Extending the Stress-Gradient hypothesis: increased local adaptation between teosinte and soil biota at the stressful end of a climate gradient

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Statement of authorship: All authors contributed substantially to the design of the study, provisioning of materials, and revising of the manuscript. AO proposed the study, collected the data, performed analyses and provided the first draft of the manuscript.
Running title: Increased co-adaptation in stressful sites
Keywords: stress-gradient, biotic interactions, climate adaptation, rhizosphere, mutualisms, local adaptation
Submitted
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Abstract

In order to predict plant responses to rapid climate change, we will need to understand the role of biotic interactions in plant climate adaptation. We extend the stress-gradient (SG) hypothesis to posit that, as interactions become more mutualistic at stressful ends of environmental gradients, so does selection for mutualistic co-adaptation. We call this the stress-gradient evolution hypothesis (SGE). We test our SGE hypothesis in the interactions of teosinte (*Zea mays* ssp. *mexicana*) with its rhizosphere soil biota in populations across a climate gradient. In support of SGE predictions, we find local adaptation of teosinte to soil biota at the stressful (cold) end of our climatic gradient but not at the benign (warm) end: sympatric combinations of plants and biota from stressful sites both increase plant fitness and generate more locally adapted plant phenotypes. Our results suggest that co-adaptation of interacting partners may be a means of ameliorating stressful environments.

Introduction

Rapid climate change poses a challenge to plant species selected to thrive under past conditions as they must adapt or colonize new territory. Climate regimes have left a strong imprint on patterns of plant adaptation (Keller *et al.*, 2010; Strasburg *et al.*, 2011), but studies have typically focused on physiological or morphological traits and less on the roles of interactions with other species. The stress-gradient hypothesis (Bertness & Callaway, 1994), predicts that biotic interactions shift from having neutral or negative outcomes under benign conditions to having mutually beneficial outcomes under stressful conditions. Here, we predict that this variation in interaction outcomes along abiotic stress gradients could also result in divergent coevolutionary outcomes. This extension of the stress-gradient hypothesis, which we call stress-gradient-evolution (SGE), predicts mutualistic evolution and local co-adaptation in stressful sites where reciprocal benefits are the strongest. Stress-ameliorating interactions have greater reciprocal benefits to fitness in high stress environments, and selection will favor efficiency and increased investments in partners to return greater benefits (see partner fidelity feedback, Sachs *et al.*, 2004). In contrast, where abiotic stress does not limit fitness, stress-ameliorating interactions may be irrelevant (weak to no selection) or antagonistic, possibly generating arms-race or 'Red Queen' dynamics (Van Valen, 1974; Gandon & Michalakis, 2002), or generating avoidance of interactions (reviewed in Thorpe *et al.*, 2011). Thus, we predict that the conditionality of the benefit of an interaction along a stress gradient will create a clinal geographic mosaic of coevolution (as described by Thompson, 2005).

For plants, many fitness-affecting biotic interactions occur below ground with a diverse community of bacteria, nematodes and fungi, collectively known as rhizosphere biota (Hiltner, 1904; Lundberg *et al.*, 2012; Toju *et al.*, 2014). Plant roots contain and exude a multitude of compounds, including a large volume of polysaccharide mucilage, providing rhizosphere biota with a highly productive habitat in and near the root (Bais *et al.*, 2006; Raaijmakers *et al.*, 2009). Some interactions involve the direct exchange of plant sugars for soil nutrients, such as phosphorous in mycorrhizal associations or nitrogen in nodule associations, while

other interactions may be more indirect, such as nematode-dependent nutrient cycling and transport of beneficial bacteria, leading to plant benefit (e.g Ingham *et al.*, 1985; Horiuchi *et al.*, 2005; Bais *et al.*, 2006). Numerous studies have shown that genetic variation among individual plants can structure rhizosphere community composition (e.g. Bouffaud *et al.*, 2014; Lundberg *et al.*, 2012; Burns *et al.*, 2015), either passively or via direct action such as favoring beneficial microbes (e.g Werner & Kiers, 2014). Individual rhizosphere species, in turn, can affect plant fitness, with outcomes ranging from pathogenicity to growth promotion (Berg & Smalla, 2009). Variation in rhizosphere community composition can have large impacts on plant growth, competition, and tolerance of stressful conditions (e.g. Klironomos, 2002; Smith & Read, 2010; Lau & Lennon, 2012), and these outcomes may vary across environmental conditions (Zhu *et al.*, 2009; Smith & Read, 2010).

Under our framework of stress-gradient-evolution, we predict that plants and rhizosphere biota will be more co-adapted at stressful sites. SGE is similar to preferential allocation and economic models of exchange used for plant-microbe provider mutualisms, in which selection on microbes increases benefits that they provide to plants in low soil nutrient environments, and decreases benefits in high nutrient environments (Johnson, 1993; Schwartz & Hoeksema, 1998; Werner *et al.*, 2014; Bever, 2015). SGE differs from such models in its focus on co-adaptation patterns, inclusion of additional benefits beyond discrete resources, and its broad applicability to stress-ameliorating interactions across species. Tests of co-adaptation and environmental gradients are still rare for plant-rhizosphere interactions (Hoeksema, 2010), but the limited results to date support our SGE hypothesis in finding variable degrees of local co-adaptation (Johnson *et al.*, 2010; Barrett *et al.*, 2012), but see (Kardol *et al.*, 2014).

Here, we use rhizosphere interactions in teosinte (*Zea mays* ssp. *mexicana*), a wild relative of maize from central Mexico (Sánchez & Corral, 1997), as a model to test some of the predictions of stress-gradient-evolution along a climatic gradient characterized by variation in mean annual temperature, seasonality, precipitation, and elevation. Cold stress is an important driver of adaptation to highland conditions in maize (Duncan & Hesketh, 1968; Mercer

et al., 2008; Skarbø & VanderMolen, 2015), and temperature gradients have shaped both genetic (Bradburd *et al.*, 2013; Pyhäjärvi *et al.*, 2013) and phenotypic (Doebley, 1984; Lauter, 2004) variation in teosinte. Because cold stress in maize can be alleviated by rhizosphere interactions (Zhu *et al.*, 2009), SGE predicts that teosinte-rhizosphere interactions in the field will vary across this climatic gradient. Specifically, SGE predicts greater co-adaptation between plants and soil biota in cold environments due to aligned fitness outcomes. In warmer, more benign climates, SGE predicts less co-adaptation, as either interactions are more neutral (with weaker selective power of the interaction on traits) or antagonistic, generating reduced interaction or temporally variable outcomes such as Red Queen or arms race dynamics.

We test our SGE hypothesis using common garden experiments of field-collected teosinte populations and associated rhizosphere biota sampled over a gradient of mean annual temperature to investigate whether teosinte are more locally adapted to rhizosphere biota in colder, more stressful climates.

Methods

Field Collections

We collected teosinte and rhizosphere materials from 10 populations along a cline spanning 6.6 degrees in mean annual temperature (MAT) and more than 1100 meters in elevation (TableS1), representing the full elevational range of *Zea mays* ssp. *mexicana*. Climatic information from each site was obtained from Bioclim (Hijmans *et al.*, 2005) and extracted using the package raster (Hijmans, 2015) in R (R Core Team, 2014). In August 2013, 2 kg of teosinte rhizosphere soil was collected from each population (multiple individuals pooled), kept refrigerated at 4°C and sent for analysis of texture, nutrients, and physical properties at INIFAP, Laboratorio Nacional de Fertilidad de Suelos y Nutricin Vegetal, revealing only weak co-correlation of fertility with MAT (Table S2, ρ MAT and: N = -0.26, P = 0.41, K =

0.54). In December 2013, seeds were collected from 12 adult plants per population, chosen to both span the spatial extent of the population and have sufficient seed quantity. Seeds were stored at 4°C until use. At the same time, rhizosphere biota were collected from additional plants selected to span the whole population, a total of approximately 6 liters (4-7 L) of roots and attached soil were collected from each site. Adult plants were unearthed and roots lightly shaken to remove non-adhering soil, and then roots and adhering soil were cut off into plastic bags. These collections were air-dried and were stored at 4°C for 5 months. To make biota inoculum treatments, samples within populations were homogenized in a blender, cutting larger roots with scissors where necessary, until root pieces were approximately ≤ 2 cm in length and well mixed with the soil.

Experiment

In May of 2014, seeds from each population were grown in six biota inoculum treatments: no inoculum, inoculum from the home site (inoculum was unique to each site), and then four separate biota inocula that were applied to all the populations. Two of these four inocula were from colder sites, two from warmer sites, such that allopatric treatments spanned the range of of teosinte climate. This design allowed us to compare degree of local adaption to rhizosphere biota in plants from cold or warm sites, our main prediction from SGE. Biota inocula were applied to sibling seeds from 12 mothers from each of the 10 populations along the elevational/climate gradient (120 mother plants $\times$ 6 treatments = 720 plants). Plants were grown in 2 L pots. Pots were filled to 1.5 L with sterilized custom potting mix (90% sand, 5% perlite 5% vermiculite 0.2% silt). This mix was steam sterilized for 4 hours at 90°C using a PRO-GROW SS60. Pots were then inoculated with 50 mL of a 4:1 mix of sterile sand and homogenized inocula, and filled to the top. In the no inoculum treatment, a separate batch of sterilized sand replaced homogenized inocula. Three seeds were added to pre-watered pots after scarification with overnight soaking, and pots were weeded after germination if more than one plant germinated. In most cases the first seed to germinate was

kept, except when this germinant died before the pot was weeded, in which case the surviving sibling germinant was kept. The planting design on the bench was randomized with respect to seed source, biota inoculum source, and mother. Plants grew in a greenhouse with an average temperature of 23.8°C over the course of the experiment. Plants were unfertilized and kept moist for the first two weeks as most plants germinated, after which pots were watered and fertilized once per week with 50 mL of Hoagland's solution at low phosphorous (100μM). Plants were treated one time with a dual application of Agri-mycin and Knack to prevent caterpillar and spider mite herbivory.

Quantification of fitness and traits

Plants were collected, washed, dried and weighed at 52 days after germination. In the related subspecies *Zea mays* ssp. *parviglumis*, vegetative biomass is significantly correlated with seed mass (pMCMC = 0.01) and number (pMCMC $<$ 0.001) (data from Piperno *et al.*, 2015, see Table S3) and thus biomass reflects fitness in this annual species. We also measured the expression of two other traits relevant to fitness in the field that show elevational clines in teosinte: stem macrohairs (Lauter, 2004), and germination fraction (Wilkes, 1977; López *et al.*, 2011). These traits are known (stem macrohairs: Hufford *et al.*, 2013; Kaur *et al.*, 1985) or hypothesized (germination fraction: Wilkes, 1977; Eagles & Lothrop, 1994) to have different adaptive values across environments in maize: where increased stem hairs and germination fraction are expected to be advantageous in cold environments, while reduced germination is expected to be advantageous in warm environments. Just prior to harvest, plant stem macrohairs were quantified as the number of hairs in 1 cm^2 below the ligule on the edge of the lowest live leaf sheath. We scored germination fraction as the fraction of the planted seeds germinating in each pot.

Data Analysis

We used linear models of plant biomass and traits as separate response variables with continuous predictor variables to test our SGE hypothesis. Our main prediction is greater local adaptation of plants to biota in cold environments than warm environments. In classic tests of local adaptation, populations and sites are treated as discrete entities (Kawecki & Ebert, 2004; Blanquart *et al.*, 2013); however, incorporating degree of local adaptation along a climate gradient requires a continuous statistical approach with two linear model parameters. We add a binary term (S) indicating whether origin of the rhizosphere biota and the plant population were the same (sympatric, $S = 1$) or mismatched (allopatric, $S = 0$) and an interaction of sympatry and MAT of the sympatric pair models (a MAT $\times$ sympatry interaction denoted as $T_S \times S$) to test the prediction that cold-sourced plant populations are more locally adapted to their rhizosphere communities than warm-sourced plant populations are to their local rhizosphere communities. The inclusion of T_S further requires plant source population temperature and inocula source temperature (T_P & T_I, MAT of plant and inocula populations along the climate gradient) to account for any main effect of plant or inocula source MAT in the T_S slope parameter. MAT main effects could capture some plant or biota sources having higher mean fitness but not necessarily being more locally adapted (see Blanquart *et al.*, 2013). We also included in all analyses a random effect of plant family within inoculated plants, and a covariate of the biomass of an uninoculated sibling for each inoculated family, as in Sawers *et al.* (2009). Briefly, the family effect takes into account differences in biomass or traits across families in the inoculated state; the uninoculated sibling effect can be interpreted as a measure of responsiveness to inoculation across all inocula. This method is analogous to using response ratios of inoculated plants relative to uninoculated siblings as the dependent variable (Sawers *et al.*, 2009). Finally, we took an information criterion approach in which we included all other two- and three-way interaction terms. We used model selection to determine which terms explain variation in our dependent variables with MCMCglmm (Hadfield, 2010) to fit the models (burn-in 8,400, then 80,000 iterations,

thinning by 50). If one of the terms testing the hypotheses is absent from the best model we concluded that the hypothesis is not supported. We used the gaussian distribution for biomass, a binomial log-link for germination probability, and a poisson log-link for stem hairs. Models were compared with Deviance Information Criterion (DIC), the extension of Akaike's Information Criterion for models fit by MCMC (Spiegelhalter *et al.*, 2002) Given that plant-biota relationships can be influenced by soil nutrient content, we also included models in which the same terms were used, except that in lieu of each MAT variable we included concentrations of N,P and K levels from the soils of population sites. In all cases, for both traits and biomass, models with MAT had better explanatory power than those with nutrients (Table S4).

Results

Our SGE framework predicted that colder sources of sympatric plants and rhizosphere biota would show a greater degree of local adaptation in benefits to teosinte plants than warmer-sourced plants and their biota. For example, increased stem hair production should have a fitness benefit for teosinte in a cold environment, but not in a warm greenhouse.

In support of SGE, we found that local adaptation of teosinte to rhizosphere biota was greater in colder, more stressful source sites (Figure 1A). In addition, plants from cold environments matched with sympatric biota were the only combination to increase expression of adaptive phenotypes matching the home climate (Figure 2). Cold-sourced sympatric combinations of plants and rhizosphere biota produce larger plants than cold-sourced plants with allopatric cold-sourced biota (predicted to be larger by 10% in best models), and biomass is closely linked to lifetime fitness (see methods, Piperno *et al.*, 2015). This signal of benefit from local biota on biomass was missing in warm-sourced plants, which grew no larger in sympatric biota than in allopatric warm-sourced biota (Figure 1, Table 1). Warm-sourced biota were overall more beneficial to plants (increased biomass) than biota from colder sites,

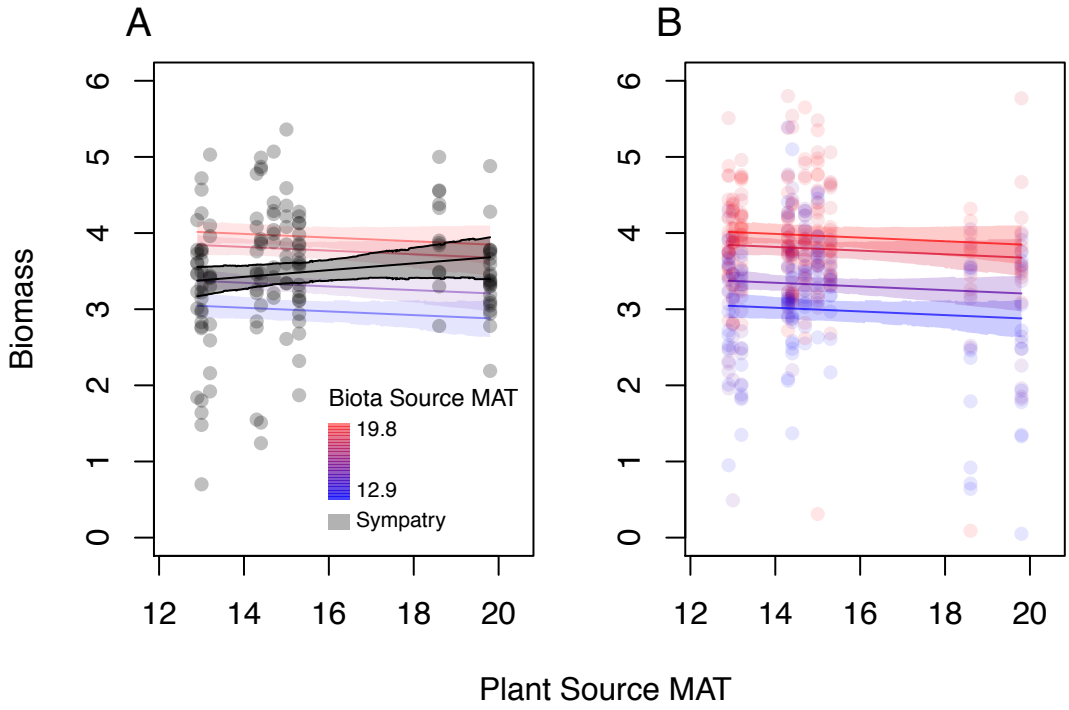

Figure 1: Local biota support larger plants than non-local biota, but only for for cold-sourced pairs. Biomass is greatest with warm-sourced biota across all plant sources. Black indicates data and model predictions for sympatric combinations across plant sources. Color indicates predictions (A and B) and data (B) for allopatric combinations of plants and biota, colored by biota source MAT from blue (coldest, 12.9°C) to red (warmest 19.8°C). Predictions include the mean and 95% highest posterior density interval (HPDI) of the mean for the four biota that were applied across plant sources.

across all populations (Figure 1B, Table 1), however, these fitness benefits decreased as uninoculated sibling size increased, indicating that warm-sourced biota are unable to provide comparable benefits to plants from otherwise phenotypically large families, or that warm-sourced biota may benefit only plants from otherwise phenotypically small families.

Stem macrohairs stem hairs are known to increase fitness in cold environments in maize (Kaur *et al.*, 1985), and cold-sourced teosinte populations produce more stem hairs when grown with sympatric rhizosphere biota (reaching >50 hairs per cm; Figure 1, see also S and $S \times T_S$ interaction in Table 1). DIC-selected best models also included an interaction term between uninoculated sibling stem hairiness and sympatry. For cold-sourced teosinte only,

Name	*parameter*	Biomass	Germ. Frac.	Hairs
Intercept	a	-0.26	2.58**	4.84**
Biota source MAT	T_I	0.23**		0.063
Plant source MAT	T_P	-0.025	-0.18**	-0.43**
Sympatry	S	**1.26****	**-2.56****	**7.40****
Source MAT × Sympatry	$T_S \times S$	**-0.071***	**0.14****	**-0.51****
Uninoculated sibling	Z	0.69**	1.61**	0.52**
Biota source MAT × Uninoculated sibling	$T_I \times Z$	-0.033**		
Plant source MAT × Uninoculated sibling	$T_P \times Z$			-0.031*
Sympatry × Uninoculated sibling	$S \times Z$		**0.66**	**-0.64***
Source MAT × Sympatry × Uninoculated sibling	$T_S \times S \times Z$			**0.044***

Table 1: For each response variable, we report estimates for only parameters included in the best model. Several possible two- and three-way terms were never included in a best model and have been omitted. **: pMCMC < 0.05,*: pMCMC < 0.1 . Parameters that test predictions of our SGE hypothesis are shown in bold.

plants with the least hairy uninoculated siblings have the greatest increase in hair expression with sympatric inocula, indicating that sympatric biota may facilitate optimal expression of adaptive phenotypes (Figure S1B, $S \times Z$, and $T_S \times S \times Z$ in Table 1).

In warm, long-season sites dormancy (reduced germination fraction) may be advantageous to avoid rotating years of weeding or otherwise non-optimal conditions, while reduced dormancy should be advantageous in cold, short season sites, though the exact optimal germination fractions are unknown (Wilkes, 1977; Eagles & Lothrop, 1994; López *et al.*, 2011). In contrast to our expectations, rhizosphere biota did not appear to increase the germination fraction of cold-sourced plants (Figure 3A), and there was a slight increase in germination fraction in warm-sourced plants inoculated with sympatric warm-sourced biota

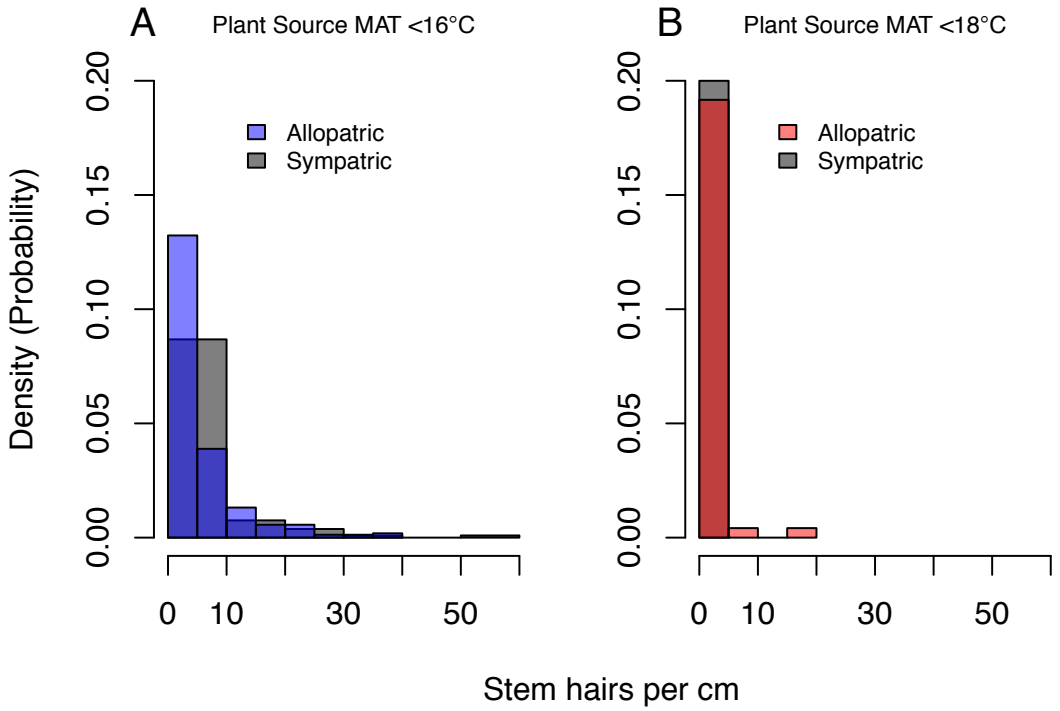

Figure 2: Cold-sourced plants in sympatric inocula have more stem hairs. Shown is a density plot of observed data for stem hairs per cm on inoculated plants split into populations of teosinte from A) colder (16 °C MAT, 8 populations) or B) warmer (18 °C MAT, 2 populations) sites, and further split into allopatric (colored) or sympatric (black) combinations of plants and biota.

($S \times T_S$ in Table 1, Figure 3B). Uninoculated sibling germination fraction is predictive of inoculated plant germination, but is more predictive for sympatrically inoculated plants than allopatrically inoculated plants (see positive $S \times Z$ term in 1, steeper slopes for sympatry in Figure 3). Biota treatments affect germination, suggesting teosinte may use cues from biota in germination decisions. Further, allopatrically inoculated plants have more unpredictable germination fraction, suggesting that germination cues may be specialized with local biota.

In sum, we found support of our SGE hypothesis in local adaptation by plants to biota in cold, stressful sites and in lack of local adaptation in more benign warm sites. Advantages of sympatry for teosinte biomass were observed only in cold-sourced combinations, and we did not find that cold-sourced biota were overall more beneficial to plant populations, suggesting

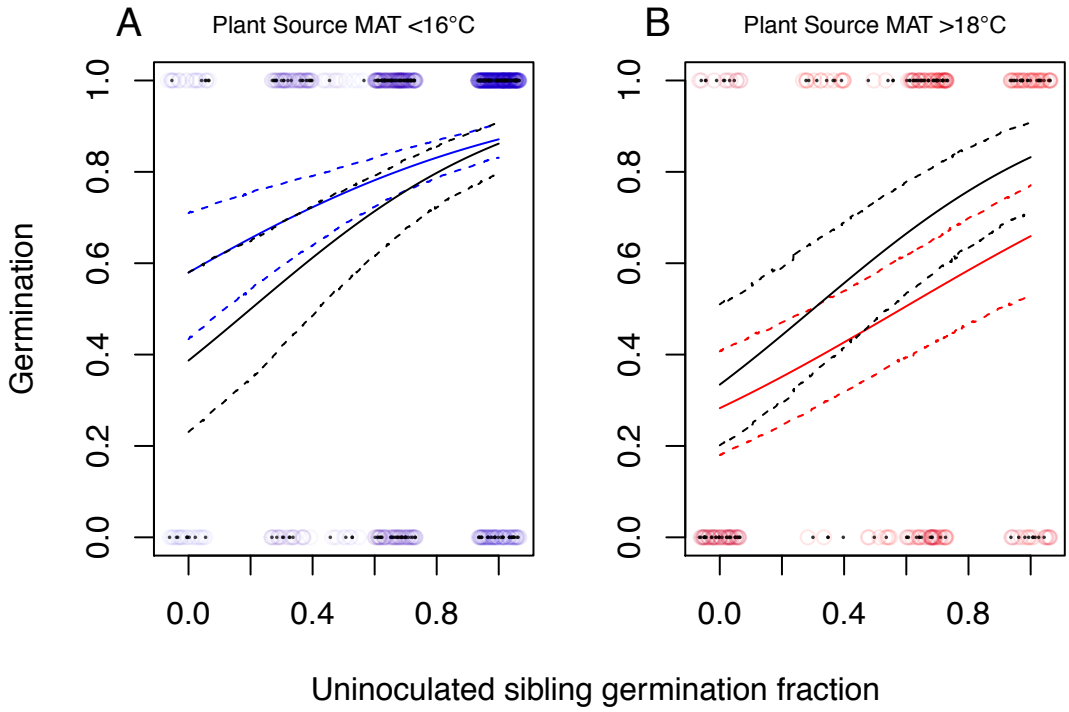

Figure 3: Fewer seeds germinate and predicted germination fraction is lower in allopatric inocula for warm-sourced teosinte populations. Points are binomial data (germinated = 1, ungerminated = 0) and lines indicate model predictions of mean germination fraction with 95% HPDI for allopatry. Panels are separated into cold- (<16 °C MAT, 8 populations) and warm- (>18 °C MAT, 3 populations) sourced teosinte plants in panels A and B respectively. Black points represent seeds in sympatric biota treatments; seeds in allopatric biota treatments are shown as open colored circles by MAT from bluest (12.9 °C) to reddest (19.8 °C). Lines are colored by plant source MAT: blue for model predictions for plant populations at 12.9 °C in panel A, red for model predictions for plant populations at 19.8 °C in panel B, and black for sympatry in both panels. Slopes are slightly steeper between predicted germination fraction and the germination fraction of uninoculated siblings in sympatric treatments across plant source temperatures.

population-specific co-adaptation between biota and plants at cold sites. We also found greater adaptive phenotypic expression of stem hairs with sympatric biota in cold-sourced plants but no effect of sympatric biota in teosinte populations from warm sites and no overall effect of cold-sourced biota on stem hairs. Allopatric biota appear to generate less predictable phenotypes across siblings than sympatric biota, further suggesting co-adaptation of plants and biota in plant phenotype expression.

Discussion

The stress-gradient hypothesis (SG) predicts that species should have more mutually beneficial outcomes of interactions when they are growing under abiotic stress, and more neutral or competitive outcomes when growing in benign conditions (Bertness & Callaway, 1994). A meta-analysis of plant-plant facilitation and competition across > 700 tests supports the SG hypothesis (He & Bertness, 2014), and while animal interactions are less commonly framed in this light, plant-animal and animal-animal interactions also show some support of SG (e.g. Daleo & Iribarne, 2009; Dangles *et al.*, 2013). Furthermore, both theory and results for some plant-microbe interactions along soil nutrient gradients support the SG prediction of more mutually beneficial outcomes in low nutrient sites (e.g. Johnson, 1993; Smith *et al.*, 2010).

Here, we extend SG to include the evolutionary outcome of interactions in response to stress gradients. When an interaction under stressful abiotic conditions ameliorates stress and increases the fitness of both species, fitness benefits to partners are aligned and selection can act on traits to maximize mutual benefit, resulting in co-adaptation. When conditions are benign, a stress-ameliorating interaction may become neutral or even antagonistic. Antagonistic interactions may generate temporally varying patterns of co-adaptation such as Red Queen or arms race dynamics which reduce the degree of observable co-adaptation. Thus SGE predicts a relationship between the degree of abiotic stress and the degree of local

adaptation of sympatric populations.

We tested for stress-gradient-evolution in teosinte interactions with rhizosphere biota along a gradient of mean annual temperature and correlated gradients of elevation, seasonality, and precipitation representative of conditions that teosinte experiences in its natural range. These gradients are known to shape genetic (Bradburd *et al.*, 2013; Pyhäjärvi *et al.*, 2013) and phenotypic (Lauter, 2004) variation in teosinte. Cold stressed environments are often unproductive (e.g. Cramer *et al.*, 1999), but the coevolutionary outcome of interactions based on environmental productivity is unclear (Thrall *et al.*, 2007). SGE predicts the evolution of increased specificity of benefit at stressful sites. Consistent with this key expectation of SGE, we observed that cold-sourced teosinte benefited more from sympatric cold-sourced biota than allopatric cold-sourced biota, while warm-sourced plant populations did equally well with both sympatric and allopatric warm-sourced biota. We cannot, however, distinguish the directionality of adaptation: whether cold-sourced teosinte are locally adapted to derive benefit from sympatric biota or if cold-sourced biota are locally adapted to provide benefit to sympatric plants. We expect that both mechanisms contribute to some extent, as SGE predicts selection on both partners to maximize beneficial exchange, and reciprocal fitness benefits should favor both increases in ability to derive benefits and increases in investment in partners that will in turn provide benefits (e.g. Sachs *et al.*, 2004; Kiers & van der Heijden, 2006; Archetti *et al.*, 2011).

Also in support of SGE, sympatric microbes increased the expression of adaptive phenotypes in cold (stressful) but not warm environments. In cold-sourced plant populations, sympatric cold-sourced biota stimulated greater expression of macrohairs, a phenotype known from maize to improve fitness in cold environments (Kaur *et al.*, 1985). Optimal germination fractions at cold and warm sites are unknown (López *et al.*, 2011), so while it appears germination cues are specialized between teosinte and local biota and influenced by site MAT, it is not clear whether this specialization is adaptive. In other systems, co-control of adaptive traits by plants and soil microbes may underlie fitness in drought conditions (Lau & Lennon,

2012), and rhizosphere biota have been regularly shown to alter fitness-affecting traits such as flowering time, herbivore defense, pathogen resistance, and morphology (Friesen *et al.*, 2011; Wagner *et al.*, 2014; Tack *et al.*, 2015)

We found that while warm-sourced biota and plants lacked local co-adaptation, warm-sourced biota benefited all plant populations equally well, and benefited plants significantly more than cold-sourced biota. Several mechanisms could explain this result. For example, we might expect that different soil nutrient status across sites could affect the relative benefit of biota. Nutrients are commonly considered to be the factor underlying microbial evolution in interactions between plants and root microbes (Johnson, 1993; Schwartz & Hoeksema, 1998; Kiers & van der Heijden, 2006; Bever, 2015). However, several lines of evidence suggest that if nutrients play a role in shaping the mutualistic nature of biota in our system, their availability is temperature-dependent. First, for all traits and biomass measured, MAT was a better predictor than soil nutrients in explaining effects biota on plants (Table S4) Second, cold sites were not different in N, P or K concentrations from warm sites (Table S2, Methods), suggesting that differnetial benefits of soil biota are not patterned by soil nutrient concentrations alone. Finally, cold stress in *Zea mays* ssp. *mays*, as well as in other plants, is thought to be primarily realized through water and nutrient stress (Bloom *et al.*, 2004; Farooq *et al.*, 2009). Maize roots in the cold are less permeable to water, nutrient pumps work less efficiently, and root systems fail to grow (Farooq *et al.*, 2009), exacerbating water and nutrient deficits. Moreover, non-tolerant maize grown in the cold, but colonized by mycorrhizal fungi, maintained turgor and photosynthesis and had increased tolerance to cold (Zhu *et al.*, 2009), suggesting a role for soil biota in ameliorating this stress.

Alternately, benefits provided by cold-sourced biota could be so strongly specialized on local hosts that other hosts cannot benefit. Further, cold-sourced biota may provide benefits specialized for cold environments, such as increased stem hair expression, and may perform poorly in the greenhouse, where our average growing temperatures and precipitation were most closely matched to average temperatures at the warmest field sites (Table S1, see Meth-

ods). Recent work observes that benefits provided by biota to plants may be contingent on experimental conditions matching the environment to which the biota are adapted (Johnson *et al.*, 2010; Lau & Lennon, 2012), but see (Kardol *et al.*, 2014). Consistent with this, when we grew the maize line B73 in the same set of biota sources, it had equivalent growth across biota source MAT, nutrients, or precipitation, at a time when night temperatures in the greenhouse were 1.5 °C cooler (Table S5). In sum, the majority of evidence supports these explanations, that temperature mediates evolution of specific benefit of biota to local hosts or in local environments. This specificty of benefits is consistent with increased co-adaptation and SGE, and has some practical applications. For example, allopatric host-panels are commonly used to detect variation in mutualistic capacity (as in Weese *et al.*, 2015), but if specificity of biota benefit varies by environment, these panels will miss strongly mutualistic partners that offer benefits only to local hosts.

Our results contribute to a growing body of literature indicating the importance of biotic interactions in setting limits of species distributions in general (e.g. HilleRisLambers *et al.*, 2013; Afkhami *et al.*, 2014), and in setting cold, montane distribution limits in particular (e.g. Brown & Vellend, 2014). Cold, high elevation, high latitude range limits are often thought to be primarily set by physiological tolerances, and not affected by biotic interactions, whereas warmer, lower limits to ranges are considered to be more strongly impacted by biotic interactions, such as diseases (Brown *et al.*, 1996; Hargreaves *et al.*, 2014). It is becoming increasingly clear that biotic interactions are relevant at physiologically stressful limits to species distributions, as interactions alter these stressful environments to ameliorate or exacerbate conditions. Numerous studies have focused on single species processes that limit ranges, such as such as source-sink dynamics or maladaptive gene flow (see Sexton *et al.*, 2009, for review); our results support calls for focusing theory and research on multi-species dynamics (Sexton *et al.*, 2009; van der Putten *et al.*, 2010). Here, we have found that fitness and climate adaptive traits are increased in sympatric pairings of high elevation, cold-site teosinte and rhizosphere biota. Other emerging results support mutualism-dependent range

limits for plants and soil biota: plants interacting with ectomycorrhizae have shown greater southern range contractions than plants associated with endomycorrhizae (Lankau *et al.*, 2015) and soil mutualists are facilitating pine invasion of novel habitat (Hayward *et al.*, 2015).

As assisted translocation is being considered as a strategy to mitigate for climate change, our results and SGE predict that co-locating sympatric heterospecifics sourced from abiotically stressful sites to similarly stressful environments might increase the likelihood of establishment of these populations compared to solo translocations, while translocations into mild sites may have less need for such considerations.

Acknowledgements

We would like to thanks Dolores Piperno for providing the data from Piperno *et al.* (2015). The project was funded by UC MEXUS, the UC Davis Center for Population Biology grants to AO, and NSF grant IOS-0922703. AO was supported by the NSF GRFP grant DGE-1148897. We would like to thank Jaime Gasca Pineda & Luis Eguiarte and the entire Eguiarte laboratory for help with collections in the field, Carlos Fabián de la Cruz, Abenamar Gordillo Hidalgo, Dario Alvarez & Arturo Chavez for greenhouse help, and Johanna Schmitt for helpful comments on the mansuscript.

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