	**0.39**	**-0.67**	**-0.62**
AP.Dist	**-0.67**	0.14	**0.72**	-0.09	0.00	0.06	-0.05	**0.44**	**-0.62**	**0.64**	-0.10	-0.02	0.04	-0.04
AP.Lat.Mov	0.08	-0.01	-0.06	**-0.98**	-0.09	0.07	-0.09	-0.07	0.08	-0.04	**-0.99**	0.03	0.05	-0.02
AP.Var.Velo	-0.05	0.02	0.04	-0.12	-0.02	**-0.74**	**0.66**	0.03	-0.04	0.03	-0.05	0.11	**-0.64**	**0.76**

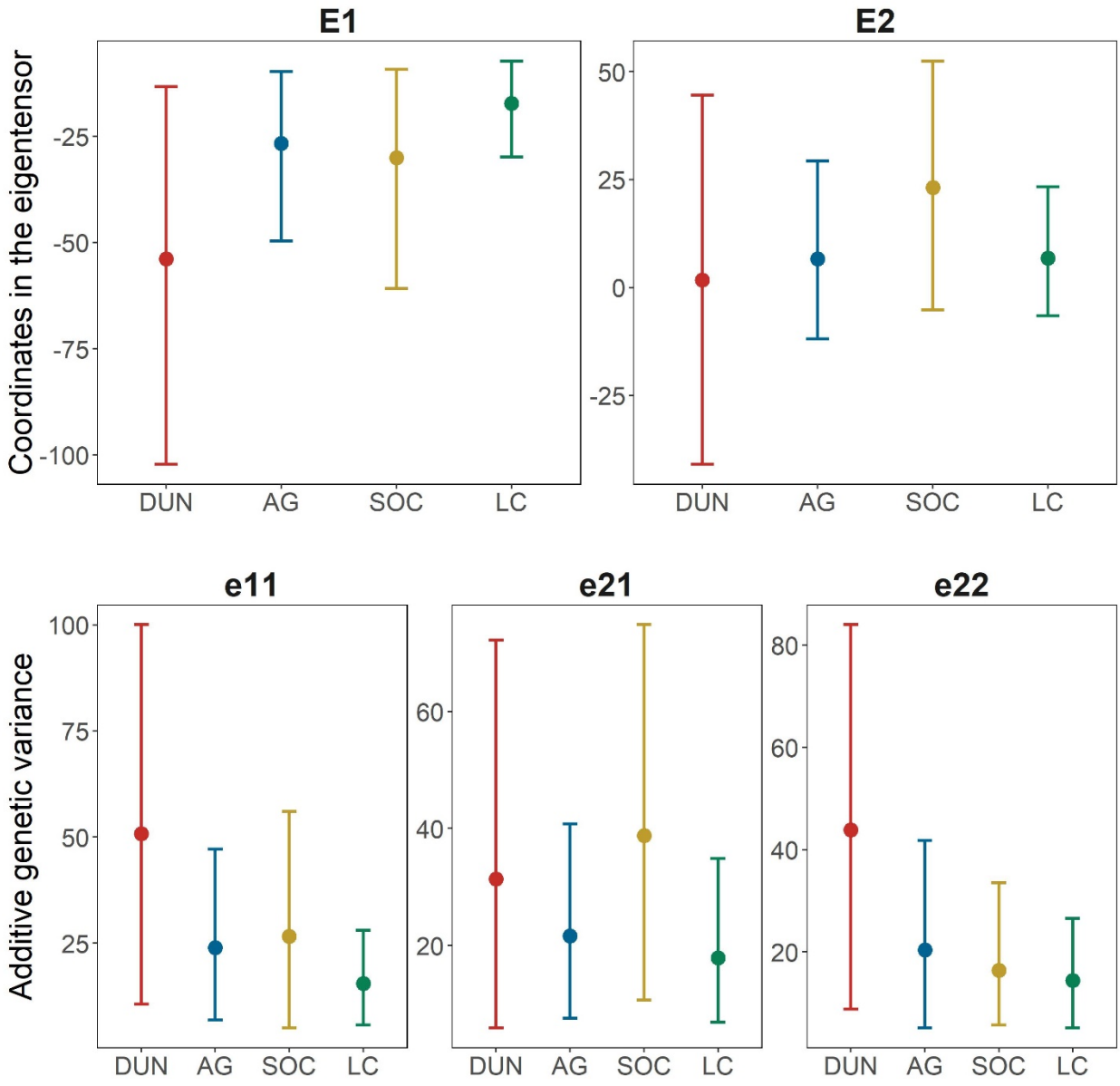

Figure S1. (a) Population coordinates along the leading eigentensors capturing most of the variation in divergence (**E**1 and **E**2). (b) Genetic variance in the direction of the leading eigenvectors of eigentensor **E**1 and **E**2. The largest population differences were found between the Dunnigan (DUN) and Las Cruces (LC) populations along **E**1 (Pmcmc > 0.85) and its leading eigenvector $\mathbf{e}_{11}$ (Pmcmc > 0.70), all other Pmcmc < 0.70.

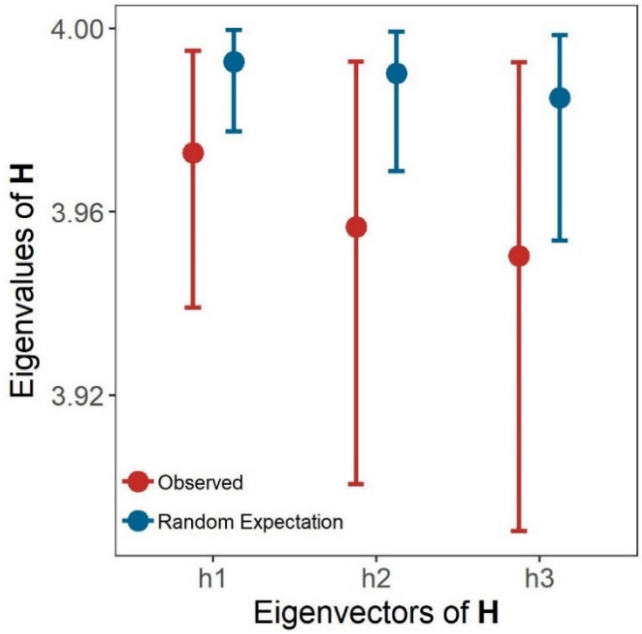

Figure S2. Eigenvalues of **H** for a comparison of the first three eigenvectors of each **G** matrix compared against random expectations. None of the eigenvectors of **H** showed significant departure from random expectations (Pmcmc < 0.65), indicating that major axes of genetic variation are highly conserved among populations. (h1: Pmcmc = 0.6, h2: Pmcmc = 0.65, h3: Pmcmc = 0.65)

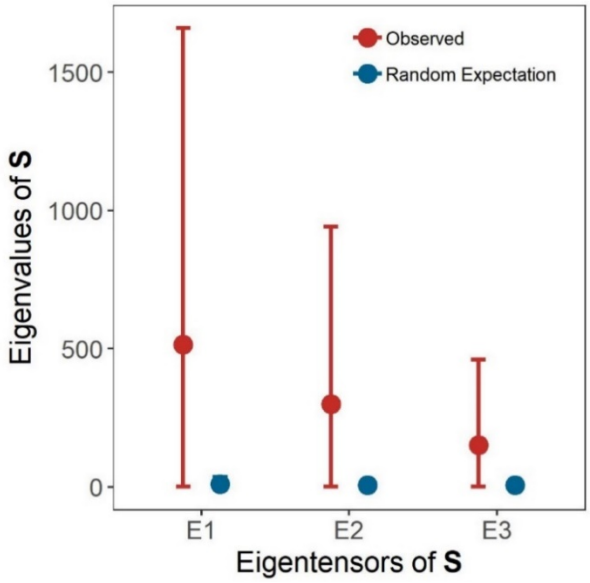

Figure S3. Eigenvalues of the first three positive eigentensors of **S**. Both $\mathbf{E}_1$, $\mathbf{E}_2$ and $\mathbf{E}_3$ were significantly different than expected from the random distribution (Pmcmc > 0.85), indicating that populations differed along $\mathbf{E}_1$, $\mathbf{E}_2$ and $\mathbf{E}_3$. (E1: Pmcmc = 0.85, E2: Pmcmc = 0. 85, E3: Pmcmc = 0. 85)