Adaptive phenotypic divergence in teosinte differs across biotic contexts

Anna M. O'Brien*[1,2,3], Ruairidh J.H. Sawers†[4], Sharon Y. Strauss‡[1,3], and Jeffrey Ross-Ibarra§[1,2,5]

[1]Center for Population Biology, University of California, Davis, CA 95616
[2]Dept. of Plant Sciences, University of California, Davis, CA 95616
[3]Dept. of Evolution and Ecology, University of California, Davis, CA 95616
[4]Laboratorio Nacional de Genómica para la Biodiversidad (LANGEBIO), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Irapuato, 36821, Guanajuato, Mexico
[5]Genome Center, University of California, Davis, CA 95616

Statement of authorship: All authors contributed substantially to the design of the study, provisioning of materials, and revising of the manuscript. A.M.O. proposed the study, collected the data, performed analyses and provided the first draft of the manuscript.

Address correspondence to Anna M O'Brien
Department of Ecology & Evolutionary Biology
25 Willcocks St
Toronto, Ontario, Canada, M5S 3B2
anna.obrien@utoronto.ca

*amobrien@ucdavis.edu
†rusawers@cinvestav.mx
‡systrauss@ucdavis.edu
§rossibarra@ucdavis.edu

Abstract

Climate is a powerful force shaping adaptation within species, often creating dramatic phenotypic clines. Yet adaptation to climate does not occur in a vacuum: species interactions filter the fitness consequences of both climatic and phenotypic variation. In other words, the translation of genotype to phenotype may be altered by biotic context, influencing the variation upon which climatic selection can act. We investigate the role of such interactions in changing the phenotypes on which selection acts using ten populations of an annual grass species (teosinte: *Zea mays* ssp. *mexicana*) sourced from along an elevational gradient, along with rhizosphere biota sourced from three of those populations. We grow teosinte families in a half-sibling design in separate biota treatments to first test whether the divergence we see among traits in teosinte populations exceeds what we would expect from genetic drift and then whether the source of rhizosphere biota affects the expression of divergent traits. We also assay the influence of these three rhizosphere biotas on contemporary additive genetic variation in teosinte traits across populations. We find that expression of most measured traits in teosinte is altered by rhizosphere biota, as well as the degree of variance and covariance among traits involved in root mass and flowering time. As a number of these traits are also found to underlie adaptive divergence across habitats, our data suggest that biota influence the expression of traits underlying local adaptation. Together, our results suggest that changes in trait expression and covariance elicited by interactor communities in root mass and flowering time may have played a historical role in local adaption of teosinte to environments, and that they would play a contemporary role in responses to changing selection pressures.

Keywords: biotic interactions, climate adaptation, rhizosphere, mutualism, local adaptation, driftsel, G matrix, phenotypic divergence

Introduction

Classic thought sees environmental variation across landscapes as a major selective force driving phenotypic differentiation (Clausen et al., 1947), and patterns of trait variation in species are concordant with this idea. For example, flowering phenology varies dramatically across latitude in plants from *Arabidopsis* to *Populus* (Stinchcombe et al., 2004; Keller et al., 2012), and climate strongly influences the fitness of species life history phenotypes in common gardens (Rehfeldt et al., 2002; Wilczek et al., 2014). Recent changes in climate have also led to numerous contemporary phenotypic responses, including animal body size (Millien et al., 2006) and plant flowering time (Franks et al., 2007; Willis et al., 2008).

Species interactions are another strong selective force shaping phenotypes. Predator-prey relationships can result in extreme trait escalations (Brodie Jr et al., 2002; Decaestecker et al., 2007; Toju, 2008), and competitive interactions may lead to phenotypic divergence that stabilizes coexistence, such as character displacement or niche partitioning (Thorpe et al., 2011; Pfennig and Pfennig, 2009; Germain et al., 2016). Mutualisms may also alter selection on traits, by either strengthening selection — such as bee pollination causing divergent selection on orchid scent (Ramírez et al., 2011) — or weakening selection, such as decay of redundant metabolism traits in insects with mutualistic gut bacteria (Bennett and Moran, 2015).

Biotic and abiotic selective forces may act conditionally (O'Brien et al., 2017): for example, plant-plant interactions often shift from negative to positive under increasingly stressful abiotic conditions (Callaway et al., 2002), or the degree of evolutionary trait escalation may depend on climate (Toju et al., 2011; Stokes et al., 2015). Interactions can even be gained or lost with changes in climate, such as expulsion or death of endosymbionts at high temperature in insects (Wernegreen, 2012) and corals (Hoegh-Guldberg, 1999), or through phenological mismatches, such as in plant-pollinator interactions (Burkle et al., 2013). In short, the interdependence of abiotic and biotic influences on trait differentiation may be pervasive.

Not all changes to phenotypes are caused by selection; plasticity in trait expression in response to changes in the biotic or abiotic environment is a pervasive and well-known phenomenon (e.g. Falconer, 1952; West-Eberhard, 1989). Environment-dependent effects of genotype on phenotype (G×E) underlie trait plasticity, and are the rule rather than the exception. Reviews of loci linked to trait variation find that observations of different effects of genotype across environments are common or even nearly ubiquitous (Hunter, 2005; Des Marais et al., 2013).

Of the biotic interactions that lead to alterations in plant phenotype, interactions with rhizosphere biota may be the most pervasive. Rhizosphere biota are the collection of bacteria, nematodes and fungi living in the vicinity of plant roots (the rhizosphere) (Hiltner, 1904; Bais et al., 2006); while it's species composition is influenced strongly by abiotic factors, plant genotype also contributes (Bulgarelli et al., 2012; Peiffer et al., 2013; Bouffaud et al., 2014; Lebeis et al., 2015) . Rhizosphere biota can alter expression of a wide-range of plant phenotypes (Friesen et al., 2011; Goh et al., 2013). Changes in traits may be caused by positive interactions, as some biota organisms provide benefits to plants such as nitrogen or phosphorus provisioning, by neutral interactions, or by negative interactions, which can reduce fitness or lead to the death of plants (Berg and Smalla, 2009). Biota effects on traits can depend on plant genotype, biota species composition or genotype, or both (Johnson et al., 2010; Wagner et al., 2014; Rúa et al., 2016). Biota effects on traits and especially on plant fitness can additionally depend on environmental conditions (Klironomos, 2002; Johnson et al., 2010; Smith and Read, 2010; Zhu et al., 2009; Smith and Read, 2010; Lau and Lennon, 2012). These responses of plant phenotypes or plant fitness to rhizosphere interactions can even be altered by the combined effects of plant genotype, biota makeup and the environment (Johnson et al., 2010; Wagner et al., 2014).

Biota-mediated trait expression may play a critical role in ecology and evolution as plant populations encounter new environmental conditions. Indeed, rhizosphere biota are already implicated in current range shifts (Lankau et al., 2015), in species invasions (Hayward et al.,

2015), and in trait responses to experimental selection on plants and soil biota for plant drought tolerance (Lau and Lennon, 2012) or flowering time (Panke-Buisse et al., 2015).

We test the importance of interactions in the expression of adaptive divergence and genetic variation in interactions between teosinte (*Zea mays* ssp. *mexicana*) — a wild annual grass species found in central Mexico and relative of domesticated maize (*Zea mays* ssp. *mays*) — and its rhizosphere biota. Local adaptation to soil biota has been documented in teosinte (O'Brien et al., 2018). Teosinte exhibits a number of phenotypes that are known to differ along elevation gradients and are suspected to be important in adaptation, including in phenology (Eagles and Lothrop, 1994; López et al., 2011), plant architecture, plant size, and stem color (Doebley, 1984; Lauter, 2004; Hufford et al., 2013). Specifically, we ask whether 1) interactions with rhizosphere biota alter how phenotypes are expressed 2) whether teosinte shows evidence of adaptive phenotypic divergence patterned by climate in rhizosphere-altered traits and 3) whether rhizosphere biota alter the potential future evolutionary responses of teosinte.

Methods

Plant and biota sources

We used seed and biota collected from 10 populations from central Mexico in 2013. Information on these populations (O'Brien et al., 2018) indicates differences in these sites in both climatic conditions (obtained using Bioclim Hijmans et al., 2005 and extracted using the package raster Hijmans, 2015 in R R Core Team, 2014) and soil characteristics, (see O'Brien et al., 2018). The sites ranged 6.6°C in mean annual temperature (MAT), more than 1100 meters in elevation, from sandy to clay soil, and the wettest site received nearly twice the annual precipitation of the driest site (O'Brien et al., 2018). We randomly selected three of these sites to use as sources of rhizosphere inocula: San Mateo Tezoquipan, San Matías Cuijingo, and South Toluca. We refer to biota sources throughout using the mean annual

temperature at the site (they become Biota15.0, Biota14.3, and Biota13.0, respectively). Biota15.0 and Biota14.3 are separated by 15.4 km, Biota15.0 and Biota13.0 by 96.1 km, and Biota14.3 and Biota13.0 by 94.6 km.

In August 2013, 2 kg of teosinte rhizosphere soil and roots were collected from adult plants at each site by unearthing roots, shaking off loose soil, and collecting the remaining soil and roots. Rhizosphere soil and roots were and kept refrigerated at 4°C until used in the experiment, when samples were homogenized in a blender. These collection and storage procedures were designed to maintain viability of both bacteria and fungi.

Experiment

In July 2014, we planted seeds from each population, inoculating them with each of the three rhizosphere biota sources (see below). For each combination of plant population and biota source, we planted 3 pots with seeds sampled from separate inflorescences from each of 10 mature plants (30 total pots per population $\times$ biota combination). Because selfing rates in teosinte are very low (Hufford et al., 2011, $\approx 3\%$), plants generally have only one male inflorescence, and stigmas from different female inflorescences on the same plant mature at different times (O'Brien, personal obs) different female inflorescences are thus likely sampling pollen from different pools of possible fathers. We therefore treat the 3 seeds from each maternal plant as half-siblings.

We grew plants in 2.83 L pots (Stuewe & Sons Treepots), with steam sterilized (4 hours at 93°C using a PRO-GROW SS60) potting mix (90% sand, 5% perlite 5% vermiculite 0.2% clay). To inoculate, we filled pots to 2 L with sterilized mix, added 50 mL of a 4:1 homogenized mix of sterile sand and inocula, and filled to the top with sterilized mix. We added seeds to pre-watered pots after scarification and overnight soaking. We randomized the bench planting design with respect to seed source, inoculum source, and maternal family. We added up to three seeds to a pot as supplies allowed, recorded the date of germination for all seeds, and weeded after germination if more than one plant germinated.

To encourage germination, we kept pots moist and unfertilized for the first two weeks, then watered and fertilized once per week with Hoagland's low P. As plants grew and demands of plant tissue for water increased, we increased water from 100 mL per week to 200 mL per week for the last 4 weeks. However, the total amount of fertilizer applied to each plant was constant such that we applied phosphate ion at a rate of 100 μmol per week (at first in 50 μM solution, and decreasing to 25 μM).

Plants began flowering in September, and we recorded first flowering date when silks or anthers were first visible. We harvested adult plants 15 days after its first inflorescence was observed. At harvest, several additional phenotypes were measured: stem width at the highest node from which aerial roots contacted the soil, the height from soil to highest ligule, the width of the penultimate leaf subtending the primary male inflorescence, and the length of the primary stem male inflorescence. A photograph of the stem was taken with a color standard, from which greenness of the oldest pre-senescence leaf sheath was measured using ImageJ (Schneider et al., 2012) and corrected as suggested in (Stevens et al., 2007). Plant roots and shoots were separated at the highest node where roots entered the soil, dried at ambient temperature until mass stabilized, and weighed. For each of these traits, we expected variation might be of adaptive importance to teosinte due to previous speculation in the literature (Doebley, 1984; Eagles and Lothrop, 1994; Lauter, 2004; López et al., 2011; Hufford et al., 2013) and obvious differences across field populations (the authors, personal obs, Figure S1).

Genotyping

An additional 9 seeds from each population were grown in a greenhouse at the University of California Davis. Young leaf tissue was sampled for DNA extraction using the DNeasy Plant Mini Kit from Qiagen. A single-nucleotide polymorphism dataset was generated from genotype-by-sequencing (GBS) (Elshire et al., 2011; Glaubitz et al., 2014) at the Biotechnology Resource Center, Cornell University, generating low coverage data at 955,690 SNPs.

The GBS dataset was filtered to include only sites with data across at least 86 of the 90 plants (95% coverage), resulting in 60,377 SNPs. We removed two individuals with more than 70% missing data (the remaining individuals after filtering ranged from 0.2% to 4.2% missing data, with an average of 0.9%).

Effects of biota on trait expression

We first asked whether biota affect the expression of our set of putatively adaptive teosinte phenotypes. Using experimental trait data we fit and compared linear models of several different structures for main effects and random effects. We fit models with main effects in three different structures: 1) intercept only, 2) intercept and biota treatment, or 3) intercept, biota treatment, and a main effect of one environmental variable describing the environment of the plant population source. Each trait was centered on the mean and scaled by the standard deviation. For this environmental variable, we compared elevation, mean annual temperature (MAT, °C $\times$ 10, for ease of fitting), total annual precipitation (TAP, in millimeters) or soil water holding capacity (SWC). We also tested four different random effects structures: 1) family within population, 2) family within biota treatment and family within population, 3) population within biota treatment and family within population, or 4) family and population within biota treatment and family within population. We ran models with MCMCglmm (Hadfield, 2010) in R (R Core Team, 2014) and compared models using the DIC (Spiegelhalter et al., 2002).

Divergence of trait means across teosinte populations and environments

We tested whether teosinte traits have adaptively diverged both across populations in general, and specifically in response to environmental variation. To develop a neutral expectation for trait divergence, we estimated coancestry (expected relatedness of a pair of individuals)

both within each population and between all pairs of populations using our SNP dataset. We computed coancestry between populations (Karhunen and Ovaskainen, 2012, using the package RAFM in R) with a random subset of 10,000 loci from the GBS dataset and parameters recommended by the authors (20,000 iterations, 10,000 burnin, and thinning by 10).

Pairing coancestry estimations with estimates of ancestral trait variance and covariance allows estimation of how much traits could shift due to neutral processes during population divergence. We estimated ancestral trait variance and covariance for all 9 traits using Driftsel (Ovaskainen et al., 2011; Karhunen et al., 2013) in R (R Core Team, 2014). Driftsel leverages phenotype information in related individuals and pairwise population coancestry to generate expectations for trait means in the full set of traits. It then uses divergence from these expectations across populations and traits to evaluate the effect of selection on the divergence of trait means across populations. Both here, and for all further trait analyses, we centered phenotype data on the mean and scaled by the standard deviation, as recommended by Hansen and Houle (2008). We ran Dritfsel for 440,000 iterations with a 40,000 iteration burn-in and thinning by 2,000 (determined by increasing iterations until MCMC samples converged). We used weak priors as recommended by the Driftsel authors (Karhunen et al., 2013). We performed tests of trait divergence for datasets in each inoculum treatment separately. We focus on results of this test for all traits collectively (S statistic), which accounts for predicted co-drift of trait means due to the structure of the ancestral trait variance and covariance (G) matrix, as well as on individual traits using only ancestral means and additive genetic variance, which acknowledges that only some traits may be under divergent selection. We also briefly explore divergence in bivariate trait space to illustrate how considering multivariate space alters expectations. To assess the contribution of effects of rhizosphere biota on trait expression, we compared the results across soil biota.

We then tested whether the pattern of trait divergence among populations was structured by abiotic variables, and thus whether local adaptation to environmental conditions

drove selection on phenotypes. We used the results from Driftsel and environmental data to perform the H test (Karhunen et al., 2014), which pools information across traits, environmental variables, and genetic variation into one statistic. An H greater than 0.95 indicates a significant correlation of phenotypes and environment beyond what would be predicted due to genetic similarity. Using population locations we extracted mean annual temperature and annual precipitation from Bioclim (Hijmans et al., 2005) with the package Raster in R (Hijmans, 2015). We also included soil water holding capacity, which was evaluated in soil samples in previous work (O'Brien et al., 2018, unpubl.). We repeated the analysis using the first two principal components of the Bioclim variables and first two principal components of the soil variables, which each include effects of many co-correlated variables and may be a more comprehensive summary of environmental variation. We performed H tests for habitat driven trait divergence in each biota treatment separately.

Variation in G matrices and response to selection across teosinte population and rhizosphere biota

To test whether interactions with rhizosphere biota might influence future responses to selection, we estimated G matrices: the additive genetic variance (diagonal elements) and covariance (off-diagonal elements) of traits. We then used these G matrices to predict responses to selection for each teosinte population. We performed G matrix estimation for each population in each rhizosphere inoculation treatment separately (using phenotype data from 30 plants), and we subset trait data to the 5 traits that showed evidence of divergence across populations. We fit animal models on centered and scaled trait data (as above). Briefly, animal models assume individual phenotypes (vector y_i) are functions of the mean trait value (μ), additive genetic breeding values (a_i) and residual effects (e_i): $y_i \sim \mu + a_i + e_i$. Linear model fitting of individual phenotypes to the animal model further assumes breeding values fit the genetic variance-covariance matrix G, where elements of G rest on the half-sibling covariance (e.g. diagonal genetic variance V_A elements are four times the estimated

covariance of that trait among half-siblings, see Falconer and Mackay, 1996). Together with a residual error variance-covariance matrix R, P the phenotypic variance-covariance matrix among traits will then be: $P \sim G + R$ (see more thorough and applied explanations in Lynch et al., 1998; Wilson et al., 2010).

We fit models with MCMCglmm() in R (Hadfield, 2010; R Core Team, 2014). We assumed normally distributed traits, used random effects for trait means, and applied a weakly informative inverse Wishart prior, biased towards very low additive genetic variance and covariance (G) (Hadfield, 2012). We fit models for 1,000,000 iterations, with 100,000 burn-in and thinning by 100. We checked trace plots of the MCMC chains for convergence (see Supplemental Figure S7) and implemented two alternate priors: reduced expected variance or both reduced expected variance and weaker bias (Hadfield, 2012). All priors yielded similar results for the analyses presented below, and had visually similar G matrices, so we present only on the results for the recommended prior.

To test whether the variation we observed in the G matrices across populations and rhizosphere inoculation treatments was greater than we would expect, we used eigentensor analysis (Aguirre et al., 2014). This analysis first calculates the pairwise variance-covariance matrix across every cell of the G matrix, calculating the variation among the set of G matrices for each cell in the matrix and covariation for each pair of cells. The analysis then uses eigendecomposition of this variance-covariance matrix to find the major axes of variation among the G matrices, known as 4th order tensors, or eigentensors. Each eigentensor contains values showing the contribution of each cell of the G matrix (with 5 traits there are 15 cells) to that eigentensor, e.g. to that axis of variance and covariance among the G matrix set. Using the breeding values estimated by the animal models, we randomized individual MCMC estimates of breeding values across the population and pedigree information and used each MCMC randomized breeding value set to generate an MCMC sample of the random G matrix set, and re-ran the eigentensor analysis across the set. For each eigentensor estimated on the real and randomized set of MCMC estimates, we compared the amount of

variation explained. If eigentensors calculated from real data explain more variation than the eigentensors calculated from randomized breeding values, this indicates significant variation among the set of G matrices (Aguirre et al., 2014).

Eigentensors consist of a matrix containing the contribution of a particular cell of the G matrix to the variation the eigentensor explains among the G matrix set. We further decomposed the eigentensors into eigenvectors, which quantifies the influence of individual traits (across all cells of the G matrix in which they are found) on the eigentensor. All eigentensor analyses include uncertainty in the estimation of the real G matrices by including the full posterior MCMC estimates.

We used a selection gradient approach to predict what responses teosinte traits would have to a certain selection gradient (abbreviated β). β is a vector representing selection on each of the traits in the G matrix. Our β included selection on only one trait (days to flowering), which we chose due to its high influence on the first eigentensor (see Results). We then calculated the predicted response of the other traits with high influence on the first eigentensor (see Results) across the posterior distribution for each G matrix, using the multivariate breeder's equation: $\Delta\bar{z} = G\beta$ (Lande, 1979). We visualized responses and assessed overlap with bivariate density kernels using the package ks (Duong, 2018) in R (R Core Team, 2014).

Testing for the role of hybridization

At low elevation, hybrids between teosinte and *Zea mays* ssp. *parviglumis* (a low elevation subspecies) can form (Pyhäjärvi et al., 2013), whereas at higher elevation introgression from maize (*Zea mays* ssp. *mays*) can be common (Hufford et al., 2013). Introgression could neutrally increase both phenotypic and genotypic divergence, or could be a source of genetic material underlying adaptive divergence. We thus evaluated population structure between our 10 populations and known individuals of ssp. *mays*, ssp. *mexicana*, ssp. *parviglumis*, and hybrids between *mexicana* and *parviglumis* to see if we could identify gene flow across

subspecies as a potential source of genetic material for adaptive divergence. Data from Swarts et al. (2017) were filtered for overlapping SNPs with our dataset using position, and subset to include approximately equal numbers of individuals known to be from each subspecies (153, 141, 144, of *mays*, *mexicana*, and *parviglumis*). We then analyzed this dataset with PCA in TASSEL (Glaubitz et al., 2014) to visually assess genetic relationships.

Results

We aimed to examine the role of biotic interactions in phenotypic divergence across abiotic environments in teosinte. We grew a common garden experiment of 10 teosinte populations in three separate rhizosphere biota treatments and measured a set of putatively adaptive phenotypic traits, including phenology, vegetative morphology, and color (Doebley, 1984; Eagles and Lothrop, 1994; Lauter, 2004; López et al., 2011; Hufford et al., 2013). Using environmental and genotype data from the same populations, our approach tested whether biota treatments affect expression of these traits, whether trait divergence is shaped by adaptation to environmental gradients or similar to neutral expectations, and whether biota treatments alter estimates genetic variation.

Do rhizosphere biota alter the expression of adaptive phenotypic variation?

Visual inspection of all phenotypes shows highly variable reaction norms across populations and families to different biota, and variable means across populations (Figure 1). Using the full set of trait data, we fit linear models for each phenotype with a range of fixed effects for source site abiotic environment and a range of random effects including genotype-by-biotic environment effects (see Methods).

Many of the putatively adaptive teosinte traits that we selected for study did indeed vary both deterministically across population source environment and plastically across biota.

Trait	Main	Random	Slope	DIC	DIC base
Days to flowering	TAP	F	1.1e-3	2305.5	2398.4
Days to germination	MAT	P	5.1e-3**	2426.7	2444.4
Tassel length	MAT	F	7.1e-3*	2346.0	2403.8
Shoot biomass	MAT	P	9.1e-3**	2404.5	2441.1
Root biomass	MAT	F + P	1.4e-3**	2373.3	2443.7
Height	–	P	–	2409.7	2418.5
Stem width	MAT	F + P	9.6e-3**	2380.5	2420.1
Leaf width	MAT	P	4.2e-3**	2423.8	2427.1
Stem greenness %	–	–	–	–	2359.3

Table 1: Best models for traits, including best environmental variable at plant source site and random effect structure based on DIC comparisons. When no environmental variable (TAP, total annual precipitation; MAT, mean annual temperature) is in the best model, cells are left blank. Soil water content and elevation do not appear in the table because they were never in best models. F indicates family in biota random effect, P is population in biota random effect. DIC base indicates the simplest model (intercept and random effect of family in population). **: pMCMC < 0.05,*: pMCMC < 0.1.

While there is often little difference in fit between the best model and the next best model for most traits (Table S1), we can nonetheless make some generalizations. Models with genotype-by-biota (either for population or family or both) interactions outperformed models without when ranked by DIC, suggesting pervasive genotype-by-biota effects on traits. The best models for most traits included significant slopes (pMCMC < 0.1) with environmental variables, validating expected patterns of traits across populations but not indicating causality. However, best models for stem greenness and height lacked significant slope terms with environment variables of the source site. Mean annual temperature was most frequently the best explanatory environmental variable for trait differences among populations (Table 1), but again, DIC differences between models with different environmental variables are often slight.

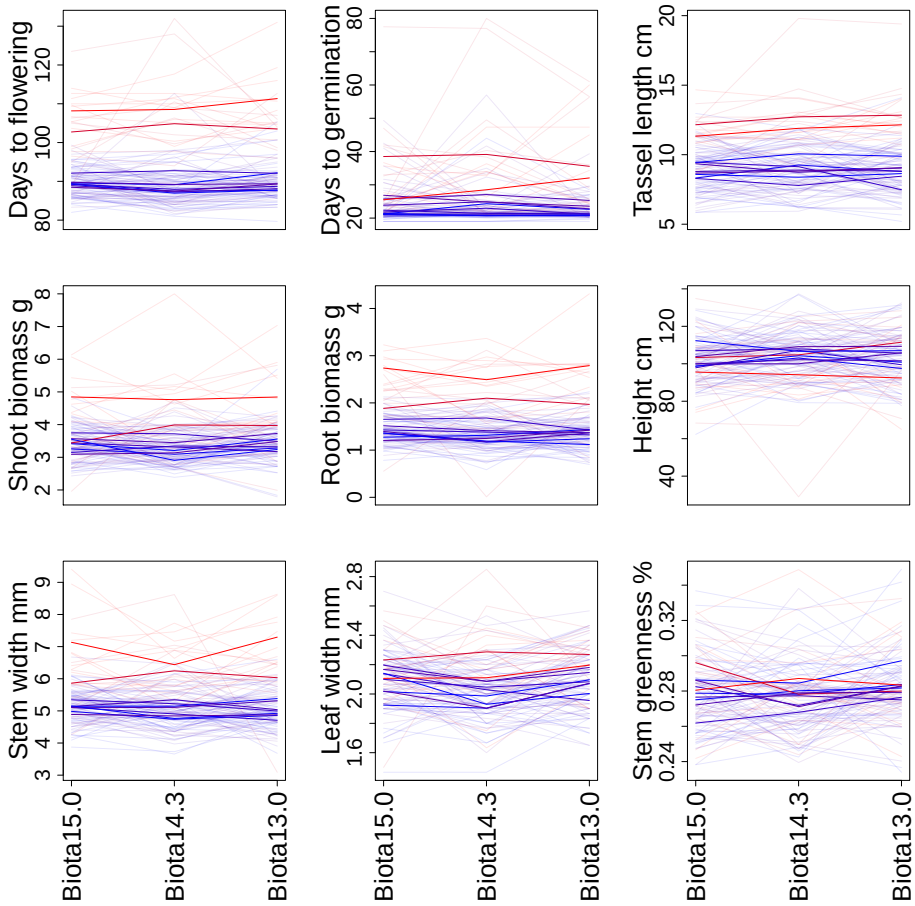

Figure 1: Average trait values (y-axis) for each trait (plots) in each biota (x-axis). Populations (bold lines) and families (faint lines) are both presented, and are colored according to mean annual temperature at their source site. Redder indicates populations from warmer sites; bluer indicates populations from colder sites.

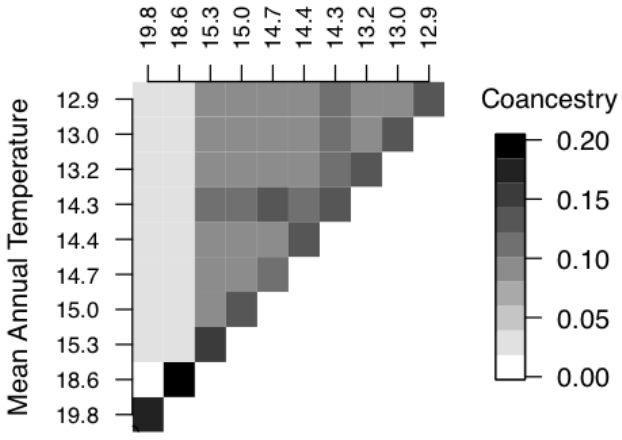

Figure 2: Coancestry within and between all teosinte populations, identified here by the mean annual temperature at their source site.

Are traits in teosinte populations the result of drift or adaptation to environment?

We first estimated the extent of genetic drift using coancestry, the probability that alleles chosen from two individuals are identical by descent. Our estimates indicated that most populations were equivalently related (coancestry ≈ 0.1) and that most populations' within population coancestry was only slightly higher than between population coancestry, indicating that there is little drift or inbreeding in these populations (Figure 2). However, two populations were not genetically similar to any other population (coancestry < 0.05), and had much higher within population coancestry (≈ 0.2), indicating much higher drift away from each other and from all other populations.

To test whether the divergence of trait means across teosinte populations may primarily reflect neutral drift or is shaped by selection, we used Driftsel (Ovaskainen et al., 2011; Karhunen et al., 2013) to compare our phenotypic data to expectations based on observed patterns of genetic coancestry. We performed these comparisons separately for each rhizosphere biota in order to assess the contribution of rhizosphere interactions to our ability to detect the signature of adaptation. Relative to the expectations from ancestral means, additive genetic variance and drift, two traits (days to flowering and root biomass) exceed expected divergence in at least one population in every biota. Three more traits (days to germination, shoot biomass, stem width) exceed expected divergence in at least one population in only one or two biota sources (Figure 3). Four traits never exceeded drift expectations (Figure 3). Populations vary in divergence as well, with only two populations exceeding expectations for one or more traits across all biota, and three population falling within expectations of phenotypic drift for all traits (Figure 3). Some traits and population combinations fall beyond neutral expectations only when compared in two or more dimensions (see Figures S2, and S3).

Assessing all traits simultaneously, we found only suggestive evidence that all putatively adaptive traits have diverged non-neutrally. The statistic S ranges between 0 and 1, and

summarizes divergence across the G matrix as a whole, where values closer to 1 indicate support for divergence. Our three biota produce S statistics 0.73, 0.87, and 0.77, suggesting only weak support for divergence of populations across the full trait dimensionality in excess of neutral drift from the ancestral means and G matrix.

In contrast, we find strong evidence that trait divergence across populations has been shaped by environment. If habitat similarity explains significant trait similarity among populations after accounting for genetic similarity among populations and habitats, this provides evidence for non-neutral trait divergence across habitat (Karhunen et al., 2014). The "H" test in Driftsel (Ovaskainen et al., 2011; Karhunen et al., 2013) compares the similarity of the population coancestry, habitat similarity, and phenotype similarity matrices, and asks whether the habitat similarity matrix explains variation in the phenotype similarity matrix, after accounting for population habitat similarity that is explained by population coancestry. Specifically, if teosinte populations from similar habitats are more similar phenotypically in the common gardens than would be expected from their genetic similarity, this is evidence that environmental variables have shaped trait divergence. The H statistic ranges from 0 to 1, with values over 0.9 or 0.95 indicating 90% and 95% confidence that environment selected on phenotypes. We observe highly significant H statistic values of 0.995, 0.985, and ≈ 1 across biota for our selected environmental variables (for the more agnostic principal component variables, H statistic values are equally significant: ≈ 1, 0.995, and 0.980). This result can be intuited by inspecting the estimated population effects in Figure 3, when compared to Figure 2: despite the fact that low elevation, warmer-sourced populations are genetically very different from each other, they have very similar trait values for most traits, and these trait values are opposite those of all higher elevation, colder-sourced populations.

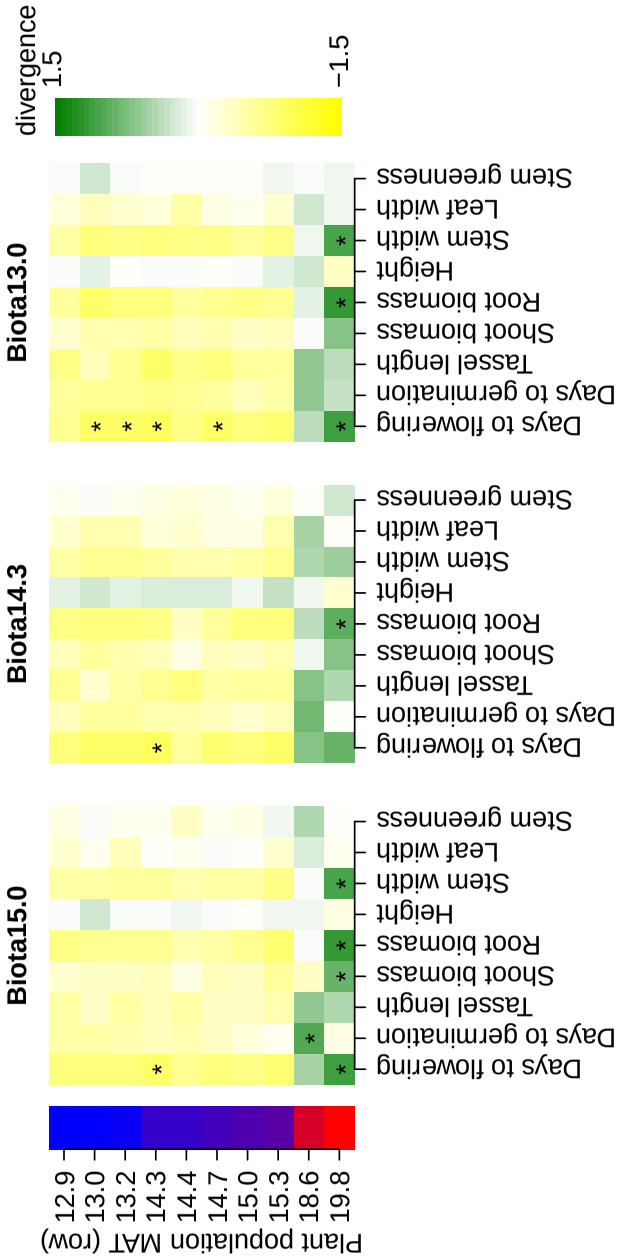

Figure 3: Population divergence from the ancestral mean breeding value for all populations (rows, sorted by MAT at source site), in all biota (separate plots). Green indicates lower values for traits than the ancestral mean breeding value, while yellow indicates higher values. Populations and traits that exceed the 95% confidence interval for neutral divergence are marked with asterisks. More populations and traits exceed expectations in multidimensional space (see Figure S2 and Results).

Do biota alter additive genetic variance and covariance?

We summarized additive genetic variance and covariance across population and rhizosphere biota in G matrices and then used eigentensor analysis to test for variation among the G matrices (Aguirre et al., 2014) and determine which subspaces of the G matrix are responsible for any variation. We performed eigentensor analysis on our 30 G matrices calculated from experimental phenotypic data (ten populations in each of three soils). The first 13 of the 15 eigentensors explain significantly more variance than eigentensors calculated on G matrices built from randomly shuffled breeding values (see Figure S4). The first and second eigentensors explain the largest portions of the variation (43 % and 17 %, respectively) in the G matrix set, with the remaining variance among G matrices split across many eigentensors. Each eigentensor consists of a matrix indicating contributions of individual cells in the G matrix to the eigentensor. Variation in estimated genetic covariance between flowering time and each of stem width, root biomass, and shoot biomass makes the strongest contributions to the first eigentensor (Figure 4). Decomposing the eigentensors into eigenvectors indicates the contribution of each trait to variance and covariance explained by the eigentensor, across all G matrix variance and covariance elements including that trait. 84 % of the variation in the first eigentensor is in its first eigenvector (11 % in the second), and 55 % and 39 % of the variation explained by the second eigentensor are in its first and second eigenvectors, respectively. The first leading eigenvector of the first two eigentensor implicates correlated changes in size and phenology, whereas the other leading eigenvectors implicate contrasts (opposite correlations to the eigenvector) especially between phenology and size traits (Figure S8). Variation among G matrices does indeed include both differences in total strength of variance-covariance and in the sign of covariance between phenology and size traits (Figure 5, and Figure S5).

If differences in additive genetic variance and covariance in traits are large enough, we might predict that populations would respond differently to selection depending on interactions with rhizosphere biota. To predict whether variation in G matrices was biologically

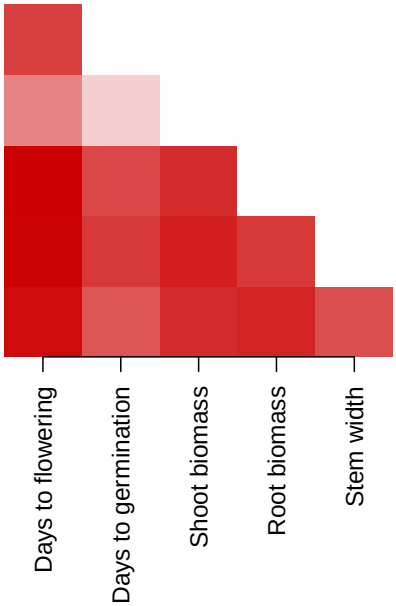

Figure 4: The first eigentensor of the set of G matrices. This eigentensor shows the contribution of each element of the G matrix (symmetric around the diagonal) to the divergence of the set of G matrices. Darker colors indicate greater contributions.

meaningful, we simulated responses of teosinte trait means to selection using a selection gradient. Briefly, we project the G matrix onto a selection gradient vector using the multivariate breeder's equation (Lande, 1979) across the posterior distribution for each G matrix ($\Delta\bar{z} = G\beta$). We chose to evaluate selection for later flowering because covariance with flowering time was identified as the greatest axis of variation among the G matrices in our eigentensor analysis. Our selection gradient β represents selection for later flowering time without direct selection on any of the other traits in the G matrix, but responses to selection will reflect the genetic covariance between other traits and flowering time as well as the additive genetic variance for flowering time. We report the predicted response of the trait with the next highest influence on the first eigentensor (root biomass).

The selection gradient approach revealed that size traits were generally predicted to increase with selection for later flowering. Most populations' predicted responses to selection overlapped across biota, and, in each biota, most populations' responses overlapped strongly with each other (no pairs of populations are significantly different in responses at the 95% probability levels with bivariate kernel density estimation). However, one population was predicted to respond to selection for later flowering quite differently depending on which biota it grew with (both compared to itself in other biota and to other populations in the same biota). In Figure 5, the population Malinalco has an especially divergent matrix in Biota14.3, where it responds with decreases in size traits with selection for later flowering, compared to Biota15.0 where it responds with increases in size traits. One additional population also has predicted decreases in size with later flowering in Biota14.3, and responses of other populations differ across biota in unique ways or do not differ (Figure S6, negative covariances in Figure S5). The dramatic average differences of Malinalco likely contribute to the significant variation detected by eigentensor analysis.

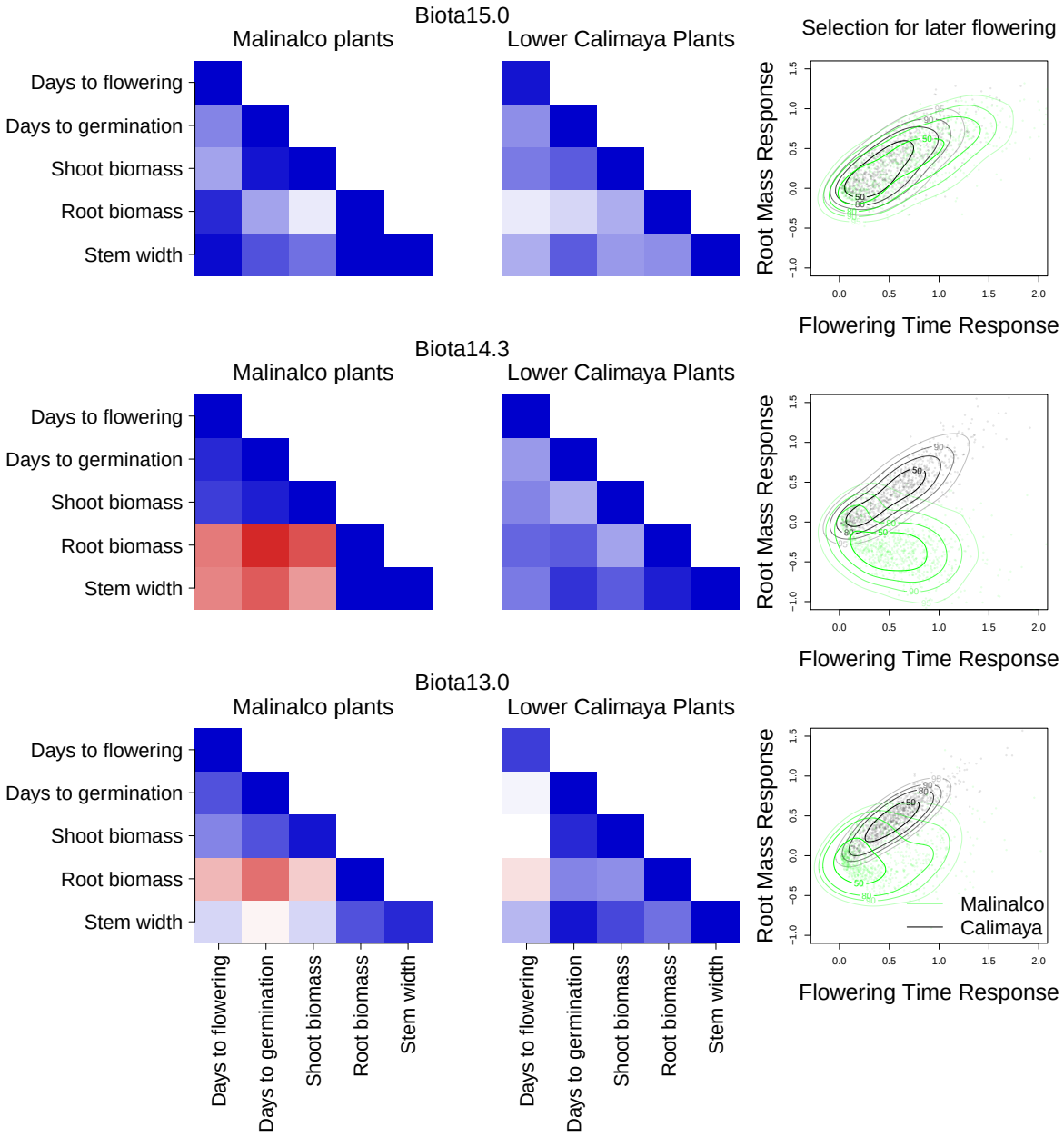

Figure 5: G matrices estimated for populations Malinalco and Lower Calimaya (left columns) across each biota (rows). Redder values indicate negative genetic covariance, and bluer values indicate more positive additive genetic covariance (off-diagonal) and variance (diagonal). Projected responses of flowering time and root biomass (right column) for G matrices in each biota to selection on flowering time (Malinalco in green, Lower Calimaya in black), with fitted bivariate probability density kernels at 50%, 80%, 90%, and 95% (lines) for responses across MCMC G matrix samples (points).

Did hybridization supply the genetic material for phenotypic differention?

We used our genotypic data and public SNP datasets to assess genetic similarity between our populations and all three different subspecies of *Zea mays*. As expected, principal component analysis finds three clusters corresponding to the three subspecies of *Zea mays* (Figure 6). Our results also show that the two lowest elevation populations from our study have slightly increased genetic similarity with ssp. *parviglumis*, relative to the other populations in our study. Specifically, our low elevation populations show more similarity to ssp. *parviglumis* on the second PCA axis and the combination of the second and third axes than individuals from the other study populations (Figure 6, top two plots), but not on axes 1, 4, or 5 (6, lower plots). While these populations do not appear to be extensively hybridized, rare or old hybridization events could be a source of the elevated genetic similarity between ssp. *parviglumis* and these populations.

Furthermore, our above coancestry analysis indicates that lower elevation populations have some of the lowest coancestry with both each other and other populations out of all the populations pairs (Figure 2), suggesting that if there is gene flow from ssp. *parviglumis*, it is with different ssp. *parviglumis* populations in each of the low elevation ssp. *mexicana* populations here. Follow-up analysis indicates that without these two populations, we do not detect a signal of phenotypic divergence patterned by climate in any biota (highly non-significant S statistics 0.42, 0.43, 0.38, and H statistics 0.42, 0.30, 0.30, for Biota15.0, Biota14.3, and Biota13.0, respectively). Regardless of where the genetic variation underlying their unique trait values originates, it is the difference between these low elevation populations and the other populations that drives the signal of adaptive divergence.

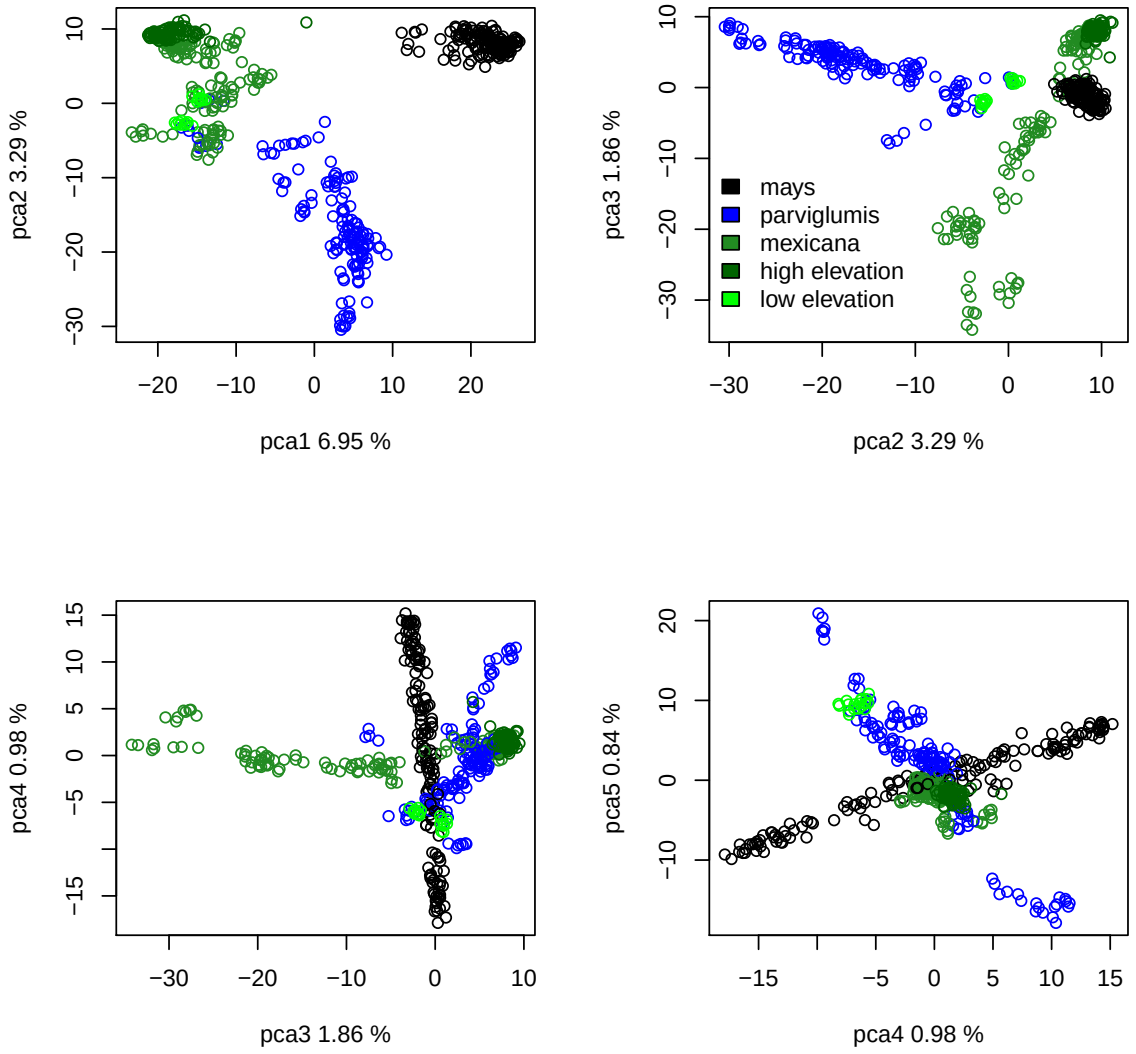

Figure 6: These four plots show the first five axes of the PCA analysis of genotypes comparing individuals (points) in the study populations (light green for low elevation populations and dark green for high elevation) to individuals from diverse populations of each subspecies in the genus *Zea*, including individuals from ssp. *mays* (black), ssp. *parviglumis* (blue), ssp. *mexicana*.

Discussion

Species interactions filter how individuals experience environments and can alter the optimum phenotypes for any given abiotic condition. Plants respond to interactions with other species by plastically and adaptively altering phenotypes. Here, we investigated whether root interactions with rhizosphere biota alter expressed divergence in adaptive phenotypes between teosinte populations, and whether such interactions have the potential to alter the course of phenotypic evolution. We found that a number of phenotypes were likely under environmentally determined divergent selection in teosinte. We discovered that biota have plastic effects on measured teosinte phenotypes, changing estimated trait divergence through effects on population breeding values. We also determined that variation in additive genetic variance and covariance that is due to both population source and root-rhizosphere interactions will likely affect the course of phenotypic evolution in teosinte.

Plastic responses and trait divergence

Whether or not divergent phenotypes across populations arise via the action of selection or drift has been the subject of intense study. Phenotypes may differ across populations due to plasticity, neutral drift, and local adaptation. Studies of phenotypic divergence across populations in a wide array of species have revealed that trait variation across populations is often, but not always, shaped by the forces of selection (Kawecki and Ebert, 2004; Leinonen et al., 2013). Plastic trait expression responses to environments at both the level of the whole organism (Falconer, 1952; West-Eberhard, 1989) and individual genes (Hunter, 2005; Des Marais et al., 2013) are another ubiquitous and well-characterized driver of phenotypic differences across environments.

In our greenhouse common environment, we find strong evidence of adaptive divergence in flowering time and root mass, regardless of the rhizosphere biota applied. However, which populations we detected as diverged in these and other traits depended on which rhizosphere

biota plants were inoculated with (Figure 3). This occurred because biota alter expression of these divergent traits, and because the direction and extent to which rhizosphere biota alter trait expression depends on teosinte population (Figure 1, Table 1).

Clearly, the rhizosphere biota communities must differ. Selection on plant traits could have altered biota communities at local sites: rhizosphere microbes can respond to selection on host phenotypes faster than hosts themselves, altering plant phenotypes either through changes in community composition or through evolution of individual members (Lau and Lennon, 2012; Mueller and Sachs, 2015). Divergence among plant genotypes across populations, and especially abiotic differences across sites are also possible causes of differences among rhizosphere biota (Bulgarelli et al., 2012; Peiffer et al., 2013; Lebeis et al., 2015; Walters et al., 2018). A number of abiotic variables from climate to soil nutrients indeed differ across the source sites for biota (O'Brien et al., 2018), but among these three sites plant populations are genetically similar (Figure 2). Furthermore, biota may simultaneously be effectors of expression variation and agents of selection. Soil biota have previously been found to alter both genotype flowering time and the fitness consequences of flowering time (Wagner et al., 2014; Lau and Lennon, 2012; Panke-Buisse et al., 2015). In teosinte as well, biota may simultaneously alter both flowering time expression (Figure 1) and genetic correlations between flowering time and shoot biomass (Figures 5 and S5), which itself is tightly correlated to teosinte fitness (data from Piperno et al, analyzed in O'Brien et al., 2018, unpubl.). Thus, variation in biota across environments may itself be a selection pressure on teosinte traits or teosinte trait plasticity in response to biota.

The selection pressures that shape phenotypic divergence among populations are difficult to detect (Karhunen et al., 2014). One way to test for the influence of environment is to compare phenotypic similarity among populations with environmental similarity, all while accounting for the amount of phenotypic similarity we expect among populations simply due to genetic similarity (Karhunen et al., 2014). We find here that environments pattern variation in teosinte phenotypes much more strongly than we expect given genetic similarity

among populations. The conclusion must be that selection on teosinte that is correlated to the environment shaped traits. We cannot exclude the possibility that the target of selection is an unmeasured, but genetically linked trait, nor the possibility that the agent of selection is an unmeasured, but strongly correlated environmental variable. However, we would expect that traits with stronger influence of past selection (days to flowering, root biomass, Figure 3), and climatic variables with stronger correlations to trait means (mean annual temperature, Table 1, Figure 1) to be more closely linked to the specific targets and agents of selection, respectively.

Detection of past selection relied on contrasting phenotypes between the two populations from warmer and colder sites (see Results). Populations from warmer sites showed low coancestry with our other populations and with each other (Figure 2) yet had similar phenotypes (Figures 1 and 3). Our analysis suggests populations from warmer sites may share some genetic diversity with the lowland subspecies (*Zea mays* ssp. *parviglumis*, Figure 6), raising the intriguing possibility that differential selection for or against shared diversity with ssp. *parviglumis* in warmer and colder sites, respectively, may have contributed to phenotypic divergence in *Zea mays* ssp. *mexicana*.

A number of traits we measured showed equivocal evidence of selection. While divergent selection has been implicated in stem color variation in other studies (Hufford et al., 2013), we saw little evidence of a clear gradient in stem greenness across populations in the greenhouse (Table 1, Figure 1), and no strong support for divergent selection (no sign in Figure 3, but see Figure S3). Leaf width and tassel length displayed clear gradients in the greenhouse, but weak evidence for divergent selection in our tests (no evidence in Figure 3, but see tassel length, Figure S3). We may have failed to detect real divergence in these, or other, traits if populations vary in plastic responses to some aspect of the abiotic environment, such that divergence of phenotypes would be more strongly expressed in certain conditions. For example, heritable differences in stem color may only be expressed in cold environments (such as the conditions in Hufford et al., 2013). Since biotic environments affect expressed

genetic variation for teosinte traits (Figure 5, Figure 1), abiotic environments likely do so as well.

Variation in G matrices - responses to changing environments

The course of adaptation to divergent environments can be strongly affected by trait variance and covariance (Schluter, 1996; Etterson and Shaw, 2001; Chenoweth et al., 2010), yet changing environmental conditions may simultaneously shift both selection on traits (Etterson and Shaw, 2001) and trait variance-covariance relationships (Wood and Brodie III, 2015). The combination of these two processes can lead to unpredictable side effects for trait evolution (Wood and Brodie III, 2016). Using eigentensor analysis, we detected some contemporary differences in additive genetic variance and covariance of size and phenology between teosinte populations and across different biota (Figures S4, 4 & S8). Differences in the G matrix matter because they can shape responses to selection. For example, differences between the direction of selection and the major axis of trait variation can be persistent across time (Chenoweth et al., 2010), and may shape trait divergence over long time scales (Schluter, 1996; McGlothlin et al., 2018), such that trait divergence among populations reflects the major axis of the G matrix in individual populations (Schluter, 1996; Chenoweth et al., 2010; McGlothlin et al., 2018). We use selection skewers (Lande, 1979) with G matrices to predict short-term multivariate responses to selection for later flowering time across teosinte populations and biota.

The differences we observe in G matrices across biota and populations could have arisen due to drift, selection, or genotype-by-environment interactions and cryptic variation. Selection can shape changes in G matrices, (e.g. G matrices may not be generated by neutral mutational inputs Arnold et al., 2008; Orr and Betancourt, 2001; Walter et al., 2017). For example, the rise in frequency of previously rare alleles at causal loci (Orr and Betancourt, 2001), can, at least temporarily, align the axis of greatest variation with the multivariate selection gradient (Walter et al., 2017). Alternatively, neutral drift can generate differences

in G matrices, but is primarily expected to generate proportional differences in total additive genetic variation and not in the relative magnitude of variances and covariances of individual traits Roff (2000); Puentes et al. (2016), but see Steppan et al. (2002); Roff et al. (2012). Estimated G matrices can differ substantially across the environment in which traits are measured due to plastic responses of organisms. Plastic responses occur when shifting environments expose genetic variants that do not have the same effect in one environment as in another (GxE loci, see Des Marais et al., 2013), including loci that may have no effect in one or several environments, but strong effects in another (cryptic variation, McGuigan and Sgrò, 2009; Paaby and Rockman, 2014). Differences between G matrices estimated in one population across environments are often similar in magnitude to the differences in estimated G matrices between two populations separated by a long evolutionary time (Wood and Brodie III, 2015).

Neutral genetic diversity in teosinte populations differs across its range due to demography (Pyhäjärvi et al., 2013; Aguirre-Liguori et al., 2017), and is lower for edge populations (Aguirre-Liguori et al., 2017), suggesting additive genetic variation may also be reduced at range edges (Sexton et al., 2009). If neutral processes drove differences in teosinte G matrices, populations near range edges that have experienced greater neutral drift, such as our two lower elevation populations, could be expected to have reduced magnitude of additive genetic variation and covariation. Our populations do not differ substantially in the amount of additive genetic variation (see Figure S9), and instead differ in orientation of the G matrix: most populations in most biota have positive average covariance for mass and phenology traits, but in others there is negative or no correlation between these traits (Figures 5 S5). Differences in orientation usually imply selection, however most differences in the direction of correlation depend on biota, suggesting that the G matrix orientation may be shaped most strongly by biotic context (GxE interactions) or adaptive divergence in biotic responses (selection on GxE).

As climate changes, we might expect selection for phenotypes to extend the patterns of

divergence we already see across climate (Etterson and Shaw, 2001): i.e. we might expect selection for later flowering time and larger root mass. Negative genetic correlations between these two traits could constrain responses to selection, positive correlations could facilitate it (Etterson and Shaw, 2001; Agrawal and Stinchcombe, 2009), and the axis of greatest variation in G matrices could cause divergence in other phenotypes as side-effects of selection (Chenoweth et al., 2010). Some matrices that we have estimated would produce different responses to selection for delayed flowering time in other traits. Specifically, we expect the Malinalco population to respond with smaller or larger roots variably across biota. Similar to our results, an experimental selection study of *Mimulus* also found that populations differ significantly in potential responses to flowering time selection under global change Sheth et al. (2016). In sum, current phenotypic variance and covariance indicates that responses to selection on phenotypes could be altered by changes in the biota that teosinte interact with, especially if populations disperse to sites with novel rhizosphere biota communities.

Accurately estimating parameters of G matrices can require large sample sizes, so studies comparing G matrices more commonly use matrices fit using large numbers of families and many total individuals (e.g. our ancestral G matrix in the divergence analysis, see also Puentes et al., 2016), but such studies are limited to comparing only very few matrices. Here, we used a few families and individuals to coarsely estimate a G matrix for each population and biota combination. We successfully detected significant variation among the set of matrices, and the traits contributing to this variation. However, as a trade-off, we have very low confidence in differences among G matrices for specific population and biota combinations (see wide variance of posterior estimates Figures S7 and S9, and in selection responses across G matrix posteriors Figure 5). Because of this low confidence, we are biased towards inferring fewer differences between G matrices than may actually exist — suggesting larger sample sizes might show further difference in G among populations.

Conclusions

We have demonstrated the potential for species interactions to be drivers of the expression of adaptive phenotypes, as well as their likely involvement in the past or future responses to environmental selection on traits. Biotic interactions are likely to play major roles in responses of species to climate change, as they may exacerbate range contractions (Lankau et al., 2015), or facilitate (HilleRisLambers et al., 2013) range expansions. Here we show that in addition to immediate ecological effects on population traits, changing biotic interactions have the potential to influence evolutionary responses – which are projected to be necessary to prevent extinctions (Shaw and Etterson, 2012). Whether changing biotic interactions are more likely to increase or decrease adaptive genetic variance visible to selection is currently unclear. We advocate that while our ability to predict changes in biotic interactions has increased, we must invest as well in understanding the evolutionary consequences of these changes.

Acknowledgements

We would like to thank Jaime Gasca Pineda & Luis Eguiarte and the entire Eguiarte laboratory for help with collections in the field, Aida Odette Avendaño-Vázquez, Carlos Fabián de la Cruz, Abenamar Gordillo Hidalgo, Dario Alvarez & Arturo Chavez for assistance in the greenhouse. The project was funded by UC MEXUS, the UC Davis Center for Population Biology, grants to A.M.O., USDA Hatch project CA-D-PLS-2066-H, and NSF grant IOS-0922703, grants to J.R.I.. A.M.O. was supported by the NSF GRFP grant DGE-1148897, and NSF grant DEB-0919559.

References

Agrawal, A. F. and J. R. Stinchcombe. 2009. How much do genetic covariances alter the rate of adaptation? Proceedings of the Royal Society of London B: Biological Sciences,

276:1183–1191.

Aguirre, J., E. Hine, K. McGuigan, and M. Blows. 2014. Comparing g: multivariate analysis of genetic variation in multiple populations. Heredity, **112**:21.

Aguirre-Liguori, J., M. Tenaillon, A. Vzquez-Lobo, B. Gaut, J. P. Jaramillo-Correa, S. Montes-Hernandez, V. Souza, and L. Eguiarte. 2017. Connecting genomic patterns of local adaptation and niche suitability in teosintes. Molecular Ecology, pages n/a–n/a.

Arnold, S. J., R. Bürger, P. A. Hohenlohe, B. C. Ajie, and A. G. Jones. 2008. Understanding the evolution and stability of the g-matrix. Evolution, **62**:2451–2461.

Bais, H. P., T. L. Weir, L. G. Perry, S. Gilroy, and J. M. Vivanco. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology, **57**:233–266.

Bennett, G. M. and N. A. Moran. 2015. Heritable symbiosis: the advantages and perils of an evolutionary rabbit hole. Proceedings of the National Academy of Sciences, **112**:10169–10176.

Berg, G. and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology, **68**:1–13.

Bouffaud, M.-L., M.-A. Poirier, D. Muller, and Y. Moënne-Loccoz. 2014. Root microbiome relates to plant host evolution in maize and other poaceae. Environmental Microbiology, **16**:2804–2814.

Brodie Jr, E. D., B. Ridenhour, and E. D. Brodie III. 2002. The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. Evolution, **56**:2067–2082.

Bulgarelli, D., M. Rott, K. Schlaeppi, E. V. L. van Themaat, N. Ahmadinejad, F. Assenza, P. Rauf, B. Huettel, R. Reinhardt, E. Schmelzer, J. Peplies, F. O. Gloeckner, R. Amann, T. Eickhorst, and P. Schulze-Lefert. 2012. Revealing structure and assembly cues for arabidopsis root-inhabiting bacterial microbiota. Nature, **488**:91–95.

Burkle, L. A., J. C. Marlin, and T. M. Knight. 2013. Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. Science, **339**:1611–1615.

Callaway, R. M., R. W. Brooker, P. Choler, Z. Kikvidze, C. J. Lortie, R. Michalet, L. Paolini, F. I. Pugnaire, B. Newingham, E. T. Aschehoug, C. Armas, D. Kikodze, and B. J. Cook. 2002. Positive interactions among alpine plants increase with stress. Nature, **417**:844–848.

Chenoweth, S. F., H. D. Rundle, and M. W. Blows. 2010. The contribution of selection and genetic constraints to phenotypic divergence. The American Naturalist, **175**:186–196.

Clausen, J., D. D. Keck, and W. M. Hiesey. 1947. Heredity of geographically and ecologically isolated races. The American Naturalist, **81**:114–133.

Decaestecker, E., S. Gaba, J. A. M. Raeymaekers, R. Stoks, L. V. Kerckhoven, D. Ebert, and L. D. Meester. 2007. Host–parasite 'red queen' dynamics archived in pond sediment. Nature, **450**:870–873.

Des Marais, D. L., K. M. Hernandez, and T. E. Juenger. 2013. Genotype-by-environment interaction and plasticity: exploring genomic responses of plants to the abiotic environment. Annual Review of Ecology, Evolution, and Systematics, **44**:5–29.

Doebley, J. F. 1984. Maize introgression into teosinte-a reappraisal. Annals of the Missouri Botanical Garden, pages 1100–1113.

Duong, T. 2018. ks: Kernel Smoothing. R package version 1.11.0.

Eagles, H. A. and J. E. Lothrop. 1994. Highland maize from central mexico—its origin, characteristics, and use in breeding programs. Crop Science, **34**:11.

Elshire, R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto, E. S. Buckler, and S. E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE, **6**:e19379.

Etterson, J. R. and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. Science, **294**:151–154.

Falconer, D. S. 1952. The problem of environment and selection. The American Naturalist, **86**:293–298.

Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics (4th Edition). Pearson, 4 edition.

Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences, **104**:1278–1282.

Friesen, M. L., S. S. Porter, S. C. Stark, E. J. von Wettberg, J. L. Sachs, and E. Martinez-Romero. 2011. Microbially mediated plant functional traits. Annual Review of Ecology, Evolution, and Systematics, **42**:23–46.

Germain, R. M., J. T. Weir, and B. Gilbert. 2016. Species coexistence: macroevolutionary relationships and the contingency of historical interactions. Proceedings of the Royal Society of London B: Biological Sciences, **283**.

Glaubitz, J. C., T. M. Casstevens, F. Lu, J. Harriman, R. J. Elshire, Q. Sun, and E. S. Buckler. 2014. Tassel-gbs: a high capacity genotyping by sequencing analysis pipeline. PloS one, **9**:e90346.

Goh, C.-H., D. F. V. Vallejos, A. B. Nicotra, and U. Mathesius. 2013. The impact of beneficial plant-associated microbes on plant phenotypic plasticity. Journal of Chemical Ecology, **39**:826–839.

Hadfield, J. 2012. Mcmcglmm course notes. See http://cran. r-project. org/web/packages/MCMCglmm/vignettes/CourseNotes. pdf.

Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. Journal of Statistical Software, **33**:1–22. Version 2.22.1.

Hansen, T. and D. Houle. 2008. Measuring and comparing evolvability and constraint in multivariate characters. Journal of evolutionary biology, **21**:1201–1219.

Hayward, J., T. R. Horton, A. Pauchard, and M. A. Nuñez. 2015. A single ectomycorrhizal fungal species can enable a pinus invasion. Ecology, **96**:1438–1444.

Hijmans, R. J. 2015. raster: Geographic data analysis and modeling. R package version 2.3-24.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology, **25**:1965–1978.

HilleRisLambers, J., M. A. Harsch, A. K. Ettinger, K. R. Ford, and E. J. Theobald. 2013. How will biotic interactions influence climate change-induced range shifts? Annals of the New York Academy of Sciences, pages 112–125.

Hiltner, L. 1904. Uber neure erfahrungen und probleme auf dem gebeit der bodenbackteriologie und unter besonderer berucksichtigung der grundungung und brache. Arb. Deut. Landwirsch Ges., **98**:5978.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research, **50**:839.

Hufford, M. B., P. Gepts, and J. ROSS-IBARRA. 2011. Influence of cryptic population structure on observed mating patterns in the wild progenitor of maize (zea mays ssp. parviglumis). Molecular ecology, **20**:46–55.

Hufford, M. B., P. Lubinksy, T. Pyhäjärvi, M. T. Devengenzo, N. C. Ellstrand, and J. Ross-Ibarra. 2013. The genomic signature of crop-wild introgression in maize. PLoS Genetics, **9**:e1003477.

Hunter, D. J. 2005. Gene-environment interactions in human diseases. Nature reviews. Genetics, **6**:287.

Johnson, N. C., G. W. T. Wilson, M. A. Bowker, J. A. Wilson, and R. M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences, **107**:2093–2098.

Karhunen, M., J. Merilä, T. Leinonen, J. Cano, and O. Ovaskainen. 2013. driftsel: an r package for detecting signals of natural selection in quantitative traits. Molecular ecology resources, **13**:746–754.

Karhunen, M. and O. Ovaskainen. 2012. Estimating population-level coancestry coefficients by an admixture f model. Genetics, **192**:609–617.

Karhunen, M., O. Ovaskainen, G. Herczeg, and J. Merilä. 2014. Bringing habitat information into statistical tests of local adaptation in quantitative traits: A case study of nine-spined sticklebacks. Evolution, **68**:559–568.

Kawecki, T. J. and D. Ebert. 2004. Conceptual issues in local adaptation. Ecol Letters, **7**:1225–1241.

Keller, S. R., N. Levsen, M. S. Olson, and P. Tiffin. 2012. Local adaptation in the flowering-time gene network of balsam poplar, populus balsamifera l. Molecular Biology and Evolution, **29**:3143–3152.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature, **417**:67–70.

Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution, **33**:402–416.

Lankau, R. A., K. Zhu, and A. Ordonez. 2015. Mycorrhizal strategies of tree species correlate with trailing range edge responses to current and past climate change. Ecology, **96**:1451–1458.

Lau, J. A. and J. T. Lennon. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proceedings of the National Academy of Sciences, **109**:14058–14062.

Lauter, N. 2004. The inheritance and evolution of leaf pigmentation and pubescence in teosinte. Genetics, **167**:1949–1959.

Lebeis, S. L., S. H. Paredes, D. S. Lundberg, N. Breakfield, J. Gehring, M. McDonald, S. Malfatti, T. G. Del Rio, C. D. Jones, S. G. Tringe, et al. 2015. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science, **349**:860–864.

Leinonen, T., R. S. McCairns, R. B. O'hara, and J. Merilä. 2013. Qst-fst comparisons: evolutionary and ecological insights from genomic heterogeneity. Nature Reviews. Genetics, **14**:179.

López, A. N. A., J. de Jesús Sánchez González, J. A. R. Corral, L. D. L. C. Larios, F. Santacruz-Ruvalcaba, C. V. S. Hernández, and J. B. Holland. 2011. Seed dormancy in mexican teosinte. Crop Science, **51**:2056.

Lynch, M., B. Walsh, et al. 1998. Genetics and analysis of quantitative traits, volume 1. Sinauer Sunderland, MA.

McGlothlin, J. W., M. E. Kobiela, H. V. Wright, D. L. Mahler, J. J. Kolbe, J. B. Losos, and E. D. Brodie III. 2018. Adaptive radiation along a deeply conserved genetic line of least resistance in anolis lizards. Evolution Letters.

McGuigan, K. and C. M. Sgrò. 2009. Evolutionary consequences of cryptic genetic variation. Trends in ecology & evolution, **24**:305–311.

Millien, V., S. Kathleen Lyons, L. Olson, F. A. Smith, A. B. Wilson, and Y. Yom-Tov. 2006. Ecotypic variation in the context of global climate change: revisiting the rules. Ecology Letters, **9**:853–869.

Mueller, U. G. and J. L. Sachs. 2015. Engineering microbiomes to improve plant and animal health. Trends in microbiology, **23**:606–617.

O'Brien, A. M., R. J. Sawers, I. Baxter, J. Ross-Ibarra, L. E. Eguiarte, J. Gasca-Pineda, and S. Y. Strauss. 2018. Variable adaptation between teosinte and soil biota across climate. In prep.

O'Brien, A. M., R. J. Sawers, J. Ross-Ibarra, and S. Y. Strauss. 2017. Evolutionary responses to conditionality in species interactions across environmental gradients. bioRxiv.

Orr, H. A. and A. J. Betancourt. 2001. Haldane's sieve and adaptation from the standing genetic variation. Genetics, **157**:875–884.

Ovaskainen, O., M. Karhunen, C. Zheng, J. M. C. Arias, and J. Merila. 2011. A new method to uncover signatures of divergent and stabilizing selection in quantitative traits. Genetics, **189**:621–632.

Paaby, A. B. and M. V. Rockman. 2014. Cryptic genetic variation: evolution's hidden substrate. Nature Reviews Genetics, **15**:247–258.

Panke-Buisse, K., A. C. Poole, J. K. Goodrich, R. E. Ley, and J. Kao-Kniffin. 2015. Selection on soil microbiomes reveals reproducible impacts on plant function. The ISME journal, **9**:980–989.

Peiffer, J. A., A. Spor, O. Koren, Z. Jin, S. G. Tringe, J. L. Dangl, E. S. Buckler, and R. E. Ley. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proceedings of the National Academy of Sciences, **110**:6548–6553.

Pfennig, K. and D. Pfennig. 2009. Character displacement: ecological and reproductive responses to a common evolutionary problem. The Quarterly Review of Biology, **84**:253–276.

Puentes, A., G. Granath, and J. Ågren. 2016. Similarity in g matrix structure among natural populations of arabidopsis lyrata. Evolution, **70**:2370–2386.

Pyhäjärvi, T., M. B. Hufford, S. Mezmouk, and J. Ross-Ibarra. 2013. Complex patterns of local adaptation in teosinte. Genome Biology and Evolution, **5**:1594–1609.

R Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Version 3.1.1.

Ramírez, S. R., T. Eltz, M. K. Fujiwara, G. Gerlach, B. Goldman-Huertas, N. D. Tsutsui, and N. E. Pierce. 2011. Asynchronous diversification in a specialized plant-pollinator mutualism. Science, **333**:1742–1746.

Rehfeldt, G. E., N. M. Tchebakova, Y. I. Parfenova, W. R. Wykoff, N. A. Kuzmina, and L. I. Milyutin. 2002. Intraspecific responses to climate in pinus sylvestris. Global Change Biology, **8**:912–929.

Roff, D. 2000. The evolution of the g matrix: selection or drift? Heredity, **84**:135–142.

Roff, D., J. Prokkola, I. Krams, and M. Rantala. 2012. There is more than one way to skin a g matrix. Journal of evolutionary biology, **25**:1113–1126.

Rúa, M. A., A. Antoninka, P. M. Antunes, V. B. Chaudhary, C. Gehring, L. J. Lamit, and *et al.* 2016. Home-field advantage? evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. BMC Evol Biol, **16**.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution, **50**:1766–1774.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. Nih image to imagej: 25 years of image analysis. Nature methods, **9**:671–675.

Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and ecology of species range limits. Annual review of ecology, evolution, and systematics, **40**:415–436.

Shaw, R. G. and J. R. Etterson. 2012. Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. New Phytologist, **195**:752–765.

Sheth, S. N., A. L. Angert, M. Vellend, and Y. Michalakis. 2016. Artificial selection reveals high genetic variation in phenology at the trailing edge of a species range. The American Naturalist, **187**:182–193.

Smith, S. E. and D. J. Read. 2010. Mycorrhizal symbiosis. Academic press.

Spiegelhalter, D. J., N. G. Best, B. P. Carlin, and A. Van Der Linde. 2002. Bayesian measures of model complexity and fit. Journal of the Royal Statistical Society: Series B (Statistical Methodology), **64**:583–639.

Steppan, S. J., P. C. Phillips, and D. Houle. 2002. Comparative quantitative genetics: evolution of the g matrix. Trends in Ecology & Evolution, **17**:320–327.

Stevens, M., C. A. PARraga, I. C. Cuthill, J. C. Partridge, and T. S. Troscianko. 2007. Using digital photography to study animal coloration. Biological Journal of the Linnean Society, **90**:211–237.

Stinchcombe, J. R., C. Weinig, M. Ungerer, K. M. Olsen, C. Mays, S. S. Halldorsdottir, M. D. Purugganan, and J. Schmitt. 2004. A latitudinal cline in flowering time in arabidopsis thaliana modulated by the flowering time gene frigida. Proceedings of the National Academy of Sciences of the United States of America, **101**:4712–4717.

Stokes, A. N., A. M. Ray, M. W. Buktenica, B. G. Gall, E. Paulson, D. Paulson, and *et al.* 2015. Otter predation on taricha granulosa and variation in tetrodotoxin levels with elevation. Northwestern Naturalist, **96**:13–21.

Swarts, K., R. M. Gutaker, B. Benz, M. Blake, R. Bukowski, J. Holland, M. Kruse-Peeples, N. Lepak, L. Prim, M. C. Romay, et al. 2017. Genomic estimation of complex traits reveals ancient maize adaptation to temperate north america. Science, **357**:512–515.

Thorpe, A. S., E. T. Aschehoug, D. Z. Atwater, and R. M. Callaway. 2011. Interactions among plants and evolution. Journal of Ecology, **99**:729–740.

Toju, H. 2008. Fine-scale local adaptation of weevil mouthpart length and camellia pericarp thickness: altitudinal gradient of a putative arms race. Evolution, **62**:1086–1102.

Toju, H., H. Abe, S. Ueno, Y. Miyazawa, F. Taniguchi, T. Sota, and T. Yahara. 2011. Climatic gradients of arms race coevolution. The American Naturalist, **177**:562–573.

Wagner, M. R., D. S. Lundberg, D. Coleman-Derr, S. G. Tringe, J. L. Dangl, and T. Mitchell-Olds. 2014. Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild arabidopsis relative. Ecology Letters, **17**:717–726.

Walter, G. M., D. Aguirre, M. W. Blows, and D. Ortiz-Barrientos. 2017. Evolution of genetic variance during adaptive radiation. bioRxiv.

Walters, W. A., Z. Jin, N. Youngblut, J. G. Wallace, J. Sutter, W. Zhang, A. González-Peña, J. Peiffer, O. Koren, Q. Shi, et al. 2018. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proceedings of the National Academy of Sciences, page 201800918.

Wernegreen, J. J. 2012. Mutualism meltdown in insects: bacteria constrain thermal adaptation. Current opinion in microbiology, **15**:255–262.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annual review of Ecology and Systematics, **20**:249–278.

Wilczek, A. M., M. D. Cooper, T. M. Korves, and J. Schmitt. 2014. Lagging adaptation to warming climate in arabidopsis thaliana. Proceedings of the National Academy of Sciences, **111**:7906–7913.

Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, and C. C. Davis. 2008. Phylogenetic patterns of species loss in thoreau's woods are driven by climate change. Proceedings of the National Academy of Sciences, **105**:17029–17033.

Wilson, A. J., D. Reale, M. N. Clements, M. M. Morrissey, E. Postma, C. A. Walling, L. E. Kruuk, and D. H. Nussey. 2010. An ecologists guide to the animal model. Journal of Animal Ecology, **79**:13–26.

Wood, C. W. and E. D. Brodie III. 2015. Environmental effects on the structure of the g-matrix. Evolution, **69**:2927–2940.

Wood, C. W. and E. D. Brodie III. 2016. Evolutionary response when selection and genetic variation covary across environments. Ecology Letters, **19**:1189–1200.

Zhu, X.-C., F.-B. Song, and H.-W. Xu. 2009. Arbuscular mycorrhizae improves low temperature stress in maize via alterations in host water status and photosynthesis. Plant Soil, **331**:129–137.

Supplementary Information

(a)

(b)

Figure S1: Variation in teosinte stem color in the field. Green at low (a) and red at high (b) elevation. Photographs from A.M.O.

Trait	Main	Random	Slope	DIC base	DIC 1st	DIC 2nd	Effects in 2nd
Days to flowering	TAP	F	0	2398.4	2305.5	2305.6	ELV, F ,− ∗ ∗
Days to germination	MAT	P	+**	2444.4	2426.7	2427.2	SWC, P, + ∗ ∗
Tassel length	MAT	F	+*	2403.8	2346.0	2346.2	ELV, F, − ∗ ∗
Shoot biomass	MAT	P	+**	2441.1	2404.5	2405.5	ELV, P, − ∗ ∗
Root biomass	MAT	F + P	+**	2443.7	2373.3	2374.4	ELV, F+P, − ∗ ∗
Height	none	P		2418.5	2409.7	2410.7	TAP, P, 0
Stem width	MAT	F + P	+**	2420.1	2380.5	2381.7	MAT, P, + ∗ ∗
Leaf width	MAT	P	+**	2427.1	2423.8	2424.3	MAT, F+P, + ∗ ∗
Stem greenness %	none	none		2359.3			

Table S1: Expanded Table 1; best models for traits. The "base" model includes only an intercept and random effect of family in population. We show the sign and the significance of the slope for simplicity. Second best model variables, slopes and significance are in the last column, except for stem greenness, where "base" model was best. Abbreviations: TAP for total annual precipitation, MAT for mean annual temperature, SWC for soil water content, ELV for elevation, F family in biota random effect, P population in biota random effect. Symbols: ** pMCMC < 0.05, * pMCMC < 0.1.

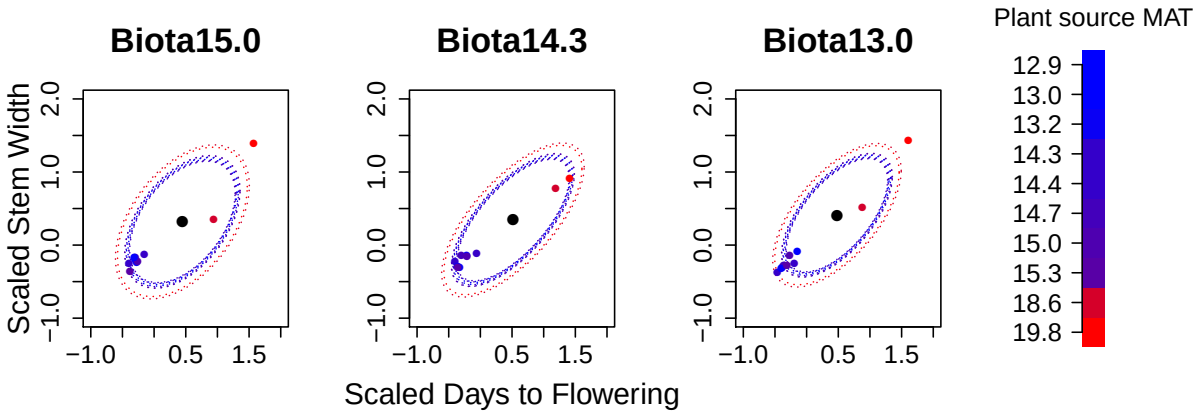

Figure S2: Standardized population means (colored points) and 95% confidence intervals for the neutral expectation of population means (matching colored circles), for flowering day and stem width, estimated by Driftsel. Redder color indicates warmer mean annual temperature at the source site. Means beyond matching confidence intervals indicates significant divergence.

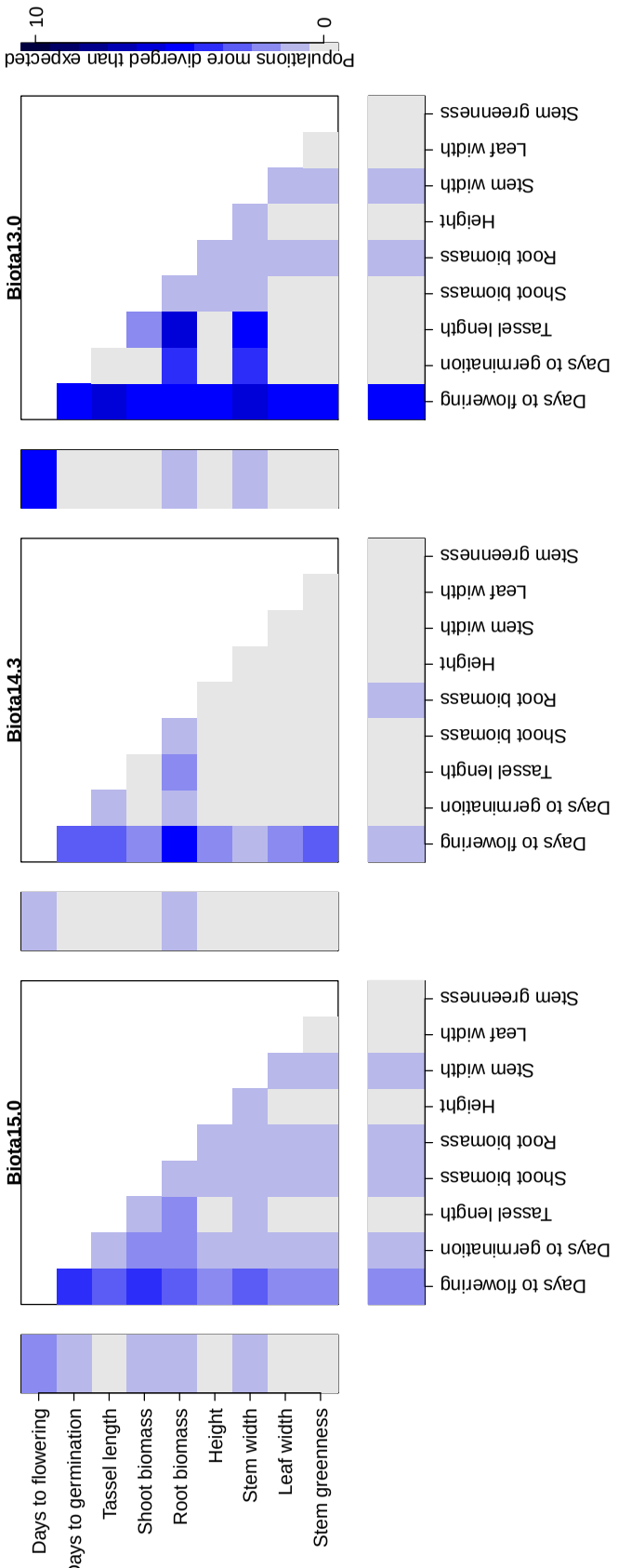

Figure S3: Populations exceeding bivariate expectations of divergence in all biota (separate plots). Darker blue squares indicate trait combinations with more populations outside expectations (each panel of figure S2 is reduced to one square here). Bars on the axes indicate the number of populations in which traits exceed divergence expectations in the univariate case (see Results for higher dimension summary S test).

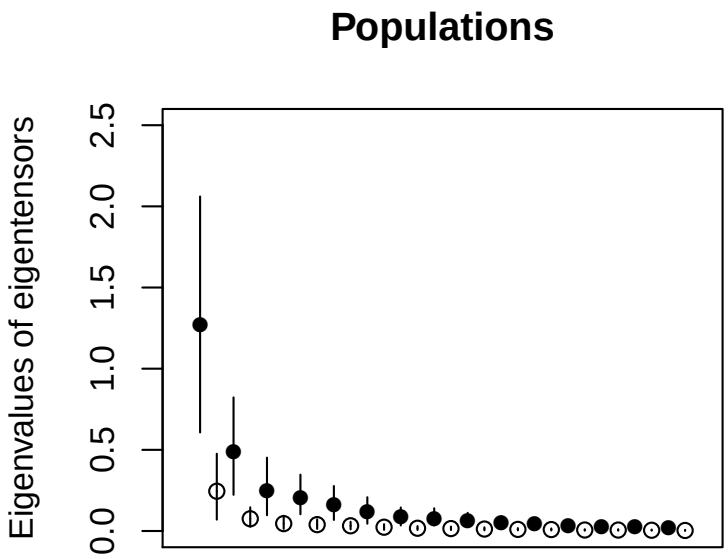

Figure S4: Eigenvalues of the first 11 eigentensors of the set of 30 G matrices (filled points), and the first 11 eigentensors from the randomized array (open points). Error bars represent confidence intervals across MCMC estimations (real set) or across the randomized array.

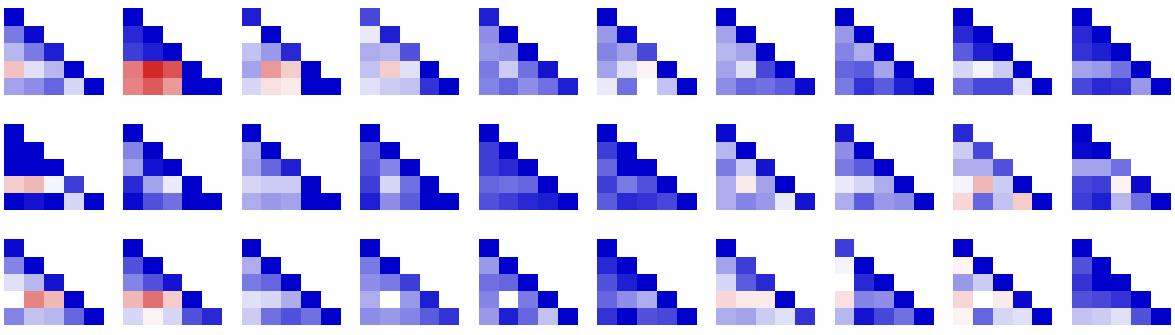

Figure S5: The full set of G matrices across populations (columns) and biotas (rows). Colors and trait organization are as in Figure 5.

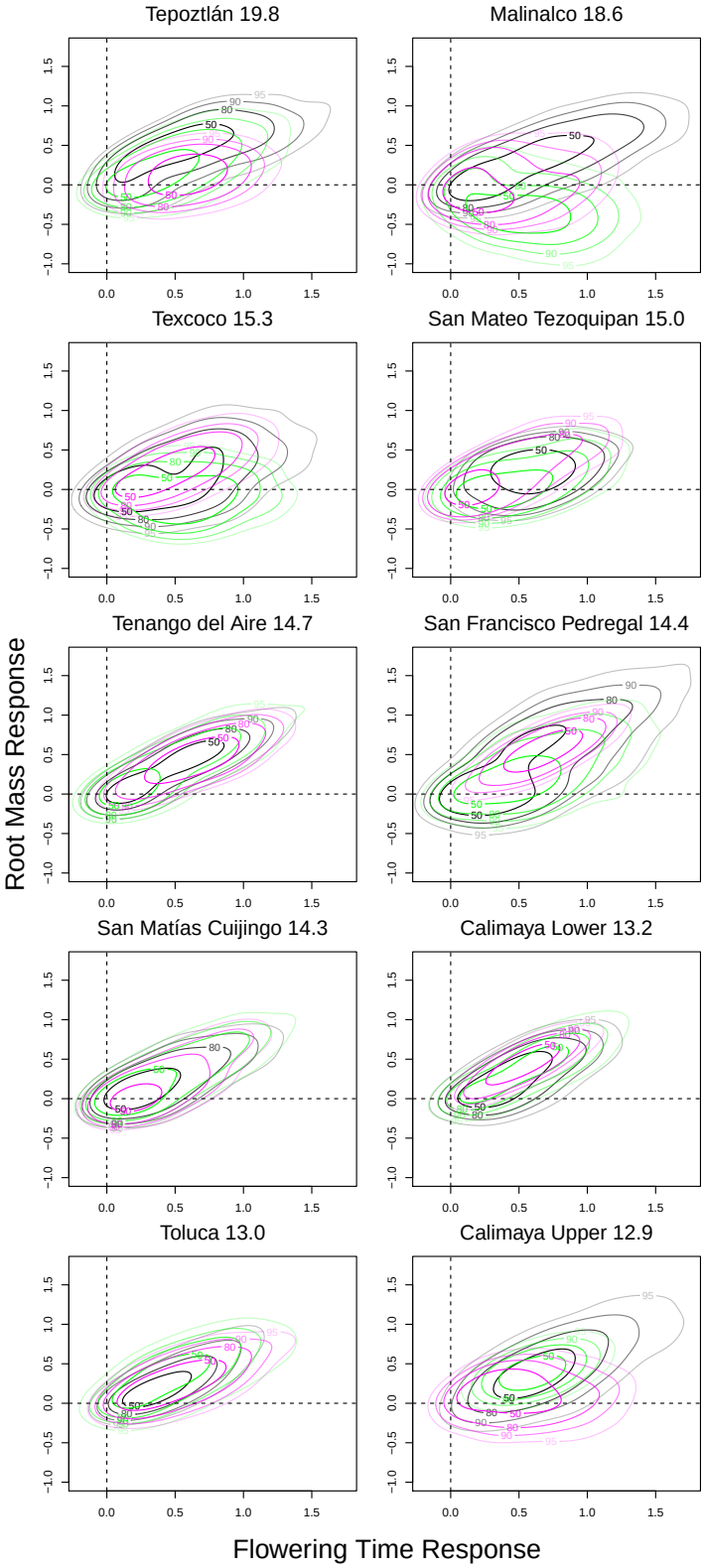

Figure S6: Responses to selection on flowering time for G matrices estimated in each population in different biota. Confidence intervals in contour lines as in Figure 5 (black, Biota15.0; green, Biota14.3 ; purple, Biota13.0). Dashed lines highlight 0 response for both traits. Plant populations are sorted by MAT °C with names.

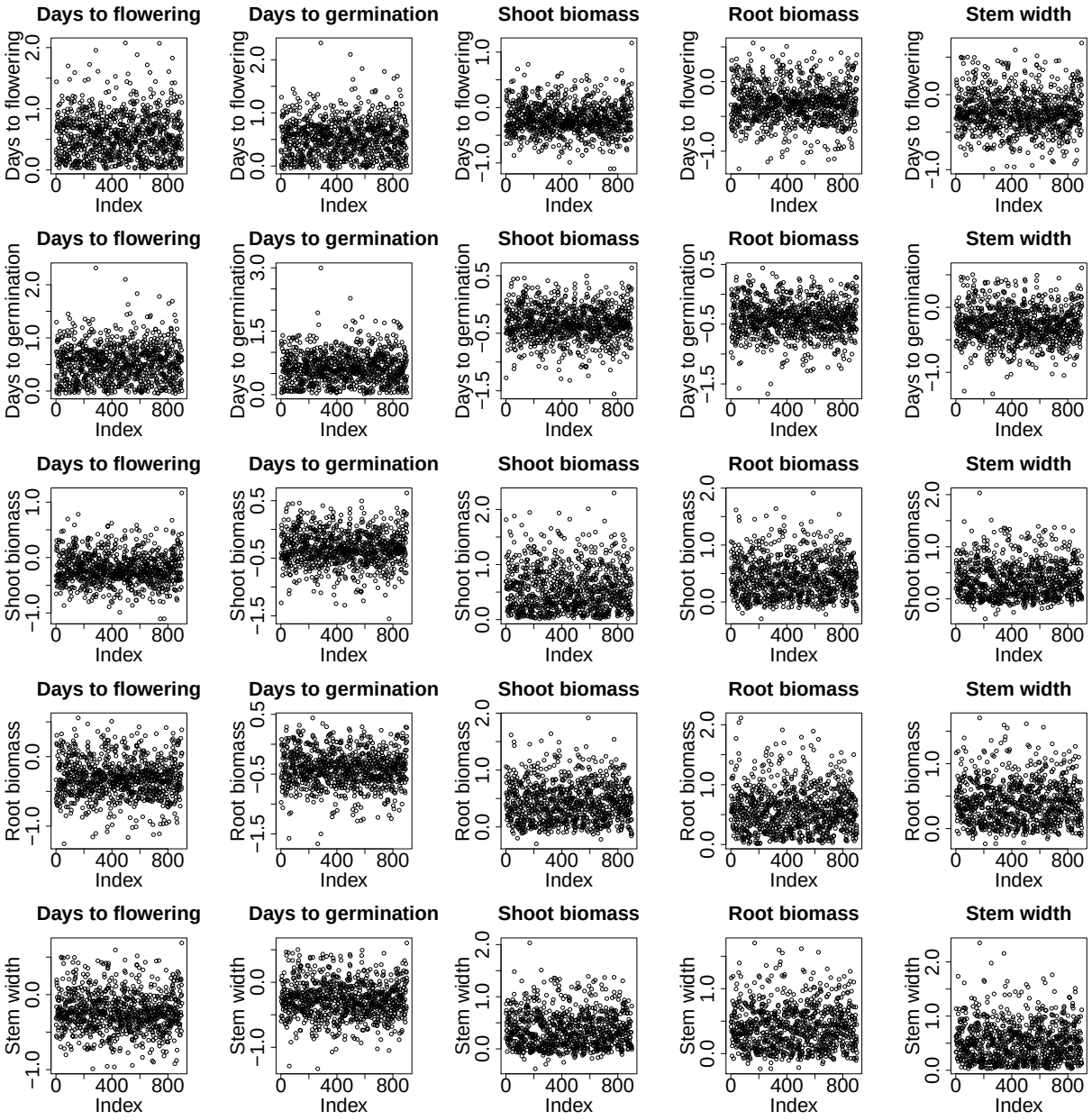

Figure S7: Trace of posterior estimates of the G matrix for Malinalco population in Biota15.0, as an example of MCMC chains.

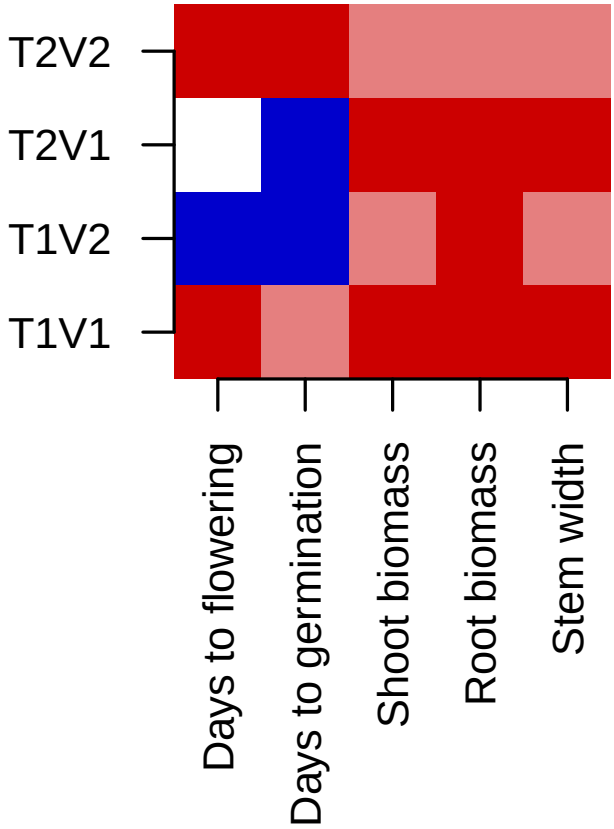

Figure S8: First and second eigenvectors of the first two eigentensors of the set of genetic variance-covariance matrices. Red indicates positive loading on the tensor, blue indicates negative loading, and color intensity indicates the strength of loading.

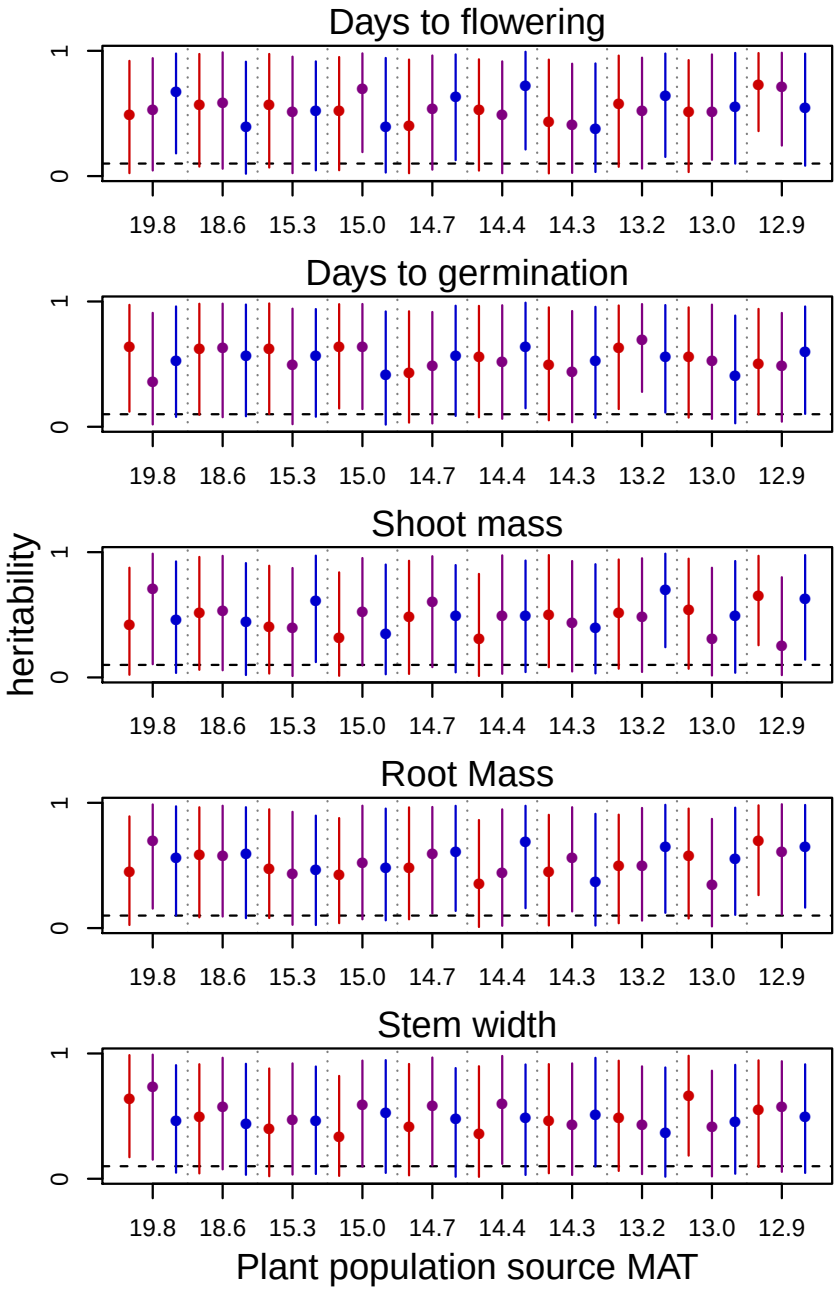

Figure S9: Estimated heritability of each trait in each population calculated from additive genetic variance and residual error (environmental variance) in fitted MCMCglmm models. Points and 95% HPDI are colored by the rhizosphere biota in which the plants were measured (red, Biota15.0; purple, Biota14.3; blue, Biota13.0). Vertical dotted lines separate plant populations.