

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

1 Introduction

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

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

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

28 in mutualistic co-adaptation. In contrast, where abiotic stress does not limit fitness, interac-
29 tions between species may become neutral or shift towards antagonism as predicted by the
30 SGH (Bertness and Callaway, 1994). Antagonism may result in selection to reduce interac-
31 tions (e.g. character displacement, Thorpe et al., 2011), or in asynchronous or oscillating
32 coevolutionary dynamics that may be difficult to detect such as arms-race or Red Queen
33 scenarios (e.g. Van Valen, 1974; Gandon and Michalakis, 2002). Finally, both theoretical and
34 empirical work (e.g. Parker, 1999; Kawecki and Ebert, 2004) suggest that as the strength of
35 selection on beneficial interactions increases, mutations favoring interaction with local part-
36 ners are more likely to fix, resulting in geographic variation in interactions (e.g. Thompson,
37 2005) where mutualistic co-adaptation between interactors increases as one moves from less
38 stressful to more stressful environments (2). This extension of the Stress-Gradient hypoth-
39 esis, which we call the Stress-Gradient-Adaptation (SGA) hypothesis, thus predicts both
40 adaptation towards increasing mutualism and greater local co-adaptation in partners from
41 stressful sites.

42 Here, we test predictions of SGA in an investigation of co-adaptation between the wild
43 grass teosinte and its root-associated biota along a climatic gradient. The diverse community
44 of bacteria, nematodes and fungi that live in and near plant roots are collectively known as
45 rhizosphere biota, (Hiltner, 1904; Bais et al., 2006; Raaijmakers et al., 2009; Lundberg et al.,
46 2012; Toju et al., 2014). Interactions between plants and their rhizosphere biota may involve
47 direct exchange of benefits, such as plant sugars for nitrogen in nodule associations, or may
48 be more indirect, such as nematode-dependent nutrient cycling (e.g Ingham et al., 1985; Bais
49 et al., 2006). The outcomes of plant-rhizosphere interactions are highly variable and may
50 range from plant death to increased plant growth or changes in relative species abundance in
51 rhizosphere biota. Such variability can be caused by environmental conditions, composition
52 of rhizosphere communities, or plant genotype (e.g. Klironomos, 2002; Berg and Smalla,
53 2009; Zhu et al., 2009; Smith and Read, 2010; Lau and Lennon, 2012; Bouffaud et al., 2014;
54 Lundberg et al., 2012; Burns et al., 2015). The conditionality of these interactions suggests

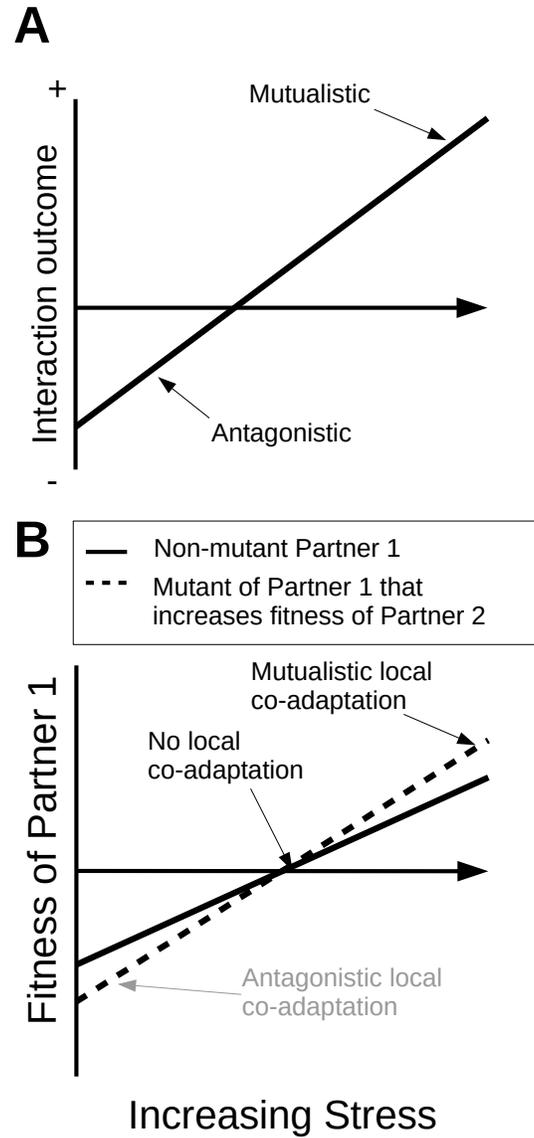


Figure 1: The stress gradient hypothesis (A) predicts ecological stress-ameliorating interactions will be mutualistic at high stress. The SGA hypothesis (B) further suggests increasing mutualistic local co-adaptation at high stress sites.

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

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

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

73 **Methods**

74 **Field Collections**

75 We collected teosinte and rhizosphere materials from 10 populations along a cline spanning
76 6.6°C in mean annual temperature (MAT) and more than 1100 meters in elevation (Table
77 S1), representing the full elevational range of teosinte. Climatic information from each site
78 was obtained from Bioclim (Hijmans et al., 2005) and extracted using the package raster
79 (Hijmans, 2015) in R (R Core Team, 2014). At high elevation, temperatures become cooler,

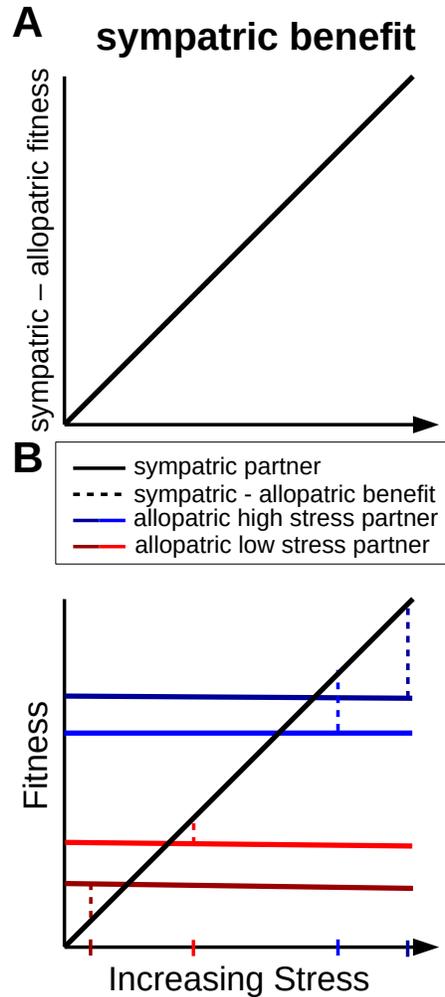


Figure 2: The SGA hypothesis predicts (A) that the expected benefit of sympatry increases with environmental stress and (B) that the fitness of hosts (y-axis) differs with sympatric (solid black line) and allopatric (solid colored lines) partners depending on stressfulness of host (x-axis) and partner (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.

80 and precipitation also decreases, especially in the growing season (Table S1), whereas at
81 low elevation, conditions are more benign (warmer and wetter). In August 2013, 2 kg of
82 teosinte rhizosphere soil were collected from each population (multiple individuals pooled),
83 kept refrigerated at 4°C and sent for analysis of texture, nutrients, and physical properties
84 at INIFAP, Laboratorio Nacional de Fertilidad de Suelos y Nutrición Vegetal. In December
85 2013, when seeds were ripe in the field, seeds were collected from 12 different mother plants
86 per population, chosen to both span the spatial extent of the population and have sufficient
87 seed quantity. These sets of sibling seeds were stored at 4°C until use. At the same time,
88 rhizosphere biota were collected from additional plants selected to span the whole population.
89 Approximately 6 liters (4-7 L) of roots and attached soil were collected from each site. Plants
90 were unearthed and roots lightly shaken to remove non-adhering soil, and then roots and
91 adhering soil were placed in bags, dried at ambient temperature, and stored at 4°C. To make
92 biota inoculum treatments, samples from each population were homogenized in a blender
93 until root pieces were approximately ≤ 2 cm in length and well mixed with the soil. These
94 were then added to pots (see below) into which seeds from teosinte populations were planted.

95 **Experiment**

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

101 Biota inocula were applied to sibling seeds from 12 mothers from each of the 10 teosinte
102 populations along the elevation/climate gradient (120 mother plants \times 6 treatments = 720
103 plants). Plants were grown in 2 L plastic grow bag pots with four drainage holes. Pots were
104 filled to 1.5 L with sterilized custom potting mix (90% sand, 5% perlite 5% vermiculite 0.2%
105 silt). This mix was steam sterilized for 4 hours at 90°C using a PRO-GROW SS60. Pots

106 were then filled with sterile potting mix, inoculated with 50 mL of a 4:1 mix of sterile sand
107 and homogenized inocula just below where seeds were to be placed, and topped off with
108 sterile mix, resulting in a small live layer of inocula sandwiched between sterilized soils in
109 each pot. In the no inoculum treatment, sterilized sand replaced homogenized inocula. As
110 only 0.5% of the pot volume is inocula, we expect any non-biotic effects of inocula to be
111 minimal relative to biotic effects. Three seeds from the same maternal plant family were
112 added to pre-watered pots after scarification with overnight soaking, and pots were weeded
113 after germination if more than one seed germinated. The planting design on the bench
114 was randomized with respect to seed source and biota inoculum source. Plants grew in a
115 temperature- and humidity- controlled greenhouse in Irapuato, Gto, Mexico with an average
116 temperature of 23.8°C over the course of the experiment (mean annual temperature at field
117 sites varied from 12.9°C to 19.8°C see Table S1). Plants were unfertilized and kept moist for
118 the first two weeks as most plants germinated, after which pots were watered and fertilized
119 once per week with 50 mL of Hoagland's solution at low phosphorous (100 μ M). Plants were
120 treated one time with a dual application of Agri-mycin and Knack to prevent caterpillar and
121 spider mite herbivory.

122 **Quantification of benefits**

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

132 stem macrohairs were quantified as the number of hairs in 1 cm² below the ligule on the
133 edge of the lowest live leaf sheath at harvest.

134 **Data Analysis**

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

140 In classic tests of local adaptation, populations and sites are treated as discrete entities
141 (Kawecki and Ebert, 2004; Blanquart et al., 2013); however, incorporating degree of local
142 adaptation along a climate gradient requires a continuous statistical approach. We model
143 benefit in a linear framework. We include a binary term (S) indicating whether origin
144 of the rhizosphere biota and the plant population were the same (sympatric, $S = 1$) or
145 mismatched (allopatric, $S = 0$) and an interaction of sympatry and MAT of the sympatric
146 pair (a MAT \times sympatry interaction denoted as $T_S \times S$) to test the prediction that colder-
147 sourced plant populations are more locally adapted with their rhizosphere communities than
148 are warmer-sourced plant populations. A term for inoculum source temperature (MAT of
149 inoculum source population, T_I) is included as a test of the prediction that colder-sourced
150 biota might be more generally mutualistic than warmer-sourced biota. The MAT of the plant
151 population source, T_P , is included to account for main effects of plant MAT when testing for
152 a sympatry effect. MAT main effects allow us to separate effects of plant or biota sources
153 having higher mean plant fitness without being locally adapted (see Blanquart et al., 2013).
154 Following Sawers et al. (2009), we also included in all analyses a random effect of plant
155 family ($F \sim N(0, \sigma)$) and the biomass of an uninoculated sibling for each inoculated family
156 (Z) as a covariate. Briefly, the family effect takes into account differences across families
157 in the inoculated state; the uninoculated sibling effect can be interpreted as a measure of

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

$$Y \sim \alpha + \beta_{TP}TP + \beta_{TI}TI + \beta_S S + \beta_Z Z + \beta_{TS}TS + F + \varepsilon$$

162 Finally, we took an information criterion approach in which we included all other two- and
163 three-way interaction terms of fixed effects. We used model selection to determine which
164 terms explain variation in our dependent variables with package MCMCglmm (Hadfield,
165 2010) to fit the models in R (R Core Team, 2014), using a burn-in of 8,400 iterations followed
166 by 80,000 iterations thinned to every 50th iteration. If a term testing a hypothesis was absent
167 from the best model, or opposite in sign from predictions, we concluded that the hypothesis
168 was not supported. We used the gaussian distribution for biomass and a poisson log-link for
169 stem macrohairs. Models were compared with Deviance Information Criterion (DIC), the
170 extension of Akaike's Information Criterion for models fit by MCMC (Spiegelhalter et al.,
171 2002).

172 Because the conditionality of plant-rhizosphere biota interactions is often influenced by
173 soil fertility, we explored the relationship between fertility and MAT across our sites. These
174 analyses revealed weak correlations between MAT and fertility (Table S2, ρ MAT and: N
175 = -0.26, P = 0.41, K = 0.54). Therefore, we also ran similar models to the one above,
176 substituting concentrations of N, P, and K from source sites of inocula and plants in lieu of
177 MAT variables. In all cases, for both stem macrohairs and biomass, models with MAT had
178 better explanatory power than those with any of the nutrient variables (Table S3), thus we
179 limit our presentation of results to effects of MAT.

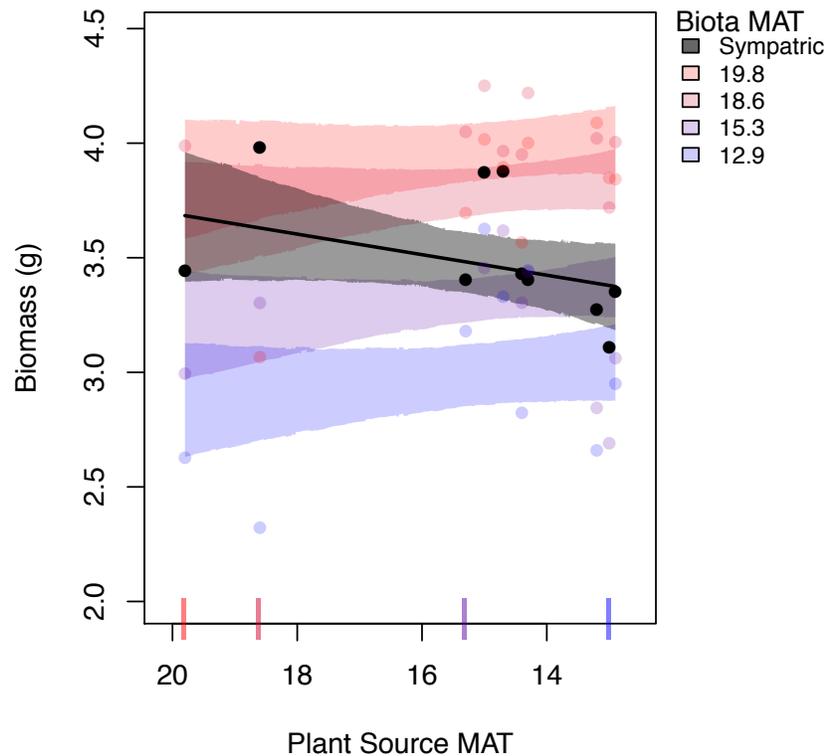


Figure 3: Observed population mean biomasses are shown as points, along with predictions (lines) and 95% highest posterior density intervals (shaded areas) for different treatments from the best fit model for biomass. Sympatric treatments are indicated by black, while allopatric treatments are separated 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.

180 Results

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

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

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

199 Plant macrohairs, thought to be adaptive in colder environments (Lauter, 2004; Hufford
200 et al., 2013; Kaur et al., 1985), also showed evidence of greater local adaptation between
201 colder-sourced plants and their biota. Plants from cold environments matched with sym-
202 patric biota were more likely to have macrohairs, and model predictions revealed a weak
203 tendency towards greater density of macrohairs (Figure 4, positive sympatry and negative
204 $T_P \times S$ terms in Table 1, pMCMC both <0.05). The best models selected by DIC also
205 included interaction terms between macrohair abundance in uninoculated siblings, source
206 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
207 Appendix S1 for interpretation).

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

Name	<i>parameter</i>	Biomass	Macrohairs
Intercept	<i>a</i>	-0.26	4.84**
Biota source MAT	T_I	0.23**	0.063
Plant source MAT	T_P	-0.025	-0.43**
Sympatry	<i>S</i>	1.26**	7.40**
Source MAT × Sympatry	$T_S \times S$	-0.071*	-0.51**
Uninoculated sib	<i>Z</i>	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.

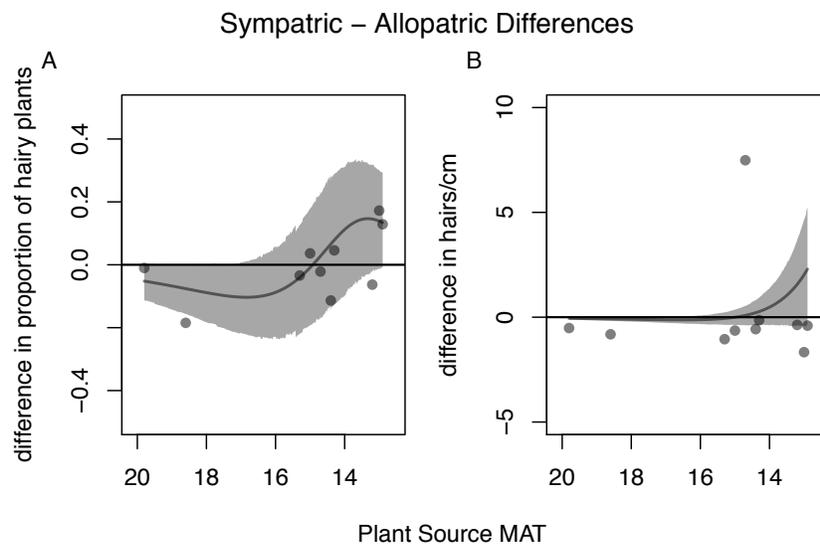


Figure 4: The difference between sympatric and allopatric plants in proportion of plants with hairs (A) and the expected number of hairs per plant (B) is shown for plant populations of different MAT. Points represent observed population average differences in sympatric and allopatric biota, with mean 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.

212 Discussion

213 Climate regimes have left a strong imprint on patterns of plant local adaptation (Keller et al.,
214 2010; Strasburg et al., 2011), but studies have typically focused on physiological or morpho-
215 logical responses and less on the roles of interactions with other species. The stress-gradient
216 hypothesis (SGH) predicts that species should have more mutually beneficial interaction out-
217 comes when they are growing under abiotic stress, and more neutral or competitive outcomes
218 when growing in benign conditions (Bertness and Callaway, 1994). Predictions of the SGH
219 are supported by a number of studies in plants (reviewed in He et al., 2013) and animals
220 (e.g. Daleo and Iribarne, 2009; Dangles et al., 2013), and by both theory (Johnson, 1993)
221 and empirical results (Smith et al., 2010) in plant-microbe interactions along soil nutrient
222 gradients.

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

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

246 In addition to finding support that teosinte from colder, high-stress environments exhibit
247 higher fitness in interactions with sympatric biota, we show that sympatric rhizosphere biota
248 increased the expression of stem macrohairs only in plants from cold environments. Stem
249 macrohairs are likely adaptive in cold environments, as macrohair abundance follows an
250 elevational cline in populations of teosinte (Hufford et al., 2013) and has been associated
251 with increased maize fitness in cold environments (Kaur et al., 1985). In other systems,
252 co-control of adaptive traits by plants and soil microbes has also been shown to underlie
253 fitness in stress conditions (e.g. drought Lau and Lennon, 2012), and rhizosphere biota are
254 known to alter fitness-affecting traits such as flowering time, herbivore defense, pathogen
255 resistance, and morphology (Friesen et al., 2011; Wagner et al., 2014; Tack et al., 2015).

256 In contrast to our prediction of more mutualistic partners from colder places, however,
257 we found that warmer-sourced biota benefited plants significantly more than colder-sourced
258 biota. It seems unlikely that we have misidentified the primary environmental stress in these
259 populations, as both genetic (Pyhäjärvi et al., 2013; Bradburd et al., 2013) and phenotypic
260 (Lauter, 2004) data suggest elevation and cold are the primary drivers of environmental
261 adaptation in teosinte. While limiting soil nutrients, for example, have frequently been
262 identified as the driving stress in the evolution of interactions with soil rhizosphere microbes
263 (Johnson, 1993; Schwartz and Hoeksema, 1998; Kiers and van der Heijden, 2006; Bever,
264 2015), we find no differences in soil nutrient availability between warm and cold sites (Table
265 S2, Methods) and source MAT was a better predictor of plant benefit in our experiment

266 (Table S3). A more plausible explanation for the observed benefit of warmer-sourced biota
267 may instead be experimental: mean greenhouse temperatures were closer to MAT of our
268 warmest sites (Table S1, see Methods), and some benefits of cold biota (such as macrohairs)
269 may be conditional on cold environments, or colder-sourced biota may simply grow poorly
270 in a warm greenhouse. Consistent with this idea, all biota sources provided the maize inbred
271 line B73 with equivalent (allopatric) benefits (Table S4 when grown in the same greenhouse
272 at slightly cooler temperatures, and other studies suggest that benefits provided by biota
273 to plants are contingent on experimental conditions matching the environment to which the
274 biota are adapted (Johnson et al., 2010; Lau and Lennon, 2012), but see (Kardol et al.,
275 2014).

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

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

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

296 Finally, our results contribute to a growing body of literature highlighting the importance
297 of biotic interactions in setting limits of species distributions in general (e.g. HilleRisLambers
298 et al., 2013; Afkhami et al., 2014), even in cold environments (e.g. Brown and Vellend, 2014)
299 where physiology has often been thought to be of greater importance (Brown et al., 1996;
300 Hargreaves et al., 2014). Emerging evidence supports mutualism-dependent range limits for
301 plants and rhizosphere biota: plants interacting with ectomycorrhizae have shown greater
302 southern range contractions than plants associated with endomycorrhizae (Lankau et al.,
303 2015), and soil mutualists are facilitating pine invasion of novel habitat (Hayward et al.,
304 2015). Numerous studies have focused on single species processes that limit ranges, such
305 as source-sink dynamics or maladaptive gene flow (see Sexton et al., 2009, for review), but
306 our results support calls for focusing theory and research on multi-species dynamics (Sexton
307 et al., 2009; van der Putten et al., 2010). As climatic conditions become more extreme
308 under global change, we predict that biotic interactions may be important components of
309 adaptation to such abiotic stress.

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