Running head: Greater co-adaptation in stressful sites

Extending the Stress-Gradient hypothesis: greater adaptation between teosinte and soil biota at higher stress sites

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Abstract

The stress-gradient hypothesis (SGH) predicts that some species interactions grade from being more mutualistic under stressful abiotic conditions to more antagonistic or neutral under benign conditions. We extend these predictions in our Stress-Gradient-Adaptation hypothesis (SGA), positing that as interactions become more mutualistic in stressful environments, fitness benefits of interactors become more aligned and selection should favor greater mutualistic co-adaptation between interacting species. We test our SGA hypothesis using the interaction between teosinte (*Zea mays* ssp. *mexicana*) and its rhizosphere soil biota across a climate gradient. In support of predictions stemming from the SGA, we find local adaptation between teosinte and rhizosphere biota at the stressful (cold) end of our climatic gradient but not at the benign (warm) end. Sympatric combinations of plants and biota from cold (stressful) sites both increase plant fitness and generate more locally adapted plant phenotypes. Counterintuitively, warmer-sourced biota provide greater benefit than colder-sourced biota, even for colder-sourced plant populations, a result we argue may be due to the environmental context of our experiment. Overall, our experiment finds some support for our SGA hypothesis and suggests that co-adaptation of interacting partners may be a means of ameliorating stressful environments.

Keywords: stress-gradient, biotic interactions, climate adaptation, rhizosphere, mutualisms, local adaptation

Introduction

The nature of biotic interactions is often shaped by the abiotic conditions in which they occur. For example, normal coral-zooxanthellae symbioses are disrupted by warming temperatures, leading corals to expel symbionts (Hoegh-Guldberg, 1999), increasing fire frequency and severity leads to the increasing success of invasive grasses in competitive interactions (D'Antonio and Vitousek, 1992), and pepper moth visibility to predators is altered by soot prevalence (Kettlewell, 1955).

The Stress-Gradient hypothesis (SGH, Bertness and Callaway, 1994) predicts that some biotic interactions will shift from having neutral or negative outcomes under benign abiotic conditions to having mutually beneficial outcomes under stressful conditions. In plants, studies testing the SGH have found consistent shifts towards facilitation or reduced competition in plant-plant interactions at high stress (He et al., 2013). Evidence from work on detritivore-detritivore (Fugère et al., 2012; Dangles et al., 2013) and plant-herbivore (Daleo and Iribarne, 2009) systems also suggests these interactions become increasingly facilitative or decreasingly costly at high abiotic stress. While not usually framed in terms of the SGH, interactions between plants and microbes also provide firm support for SGH, as, for example mycorrhizal fungi often provide net benefits to plants in low nutrient (stressful) conditions, but net costs in high nutrient conditions (Smith et al., 2010).

We extend this ecological hypothesis to include effects on co-adaptation between mutualists across abiotic gradients of stress. If high stress environments limit the fitness of both partners in a stress-ameliorating biotic interaction, then a mutation in one organism that ameliorates the stress of its partner will result in positive fitness feedbacks (Sachs et al., 2004) and be favored by selection (Fig 1). Such positive feedbacks could occur via mutations that affect the efficiency of resource exchange (Connor, 1995) or when mutations that increase investment in partners result in increased reciprocation (Sachs et al., 2004; Kiers and van der Heijden, 2006; Archetti et al., 2011). As mutations occur and are selected, reciprocal stress-ameliorating mutations will be fixed in partners in high stress environments, resulting

in mutualistic co-adaptation. In contrast, where abiotic stress does not limit fitness, interactions between species may become neutral or shift towards antagonism as predicted by the SGH (Bertness and Callaway, 1994). Antagonism may result in selection to reduce interactions (e.g. character displacement, Thorpe et al., 2011), or in asynchronous or oscillating coevolutionary dynamics that may be difficult to detect such as arms-race or Red Queen scenarios(e.g. Van Valen, 1974; Gandon and Michalakis, 2002). Finally, both theoretical and empirical work (e.g. Parker, 1999; Kawecki and Ebert, 2004) suggest that as the strength of selection on beneficial interactions increases, mutations favoring interaction with local partners are more likely to fix, resulting in geographic variation in interactions (e.g. Thompson, 2005) where mutualistic co-adaptation between interactors increases as one moves from less stressful to more stressful environments (2). This extension of the Stress-Gradient hypothesis, which we call the Stress-Gradient-Adaptation (SGA) hypothesis, thus predicts both adaptation towards increasing mutualism and greater local co-adaptation in partners from stressful sites.

Here, we test predictions of SGA in an investigation of co-adaptation between the wild grass teosinte and its root-associated biota along a climatic gradient. The diverse community of bacteria, nematodes and fungi that live in and near plant roots are collectively known as rhizosphere biota, (Hiltner, 1904; Bais et al., 2006; Raaijmakers et al., 2009; Lundberg et al., 2012; Toju et al., 2014). Interactions between plants and their rhizosphere biota may involve direct exchange of benefits, such as plant sugars for nitrogen in nodule associations, or may be more indirect, such as nematode-dependent nutrient cycling (e.g Ingham et al., 1985; Bais et al., 2006). The outcomes of plant-rhizosphere interactions are highly variable and may range from plant death to increased plant growth or changes in relative species abundance in rhizosphere biota. Such variability can be caused by environmental conditions, composition of rhizosphere communities, or plant genotype (e.g. Klironomos, 2002; Berg and Smalla, 2009; Zhu et al., 2009; Smith and Read, 2010; Lau and Lennon, 2012; Bouffaud et al., 2014; Lundberg et al., 2012; Burns et al., 2015). The conditionality of these interactions suggests

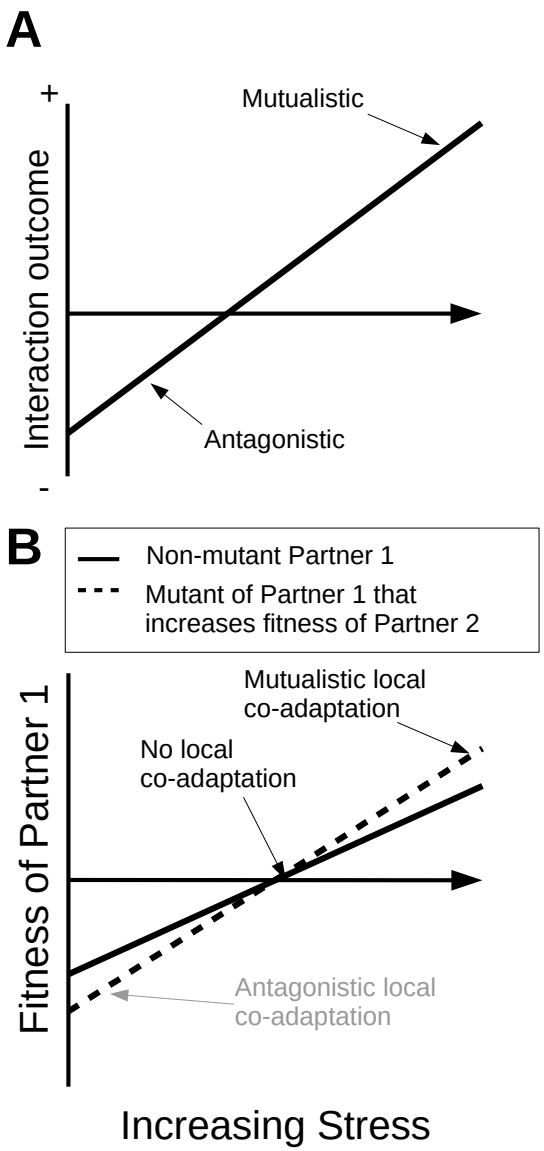

Figure 1: The stress gradient hypothesis (A) predicts ecological stress-ameliorating interactions will be mutualistic at high stress. The SGA hypothesis (B) further suggests increasing mutualistic local co-adaptation at high stress sites.

that SGA could predict plant-rhizosphere interactions across environmental gradients.

Teosinte (*Zea mays* ssp. *mexicana*) is an annual grass found in central Mexico (Sánchez and Corral, 1997) and a wild relative of domesticated maize (*Zea mays* ssp. *mays*). Both genetic (van Heerwaarden et al., 2011) and phenotypic (Hufford et al., 2013) variation in maize has been shaped by adaptation along elevational gradients. Cold stress inhibits water and nutrient uptake in maize (Bloom et al., 2004; Farooq et al., 2009) and is likely a driving factor in adaptation to highland conditions (Duncan and Hesketh, 1968; Mercer et al., 2008; Skarbø and VanderMolen, 2015). Elevational and temperature gradients have also played an important role in adaptation in teosinte (Bradburd et al., 2013; Pyhäjärvi et al., 2013), and phenotypic differences between high and low elevation populations are thought to be adaptations to cold (Doebley, 1984; Lauter, 2004).

Because cold stress in maize can be alleviated by interactions with rhizosphere biota (Zhu et al., 2009), our SGA hypothesis predicts adaptive shifts towards greater mutualism and co-adaptation with sympatric (local) teosinte in rhizosphere biota from colder, more stressful sites (Figure 2). To test these predictions, we collected ten populations of teosinte along a climatic gradient characterized by variation in mean annual temperature and inoculated them with rhizosphere biota sourced from sympatric and allopatric populations along the gradient.

Methods

Field Collections

We collected teosinte and rhizosphere materials from 10 populations along a cline spanning 6.6°C in mean annual temperature (MAT) and more than 1100 meters in elevation (Table S1), representing the full elevational range of teosinte. 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). At high elevation, temperatures become cooler,

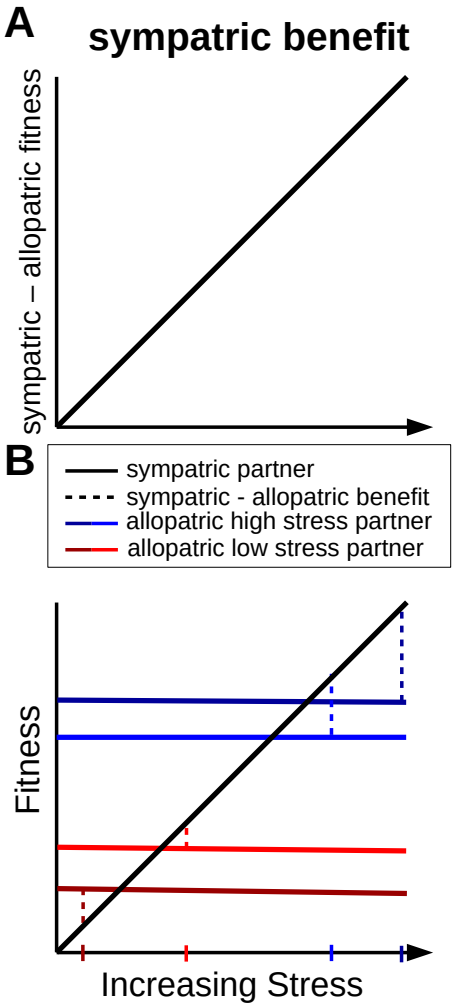

Figure 2: The SGA hypothesis predicts (A) that the expected benefit of sympatry increases with environmental stress and (B) that the fitness of hosts (y-axis) differs with sympatric (solid black line) and allopatric (solid colored lines) partners depending on stressfulness of host (x-axis) and partner (line color) source sites. Dashed lines show the effect of sympatry over allopatry when partners are sourced from similarly stressful sites. For comparison, tick marks on the x-axis match the stress level of allopatric partner source sites to stress level of host source site.

and precipitation also decreases, especially in the growing season (Table S1), whereas at low elevation, conditions are more benign (warmer and wetter). In August 2013, 2 kg of teosinte rhizosphere soil were 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 Nutrición Vegetal. In December 2013, when seeds were ripe in the field, seeds were collected from 12 different mother plants per population, chosen to both span the spatial extent of the population and have sufficient seed quantity. These sets of sibling 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. Approximately 6 liters (4-7 L) of roots and attached soil were collected from each site. Plants were unearthed and roots lightly shaken to remove non-adhering soil, and then roots and adhering soil were placed in bags, dried at ambient temperature, and stored at 4°C . To make biota inoculum treatments, samples from each population were homogenized in a blender until root pieces were approximately ≤ 2 cm in length and well mixed with the soil. These were then added to pots (see below) into which seeds from teosinte populations were planted.

Experiment

In May of 2014, seeds from each teosinte population were grown in each of six inoculum treatments: no inoculum, sympatric inoculum (collected from same site), and inocula from four sites spanning the climatic range (see Figure S1). Each teosinte population thus experienced biota originating from both its home site and biota originating from both warm allopatric (collected from a different site than the teosinte) and cold allopatric sites.

Biota inocula were applied to sibling seeds from 12 mothers from each of the 10 teosinte populations along the elevation/climate gradient (120 mother plants $\times$ 6 treatments = 720 plants). Plants were grown in 2 L plastic grow bag pots with four drainage holes. 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 filled with sterile potting mix, inoculated with 50 mL of a 4:1 mix of sterile sand and homogenized inocula just below where seeds were to be placed, and topped off with sterile mix, resulting in a small live layer of inocula sandwiched between sterilized soils in each pot. In the no inoculum treatment, sterilized sand replaced homogenized inocula. As only 0.5% of the pot volume is inocula, we expect any non-biotic effects of inocula to be minimal relative to biotic effects. Three seeds from the same maternal plant family were added to pre-watered pots after scarification with overnight soaking, and pots were weeded after germination if more than one seed germinated. The planting design on the bench was randomized with respect to seed source and biota inoculum source. Plants grew in a temperature- and humidity- controlled greenhouse in Irapuato, Gto, Mexico with an average temperature of 23.8°C over the course of the experiment (mean annual temperature at field sites varied from 12.9°C to 19.8°C see Table S1). 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 benefits

After 52 days of growth whole plants were harvested, washed of adhering soil, dried (at approximately 45 °C until mass stabilized), and weighed to measure pre-reproduction vegetative biomass. In the related subspecies *Zea mays* ssp. *parviglumis*, vegetative biomass is significantly correlated with seed mass and number (both pMCMC < 0.001, data from Piperno et al., 2015, see Figure S2), and thus biomass is a reasonable fitness surrogate in this annual species. We also quantified the expression of stem macrohairs, a trait relevant to fitness in the field that also shows an elevational cline in teosinte (Lauter, 2004). Stem macrohairs are known to have different adaptive values across environments in maize, and are advantageous only in cold environments (Hufford et al., 2013; Kaur et al., 1985). 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 at harvest.

Data Analysis

We used linear models of plant biomass and stem macrohairs as separate response variables with continuous predictor variables to test our SGA hypothesis. Our main predictions are that colder-sourced plant populations should be more locally adapted with their rhizosphere biota than warmer-sourced populations and that colder-sourced biota are better mutualists than warmer-sourced biota (Figure 2).

In classic tests of local adaptation, populations and sites are treated as discrete entities (Kawecki and Ebert, 2004; Blanquart et al., 2013); however, incorporating degree of local adaptation along a climate gradient requires a continuous statistical approach. We model benefit in a linear framework. We include 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 (a MAT $\times$ sympatry interaction denoted as $T_S \times S$) to test the prediction that colder-sourced plant populations are more locally adapted with their rhizosphere communities than are warmer-sourced plant populations. A term for inoculum source temperature (MAT of inoculum source population, T_I) is included as a test of the prediction that colder-sourced biota might be more generally mutualistic than warmer-sourced biota. The MAT of the plant population source, T_P, is included to account for main effects of plant MAT when testing for a sympatry effect. MAT main effects allow us to separate effects of plant or biota sources having higher mean plant fitness without being locally adapted (see Blanquart et al., 2013). Following Sawers et al. (2009), we also included in all analyses a random effect of plant family ($F \sim N(0, \sigma)$) and the biomass of an uninoculated sibling for each inoculated family (Z) as a covariate. Briefly, the family effect takes into account differences across families in the inoculated state; the uninoculated sibling effect can be interpreted as a measure of

overall responsiveness to inoculation across all inocula and plant sources. This method is analogous to using response ratios of inoculated plants relative to uninoculated siblings as the dependent variable (Sawers et al., 2009). Including an error term ε, the response variable Y can thus be modeled using the following equation:

$$Y \sim \alpha + \beta_{T_P} T_P + \beta_{T_I} T_I + \beta_S S + \beta_Z Z + \beta_{T_S S} T_S S + F + \varepsilon$$

Finally, we took an information criterion approach in which we included all other two- and three-way interaction terms of fixed effects. We used model selection to determine which terms explain variation in our dependent variables with package MCMCglmm (Hadfield, 2010) to fit the models in R (R Core Team, 2014), using a burn-in of 8,400 iterations followed by 80,000 iterations thinned to every 50th iteration. If a term testing a hypothesis was absent from the best model, or opposite in sign from predictions, we concluded that the hypothesis was not supported. We used the gaussian distribution for biomass and a poisson log-link for stem macrohairs. Models were compared with Deviance Information Criterion (DIC), the extension of Akaike's Information Criterion for models fit by MCMC (Spiegelhalter et al., 2002).

Because the conditionality of plant-rhizosphere biota interactions is often influenced by soil fertility, we explored the relationship between fertility and MAT across our sites. These analyses revealed weak correlations between MAT and fertility (Table S2, ρ MAT and: N = -0.26, P = 0.41, K = 0.54). Therefore, we also ran similar models to the one above, substituting concentrations of N, P, and K from source sites of inocula and plants in lieu of MAT variables. In all cases, for both stem macrohairs and biomass, models with MAT had better explanatory power than those with any of the nutrient variables (Table S3), thus we limit our presentation of results to effects of MAT.

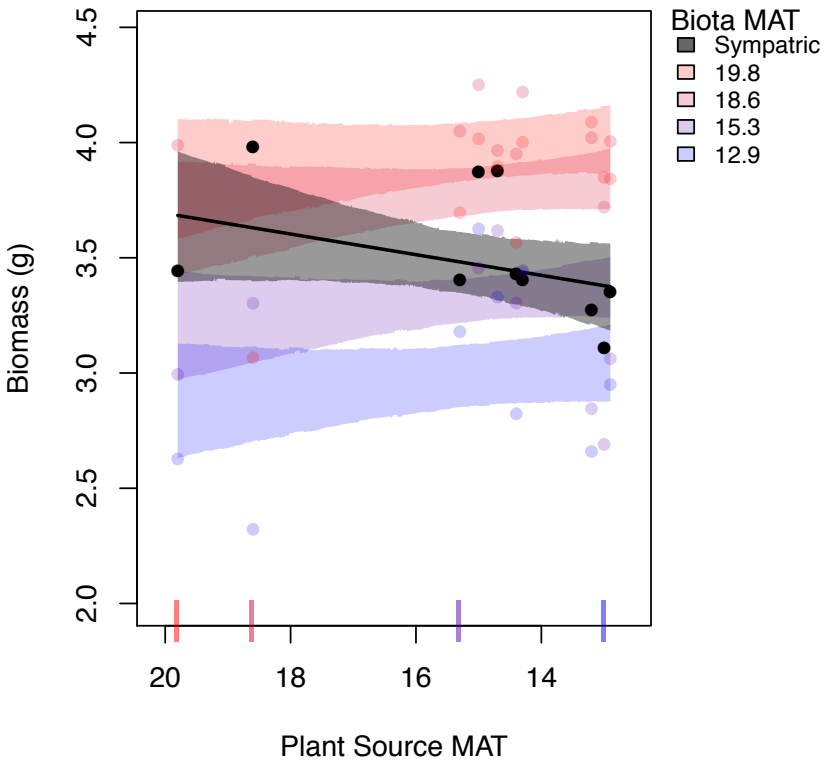

Figure 3: Observed population mean biomasses are shown as points, along with predictions (lines) and 95% highest posterior density intervals (shaded areas) for different treatments from the best fit model for biomass. Sympatric treatments are indicated by black, while allopatric treatments are separated by colors representing the mean annual temperature (MAT) of the inoculum source. Tick marks on the x-axis indicate the plant source MAT where the four main biota treatments are sympatrically paired.

Results

Our SGA hypothesis predicted that colder sources of sympatric teosinte and rhizosphere biota would show a greater degree of local co-adaptation in benefits to plants than warmer-sourced sympatric plants and biota, and that colder sources of biota would provide more benefits on average.

In support of the SGA hypothesis, we found that colder-sourced teosinte received greater benefit from their sympatric biota than warmer-sourced teosinte received from their sym-

patric biota. Our best model for biomass (a good proxy for fitness, see Methods, Piperno et al., 2015) includes both a significant positive sympatry term and a significant negative MAT by sympatry term ($T_S \times S$, Table 1, pMCMC <0.05 and <0.1, respectively). Plant populations from colder sites are predicted to be larger by 10% when grown with local, sympatric biota than when grown with colder-sourced allopatric biota (Figure 3; see Figure S3 for presentation of data that corrects for T_I). In contrast, for plant populations from warmer sites, there was no significant difference in biomass between sympatric and allopatric warmer-sourced biota. The best model explaining biomass attained also included an interaction term between uninoculated sibling size and biota source MAT ($T_I \times Z$), indicating that teosinte families are more similarly sized when inoculated with warmer-sourced biota and that teosinte families with higher biomass have smaller differences across biota sources (see negative $T_I \times Z$ parameter for biomass in Table 1, pMCMC < 0.05, and Figure S4).

Plant macrohairs, thought to be adaptive in colder environments (Lauter, 2004; Hufford et al., 2013; Kaur et al., 1985), also showed evidence of greater local adaptation between colder-sourced plants and their biota. Plants from cold environments matched with sympatric biota were more likely to have macrohairs, and model predictions revealed a weak tendency towards greater density of macrohairs (Figure 4, positive sympatry and negative $T_P \times S$ terms in Table 1, pMCMC both <0.05). The best models selected by DIC also included interaction terms between macrohair abundance in uninoculated siblings, source MAT, and sympatry ($T_P \times Z$, $S \times Z$, and $T_S \times S \times Z$ in Table 1, all pMCMC <0.1, see Appendix S1 for interpretation).

Finally, while the SGA hypothesis predicts that rhizosphere biota from higher-stress cold sites should be better mutualists, we find that warmer-sourced biota stimulated greater growth across all plant populations (Figure 3, Table 1, pMCMC <0.05). Colder-source biota nonetheless provided benefit compared to uninoculated controls (Table S5).

Name	*parameter*	Biomass	Macrohairs
Intercept	a	-0.26	4.84**
Biota source MAT	T_I	0.23**	0.063
Plant source MAT	T_P	-0.025	-0.43**
Sympatry	S	**1.26****	**7.40****
Source MAT × Sympatry	$T_S \times S$	**-0.071***	**-0.51****
Uninoculated sib	Z	0.69**	0.52**
Biota source MAT × Uninoculated sib	$T_I \times Z$	-0.033**	
Plant source MAT × Uninoculated sib	$T_P \times Z$		-0.031*
Sympatry × Uninoculated sib	$S \times Z$		**-0.64***
Source MAT × Sympatry × Uninoculated sib	$T_S \times S \times Z$		**0.044***

Table 1: Parameter estimates from the best model (see Methods) for biomass and macrohairs. Parameters that test predictions of our SGA hypothesis are shown in bold. **: pMCMC < 0.05,*: pMCMC < 0.1.

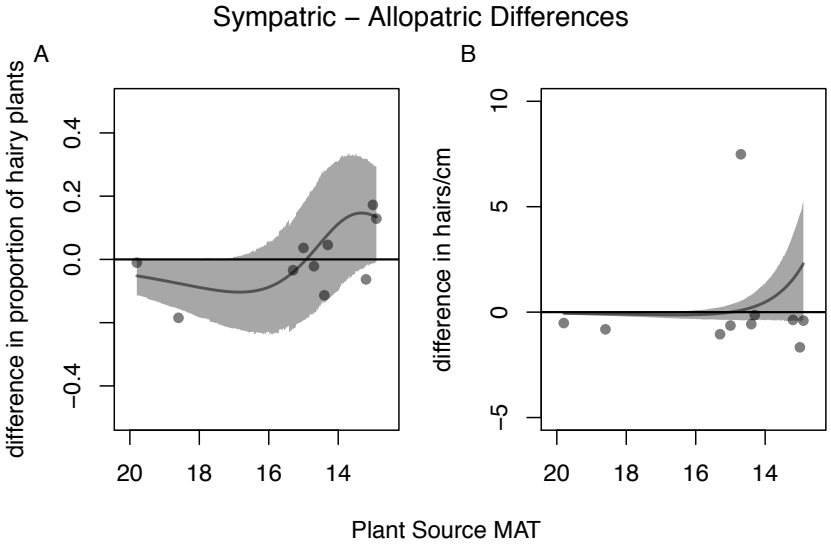

Figure 4: The difference between sympatric and allopatric plants in proportion of plants with hairs (A) and the expected number of hairs per plant (B) is shown for plant populations of different MAT. Points represent observed population average differences in sympatric and allopatric biota, with mean predicted differences (lines) and 95% highest posterior density intervals (shading) from the best fit model also shown. Predictions are for plants with average uninoculated siblings and points are adjusted for sibling effects for consistency, but points uncorrected for sibling effects are not qualitatively different. Note that poisson fitting creates curvilinear lines on a normal scale.

Discussion

Climate regimes have left a strong imprint on patterns of plant local adaptation (Keller et al., 2010; Strasburg et al., 2011), but studies have typically focused on physiological or morphological responses and less on the roles of interactions with other species. The stress-gradient hypothesis (SGH) predicts that species should have more mutually beneficial interaction outcomes when they are growing under abiotic stress, and more neutral or competitive outcomes when growing in benign conditions (Bertness and Callaway, 1994). Predictions of the SGH are supported by a number of studies in plants (reviewed in He et al., 2013) and animals (e.g. Daleo and Iribarne, 2009; Dangles et al., 2013), and by both theory (Johnson, 1993) and empirical results (Smith et al., 2010) in plant-microbe interactions along soil nutrient gradients.

We present here an extension of the SGH, which we term the stress-gradient adaptation (SGA) hypothesis. SGA posits that selection in stressful sites will act to increase the frequency of alleles that increase the functionality of a stress-ameliorating mutualism. SGA predicts that organisms in high stress environments will be adapted to interact with local biota and that such organisms should be better mutualists. SGA is related to, but different from, other frameworks for predicting adaptation in species interactions. In contrast to models of co-adaptation that are predicated on levels of environmental productivity and biological diversity (Thrall et al., 2007), SGA predicts selection for increasingly mutualistic phenotypes and increasing local co-adaptation at stressful sites without regard to diversity of partners. For plant-rhizosphere interactions involving nutrient exchange, SGA and economic models (where resources are preferentially allocated to better partners, increasing their fitness) both predict that selection on microbes in low soil nutrient environments should favor increased benefits provided to plants (Johnson, 1993; Schwartz and Hoeksema, 1998; Werner et al., 2014; Bever, 2015), but SGA differs in its focus on adaptation patterns in both partners, its inclusion of stresses beyond soil resources, and its applicability to a wide variety of conditional interactions.

We tested predictions of our SGA hypothesis using populations of teosinte and its associated rhizosphere biota occurring along a gradient of mean annual temperature and correlated gradients of elevation, seasonality, and precipitation. Consistent with the key expectation of SGA, we find greater local adaptation between colder-sourced biota and teosinte. Colder-sourced teosinte benefited more from sympatric colder-sourced biota than allopatric colder-sourced biota, while warmer-sourced plant populations did equally well with both sympatric and allopatric warmer-sourced biota.

In addition to finding support that teosinte from colder, high-stress environments exhibit higher fitness in interactions with sympatric biota, we show that sympatric rhizosphere biota increased the expression of stem macrohairs only in plants from cold environments. Stem macrohairs are likely adaptive in cold environments, as macrohair abundance follows an elevational cline in populations of teosinte (Hufford et al., 2013) and has been associated with increased maize fitness in cold environments (Kaur et al., 1985). In other systems, co-control of adaptive traits by plants and soil microbes has also been shown to underlie fitness in stress conditions (e.g. drought Lau and Lennon, 2012), and rhizosphere biota are known 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).

In contrast to our prediction of more mutualistic partners from colder places, however, we found that warmer-sourced biota benefited plants significantly more than colder-sourced biota. It seems unlikely that we have misidentified the primary environmental stress in these populations, as both genetic (Pyhäjärvi et al., 2013; Bradburd et al., 2013) and phenotypic (Lauter, 2004) data suggest elevation and cold are the primary drivers of environmental adaptation in teosinte. While limiting soil nutrients, for example, have frequently been identified as the driving stress in the evolution of interactions with soil rhizosphere microbes (Johnson, 1993; Schwartz and Hoeksema, 1998; Kiers and van der Heijden, 2006; Bever, 2015), we find no differences in soil nutrient availability between warm and cold sites (Table S2, Methods) and source MAT was a better predictor of plant benefit in our experiment

(Table S3). A more plausible explanation for the observed benefit of warmer-sourced biota may instead be experimental: mean greenhouse temperatures were closer to MAT of our warmest sites (Table S1, see Methods), and some benefits of cold biota (such as macrohairs) may be conditional on cold environments, or colder-sourced biota may simply grow poorly in a warm greenhouse. Consistent with this idea, all biota sources provided the maize inbred line B73 with equivalent (allopatric) benefits (Table S4 when grown in the same greenhouse at slightly cooler temperatures, and other studies suggest that benefits provided by biota to plants are contingent on experimental conditions matching the environment to which the biota are adapted (Johnson et al., 2010; Lau and Lennon, 2012), but see (Kardol et al., 2014).

Tests of co-adaptation and environmental gradients are still rare for plant-rhizosphere interactions (Hoeksema, 2010), but limited results to date tend to also support our SGA hypothesis. Variable degrees of local co-adaptation were found for a grass and associated arbuscular mycorrhizal fungi across a nutrient gradient, and the combinations with greater local benefit came from sites where interactions with mutualists would ameliorate the primary plant nutrient stress (Johnson et al., 2010). Variable effects of local rhizobia are also found in acacia (Barrett et al., 2012), though the authors did not present any test of sympatric effects across environment of origin. And while in some systems, selection appears to have modified the mutualistic benefits provided by microbes to hosts (and vice versa) in accordance with predictions of SGA (Weese et al., 2015; Johnson et al., 2010), in others authors have found no evidence of local adaptation with rhizosphere biota across putatively stressful environmental gradients (Kardol et al., 2014).

Our findings of variable local adaptation, though focusing only on benefits to a single partner, have implications for experimental design in mutualism research. In attempts to quantify partner quality, studies frequently compare partners on hosts that are allopatric for all partners (as in Weese et al., 2015). However, if benefits between hosts and biota vary by environment, these treatments may miss strongly mutualistic partners that offer

benefits only to local hosts. While we cannot determine here whether sympatric benefits are derived from plants or provided by biota, it is likely that both contribute, suggesting that fully allopatric panels are inadequate for testing either hosts or symbionts.

Finally, our results contribute to a growing body of literature highlighting the importance of biotic interactions in setting limits of species distributions in general (e.g. HilleRisLambers et al., 2013; Afkhami et al., 2014), even in cold environments (e.g. Brown and Vellend, 2014) where physiology has often been thought to be of greater importance (Brown et al., 1996; Hargreaves et al., 2014). Emerging evidence supports mutualism-dependent range limits for plants and rhizosphere 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). Numerous studies have focused on single species processes that limit ranges, such as source-sink dynamics or maladaptive gene flow (see Sexton et al., 2009, for review), but our results support calls for focusing theory and research on multi-species dynamics (Sexton et al., 2009; van der Putten et al., 2010). As climatic conditions become more extreme under global change, we predict that biotic interactions may be important components of adaptation to such abiotic stress.

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