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Research

Evidence for functional divergence in arbuscular mycorrhizal fungi from contrasting climatic origins

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Summary

- A considerable amount of phenotypic, genetic and symbiotic functional variability has been documented in arbuscular mycorrhizal fungi (AMF). However, little is known about whether distinct AMF ecotypes have evolved within their geographic range. We tested the hypothesis that AMF growing at temperatures closer to those prevalent within their origin would benefit their host and grow more than isolates distant from their native conditions.
- For each of six AMF species, we chose pairs of isolates that originated from distant areas with contrasting climates. Each isolate was grown in association with two grass species of different thermal optima at two temperature settings. Thus, we also tested whether AMF from different climatic origins were dependent on the thermal adaptation of the host plant species or to temperature *per se*.
- Although fungal growth was not directly affected by temperature, we found that AMF isolates originating from contrasting climates consistently and differentially altered plant growth.
- Our results suggest that AMF from contrasting climates have altered symbiotic function, thus linking an abiotic factor to ecotypic differentiation of putatively important symbionts.

Introduction

Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) have co-evolved with terrestrial plants from the Devonian (Remy *et al.*, 1994; Redecker *et al.*, 2000). In return for carbon, AMF may provide benefits to the host plant, such as increased nutrient acquisition (Marschner & Dell, 1994), enhanced water use efficiency (Auge, 2001), pathogen protection (Borowicz, 2001; Wehner *et al.*, 2010) or increased stress tolerance (Feng *et al.*, 2002; Schutzendubel & Polle, 2002). However, AMF symbiotic interactions range from parasitic to mutualistic depending on environmental factors and genotype identities of associating plant and fungal symbionts (Johnson *et al.*, 1997).

Despite the ecological importance of AMF, little is known about the factors that control their fitness and symbiotic functioning, which ultimately determine their evolution (Rosendahl, 2008). AMF are widespread, found in a wide variety of host species and soils, and several AMF species have global distributions (Öpik *et al.*, 2006). The higher AMF richness found in tropical habitats (Öpik *et al.*, 2006) may result from a lack of genetic variation in ecologically important traits, which, in turn, could limit the dispersal ability of tropical species (Kellermann *et al.*, 2009). A recent global data analysis on the small subunit (SSU) rRNA gene suggests that many AMF taxa are restricted to limited geographic ranges (Öpik *et al.*, 2010). It is therefore possible that undescribed 'thermal specialists' with narrow geographic distributions exist within or among species in the Glomeromycota. Currently, only *c.* 220 morphologically distinct species of AMF have been described, although molecular studies suggest that the number of actual species may be significantly higher (Öpik *et al.*, 2009). Furthermore, genetic diversity is high within populations of

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a species (Koch *et al.*, 2004) and even within or among spores of a population (Kuhn *et al.*, 2001; Pawlowska & Taylor, 2004; Hijri & Sanders, 2005). Intraspecific genetic diversity is also expressed as a variation in fungal and host plant growth (Koch *et al.*, 2004; Munkvold *et al.*, 2004; Koch *et al.*, 2006; Ehinger *et al.*, 2009).

Although natural selection within (meta-) communities can result in locally adapted genotypes and populations, the contribution of environmental adaptation (i.e. genotypes imparting greater fitness in their local environment than in nonlocal environments) to such variation is still unclear (Kawecki & Ebert, 2004). Climate is considered to be a major driver of adaptive differentiation for many organisms, including other fungi (Robinson, 2001; López-Gutiérrez *et al.*, 2008) and plants (Reich *et al.*, 1996; Rutter & Fenster, 2007). Specifically, temperature is among the strongest selective pressures for many species (Hochdka, 1984; Bennett *et al.*, 1990), and is an ecological variable that affects AMF (Helgason & Fitter, 2009). Most organisms, including fungi, exhibit optimal performance within a temperature range that appears to correspond with that found in their climates of origin (López-Gutiérrez *et al.*, 2008).

In the light of the high within-species' variability and global distribution of many AMF species, ecotypes probably evolved that were adapted to local temperatures. There is some evidence of phenotypic plasticity within certain AMF species in response to abiotic conditions (Stahl & Christensen, 1991). Ecotypic differentiation of AMF may result from responses to soil metal contamination (Weissenhorn *et al.*, 1993; del Val *et al.*, 1999; Toler *et al.*, 2005), salinity stress (Carvalho *et al.*, 2004) or edaphic nutrient availability (Johnson *et al.*, 2010). Evidence for climatic adaptation is rather limited. For example, Lekberg & Koide (2008) concluded that 'wide environmental tolerances seem to be present' within two AMF populations based on the study of two *Glomus* species. Alternatively, general purpose genotypes could be widespread among AMF, enabling them to spread globally and tolerate a wide range of environments (van Doninck *et al.*, 2002; Rosendahl *et al.*, 2009). The scarce existing research on ecotypic differentiation has tested a limited number of AMF, usually the most commonly found globally (Lekberg & Koide, 2008). Moreover, studies performed to date have not accounted for co-evolved functional effects involving host plant and fungal symbionts, which may be important for symbioses that are not host specific (Ravnskov & Jakobsen, 1995; van der Heijden *et al.*, 2003).

In this study, we compared the growth responses of host species inoculated by individual AMF isolates or assembled communities representing species in different clades in the phylum Glomeromycota from a wide range of provenances (Supporting Information Table S1). We used two isolates from areas with contrasting climates for each of six AMF species, as well as two isolate mixtures, each comprising AMF from the same climatic origin. In particular, we chose isolates from climates that differed markedly in their mean annual temperatures (Table S1). All fungal isolates were grown previously in a common environment for several years, thus avoiding environmental maternal effects. We tested the hypothesis that, under a given set of temperature conditions, isolates growing at temperatures closer to those more prevalent within their geographic origin would perform better (i.e. benefit their host and grow more profusely inside and outside the root system) than isolates growing further away from their native temperature conditions. We further tested whether fungal performance would be enhanced if the plant host was a species adapted to similar temperature conditions as the AMF isolates.

Materials and Methods

The study comprised 360 replicate 'conetainers' divided into two controlled environment (CE) units (Conviron E15, Winnipeg, MN, Canada) set at two contrasting temperature settings, which were continuously monitored (data not shown). Because we did not have replicate CE units for each temperature setting, all replicates from each thermal treatment were switched between the two CE units biweekly, and the CE units were reprogrammed accordingly. The experimental design comprised the crossed factors 'AMF species', 'AMF origin', plant 'host' and 'temperature'. Specifically, the two host species consisted of the C_3 'cool season' grass Alpine Kentucky Bluegrass (*Poa pratensis* L.) and the C_4 'warm season' grass Sundevil II Bermudagrass [*Cynodon dactylon* (L.) Pers.] that were obtained from Pickseed® (Tangent, OR, USA) and J.R. Simplot Co., Jacklin Seed Division (Post Falls, ID, USA), respectively. *Poa pratensis* is best adapted for temperature conditions in the range of 15–24°C, whereas *C. dactylon* is best adapted to grow in the range of 26–35°C (Baker & Jung, 1968; Beard, 1973; Barnes *et al.*, 2007). Host plants were grown at two temperature settings with or without (controls) inoculation by AMF. Six AMF species were used, and two fungal strains of each species (i.e. culture isolates from contrasting climatic origins – ICCOs) were selected (Table S1). Thus, each AMF species was represented by one isolate from a cooler and one isolate from a relatively warmer climatic origin (Table S1). In addition, all six isolates from 'warm' and 'cool' climatic origins were mixed separately, and the two mixtures were considered as another AMF 'species'. In our statistical analyses, the climatic origin of the isolates (or their respective mixtures) was nested within species and treated as a fixed effect, as isolates were not chosen randomly, but according to their known and contrasting climatic origins. Each treatment combination was replicated six times. The settings of the two CE units were 23.0 : 26.0°C ('warm') and 13.0 : 16.0°C ('cool')

mean day : night temperatures, 50% mean day : night relative humidity and a 16 h photoperiod. Light conditions were attained using a combination of T12 lamps and compact fluorescent bulbs. Before starting, we measured for CE units 1 and 2 an average of 402 and 401 μmol m^{-2} s^{-1}, respectively, at a distance of *c.* 115 cm. A week later, we measured, at the same distance, an average photosynthetically active radiation of 432 and 440 μmol m^{-2} s^{-1}, respectively.

Experimental units consisted of 4 cm × 20.5 cm 'conetainers' (Stuewe and Sons Inc., Corvallis, OR, USA). Each conetainer was filled with 80 ml of sterilized (121°C for 20 min) Sunshine mix #2 (Sun Gro Horticulture, Vancouver, BC, Canada), pressed tightly to cover the openings at the bottom, 50 ml of a sterilized (121°C for 20 min) substrate comprising Turface (montmorillonite clay, Turface Athletics MVP, Profile Products LLC, Buffalo Grove, IL, USA), soil and sand (3 : 1 : 1, v : v), 25 ml of each AMF inoculum (containing spores, roots and hyphae) and covered with 50 ml of the same substrate. The soil used in the mixture was collected from the long-term mycorrhizal research site (LTMRS) on the campus of the University of Guelph (total N = 140 mmol kg^{-1}; available P = 0.065 mmol kg^{-1}; pH 7.7), passed through a 5 mm mesh sieve and air-dried before being stored at room temperature. AMF inocula were obtained from the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM; http://invam.caf.wvu.edu/index.html) (Table S1). Inoculum of each isolate was bulked for experiments by mixing the starting material with sterile Turface : sand (1 : 1, v : v), seeding with Sudan grass (*Sorghum vulgare* var. *sudanense*) and growing the pot cultures for approximately 1 yr in a glasshouse. As these isolates were also cultured under standardized conditions at INVAM for many years, our set-up avoided potential confounding effects caused by environmental maternal effects. Inocula were carefully examined to verify that spores were abundant, appeared healthy, and that no spores of nontarget species were present. On 5 July 2007, approximately 100 seeds of *P. pratensis* and 20 seeds of *C. dactylon* were added to each 'conetainer' and covered with 25 ml of sterile sand to conserve moisture. To correct for differences in non-AM microbial communities, each conetainer received a 1 ml filtered (20 μm mesh) washing comprising an extract from a mixture of subsamples from all AMF inocula. All experimental units were maintained at 21°C until 1 August 2007 to enable equal seed germination among treatments. Plants were watered daily to field capacity and 30 and 20 mg of 17–5–19 fertilizer were added to each 'conetainer' on 4 September and 12 November 2007, respectively. All plants were clipped at rim level of each conetainer (*c.* 2 cm above the surface of the soil) once each month, and the clippings were dried at 60°C for 48 h. Conetainers were destructively harvested on 12 December 2007 and the total shoot dry weight was measured by pooling all clippings and shoots from the final harvest. Subsets of entire root systems were washed, dried and weighed for both plant species (n = 5 for *P. pratensis*, which had extremely dense and fine root systems, and n = 176 for *C. dactylon*). For all replicates (n = 360), the percentage of root length colonized was determined according to the methodology developed by McGonigle *et al.* (1990). Subsamples of conetainer contents (*c.* 40 cm^3) were air dried for 6 wk, after which hyphal lengths and spore counts were measured from all replicates (Klironomos *et al.*, 1993; Miller *et al.*, 1995).

Statistical analysis

The shoot weight was correlated with the total plant dry weight (roots and shoots) for both *P. pratensis* (R = 0.8175, P < 0.1) and *C. dactylon* (R = 0.9559, P < 0.0001), thus suggesting that shoot weight is a good estimator of total plant dry weight. Plant growth and AMF characters (percentage root length colonized by arbuscules and hyphae, soil hyphal length and spore number) were analyzed by multivariate analysis of variance (MANOVA), followed by univariate analysis of variance (ANOVA). Root colonization by vesicles was analysed by univariate ANOVA only; nonvesicle-forming isolates (Gigasporaceae) were excluded. Data were analysed using a factorial model with the fixed factor 'AMF origin' nested within 'AMF species', crossed with the factors 'temperature' and 'host' plant species. The factor 'AMF origin' should be considered as climatic origin, as we deliberately chose isolates from contrasting climates (Table S1) and were unable to use replicate isolates from the different locations. Comparisons between temperature treatments should be interpreted with caution because of the lack of replicate CEs for each temperature setting. To test whether 'AMF origin' as a main effect or interaction term was consistent across all AMF species, additional (P value-protected) contrast analyses were performed on significant F ratios (by pooling isolates according to climatic origins). The AMF mixtures were tested against the pooled monostrain treatments in a model with 'AMF' (monostrains vs mixtures), 'host' and 'temperature' as factors. We also reran the main analysis with the nested model omitting the mixtures to test whether the reported treatments were still present. Because of the fixed contrast between ICCOs, the factor 'AMF origin' also had a 'crossed' character, and results from fully crossed (M)ANOVA models were qualitatively similar (data not shown). Indeed, whether crossed or nested, these models had identical R^2 values, but partitioned the explained variance differently. Nonmycorrhizal controls were evaluated by a three-way ANOVA with the main factors 'AMF treatment' with two levels (i.e. control and inoculated), 'host' plant species and 'temperature'. To meet the requirements of the statistical tests, variables were log or

arcsine transformed (percentage root length colonized) when necessary (Zar, 1984). Where appropriate, and unless otherwise specified, least-square means contrasts within treatments were performed or means were compared using Tukey's honestly significant difference (HSD) test ($P < 0.05$). Statistical analyses were performed with the software JMP®7.0 (SAS Institute Inc., Cary, NC, USA).

Results

Treatment effects on plant growth

Overall, shoot weight was greater under warm than under cool growth conditions (Table 1, Fig. 1). As hypothesized, cool-adapted *P. pratensis* grew significantly more than warm-adapted *C. dactylon* in cool temperatures, whereas the growth of the two plant species did not differ under warm conditions (i.e. a significant host × temperature interaction: Table 1, Fig. 1).

We consistently detected a significant AMF origin × host interaction (Table 1, Fig. 1). Irrespective of temperature conditions, AMF isolates from cooler climates consistently enhanced plant growth of warm-adapted *C. dactylon* relative to isolates from warmer climates (contrast analysis, $F_{1,280} = 15.49$, $P < 0.0001$). Conversely, *P. pratensis* grew larger, on average, when colonized by all AMF genotypes from warm climates (contrast analysis, $F_{1,280} = 14.30$, $P < 0.0002$).

Plant growth responses to AMF colonization were similar under both thermal regimes. Mycorrhizal plants generally did not differ significantly from nonmycorrhizal controls (Fig. 1). The *P* values of 'AMF treatment' and all interactions with 'host' and 'temperature' were > 0.143. Overall, the shoot weight of the two AMF mixtures did not differ significantly from their pooled cool or warm monoculture counterparts. Treatment effects were similar when the mixtures were removed from the statistical analysis (data not shown).

AMF phenotypes under cool and warm conditions

No visible signs of AMF colonization in the form of arbuscules or vesicles were detected in any control plants (data not shown). AMF species differed in all three variables measuring internal colonization (Table 1, Fig. S1). All hosts colonized by isolates of the Gigasporaceae lacked vesicles, a trait that is inherently absent in this AMF family. Mycorrhizal colonization of roots by isolates of the Gigasporaceae family was generally lower than that by AMF from other families. Overall, arbuscular colonization was significantly higher in *P. pratensis* than in *C. dactylon* (Table 1, Fig. 2). Although temperature did not affect significantly the levels of root colonization, we measured a significant temperature × AMF species' interaction for arbuscular root colonization (Table 1, Fig. S1). Colonization rates by *A. leptoticha* were higher than those of *G. claroideum* and *G. mosseae* isolates under warm conditions, whereas this pattern was reversed under cooler conditions. The amount of arbuscule development per root length also differed among isolates (Table 1, Fig. S1). However, a significant AMF origin × host species' effect was detected. Contrasting the shoot weight data, the effect of origin on arbuscule development was less pronounced. In general, isolates originating from cool climates produced

Table 1 Summary of statistical analysis. *F* statistics of MANOVA (all traits combined) and the respective univariate ANOVAs are shown. Data on vesicular colonization of roots were analyzed by ANOVA only, and isolates of Gigasporaceae were excluded. As all main factors were considered to be fixed, the residual mean square term was used to calculate all *F* ratios

	MANOVA			Biomass[2]		AC		VC		HC		HL		SP	
Treatment[1]	N. df	D. df	D. df *F*	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
AMF Sp.	30	1106	**22.56*****	6	**4.63*****	6	**30.40*****	4	**21.45*****	6	**85.33*****	6	**18.15*****	6	**62.33*****
AMF Or. [isol.]	35	1164	**3.33*****	7	1.22	7	**2.10***	5	**9.12*****	7	**9.57*****	7	**2.94****	7	*1.82*+
Host	5	276	**16.95*****	1	**29.50*****	1	**57.62*****	1	0.09	1	0.34	1	2.53	1	1.44
Temp.	5	276	**85.22*****	1	**415.37*****	1	1.94	1	1.01	1	0.49	1	0.38	1	0.29
AMF Sp. × Host	30	1106	*1.43*+	6	0.64	6	**3.14****	4	1.91	6	1.48	6	1.50	6	0.69
AMF Or. [isol.] × Host	35	1164	**2.19*****	7	**5.27*****	7	**2.13***	5	0.48	7	1.14	7	0.72	7	1.62
AMF Sp. × Temp.	30	1106	0.89	6	0.58	6	**2.58***	4	0.40	6	0.96	6	0.10	6	0.38
AMF Or. [isol.] × Temp.	35	1164	0.99	7	*1.87*+	7	0.61	5	1.19	7	0.96	7	0.36	7	1.27
Host × Temp.	5	276	**12.34*****	1	**60.7*****	1	0.01	1	0.42	1	0.11	1	0.01	1	0.66
AMF Sp. × Host × Temp.	30	1106	*1.38*+	6	1.12	6	*1.96*+	4	0.81	6	0.94	6	*1.92*+	6	1.08
AMF Or. [isol.] × Host × Temp.	35	1164	0.78	7	0.21	7	*2.01*+	5	1.30	7	0.57	7	1.07	7	0.22
Residual				280	0.000692	280	0.00957	200	0.01307	280	0.01557	280	0.01917	280	0.15539

[1]AMF Or., AMF origin; AMF Or. [isol.], isolates from cool or warm origin nested within species; AMF Sp., AMF species; Temp., temperature.
[2]Biomass corresponds to shoot biomass and AC, VC, HC, HL and SP correspond to root colonization by arbuscules, vesicles and hyphae, extraradical hyphal lengths and arbuscular mycorrhizal fungi (AMF) spore densities, respectively.
Significance levels of *F* statistics are *, ** and *** corresponding to $P < 0.05$, 0.01 and 0.001, respectively, and are in bold. Values in italics are marginally significant effects (+, $P < 0.1$). The residual mean squares of the ANOVA models are shown in the bottom row.

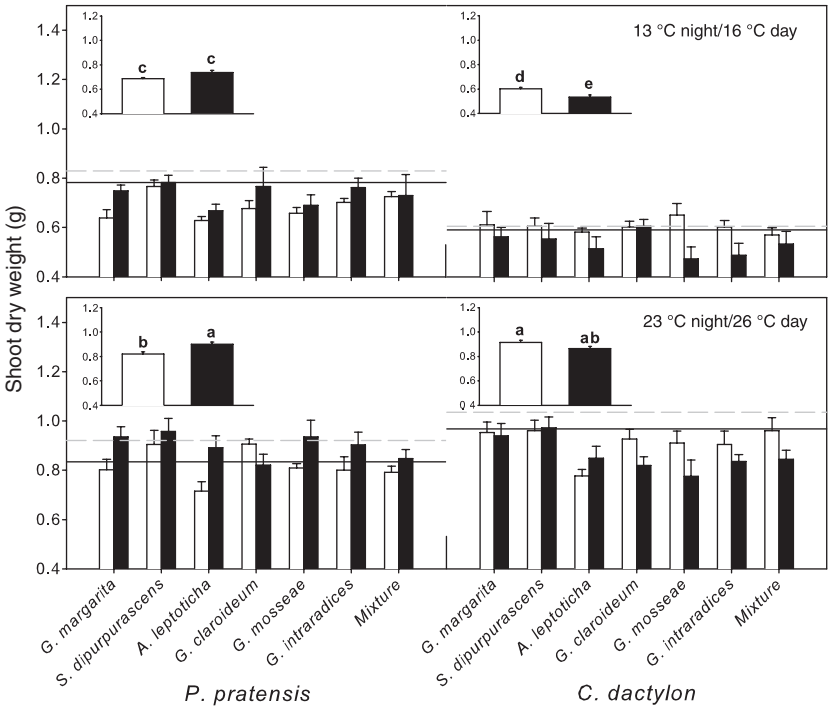

Fig. 1 Shoot weight produced by the cool-adapted (*Poa pratensis*) and warm-adapted (*Cynodon dactylon*) plants colonized by individual arbuscular mycorrhizal fungi (AMF) isolates from cool (white) or relatively warmer (black) climatic origin under cool (top) and relatively warmer (bottom) temperature conditions. Horizontal lines represent the shoot weight produced by nonmycorrhizal controls. Inner graphs represent the pooled data by climatic origin. Error bars and the dashed line (for the nonmycorrhizal controls) are standard errors of the mean (SEM). For the inner graphs, bars with the same letter are not different according to Tukey's honestly significant difference (HSD) text, $P > 0.05$. AMF species and isolates are listed in Supporting Information Table S1.

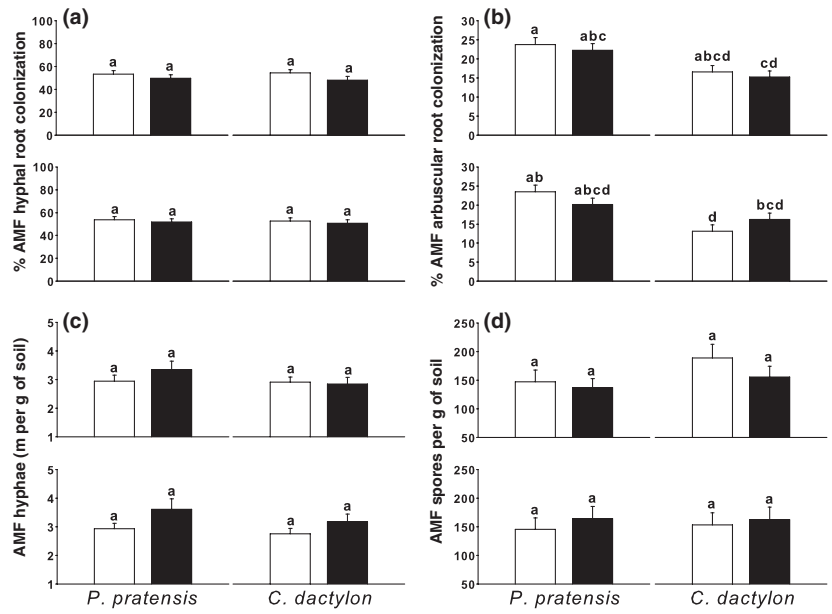

Fig. 2 Overall root (a, b) and soil (c, d) colonization levels of arbuscular mycorrhizal fungi (AMF) pooled from either cool or warm origins (open and black bars, respectively). Top and bottom graphs in each quadrant correspond to the cool and warm growth conditions, respectively. Error bars are standard errors of the mean (SEM). Bars with the same letter are not different according to Tukey's honestly significant difference (HSD) test, $P > 0.05$.

more arbuscules in roots of *P. pratensis* than did isolates of warm climatic origin, and vice versa for *C. dactylon* (Figs 2b, S1).

Temperature did not affect significantly either extraradical AMF hyphal length or the number of spores produced (Table 1, Fig. S2). However, the amount of extraradical hyphae and spore numbers varied significantly among AMF species (Table 1). AMF in Gigasporaceae generally produced more extraradical hyphae and fewer spores that those in other families (Fig. S2). We did not detect a consistent difference in AMF growth (i.e. extraradical hyphae and spore production) between ICCOs (Fig. 2c,d; contrast analyses, $F_{1,280} = 2.26$, $P < 0.13$ and $F_{1,280} = 1.48$, $P < 0.22$, respectively). However, the significant AMF origin effects revealed intraspecific variability on both of these AMF fitness-related traits (Fig. S1, Table 1). Overall, the reported treatment effects (Table 1) were similar when the isolate mixtures were removed from the statistical analysis

(data not shown). Except for vesicular colonization ($F_{1,239} = 0.43$, $P < 0.513$), AMF mixtures produced significantly more arbuscules, internal hyphae, extraradical hyphae and spores than the pooled AMF single-isolate treatments ($F_{1,335} = 6.51$, $P < 0.011$, $F_{1,335} = 17.96$, $P < 0.0001$, $F_{1,335} = 11.86$, $P < 0.0006$ and $F_{1,335} = 7.76$, $P < 0.006$, respectively).

Discussion

The results of our climate chamber study showed that AMF that originated from contrasting climates consistently and differentially altered the growth responses of two grass species. The AMF isolates used in this study were chosen solely by their taxonomic affiliation and climatic origin, without any *a priori* knowledge of their symbiotic function, or other biotic or abiotic factors that may have been different among their locations of origin. The significant AMF origin by host species' interaction on shoot weight rejects the null hypothesis that functional variability within AMF is unrelated to their climatic origin. Across several AMF species, isolates from different climatic origins caused similar effects on the two host plants, suggesting that climate may have altered the function of these plant symbionts in similar ways. Qualitatively, the two host species responded differently to inoculation by isolates of cool or warm origins (or their mixtures), as captured by the significant AMF origin × host interaction term (Fig. 1). AMF origin significantly affected each plant species, and these effects were replicable, as they were qualitatively similar under both temperature conditions. However, overall shoot weight was not altered by the main effect AMF origin, as contrasting 'cool' and 'warm' climatic origins of AMF had opposite effects on each of the two host species. Although other studies have reported evidence of ecotypic differentiation and local adaptation (Stahl & Christensen, 1991; Johnson *et al.*, 2010), our study links climate to functional divergence among related AMF. Importantly, we used morphologically defined taxa across the Glomeromycota. These were sampled from distant communities distributed globally and had been maintained for many years under a common culture regime. Thus, our findings suggest that any potential selection or genetic change that may have occurred in this common environment did not overcome the environmental signature from their climates of origin and probably reflected climate-driven ecological specificity. Considering that other abiotic or biotic factors probably also differed among the locations from which AMF were isolated, and AMF symbiotic traits are also variable within populations (Munkvold *et al.*, 2004; Koch *et al.*, 2006), the finding of such consistent AMF 'origin' interaction is unlikely to have been caused by such unmeasured random factors. However, from our study, we cannot determine the mechanism(s) responsible for how different climates affect AMF symbiotic functioning. We speculate that the length of the growth season, the frequency of droughts or other factors associated with climates may be more important than just average temperature *per se*. In the light of predicted and ongoing climate change and differential dispersal rates of plants and AMF, our results imply that co-adapted plant–AMF associations could potentially break up and affect successional dynamics through complex feedbacks.

Parallels are found in other plant–microbial interactions, where plant–parasite co-evolution is tightly coupled with adaptation of an obligate parasite to temperature (Laine, 2008). In the case of ectomycorrhizal (ECM) symbioses, López-Gutiérrez *et al.* (2008) conducted studies that suggest that the metabolism of congeneric ECM fungi from contrasting latitudes is inherently different. Specifically, they observed that, irrespective of the incubation temperatures used, the mean respiration rate was significantly higher for ECM isolates from Alaska than for isolates from Pennsylvania. In this case, shorter growing seasons may have selected ECM with higher metabolic rates. However, we have no such measurements and no clear evidence of consistent differences in fungal growth rates among ICCOs. The physiological mechanism of how different fungi alter the growth of their hosts remains unclear.

Differences in growth by isolates of AMF species were similar overall to those reported by Hart & Reader (2002). Compared with isolates of other AMF genera used in our study, *Gigaspora* and *Scutellospora* isolates enhanced host growth, produced fewer spores and had the lowest levels of root colonization. Levels of arbuscule colonization by AMF isolates were modulated by host species and growth temperature, highlighting complex interactions of AMF with biotic and abiotic factors. When analysing the isolate pairs of the different AMF species individually, some patterns of ecotypic differentiation were observed for certain traits (e.g. a marked differential in spore numbers produced by the isolates of *G. intraradices* under cool vs warm temperatures for *C. dactylon*), giving some support for thermal adaptation in AMF growth. However, a consistent general pattern in root and soil colonization and spore production among isolates, indicative of widespread thermal adaptation, was not detected. If such thermal adaptation does exist, it may be weak relative to the functional variability observed in and among AMF species and communities, which may be more strongly influenced by factors other than temperature. Lekberg & Koide (2008) specifically investigated adaptation to extreme climatic conditions, including temperature, and their findings suggested that AMF are rather general purpose genotypes. Ecotypic differentiation may be widespread among AMF in relation to environmental factors other than temperature, as suggested by their responses to salinity or pollution (Stahl & Christensen, 1991; Weissenhorn *et al.*, 1993; Galli *et al.*, 1994; del Val *et al.*, 1999; Carvalho *et al.*, 2004; Toler *et al.*, 2005). However, we avoided extreme

conditions and instead used temperature settings optimal for host species and approximating climates of ICCOs. Although our results corroborate the thermal adaptation of the host plants, little is known about the thermal optima of AMF (see Tibbett & Cairney, 2007). As AMF growth was not directly affected by temperature differences of 10°C, these fungi may not be greatly affected by a wide temperature range when other growing conditions are otherwise similar. More contrasting thermal or hydric conditions will probably yield different results (Koch *et al.*, 2006).

In conclusion, our data on plant growth responses support the hypothesis that AMF in regions of contrasting climates evolve ecotypes, as evidenced by altered symbiotic function in contrasting cool vs warm climates. However, there are very limited empirical data testing the context in which selective pressures imposed by climate are compared with other factors. Many ecophysiological processes are tightly coupled to climate in natural ecosystems, and future work should focus on how different environmental factors are acting as selective forces and mediate the interactions between soil biota and plants in communities.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Percentage colonization of roots of *Poa pratensis* and *Cynodon dactylon* by arbuscules, vesicles and hyphae produced by arbuscular mycorrhizal fungi (AMF) isolates of different species or mixtures from cool and warm climatic origins under either cool or warm growth conditions.

Fig. S2 Number of spores and soil hyphal colonization produced by arbuscular mycorrhizal fungi (AMF) isolates from cool and relatively warmer origins under either cool or relatively warmer environmental conditions.

Table S1 Mean annual temperatures for the 30-yr period 1971–2000 in regions that correspond to the geographic origin of the arbuscular mycorrhizal fungi (AMF) isolates used in this study

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