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Fungal endophyte–grass symbioses are rare in the California floristic province and other regions with Mediterranean-influenced climates

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ABSTRACT

Research on agronomic grasses has shown that Class 1 fungal endophytes (*Neotyphodium/Epichloë*; Clavicipitaceae) can have profound effects on host plant fitness. However, in natural systems, even basic ecological knowledge of most endophyte symbioses is lacking. Here, I describe the distribution and abundance of endophytes across 36 native (or naturalized) grasses in a previously unsurveyed region, the California Floristic Province. Symbiosis was generally low: 8.33 % of species and 18.75 % of genera hosted endophytes. I then compared the proportions of symbiotic species and genera found in California and other Mediterranean regions to the proportions found in non-Mediterranean regions. Surveys of Mediterranean-influenced regions showed significantly lower proportions of species (~66 % lower) and genera (~65 % lower) hosting endophyte than surveys of non-Mediterranean regions. This pattern suggests that selection in Mediterranean climates may not favor endophyte symbioses.

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Research over the last few decades has generated an increasing awareness of the importance of microbial associates of plants. For example, the diversity of mycorrhizal fungi has been shown to greatly impact plant diversity and ecosystem productivity, and N-fixing bacteria have been shown to facilitate everything from primary succession to invasional meltdown (van der Heijden *et al.* 1998; Uliassi & Ruess 2002; Hughes & Denslow 2005). Yet, in most cases far less is known about interactions of plants with microbial

organisms than with macro-organisms, such as pollinators, competitors, and herbivores. In some cases, even basic ecological knowledge on the distribution and abundance of the organisms is limited. Improving our understanding of the ecology of these microbes is a critical step towards unraveling how they impact important ecological and evolutionary processes.

Systemic fungal endophytes of the genera *Neotyphodium* and *Epichloë* (Class 1 endophytes; Clavicipitaceae; Rodriguez

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et al. 2009) can confer substantial benefits – drought tolerance, resistance to herbivory and pathogens, enhanced nutrient uptake, and increased competitive ability – to agronomic grass hosts (Malinowski *et al.* 2000; Clay & Schardl 2002; Clay *et al.* 2005). Importantly, these benefits can have community level effects and can significantly impact key ecological processes; for example, the endophyte in tall fescue (*Schedonorus phoenix*) alters plant diversity, succession, and the abundance and diversity of arthropods in U.S. habitats where it has been introduced (Clay & Holah 1999; Rudgers *et al.* 2007; Rudgers & Clay 2008). In fact, endophyte-mediated neurotoxic effects on livestock (e.g. increased gestation period, lower fertility, and reduced weight gain) have been estimated to cost this industry a billion dollars annually in the U.S. alone (Panaccione *et al.* 2001). While the large economic impact of endophytes in agronomic, forage grasses (e.g. tall fescue and perennial ryegrass) has motivated a great deal of research in agricultural systems, even basic knowledge of symbioses between fungal endophyte and most native, wild grasses has received little or no attention (Saikkonen *et al.* 2006).

Here, I first investigate one aspect of the ecology of Class 1 endophytes in native, wild grasses – their distributions and abundances – across 16 genera, 36 species, and 207 populations in a previously unsurveyed region, the California floristic province. Using these data and those from the literature, I then explore whether Mediterranean climate is associated with the frequency of grass–endophyte symbioses.

While White & Cole (1985) examined a few herbarium specimens from California, to my knowledge, no large-scale survey of endophytes has been conducted in the California floristic province, and in general, much less work has been published on endophytes in the western United States compared to the rest of the country. The California floristic province, which has been characterized by Conservation International as a biodiversity hotspot with high levels of plant endemism (<http://www.biodiversityhotspots.org/Pages/default.aspx>), has many endemic grass species for which we have little information on endophyte symbiosis. Moreover, unlike the rest of North America, California experiences a Mediterranean climate with hot, dry summers and cool, wet winters. This climate may exert different selective pressures on plants or endophytes than do temperate climates, perhaps altering the frequency of grass–endophyte symbiosis. A synthesis of previous large-scale endophyte surveys demonstrates that the frequency of grass species and genera with endophyte varies markedly among regions (Fig 1), but it remains unclear what factors underlie these large-scale differences in the frequency of endophyte symbiosis. In this paper, I take a first step toward addressing the role of climate in the distribution of endophyte symbiosis by combining my survey data of California with published surveys to determine whether the frequency of species and genera with endophyte significantly differs between regions with Mediterranean-influenced climates and regions with other climate types.

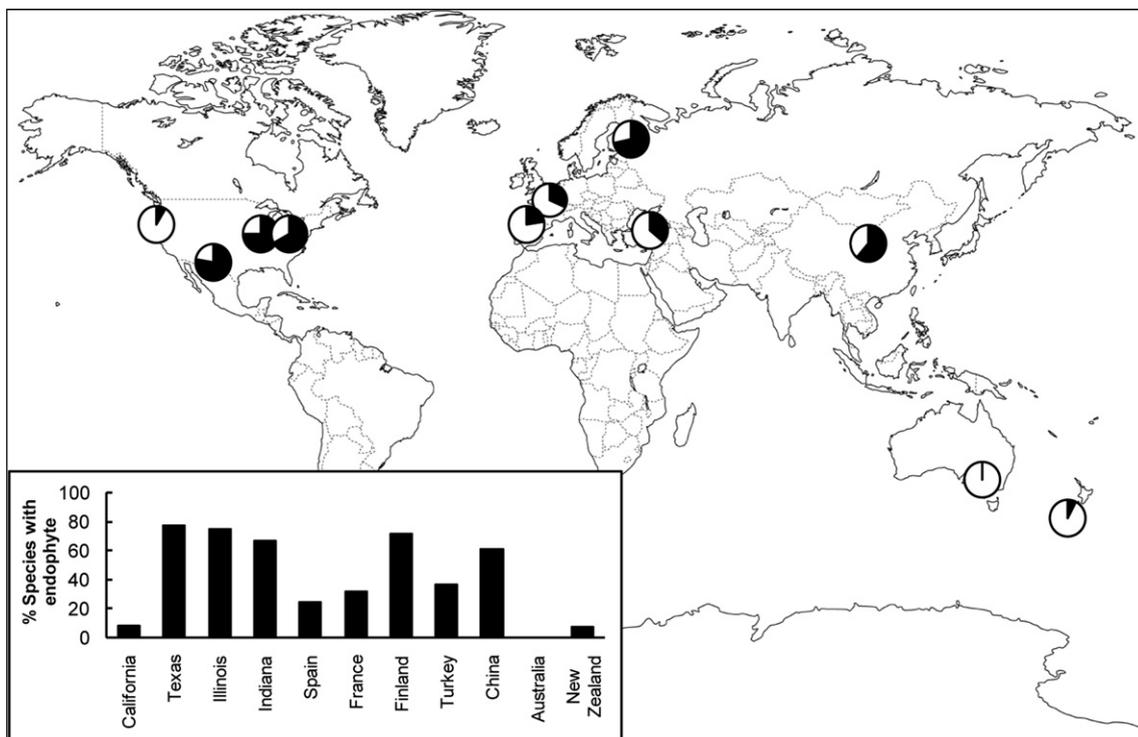


Fig 1 – Surveys result for Class 1 fungal endophytes across the world. Each pie graph is placed at the approximate location where a survey was conducted. For each pie graph, the dark area indicates the proportion of species with endophyte and light area indicates the proportion of species without endophyte in that region. INSET: Percentage of species surveyed that host endophyte in each of the 11 regions. The percentage of genera with endophyte shows a similar pattern across the surveyed areas.

Methods

California field survey

I surveyed 3 800 individual plants spanning 16 genera, 36 species, and 207 populations throughout California for endophyte frequency in Jan.–May 2009 and Jan.–Apr. 2010. Grass species surveyed were chosen from genera known to host endophyte in other regions, and thus were potentially biased towards finding endophyte. Seventy-two percent of species examined were native, wild grasses, but a small number of populations of naturalized, non-native species were examined as well (Tables 1 and 2). Twelve (33 %) of the species examined are endemic to California or the West Coast (Tables 1 and 2). Herbarium records were used to locate populations (*Consortium of California Herbaria*, ucjeps.berkeley.edu/consortium/); additional populations were surveyed when observed during other field research. Sampling focused on the Coast Range, Sierra Nevada, and coastal areas of northern and central California, but some collections were also made in southern California's Peninsular Range. Samples were also collected in the Central Valley, but surveys of this region were limited because of the pervasiveness of invasive annual grass species and relative lack of native species. The Coast Range and Sierra Nevada were surveyed more thoroughly than the coast because coastal areas contain a great deal more private or developed land than the more wild mountainous regions, which are less densely populated and include more public protected areas.

For each population, I collected ~3 stems (<3 stems if plants were very small) from each of ~20 plants (see Tables 1 and 2 for sample sizes). When populations were very small (<20 individuals), I collected tissue from all plants present. The collected tissue was stored in the lab at 4 °C for up to 48 hr to avoid decomposition. I scored samples for endophyte presence by staining leaf sheaths with aniline blue-lactic acid dye and examining tissue for fungal hyphae at 200× magnification with a compound microscope as in Bacon & White (1994). Other studies have found that staining produces results consistent with immunoassay or polymerase chain reaction methods of detection (Dombrowski et al. 2006; Trento et al. 2007).

Mediterranean climate

To determine whether the frequency of species and genera with endophyte is significantly different for regions with Mediterranean-influenced climates, I used the data from my survey of California and 10 previously published surveys (four surveys conducted in Mediterranean-influenced climates and six surveys conducted in other climate types). To be classified in the Mediterranean-influenced climate group, at least some part of the survey region described by the authors must have been in a Mediterranean-influenced climate, and to be classified in the non-Mediterranean climate group, none of the survey region could be in a Mediterranean climate. While this is a rough classification, it provides a first step towards investigating global-scale patterns of endophyte symbiosis. The Mediterranean survey regions included Ankara, Turkey and Salamanca, Spain which experience Continental Mediterranean climates (Tunali et al. 2000; Zabalgozeazcoa et al.

2003), France, which included the Mediterranean Southern region (Leyronas & Raynal 2001), and Victoria, Australia, the western part of which experiences a Mediterranean climate (Aldous et al. 1999). The non-Mediterranean regions included Texas, Illinois, and Indiana in the United States as well as Finland, northern China, and New Zealand (mostly South Island) (White & Cole 1985; Clay & Leuchtman 1989; Saikkonen et al. 2000; Spyreas et al. 2001; Rolston et al. 2002; Wei et al. 2006). These surveys were selected because of their similarity in methodology to my California survey, which facilitated comparisons across regions. To be included in the analysis, survey papers had to meet four criteria: (1) The paper reported information on both symbiotic and non-symbiotic species and populations. Many studies focus only on symbiotic species but fail to report endophyte-free species making it impossible to estimate the proportion of symbiotic species or genera. (2) The samples examined in the study were field collections of wild plants rather than germplasm or botanical garden material. The latter may be subject to different selective pressures that could alter endophyte frequency. (3) The survey focused on one region or included sufficient geographic information, such that its geographic and climatic attributes could be accessed with reasonable accuracy (e.g. "Salamanca, Spain" rather than "Europe"). (4) The survey focused on native species and tested multiple genera, species, and populations. Unfortunately, some regions where ongoing research is investigating endophytes in native grasses are not included here (e.g. Argentina; Novas et al. 2007), because no surveys could be identified that met these criteria. See Online Appendix 1 for details on the findings of the included surveys.

For each survey, I calculated the percentage of species and genera examined that hosted endophyte. To test whether endophyte prevalence is significantly different between climate types, I used Welch ANOVA with the fixed factor of climate type (Mediterranean vs non-Mediterranean) and the response variable of percentage of species or genera with endophyte (JMP 2007–2010, v. 8.0). For each of the 21 genera present in both Mediterranean and non-Mediterranean surveys, I calculated the percentages of species that hosted endophyte in each climate type. I then used a two-tailed, paired t-test with the independent factor of climate type and the response variable of percentage of species hosting endophyte (within each genus). By using a paired test and only including genera that occur in both climate types, this test roughly accounts for phylogenetic differences in plant taxa between Mediterranean and non-Mediterranean climates.

Results and discussion

California field survey

Endophyte symbiosis in the California floristic province was generally low with only 8.33 % of all species and 18.75 % of all genera examined hosting a systemic endophyte (3 of 36 species; 3 of 16 genera; Tables 1 and 2). Leuchtman (1992) estimated that, worldwide, 20–30 % of grass species (Poaceae) host these systemic endophytes. If this estimate is accurate, my results indicate that California may have ~60–70 % fewer species with endophyte than expected. The

Table 1 – California grass species that were not found to host fungal endophytes

Species	Locations ^a	Native status ^b	Host reprod. strategy ^b	Populs. (samples) ^c
<i>Achnatherum stillmanii</i>	Foresthill	CA endemic	Perennial	1 (22)
<i>Aegilops triuncialis</i>	Lower Lake	Invasive	Annual	1 (20)
<i>Agrostis blasdalei</i>	Bodega Bay	CA endemic	Perennial	1 (8)
<i>Avena barbata</i>	Davis, Napa, Willows	Invasive	Annual/perennial	3 (64)
<i>Bromus carinatus</i>	Bodega Bay	WC endemic	Annual	1 (18)
<i>B. catharticus</i>	Somerset (4), Stonyford	Non-native	Perennial	5 (79)
<i>B. diandrus</i>	Napa	Invasive	Annual	1 (31)
<i>B. grandis</i>	Carmel Valley (6)	CA endemic	Perennial	6 (99)
<i>Danthonia californica</i>	Bodega Bay	Native	Perennial	1 (22)
<i>Elymus elymoides</i>	Colfax, Colfax-Summit, Elk Creek (2), Lower Lake, Napa, North El Dorado	Native	Perennial	7 (110)
<i>E. glaucus</i>	Bodega Bay, Colfax, Colfax-Summit (2), Davis (2), Elk Creek, Lower Lake (3), Napa (3), Nevada City, Oroville (3), Penn Valley, San Luis Obispo, Santa Margarita	Native	Perennial	20 (323)
<i>E. multisetus</i>	Anza, North San Juan	Native	Perennial	2 (13)
<i>E. stebbinsii</i>	Colfax, Idyllwild, Nevada City	CA endemic	Perennial	1 (26)
<i>E. trachycaulus</i>	Anza, Gridley, Julian (2), Willows	Native	Perennial	5 (94)
<i>Festuca californica</i>	Chico, Lasslys, Lower Lake (3), Napa (3)	WC endemic	Perennial	8 (181)
<i>F. occidentalis</i>	Oroville, Santa Margarita	Native	Perennial	2 (25)
<i>Holcus lanatus</i>	Bodega Bay (2)	Invasive	Perennial	2 (56)
<i>Hordeum brachyantherum</i>	Bodega Bay	Native	Perennial	1 (5)
<i>H. murinum</i>	Chico, Davis	Invasive	Annual	2 (33)
<i>Leymus triticoides</i>	Davis	Native	Perennial	1 (20)
<i>Lolium multiflorum</i>	Carmel Valley, Davis	Invasive	Annual/biennial	2 (35)
<i>L. perenne</i>	Bradley, Chico	Non-native	Perennial	2 (35)
<i>Melica californica</i>	Colfax-Summit, Elk Creek, Napa, Oroville, Willows	CA endemic	Perennial	5 (68)
<i>M. geyeri</i>	Elk Creek	Native	Perennial	1 (20)
<i>M. torreyana</i>	Napa	CA endemic	Perennial	1 (10)
<i>Nassella pulchra</i>	Bradley (2), Fremont, Livermore (2), Lower Lake, Napa	WC endemic	Perennial	7 (128)
<i>Poa bolanderi</i>	Banning, Idyllwild, Mountain Center, Santa Margarita	Native	Annual	4 (91)
<i>P. bulbosa</i>	Colfax	Non-native	Perennial	1 (15)
<i>P. douglasii</i>	Bodega Bay	CA endemic	Perennial	1 (8)
<i>P. secunda</i>	Colfax, Elk Creek (2), Napa (3), Nevada City, North El Dorado, Oroville, Willows	Native	Perennial	10 (224)
<i>P. unilateralis</i>	Bodega Bay	WC endemic	Perennial	1 (10)
<i>Vulpia microstachys</i>	Napa	Native	Annual	1 (20)
<i>V. myuros</i>	Napa	Invasive	Annual	1 (22)

a Number of populations examined within each city/town indicated in () if greater than one.

b Native status and host reproductive strategy based on www.cal-ipc.org, www.calflora.org, and <http://plants.usda.gov>. "WC endemic" indicates West Coast endemic.

c Number of populations examined with total number of plants examined across all populations of the species in ().

three species identified to host endophyte were *Achnatherum speciosum* (Desert needlegrass), *Bromus laevipes* (Chinook brome), and *Festuca idahoensis* (Idaho fescue) (Table 2). *A. speciosum* and *F. idahoensis* are native grasses that grow throughout western North America, while *B. laevipes* is endemic to the west coast (mostly occurring in California; Hickman 1993). For the species I surveyed most broadly,

B. laevipes, I observed substantial variation in endophyte frequency among populations. However, the majority of populations were fixed for endophyte status (i.e. 0 % or 100 % symbiotic) and endophyte frequency was greater in the Coast and Coast Range regions than in the Sierra Nevada, which had only 11.5 % of populations with endophyte (Table 2). Symbiont frequency also varied among the three *A. speciosum*

Table 2 – California grass species that host fungal endophytes

Species	Locations	Region	Sample size	% E+ samples
<i>Achnatherum speciosum</i> (native, perennial)	Mountain Center	Peninsular Range	26	84.6
	Mountain Center	Peninsular Range	13	84.6
	Mountain Center	Peninsular Range	11	63.6
<i>Bromus laevipes</i> (West Coast endemic, annual/perennial)	Alamo	Coastal Range	30	0.0
	Alleghany	Sierra Nevada	20	0.0
	Alleghany	Sierra Nevada	20	0.0
	Branscomb	Coast	4	0.0
	Branscomb	Coast	22	0.0
	Branscomb	Coast	4	0.0
	Carmel Valley	Coast	5	100.0
	Carmel Valley	Coast	10	90.0
	Carmel Valley	Coast	21	71.4
	Carmel Valley	Coast	7	100.0
	Carmel Valley	Coast	18	88.9
	Carmel Valley	Coast	11	100.0
	Chico	Sierra Nevada	28	0.0
	Chico	Sierra Nevada	20	0.0
	Chico	Sierra Nevada	15	0.0
	Chico	Sierra Nevada	15	0.0
	Clearlake Oaks	Coastal Range	18	0.0
	Clearlake Oaks	Coastal Range	19	0.0
	Clearlake Oaks	Coastal Range	7	0.0
	Clearlake Oaks	Coastal Range	14	100.0
	Clearlake Oaks	Coastal Range	47	0.0
	Clearlake Oaks	Coastal Range	22	100.0
	Clearlake Oaks	Coastal Range	9	33.3
	Clearlake Oaks	Coastal Range	7	100.0
	Colfax	Sierra Nevada	23	39.1
	Colfax	Sierra Nevada	9	100.0
	Colfax	Sierra Nevada	24	0.0
	Colfax	Sierra Nevada	24	0.0
	Colfax	Sierra Nevada	8	25.0
	Diablo Range	Coastal Range	12	0.0
	El Dorado	Sierra Nevada	10	0.0
	El Dorado	Sierra Nevada	10	0.0
	Elk Creek	Coastal Range	38	0.0
	Elk Creek	Coastal Range	28	0.0
	Elk Creek	Coastal Range	39	41.0
	Elk Creek	Coastal Range	10	0.0
	Elk Creek	Coastal Range	20	0.0
	Elk Creek	Coastal Range	20	0.0
	Elk Creek	Coastal Range	14	0.0
	Elk Creek	Coastal Range	33	0.0
	Elk Creek	Coastal Range	42	66.7
Elk Creek	Coastal Range	19	0.0	
Elk Creek	Coastal Range	27	0.0	
Elk Creek	Coastal Range	51	49.0	
Elk Creek	Coastal Range	20	0.0	
Elk Creek	Coastal Range	6	0.0	
Elk Creek	Coastal Range	29	58.6	
Foresthill	Sierra Foothills	15	0.0	
Foresthill	Sierra Foothills	20	0.0	
Foresthill	Sierra Foothills	20	0.0	
Lower Lake	Coastal Range	35	74.3	
Lower Lake	Coastal Range	16	37.5	
Lower Lake	Coastal Range	13	0.0	
Lower Lake	Coastal Range	16	0.0	
Lower Lake	Coastal Range	20	0.0	
Lucerne	Coastal Range	19	94.7	
Lucerne	Coastal Range	20	100.0	
Lucerne	Coastal Range	20	0.0	

(continued on next page)

Table 2 – (continued)

Species	Locations	Region	Sample size	% E+ samples
	Lucerne	Coastal Range	8	87.5
	Lucerne	Coastal Range	24	100.0
	Magalia	Sierra Nevada	22	0.0
	Magalia	Sierra Nevada	18	0.0
	Napa	Coastal Range	13	0.0
	Napa	Coastal Range	25	0.0
	Napa	Coastal Range	5	0.0
	Napa	Coastal Range	13	0.0
	Napa	Coastal Range	21	0.0
	Napa	Coastal Range	13	0.0
	Napa	Coastal Range	12	0.0
	Napa	Coastal Range	14	0.0
	Nevada City	Sierra Nevada	15	0.0
	Nevada City	Sierra Nevada	12	0.0
	Nice	Coastal Range	20	100.0
	Nice	Coastal Range	30	100.0
	North San Juan	Sierra Nevada	20	0.0
	Oroville	Sierra Nevada	21	0.0
	Penn Valley	Sierra Nevada	19	0.0
	Penn Valley	Sierra Nevada	6	0.0
	Placerville	Sierra Nevada	12	0.0
	Somerset	Sierra Nevada	25	0.0
	Stonyford	Coastal Range	20	0.0
	Stonyford	Coastal Range	16	100.0
	Stonyford	Coastal Range	30	76.7
	Stonyford	Coastal Range	36	27.8
	Stonyford	Coastal Range	26	100.0
	Stonyford	Coastal Range	10	0.0
	Ukiah	Coast	13	76.9
	Ukiah	Coast	12	100.0
	Upper Lake	Coastal Range	18	72.2
	Willows	Coastal Range	68	48.5
<i>Festuca idahoensis</i> (native, perennial)	Napa	Coastal Range	40	0.0
	Napa	Coastal Range	14	0.0
	Nevada City	Sierra Nevada	6	0.0
	Nevada City	Sierra Nevada	15	100.0

populations but generally remained high (Table 2). Endophytes were absent from three populations of *F. idahoensis* (two Coast Range populations and one Sierra Nevada population) but were present in 100 % of plants from another Sierra Nevada population (Table 2).

A. speciosum and *B. laevipes* were not previously known as endophyte hosts but are in genera that commonly host endophytes in other regions. While White & Cole (1986) examined herbarium specimens of *B. laevipes* without finding endophyte, to my knowledge, no specimens of *A. speciosum* have been examined previously. Endophyte symbiosis has previously been documented for *F. idahoensis*. White & Cole (1985) did not find endophyte in herbarium specimens of *F. idahoensis* from San Francisco, California as well as Colorado, Montana, and Wyoming, but Siegel et al. (1995) found endophytes in 2 of 14 seed accessions. The endophytes appear to be exclusively vertically transmitted for all three species as no stromata were ever observed in the field or greenhouse (across multiple years) and offspring produced by symbiotic parents were generally symbiotic. For example, I have examined >4 000 *B. laevipes* plants from >200 populations in nature and >8 000 plants from ~40 populations in the greenhouse and never observed any indication of

horizontal transmission (i.e. no stromata on E+ plants nor endophytic offspring produced by E- neighboring plants in common gardens). Further, when I grew seeds from three Coast Range populations of *B. laevipes*, 100 % of offspring produced by parents with endophyte also had endophyte (20 plants/population and 5 seedlings/plant were scored).

Mediterranean climate

Interestingly, the proportion of genera and species of California grasses with endophyte was more similar to the findings of surveys conducted in distant regions of the world (e.g. Australia, France, Turkey, Spain, and New Zealand) than to surveys conducted in the geographically closest survey regions (i.e. Texas, Indiana, and Illinois) (Fig 1). The proportion of species with endophyte was ~88–89 % less in the survey of California than in these other N. American surveys. These findings suggest that a factor that may be important in explaining the low frequency of endophyte in California is its Mediterranean climate, which is a key feature that not only distinguishes the California floristic province from the rest of North America, but is also present in four of the five regions with similar frequencies of endophyte (i.e. Australia, France, Turkey, and Spain).

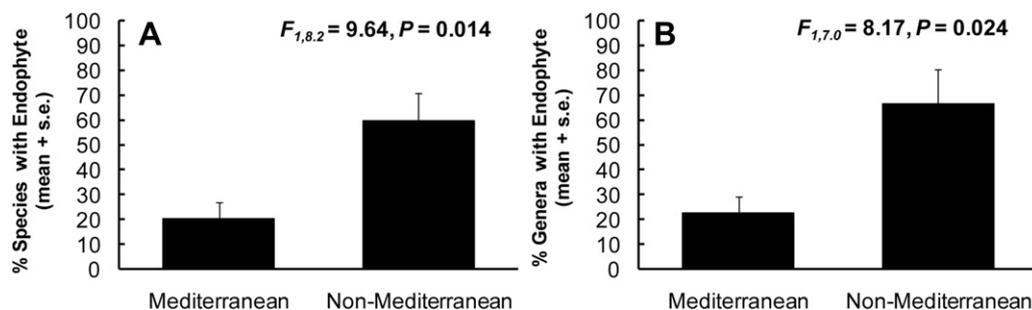


Fig 2 – Frequency of endophyte symbiosis in Mediterranean-influenced versus non-Mediterranean climates. The mean percentage of species (A) and genera (B) with endophyte is significantly lower in Mediterranean climate.

Surveys conducted in Mediterranean climates found ~66% lower proportion of grass species hosting endophyte than surveys conducted in non-Mediterranean climates (Fig 2A; $F_{1,8.2} = 9.64$, $P = 0.014$). Similarly, Mediterranean surveys had a ~65% lower proportion of grass genera hosting endophyte (Fig 2B; $F_{1,7.0} = 8.17$, $P = 0.024$). The significantly lower frequency of grass species and genera with endophyte in Mediterranean-influenced climates suggests that the dry summers and wet winters of a Mediterranean climate may select against grass–endophyte symbioses. Another possible explanation for lower endophyte frequencies in Mediterranean regions is historical differences in plant taxa between climate types (with clades of non-Mediterranean grasses more likely to host endophytes than clades of Mediterranean grasses). However, Mediterranean regions still had a 61% lower frequency of endophytes when roughly controlling for phylogenetic differences between climate types at the genus-level ($t_{1,20} = 3.35$; $P = 0.003$; paired *t*-test that compared the percentages of species hosting endophyte in genera represented in both climate types). Further, I found no obvious phylogenetic autocorrelation between climate type and endophyte status when I mapped them onto a grass supertree (Salamin et al. 2002; see Online Appendix 2, Fig 1), nor did I detect a significant phylogenetic signal in the character distribution of either trait on the phylogeny ($P \geq 0.8$ for both traits; see Online Appendix 2 for details). These analyses suggest that the lower frequency of endophytes in Mediterranean climates is unlikely to result from plant clades with endophyte simply being more common in non-Mediterranean climates, but when more survey and phylogenetic data becomes available, a more rigorous examination of the relationship between endophyte and climate in a phylogenetic context would be valuable.

While my analyses found endophyte frequency to be generally lower in Mediterranean climates, Clement et al. (2001) found high frequencies of endophytes in germplasm collections of tall fescue from a Mediterranean region (i.e. Tunisia, Morocco, and Sardinia), indicating that endophyte can be prevalent in some cases. The high endophyte frequency may be unique to this forage species in which the endophyte-mediated benefits have been shown to be extraordinary (Saikkonen et al. 2006). Clearly, much more work is needed to know whether Mediterranean climate selects against grass endophyte symbioses, and what may be the underlying mechanisms for this pattern. To further explore this pattern in nature, we need to conduct more surveys that look across many genera and species in both new

Mediterranean (e.g. central Chile, western Australia, and southwestern South Africa) and non-Mediterranean regions. These data would greatly aid in a more rigorous study of phylogenetic correlation between endophyte symbiosis and climate type (as well as other environmental factors). Phylogenetic analyses could be used to distinguish among alternative explanations for the lower frequency of endophyte in Mediterranean climates and could serve as a valuable contribution to our understanding of the factors affecting endophyte symbiosis at a large scale. Additionally, greenhouse or growth chamber experiments manipulating water and/or temperature could be used to investigate possible mechanisms underlying this pattern, and field experiments could directly measure selection on endophytes and symbiotic plants in regions with Mediterranean and other climates. Some such experiments are currently underway for *B. laevipes* in California.

Conclusions

In this study, I demonstrated that Class 1 endophyte–grass symbioses are less common in the California floristic province than expected based on global frequencies of symbiosis, and that regions with Mediterranean-influenced climates generally have lower frequencies of endophytes, suggesting that this climate type may select against endophytes or endophyte-symbiotic plants. While previous greenhouse and lab research investigating the relationship between endophyte presence and aspects of climate (e.g. water availability and to a lesser extent temperature; Breen 1992; Clay & Schardl 2002; Ju et al. 2006; Kannadan & Rudgers 2008; Rudgers & Swafford 2009) has been invaluable, to gain a complete understanding of climate's role, research investigating this relationship at a large geographic scale is also needed. Given current concern with global climate change, developing a thorough understanding of how climate affects fungal endophytes and other important plant symbionts is crucial.

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Supplementary data

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REFERENCES

- Aldous DE, Isaacs S, Mebalds MI, 1999. Endophytes in the genus *Neotyphodium* are not found in Australian native grasses. *Australasian Plant Pathology* **28**: 183–186.
- Bacon CW, White Jr JF, 1994. Stains, media, and procedures for analyzing endophytes. In: Bacon CW, White Jr. JF (eds), *Biotechnology of Endophytic Fungi of Grasses*. CRC Press Boca Raton, FL, USA, pp. 47–56.
- Breen J, 1992. Temperature and seasonal effects on expression of acremonium endophyte-enhanced resistance to *Schizaphis graminum* (Homoptera: Aphididae). *Environmental Entomology* **21**: 68–74.
- Clay K, Holah J, 1999. Fungal endophyte symbiosis and plant diversity in successional fields. *Science* **285**: 1742–1744.
- Clay K, Holah J, Rudgers JA, 2005. Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 12465–12470.
- Clay K, Leuchtman A, 1989. Infection of woodland grasses by fungal endophytes. *Mycologia* **81**: 805–811.
- Clay K, Scharndl C, 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *The American Naturalist* **160**: S99–S127.
- Clement SL, Elberson LR, Youssef NN, Davitt CM, Doss RP, 2001. Incidence and diversity of *Neotyphodium* fungal endophytes in tall fescue from Morocco, Tunisia, and Sardinia. *Crop Science* **41**: 570–576.
- Dombrowski JE, Baldwin JC, Azevedo MD, Banowitz GM, 2006. A sensitive PCR-based assay to detect *Neotyphodium* fungi in seed and plant tissue of tall fescue and ryegrass species. *Crop Science* **46**: 1064–1070.
- Hickman JC, 1993. *The Jepson Manual: higher plants of California*. University of California Press, Berkeley, CA.
- Hughes RF, Denslow JS, 2005. Invasion by a N_2 -fixing tree alters function and structure in a wet lowland forest of Hawaii. *Ecological Applications* **15**: 1615–1628.
- JMP. Version 8. SAS Institute Inc., Cary, NC, 2007–2010.
- Ju H-J, Hill NS, Abbott T, Ingram KT, 2006. Temperature influences on endophyte growth in tall fescue. *Crop Science* **46**: 404–412.
- Kannadan S, Rudgers JA, 2008. Endophyte symbiosis benefits a rare grass under low water availability. *Functional Ecology* **22**: 706–713.
- Leuchtman A, 1992. Systematics, distribution, and host specificity of grass endophytes. *Natural Toxins* **1**: 150–162.
- Leyronas C, Raynal G, 2001. Presence of *Neotyphodium*-like endophytes in European grasses. *Annals of Applied Biology* **139**: 119–127.
- Malinowski DP, Alloush GA, Belesky DP, 2000. Leaf endophyte *Neotyphodium coenophialum* modifies mineral uptake in tall fescue. *Plant and Soil* **227**: 115–126.
- Novas VM, Collantes M, Cabral D, 2007. Environmental effects on grass–endophyte associations in the harsh conditions of south Patagonia. *FEMS Microbiology Ecology* **61**: 164–173.
- Panaccione DG, Johnson RD, Wang J, Young CA, Damrongkool P, Scott B, Scharndl CL, 2001. Elimination of ergovaline from a grass–*Neotyphodium* endophyte symbiosis by genetic modification of the endophyte. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 12820–12825.
- Rodriguez RJ, White Jr JF, Arnold AE, Redman RS, 2009. Fungal endophytes: diversity and functional roles. *New Phytologist* **182**: 314–330.
- Rolston MP, Stewart AV, Latch GCM, Hume DE, 2002. Endophytes in New Zealand grass seeds: occurrence and implications for conservation of grass species. *New Zealand Journal of Botany* **40**: 365–372.
- Rudgers JA, Clay K, 2008. An invasive plant–fungal mutualism reduces arthropod diversity. *Ecology Letters* **11**: 831–840.
- Rudgers JA, Holah J, Orr SP, Clay K, 2007. Forest succession suppressed by an introduced plant–fungal symbiosis. *Ecology* **88**: 18–25.
- Rudgers JA, Swafford AL, 2009. Benefits of a fungal endophyte in *Elymus virginicus* decline under drought stress. *Basic and Applied Ecology* **10**: 43–51.
- Saikkonen K, Ahlholm J, Helander M, Lehtimäki S, Niemeläinen O, 2000. Endophytic fungi in wild and cultivated grasses in Finland. *Ecography* **23**: 360–366.
- Saikkonen K, Lehtonen P, Helander M, Koricheva J, Faeth SH, 2006. Model systems in ecology: dissecting the endophyte–grass literature. *Trends in Plant Science* **11**: 428–433.
- Salamin N, Hodkinson TR, Savolainen V, 2002. Building supertrees: an empirical assessment using the grass family (Poaceae). *Systematic Biology* **51**: 136–150.
- Siegel MR, Scharndl CL, Phillips TD, 1995. Incidence and compatibility of Nonclavicipitaceous fungal endophytes in *Festuca* and *Lolium* grass species. *Mycologia* **87**: 196–202.
- Spyreas G, Gibson DJ, Basinger M, 2001. Endophyte infection levels of native and naturalized fescues in Illinois and England. *Journal of the Torrey Botanical Society* **128**: 25–34.
- Trento S, Elias S, Garay A, Zavala J, 2007. Comparison of endophyte detection in fescue and ryegrass seeds using an immunoblot assay and a microscopic method. *Seed Science and Technology* **35**: 65–74.
- Tunali B, Shelby R, Morgan-Jones G, Kodan M, 2000. Endophytic fungi and ergot alkaloids in native Turkish grasses. *Phytoparasitica* **28**: 375–377.
- Uliassi DD, Ruess RW, 2002. Limitations to symbiotic nitrogen fixation in primary succession on the Tanana River Floodplain. *Ecology* **83**: 88–103.
- van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR, 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69–72.
- Wei YK, Gao YB, Xu H, Su D, Zhang X, Wang YH, Lin F, Chen L, Nie LY, Ren AZ, 2006. Occurrence of endophytes in grasses native to northern China. *Grass and Forage Science* **61**: 422–429.
- White Jr JF, Cole GT, 1985. Endophyte–host associations in forage grasses. I. Distribution of Fungal endophytes in some species of *Lolium* and *Festuca*. *Mycologia* **77**: 323–327.
- White Jr JF, Cole GT, 1986. Endophyte–host associations in forage grasses. V. Occurrence of fungal endophytes in certain species of *Bromus* and *Poa*. *Mycologia* **78**: 846–850.
- Zabalgoeazcoa I, De Aldana BRV, Ciudad AG, Criado BG, 2003. Fungal endophytes in grasses from semi-arid permanent grasslands of western Spain. *Grass and Forage Science* **58**: 94–97.