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Key words: ascorbate, glutathione, oxidative stress, peroxide, reactive oxygen species (ROS).

Letters

Ectomycorrhizal fungi from Alaska and Pennsylvania: adaptation of mycelial respiratory response to temperature?

Despite the importance of ectomycorrhizal (ECM) fungi to both the carbon economy of their hosts (Smith & Read, 1997; Wallander *et al.*, 2004) and ecosystem respiration (Rygiewicz & Andersen, 1994; Bååth & Wallander, 2003; Hobbie & Hobbie, 2006; Heinemeyer *et al.*, 2007; Moyano *et al.*, 2008), little is known about the factors that control their rates of respiration. We have recently found that substantial variation exists among ECM fungal isolates in the ability of respiration to acclimate to temperature (Malcolm *et al.*, 2008). The potential for respiratory adaptation to temperature, an evolutionary response, has yet to be shown for ECM fungi. Therefore, in this study, we explored variation in respiration for ECM fungi in culture from two sites located at different latitudes.

Fungi are presumed to adapt to prevailing temperatures because species taken from different climates exhibit temperature ranges for optimal growth, or exhibit distinct minimum or maximum temperatures for survival that correspond to their climates of origin (Cooke & Whipps, 1993; Tibbett *et al.*, 1998; Robinson, 2001). The same is true for plants. With the presumption that temperature exerts a strong selective effect on respiration along environmental gradients (Reich *et al.*, 1996), studies utilizing elevational or latitudinal gradients have shown repeatedly that plant respiration rates at a given measurement temperature are frequently higher for ecotypes from northern latitudes or higher elevations than for those from southern latitudes or lower elevations (Sowell & Spomer, 1986; Mariko & Koizumi, 1993; Reich *et al.*, 1996).

Therefore, we hypothesized that cultures of ECM fungi from near Fairbanks, Alaska would have higher respiration rates at a given measurement temperature than those from near State College, Pennsylvania, which, if found, would be suggestive of adaptation to temperature, as has been found for plants along environmental gradients. We also determined whether isolates from contrasting sites differed in respiratory sensitivity to temperature (Q_{10}) in order to help predict whether the effects of future shifts in soil temperature on the respiration rates of ECM fungi depend on site.

Because different fungal lineages have different evolutionary histories, it may not be valid to contrast an isolate of one lineage from higher latitude with another isolate of a different lineage from lower latitude (Burt, 1989). Thus, while we were not able to collect isolates of the same species from contrasting sites, we did make comparisons using congeneric contrasts. Specifically, we collected ECM fungi from four genera and three families: Amanitaceae (*Amanita* spp.), Russulaceae (*Lactarius* spp.) and Boletaceae (*Leccinum* spp. and *Suillus* spp.), each from both Alaska (latitude 65°07′N, longitude 147°30′) and Pennsylvania (latitude 40°48′N, longitude 77°54′) (Table 1).

Fungi were cultured from field-collected sporocarps and maintained on a growth medium consisting of 19.5 g of potato dextrose agar (Difco; Becton, Dickinson & Co., Sparks, MD, USA), 7.5 g of Bacto Agar (Difco) and 0.375 g of NH_4Cl per liter of water. Fungal cultures were initially maintained in incubators set to temperatures reflective of those experienced by the fungi during the growing season at their site of origin: 17°C for Pennsylvanian isolates and 11°C

Table 1 Details of collection of eight isolates of ectomycorrhizal (ECM) fungi from four different genera and three different basidiomecete families: Amanitaceae (*Amanita* spp.), Russulaceae (*Lactarius* spp.) and Boletaceae (*Leccinum* spp. and *Suillus* spp.)

ECM fungal species	Isolate	Collected	Vegetation
Amanita muscaria var. *muscaria*	GL015	South Fairbanks, AK	*Betula papyrifera*, *Populus balsamifera*, *Picea glauca*
Amanita citrina	SC070	State College, PA	*Pinus resinosa*
Lactarius cf. *pubescens*	AK010	Chatanika, AK	*Betula papyrifera, Populus balsamifera*
Lactarius chrysorrheus	SC016	State College, PA	*Pinus resinosa*
Leccinum cf. *alaskanum*	GL005	South Fairbanks, AK	*Betula papyrifera, Populus balsamifera, Picea glauca*
Leccinum aurantiacum	SC014	State College, PA	*Pinus resinosa*
Suillus intermedius	BX007	State College, PA	*Pinus resinosa*
Suillus cf. *grevillei*	AK014	South Fairbanks, AK	*Picea mariana*

for Alaskan isolates (for more information about environmental temperatures, see Malcolm *et al.*, 2008).

Two isolates (one from Pennsylvania and one from Alaska) from each of the four genera were propagated using the growing medium described above in each of 10 100 × 20 mm Petri dishes, for a total of 80 dishes. Each isolate was initially grown at 11°C (Alaskan isolates) or 17°C (Pennsylvanian isolates) for 2–3 wk in order for each fungal colony to initiate new growth (*c.* 2–4 mm increase in diameter). After new growth had been established, half of the 10 dishes of each isolate were maintained in the initial incubator. The other half were shifted to the other incubator. Each of the replicate dishes was maintained for 1 wk in these incubators before respiration was assessed in order to allow for the potential for physiological acclimation to temperature (Malcolm *et al.*, 2008).

Steady-state respiration rates (CO_2 exchange rates) were determined at 11, 17, and 23°C for each of the 80 dishes using a custom-built gas exchange system (Malcolm *et al.*, 2008). In order to standardize respiration rates by fungal dry weight, fungal tissues were separated from agar by melting in test tubes held in boiling water immediately following the respiration measurements. The tissues were placed in a drying oven (60°C) until constant weight was achieved.

The proportional change in respiration rate over a 10°C interval (Q_{10}) was calculated by plotting the log-transformed respiration rates against measurement temperature, obtaining the slopes of the linear regression, and using the following equation (Atkin *et al.*, 2000):

$$Q_{10} = 10^{(10 \times \text{slope})}$$

Because respiration rates were obtained across a range of measurement temperatures on the same set of individual cultures, we used the Repeated Measures Proc Mixed ANOVA Procedure in SAS (version 9.1, 2002–2003; SAS Institute Inc., Cary, NC, USA) to analyze the respiration responses to temperature. We determined the effects of site, genus, measurement temperature and the interaction between site and measurement temperature on respiration rate when: isolates from Alaska were incubated at 11°C and isolates from Pennsylvania were incubated at 17°C; all isolates were incubated at a common temperature of 11°C; and all isolates were incubated at a common temperature of 17°C. To analyze Q_{10} we used the Proc GLM ANOVA Procedure in SAS with site and genus as main effects. Differences were analyzed in the same cases as above.

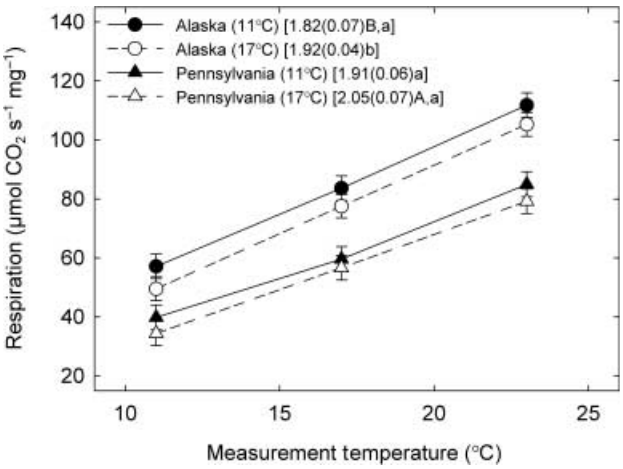

Fig. 1 Mean (± 1 SEM) of respiration at three measurement temperatures for ectomycorrhizal fungi originating from Alaska and Pennsylvania incubated at 11 and 17°C. Mean (SEM) Q_{10} values are displayed for each site of origin at each incubation temperature. Q_{10} values followed by different uppercase letters are significantly different for the comparison of fungi from Alaska and Pennsylvania incubated at 11°C and 17°C, respectively; and Q_{10} values followed by different lowercase letters are significantly different for the comparison of fungi from both sites incubated at a common temperature of 11 or 17°C. n = 4 genera.

Irrespective of whether the isolates were incubated at temperatures reflective of their sites of origin (Alaskan isolates at 11°C and Pennsylvanian isolates at 17°C) or at common temperatures (either 11 or 17°C), the mean respiration rate was significantly higher at a given measurement temperature for isolates from Alaska than for isolates from Pennsylvania over the range 11–23°C (Fig. 1, Supporting information Table S1). The significant effect of site of origin was observed in all

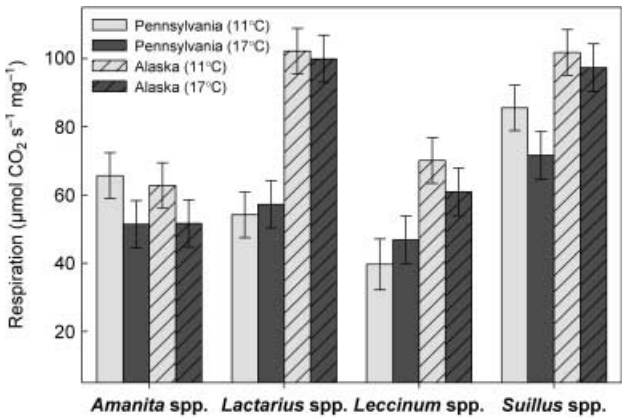

Fig. 2 Mean (± 1 SEM) of respiration, averaged over measurement temperatures of 11, 17 and 23°C, for each of four genera of fungi originating from Alaska and Pennsylvania. Fungi were incubated at 11 or 17°C. n = 5 Petri dishes for each combination of site × genus × treatment.

genera except for *Amanita* (Fig. 2). Furthermore, the average respiration rate of Alaskan isolates at a measurement temperature of 11°C (57.16 ± 4.17 μmol CO_2 s^{-1} mg^{-1}) was very close to that of Pennsylvanian isolates at a measurement temperature of 17°C (56.70 ± 4.17 μmol CO_2 s^{-1} mg^{-1}), irrespective of incubator temperature (Fig. 1).

These results are consistent with the hypothesis that the metabolism of ECM fungi can adapt to some factor related to site of origin. Because the two sites differ markedly in latitude, this factor may be temperature, but this is impossible to determine unequivocally because, in addition to temperature, vegetation type, soil chemistry and moisture may also differ between sites. Nevertheless, temperature differences along latitudinal gradients appear to create a strong selective pressure on respiration responses in plants (Reich *et al.*, 1996), so it is possible that adaptation to temperature contributed at least partly to the observed phenomenon in ECM fungi. The large number of studies performed on plant latitudinal and altitudinal gradients with results that are consistent with adaptation to temperature (Sowell & Spomer, 1986; Mariko & Koizumi, 1993; Reich *et al.*, 1996), as are ours, suggests that, if temperature is not the only factor to which organisms have adapted along such gradients, it may certainly be an important contributing factor.

The Q_{10} values reported for the fungi in this study, between 1.82 and 2.05 for measurement temperatures between 11 and 23°C (Fig. 1), were close to the often-assumed value of 2.0 for most biological systems (Cox *et al.*, 2000; Potter *et al.*, 2001; Atkin *et al.*, 2005). We have shown, however, that site may influence the sensitivity to temperature of ECM fungi. Overall, values of Q_{10} were significantly lower for isolates from Alaska than for isolates from Pennsylvania, except when the isolates were incubated at a common temperature of 11°C (Fig. 1, Table S2). Thus, as global temperatures increase, proportional increases in respiration for a given temperature shift could be lower for the fungi at high latitude than for those at lower latitude. Few comparable data exist for ECM fungi, but in studies of plants, Sowell & Spomer (1986) and Mariko & Koizumi (1993) indicated that no significant variation in Q_{10} was seen among ecotypes from different elevations. Further, the sensitivity of the respiration response to temperature is similar to that of roots, mycorrhizal fungal hyphae, and soil microbes, as previously shown by Bååth & Wallander (2003) in microcosm.

We acknowledge that the respiration rates reported herein may not necessarily reflect absolute rates for fungi *in symbio* because of potential host effects on carbon supply. However, the respiration rates obtained in this study do agree with those obtained in previous studies of ECM fungi in culture (Taber & Taber, 1987; Souto *et al.*, 2000; Malcolm *et al.*, 2008), with those obtained *in symbio* (Rygiewicz & Andersen, 1994), and also with a field estimate calculated by Malcolm *et al.* (2008). Still, we urge modelers to use caution if utilizing data from this study, but to take note of the broader ecological pattern that we have shown.

In summary, our results suggest that the metabolisms of ECM fungi from two widely divergent sites from varying latitudes may be inherently different which could, in turn, have far-reaching implications for the carbon economy of the host plant and ecosystem respiration. For example, if the host does have less control over carbohydrate transfer to the fungus than the fungus itself, then for plant hosts from lower latitudes, fungal isolates from higher latitudes may be inappropriate symbionts because of their disproportionately high respiration costs. Moreover, because ECM fungi contribute significantly to total soil respiration (Heinemeyer *et al.*, 2007; Moyano *et al.*, 2008), adaptation to prevailing temperatures would tend to equalize their contribution to total soil respiration rates along temperature gradients. Finally, our use of congeneric contrasts from a variety of families suggests a general propensity for physiological adaptation among the Basidiomycota. Further studies that include more latitudes and/or more replication within latitudes, and studies that consider the respiration response of ECM fungi *in symbio*, appear to be warranted.

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Key words: adaptation, carbon demand, ectomycorrhizal fungi, latitude, Q_{10}, respiration, temperature.

Supporting Information

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

Table S1 Analysis of variance of respiration of ectomycorrhizal fungi incubated at temperatures reflective of the litter of their sites of origin during the growing season (11°C for Alaskan isolates and 17°C for Pennsylvanian isolates) or at common temperatures (either 11 or 17°C).

Table S2 Analysis of variance for the respiration Q_{10} value of four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania

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Supporting Information

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Treatment	Factor	Numerator df	Denominator df	*F*-value	*P*-value
Alaska (11°C) Pennsylvania (17°C)	Site	1	35	23.98	<0.0001
	Genus	3	35	7.14	0.0007
	Measurement temperature	2	76	481.82	<0.0001
	Site × Measurement temperature	2	76	4.76	0.0113
Alaska (11°C) Pennsylvania (11°C)	Site	1	34	16.48	0.0003
	Genus	3	34	8.87	0.0002
	Measurement temperature	2	74	453.41	<0.0001
	Site × Measurement temperature	2	74	4.40	0.0156
Alaska (17°C) Pennsylvania (17°C)	Site	1	35	14.7	0.0005
	Genus	3	35	9.74	<0.0001
	Measurement temperature	2	76	521.43	<0.0001
	Site × Measurement temperature	2	76	6.15	0.0033

There were three measurement temperatures and four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania. $n = 5$ Petri dishes for each combination of Site × Genus × Treatment.

Table S2 Analysis of variance for the respiration Q_{10} value of four genera of fungi, each comprising two isolates, one from Alaska and one from Pennsylvania

Treatment	**Factor**	**df**	**Sum of squares**	***F*-Value**	***P*-Value**
Alaska (11°C)	Site	1	0.53	5.61	0.0235
Pennsylvania (17°C)	Genus	3	0.18	0.64	0.5949
	Error	35	3.28	~	
Alaska (11°C)	Site	1	0.07	0.86	0.36
Pennsylvania (11°C)	Genus	3	0.41	1.76	0.1736
	Error	34	2.67	~	
Alaska (17°C)	Site	1	0.17	4.99	0.032
Pennsylvania (17°C)	Genus	3	0.29	2.78	0.0557
	Error	35	1.21	~	

Fungi were either incubated at separate temperatures: 11°C (Alaska isolates) and 17°C (Pennsylvania isolates), or at common temperatures of either 11°C or 17°C. n = 5 Petri dishes for each combination of Site × Genus × Treatment.