

Local Adaptation in *Festuca arizonica* Infected by Hybrid and Nonhybrid *Neotyphodium* Endophytes

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Abstract Cool-season grasses often harbor obligate fungal symbionts from the genus *Neotyphodium*, and these symbiota can function as a single ecological unit. Previous studies have shown that gene flow in *Neotyphodium* in *Festuca arizonica* is low enough such that populations could diverge and form local adaptations. A reciprocal transplant experiment was performed between two *F. arizonica/Neotyphodium* populations in Arizona, Clint's Well and Flagstaff, using symbiota with the most common *Neotyphodium* genotypes in each population, to test for local adaptations. The genetic difference between populations is potentially large as *Neotyphodium* from Clint's Well are of hybrid origin. Local environmental variation was the most important source of variation for *F. arizonica/Neotyphodium* symbiota growth, with individuals at Flagstaff growing larger and individuals at Clint's Well not reproducing. Local environment and the source population of the symbiota interacted to affect vegetative growth. Symbiota from Clint's Well, which harbor hybrid *Neotyphodium*, had higher volume/wet mass and volume/dry mass ratios but only in the marginal Clint's Well habitat. The local environment also affected *F. arizonica/Neotyphodium* reproduction because only symbiota transplanted to Flagstaff reproduced. Symbiota from Clint's Well produced more panicles, whereas symbiota from Flagstaff with nonhybrid *Neotyphodium* produced greater seed mass per panicle. Overall seed mass production was not

different, suggesting that the two strategies are functionally equivalent. We find that *F. arizonica/Neotyphodium* symbiota vary geographically, but potential local adaptations are only apparent in marginal habitats and may be related to the evolutionary history of the *Neotyphodium* part of the symbiota.

Introduction

The observation that interspecific interactions vary depending upon environmental and genetic factors led to the development of the geographical mosaic theory of coevolution [1]. This theory posits that interactions evolve in metapopulations, with the interaction between local selection pressures, migration, and drift creating a range of possible outcomes including stable and dynamic antagonisms and mutualisms and equilibrium points where both may exist [2–6].

Recent empirical studies of the coevolutionary process support the importance of genetic and environmental variation on interspecific interactions. For example, seed production by *Amphicarpaea bracteata* (Leguminosae) is strongly affected by the genotype of its symbiotic bacteria *Bradyrhizobium* [7, 8], and the interaction between the fungus *Colletotrichum magna* and their plant hosts can change from mutualistic to parasitic by a mutation at a single locus [9]. Environmental variation can switch mutualistic interactions into parasitic ones [10, 11], and changes in population demographics [12–14] can also change the outcomes of interspecific interactions.

In this study, we tested variation in a grass/endophyte symbiosis in two environments. *Neotyphodium* endophytes are obligate, systemic, asexual, and vertically transmitted symbionts of the grass subfamily Pooideae and are closely related to the *Epichloë* endophytes. Because the interaction

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is vertically transmitted, the reproductive success for both plant and endophyte are directly linked, and an individual host will carry the same symbiotic *Neotyphodium* for its entire life. As such, an individual host/*Neotyphodium* combination (hereafter, we use symbiotum to designate the host/endophyte combination) will function as a single ecological unit. In some host species, *Neotyphodium* provides its hosts with a suite of herbivore-detering alkaloids making the interaction highly mutualistic [15]. Herbivore deterrence is particularly well studied in the agronomic grasses tall fescue (*Lolium arundinaceum*=*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*). *Neotyphodium* does not produce alkaloids at herbivore-detering levels in all its hosts, however [16]. Some *Neotyphodium* have also been shown to benefit their hosts by increasing drought resistance [17–20], herbicide resistance [21], and by increasing phosphorous [22, 23] and nitrogen uptake [24], although it is not known if these benefits are as variable between hosts as alkaloid production. These benefits are also context dependent; both environmental and genetic factors alter the outcome [25]. *Neotyphodium*'s effects on its hosts can be altered by nutrient availability [26, 27], herbivory [28], the surrounding community [29], and by genetic interactions between the host and endophyte [21, 30–32]. These variable outcomes because of genetic interactions are often seen in native grass–endophyte associations leading to variation in herbivore deterrence [33, 34], geographic variation in the symbiosis [35], nonrandom host–endophyte genotype combinations [36], and local adaptations [37]. These variable outcomes can even produce grass–endophyte combinations with negative outcomes for the grass host [32, 38, 39].

Genetic variation within the genus *Neotyphodium* is likely to be large because about two thirds of all known species are the result of hybridization between either two *Epichloë* spp. (a closely related, sexually reproducing grass choke pathogen) or a *Neotyphodium* sp. and *Epichloë* sp., presumably via a parasexual process [40, 41]. Hybridization has occurred frequently, which is unusual for fungi [42], and some individuals within host species can harbor either hybrid or nonhybrid endophytes [43–46], sometimes within the same host population [47]. Hybridization is hypothesized to create new asexual vertically transmitted *Neotyphodium* spp. because of the new hybrid's inability to form ascospores [48], and these new endophyte species could then increase in frequency by providing a selective advantage either by masking deleterious mutations (avoiding Müller's ratchet) and/or the acquisition of new genes or gene combinations that increase benefits to their hosts [42, 49, 50]. However, to our knowledge, the hypothesis that hybrid *Neotyphodium* provides a selective advantage has not yet been tested other than the observation that such hybrids are common.

Festuca arizonica is the predominant range grass in the semiarid grasslands of the southwestern USA [51].

Neotyphodium commonly inhabits *F. arizonica* with many populations having more than 90% of the individuals infected, although the infection rate is variable (60–100% [52]). Alkaloids are generally produced at low and variable levels [31], such that neither vertebrate [53] nor invertebrate [53–55] herbivores are deterred. Three distinct genotypes of *Neotyphodium* have been found, and one of them is of hybrid origin [47, 56, 65]. The geographic distribution of these genotypes is highly structured [47].

To test for geographic variation in the *F. arizonica*/*Neotyphodium* symbiotum, we reciprocally transplanted symbiota from two populations; one of which has relatively high soil nitrogen and precipitation (designated Flagstaff), whereas the other is relatively nitrogen and moisture poor (Clint's Well). These sites also differ in the evolutionary history of the *Neotyphodium* part of the symbiota. The majority of *Neotyphodium* at Clint's Well is of hybrid origin, whereas all of the *Neotyphodium* at Flagstaff are nonhybrid [47]. Multiple species of *Neotyphodium* have been described in *F. arizonica*, and the *Neotyphodium* used in this study likely correspond to the hybrid *N. tembladerae* [42, 57] and the nonhybrid *N. huerfanum* [42, 58]. We tested how the most common symbiota types in each population, *F. arizonica* with nonhybrid *Neotyphodium* from Flagstaff and *F. arizonica* with hybrid *Neotyphodium* from Clint's Well, vary geographically by reciprocally transplanting these symbiotum types from each population to each respective geographic locale. We then compared relative growth, reproduction, and long-term survival of each type in each geographic location to ascertain whether symbiota are locally adapted.

Materials and Methods

Field Populations

The two populations used were Clint's Well, located in Arizona's Coconino National Forest, and a natural area within the Arboretum at Flagstaff, AZ. These sites are approximately 90 km apart and vary in several abiotic characteristics. The Flagstaff soil is comprised primarily of shallow to moderately deep gravelly clay loam over volcanic cinders and basalt flows [59] and is relatively nutrient rich containing 0.20 mg/100 g soil nitrogen [52], whereas the Clint's Well soil has a loamy surface and clay subsoil moderately deep to deep over limestone and sandstone [59] and contains lower nitrogen concentrations (0.09/100 g soil) [52]. The Clint's Well site is heavily forested with Ponderosa pine (*Pinus ponderosa*), whereas the Flagstaff site is open grassland with few trees. Precipitation as measured at nearby stations is lower at Clint's Well (49.23 cm/year, 37 year average) compared to Flagstaff (53.87 cm/year, 54 year average);

Western Regional Climate Center, <http://www.wrcc.dri.edu/>). When examined by month, the station near the Flagstaff site has a higher mean precipitation in 9 of the 12 months and is significantly higher overall ($p=0.03$, Wilcoxon signed-rank test for paired samples). Both sites receive most of their moisture twice a year, snow melt in the spring from winter precipitation, and summer rains from late July–August.

Experimental Design

Fifty symbiota from each site were randomly selected without *a priori* knowledge of their infection status, or if infected, the genotype of the *Neotyphodium* symbiont, and removed in late May to early June 1997 and taken to the greenhouse at Arizona State University. *F. arizonica* is a perennial bunch grass that can easily be divided into equally sized ramets, so plants were split into approximately equal sections. Two ramets from each plant were weighed and planted in soil either from their native site or from the foreign site. The plants were returned to Clint's Well and Flagstaff in July of 1997, replanted approximately 0.5 m apart, and initial height and diameter measurements were taken. With the exception of two water treatments given in the first 14 days after transplantation to aid establishment, plants received only ambient precipitation. Height and diameter were measured in the fall of 1997 and twice a year thereafter, when the plants broke their winter dormancy (late May/early June) and when the plants reproduced (late August/early September), for 3 years. Plant size was estimated from the formula for a cylinder (volume= $\pi r^2 h$) as the growth form of bunch grasses approximates a cylinder [60]. For a given time point, both populations were measured within 3 days of each other. Panicles (culms with inflorescences) were counted, and seeds were collected at the end of every growing season starting in 1998 as the plants did not produce seeds in their first post-transplant year. Survivorship of each plant was also assessed at this time. A later assessment of long-term survivorship took place in May 2004. In the fall of 2000, the aboveground tissue was removed, the plants were dried at 70°C for 2 days, and dry mass was measured.

During the summer of 1998, each individual was screened for the presence of *Neotyphodium* by culturing leaf samples as described in Schulthess and Faeth [52]. Microsatellites were first used to determine *Neotyphodium* haplotypes (as described in [47]). A hybrid origin was suspected for some of the haplotypes because of the presence of multiple genes for some microsatellite loci, and subsequent analysis of β -tubulin intron sequence (as described in [41]) confirmed these haplotypes as hybrids ([65] Geographic and genetic variation in the *Neotyphodium/Festuca arizonica* interaction). We grouped these haplotypes into a single hybrid group, as all the haplotypes differed by only a

single microsatellite mutation from the most common haplotype [47] and because sequences from the internal transcribed spacer (ITS) region of the ribosomal deoxyribonucleic acid indicate that the hybrid haplotypes are a single clade ([65] Geographic and genetic variation in the *Neotyphodium/Festuca arizonica* interaction). A single, non-hybrid *Neotyphodium* genotype was found in Flagstaff symbiota (hereafter referred to as FL/NH) whereas the Clint's Well population was 81% hybrid and 19% non-hybrid [47]. From the Clint's Well population, only symbiota with hybrid *Neotyphodium* (hereafter referred to as CW/H) were used in the analysis. There are three nonhybrid haplotypes found at Clint's Well [47], and according to both ITS and *b*-tubulin sequences, the hybrid haplotypes are not closely related ([65] Geographic and genetic variation in the *Neotyphodium/Festuca arizonica* interaction). Only nine individuals with nonhybrid *Neotyphodium* from Clint's Well identical to the nonhybrid *Neotyphodium* at Flagstaff survived the entire course of the experiment, and this sample size was deemed too small to be informative.

We analyzed alkaloid levels and types for samples from five CW/H and four FL/NH randomly selected symbiota at the end of the growing season in September 2002. The tissue was freeze dried, and extraction and analysis were done as described in Faeth *et al.* [31]. Samples were analyzed for levels of peramine, the only alkaloid type heretofore found in *F. arizonica/Neotyphodium* [31], and for the presence of loline and ergovaline, two other major alkaloid types known from *Epichloë*- or *Neotyphodium*-infected grasses [61].

Statistical Analysis

The source population and the *Neotyphodium* genotype (hereafter referred to as symbiota type) of the symbiota was treated as a single treatment, acknowledging that variation in this factor could be due to plant genotype, *Neotyphodium* genotype, their interaction, and/or maternal effects. We used repeated-measures analysis of covariance (ANCOVA) [62] to test for effects of local environment, symbiota type on plant volume, panicle number, seed mass, and the seed mass/panicle ratio. Final wet mass, dry mass, volume/wet mass, and volume/dry mass were first analyzed using a multivariate analysis of variance (MANOVA) with local environment, symbiota type, and their interaction as factors and then using analyses of variance (ANOVAs) on each variable individually. The reproductive variables, panicle number, seed mass, and the seed mass/panicle ratio were analyzed using repeated-measures ANCOVA with symbiota type as the factor. Not all individuals were used in the analysis because some were determined to be *Neotyphodium*-free, died during the course of the experiment, or could

Table 1 Repeated-measures ANCOVA and ANCOVA table describing differences in *F. arizonica*/*Neotyphodium* symbiota volume

Effect	Volume		
	df	F	p value
Between subjects			
local environment	1	148.08	<0.0001
Symbiotum type	1	7.30	0.008
Local environment× symbiotum type	1	0.91	0.34
Within subjects			
Time	3.31	11.27	<0.0001
Time×local environment	3.31	125.65	<0.0001
Time×symbiotum type	3.31	1.82	0.14
Time×local environment× symbiotum type	3.31	5.07	0.001
Covariate	1	9.34	0.003
Covariate×time	3.31	4.29	0.004

Values in bold are significant at $\alpha = 0.05$.

not be unambiguously genotyped. Because *Neotyphodium* genotyping was done after the transplanting, the final sample sizes were unequal with 22 CW/H and 33 FL/NH at the Flagstaff site and 21 CW/H and 20 FL/NH at the Clint's Well site. The response variables were non-normally distributed, so the data were Box-Cox transformed. Plant volume was analyzed with repeated-measures ANCOVA using the factors symbiotum type, local environment, and time. Plant volume at the initial transplanting was used as a covariate. Wet mass, dry mass, the volume/wet mass ratio, and the volume/dry mass ratio were analyzed using the same factors in an ANCOVA but not as a repeated measures design, as these variables were only measured once, with the symbiotum mass at the start of the experiment as a covariate. For the tests on reproductive growth and output, only the plants at the Flagstaff site were used because plants at Clint's Well did not reproduce during the course of the experiment. We used ANCOVA with the same factors as above with the exception of local environment. Initial volume was used as a covariate, except in the analysis of the seed mass per panicle ratio.

Results

Local environment had a strong effect on *F. arizonica* growth. All symbiota, regardless of source location, grew better at Flagstaff, the site with higher soil nitrogen and precipitation, than plants at Clint's Well in terms of both volume ($p < 0.0001$; Table 1) and dry biomass ($p < 0.0001$; Table 2). The symbiotum type also significantly affected volume ($p = 0.008$, Table 1) and interacted with the local environment over time ($p = 0.001$, Table 1, Fig. 1).

As expected from the large environmental effects on volume, the local environment was a significant source of variation for variables related to mass for both the overall MANOVA and for the individual ANOVAs for each variable (Table 2). However, despite its effects on plant volume, there was no symbiotum type effect in the overall MANOVA ($p = 0.08$, Table 2). Symbiotum type did, however, interact with the local environment in the overall MANOVA ($p = 0.04$, Table 2) and was a significant source of variation for both the volume/wet mass ($p = 0.01$; Table 2) and volume/dry mass ($p = 0.009$, Fig. 2) ratios. Plants transplanted to Flagstaff were equivalent in both volume/wet mass and volume/dry mass ratios. However, at Clint's Well, CW/H plants had a larger ratio than FL/NH. Whereas CW/H individuals were larger for both height (t test: t ratio = 3.13, $p = 0.002$) and diameter (t test: t ratio = 7.20, $p < 0.0001$) than FL/NH individuals at Clint's Well, the difference in volume is more strongly influenced by the changes in diameter (measurements in fall, 2000: CW/H = 3.42 cm, 95% confidence interval [CI] = 2.63–4.21; FL/NH = 2.31 cm, 95% CI = 1.83–2.80), than height (measurements in fall, 2000: CW/H = 29.15 cm, 95% CI = 25.95–32.36; FL/NH = 26.67 cm, 95% CI = 23.15–30.19). Number of tillers is strongly and positively correlated with biomass [32], suggesting that the CW/H symbiotum have larger volumes or sizes but lower density of tillers per unit volume than the FL/NH symbiotum.

Neither CW/H nor FL/NH symbiota reproduced at Clint's Well. A logistic regression using local environment, symbiotum type, and the associated interaction on *F. arizonica* reproductive success found local environment as the only significant factor (likelihood ratio tests: local environment,

Table 2 MANOVA and individual ANOVAs describing differences in *F. arizonica*/*Neotyphodium* symbiota vegetative growth

Effect	MANOVA			Wet mass			Dry mass			Volume/wet mass			Volume/dry mass		
	df	F	p value	df	F	p value	df	F	p value	df	F	p value	df	F	p value
Local environment	4	77.23	<0.0001	1	221.31	<0.0001	1	227.66	<0.0001	1	26.33	<0.0001	1	35.41	<0.0001
Symbiotum type	4	2.16	0.08	1	0.05	0.83	1	0.39	0.53	1	2.91	0.09	1	1.96	0.16
Local environment× symbiotum type	4	2.63	0.04	1	0.001	0.97	1	0.18	0.67	1	6.20	0.01	1	7.21	0.009

Values in bold are significant at $\alpha = 0.05$.

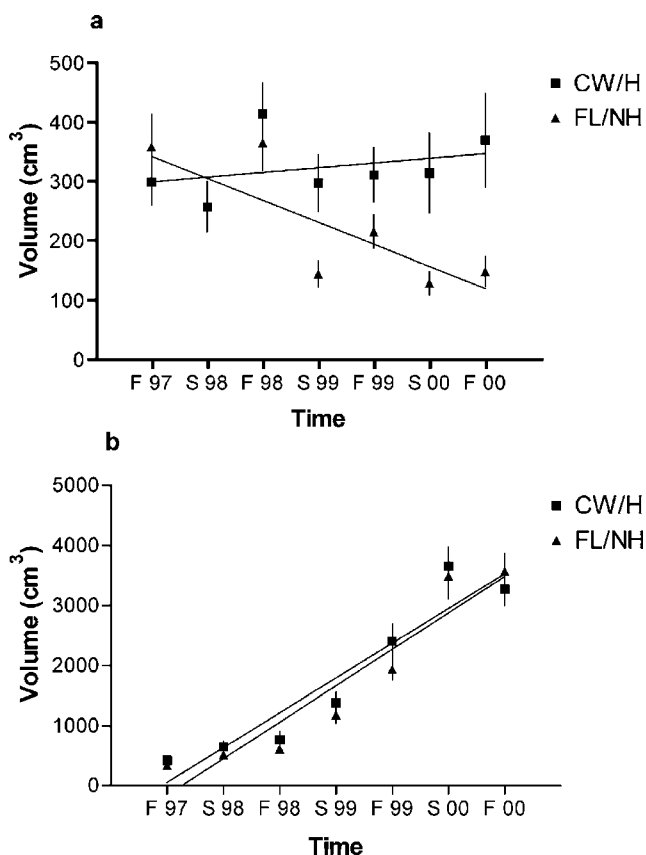


Fig. 1 a, b Difference in mean plant volume (± 1 standard error) between symbiotum types Clint's Well/hybrid (CW/H) and Flagstaff/non-hybrid (FL/NH) over time (Fall or Summer with year) at Clint's Well (a) and Flagstaff (b) shown with a simple linear regression line. The interaction between time, symbiota type, and local environment was significant by repeated-measures ANCOVA ($p=0.001$). 167 \times 234 mm (600 \times 600 DPI)

$p < 0.0001$; symbiotum type, $p=0.99$; local environment \times symbiotum type, $p=0.99$). There was no effect of symbiota type when only symbiota at Flagstaff were considered (likelihood ratio test, symbiotum type, $p=0.80$). Lack of reproduction was likely due to the local environment rather than a consequence of the transplanting process because other naturally occurring *F. arizonica* plants in the area did not reproduce either (Sullivan, personal observation), whereas the symbiota transplanted to Flagstaff did. Therefore, none of the symbiota transplanted to Clint's Well were used in the analysis of reproductive growth. Most symbiota at Flagstaff reproduced. For the ones that did not reproduce, symbiotum type was not a significant factor explaining the lack of reproduction (for panicles, $\chi^2=0.014$, $df=1$, $p=0.91$; for seed production, $\chi^2=0.845$, $df=1$, $p=0.38$). The subsequent analyses were performed only using data from individuals that reproduced in all 3 years (N for CW/H=17, N for FL/NH=23).

Symbiotum type explained some of the variation in reproduction but not in a consistent manner (Table 3).

CW/H individuals produced more panicles than FL/NH individuals ($p=0.04$, Table 3, Fig. 3). However, FL/NH individuals produced a greater mass of seeds per panicle ($p=0.01$, Table 3, Fig. 3). Reproduction varied over time (1998: mean number of panicles (± 1 standard error)= 20.15 ± 2.12 ; mean seed mass= 1.53 ± 0.19 g; 1999: mean number of panicles= 55.0 ± 5.34 ; mean seed mass= 3.24 ± 0.32 g; 2000: mean number of panicles= 44.86 ± 4.47 ; mean seed mass= 1.47 ± 0.18 g) probably because of accumulating growth and environmental variation between years.

Peramine alkaloid levels for *F. arizonica* infected with either hybrid (1.96 ppm ± 1.05 [1 SD]) and nonhybrid (1.48 ppm ± 2.45) *Neotyphodium* strains were consistent with levels previously found for this symbiosis [31], which are likely too low to have any herbivore-detering effect [61].

Discussion

We found geographic differences between populations of *F. arizonica*/*Neotyphodium* symbiota, but these differences are most pronounced in the more marginal habitat of Clint's Well and suggest that the native symbiota are locally adapted. The architectural difference in *F. arizonica* vegetative growth appears to be due to larger sizes but reduced tiller density in the CW/H symbiotum given that volumes or sizes were greater but biomass was not different than the FL/NH symbiotum. We do not know if altered plant architecture translates into increased persistence or reproduction for CW/H symbiota in marginal habitats such as Clint's Well.

This experiment could not test the effects of *Neotyphodium* hybridization independently of the original populations of

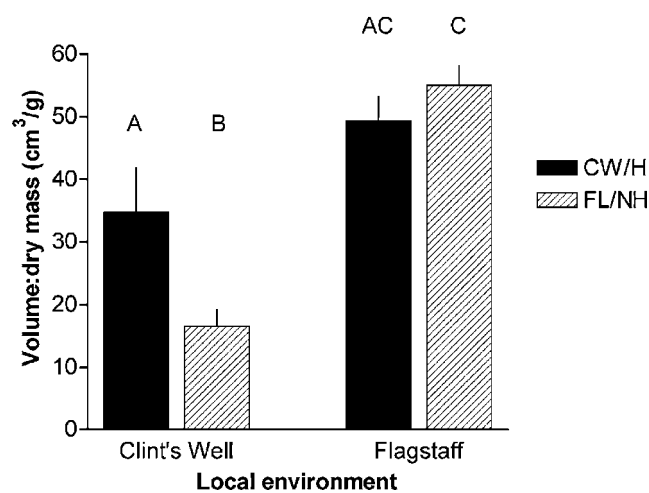


Fig. 2 Interaction between symbiotum type and the local environment (Clint's Well [CW] and Flagstaff [FL]) on mean symbiotum volume/dry mass ratio (± 1 standard error). The interaction was significant by ANCOVA ($p=0.01$). Differences in pairwise comparisons by Tukey's HSD are indicated by letters above bars

Table 3 Repeated measures ANCOVA table describing *F. arizonica*/*Neotyphodium* symbiota reproductive output

Effect	Panicles			Seed mass			Seed mass/panicle		
	df	F	p value	df	F	p value	df	F	p value
Between subjects									
Symbiotum type	1	4.40	0.04	1	0.14	0.71	1	7.35	0.01
Within subjects									
Time	1.56	24.20	<0.0001	1.59	16.17	<0.001	1.71	13.73	<0.0001
Time×symbiotum type	1.56	2.68	0.08	1.59	0.45	0.59	1.71	2.78	0.08
Covariate	1	6.69	0.01	1	3.98	0.05	1	0.001	0.97
Time×covariate	1.56	0.61	0.52	1.59	0.78	0.44	1.71	0.04	0.94

Initial volume is used as a covariate. Only plants transplanted to the Flagstaff site that reproduced in all 3 years were used in the analysis. Within subject tests use the Geisser–Greenhouse conservative *F* test, as the assumption of sphericity was not met. Values in bold are significant at $\alpha = 0.05$.

the symbiota, as the Flagstaff population does not contain any symbiota with hybrid *Neotyphodium*. The prevalence of hybridization in *Neotyphodium* is hypothesized to be due to the fitness advantage of gaining novel genes for defense (e.g. [42, 50]), although this hypothesis has heretofore not been tested. This hypothesis probably does not explain the presence of hybrid *Neotyphodium* in *F. arizonica* as it does not produce either increased levels or different types of alkaloids, but then again, herbivory is not an important source of selection for *F. arizonica* [16]. Alternative theories on hybridization predict that it may be most advantageous in marginal habitats where each respective hybridizing population is at the edge of its range [50, 63]. This hypothesis would better explain the distribution of hybrid *Neotyphodium* in *F. arizonica*, although tests relating hybrid *Neotyphodium* to specific fitness advantages (e.g., drought tolerance, nutrient uptake, herbivory) still need to be done.

There are several possibilities for selective advantages for CW/H at Clint's Well. The increased volume might aid in future reproduction. For the symbiotum transplanted to Flagstaff, volume is positively correlated with reproduction

(Kendall's tau between plant volume and panicle number = 0.449, $n=224$, $p<0.0001$; Kendall's tau between plant volume and seed mass = 0.261, $n=224$, $p<0.0001$). In the marginal conditions at Clint's Well, CW/H may reach the minimum size required for reproduction more quickly. Alterations in architecture (i.e., volume to mass ratio) may also influence photosynthesis via differences in light capture or may be associated with different nitrogen levels [64]. It is interesting to note that the Clint's Well site is forested, and *F. arizonica* grows underneath tall and often dense Ponderosa pine trees. Alternatively, the Flagstaff site is generally unforested, open grassland. Increased volume at the expense of tiller density may be advantageous at Clint's Well to maximize photosynthesis under lower light conditions. In addition, nitrogen acquisition can also be affected by the presence of *Neotyphodium* [24], although the role that genetic variation via hybridization may play in this process is not known. It is interesting to note that *F. arizonica* plants associated with hybrid *Neotyphodium* are also common in another site in Arizona, Buck Springs [47] that is also nitrogen poor [52], suggesting that for *F. arizonica*/*Neotyphodium*, the occasional genetic input from a pathogen may help the symbiota acquire genetic diversity providing them an advantage in marginal habitats because of the greater possibility for local adaptations.

Symbiota with hybrid *Neotyphodium* are not found naturally at Flagstaff, the population with higher soil nitrogen and precipitation [47], although transplanted symbiota from Clint's Well with hybrid *Neotyphodium* grew as well or better at Flagstaff than native symbiota. Previous work on the *Neotyphodium* gene flow between Clint's Well and Flagstaff indicates that *Neotyphodium* migration between Flagstaff and Clint's Well is low [47]. As such, rare migrant seeds carrying hybrid symbionts to Flagstaff may be lost because of stochastic processes. Another interesting possibility is that CW/H plants transplanted to Flagstaff

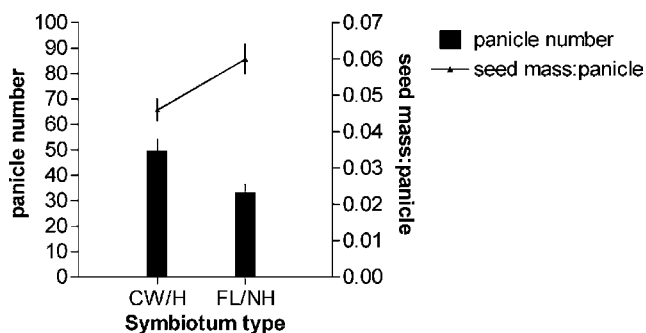


Fig. 3 Effects of symbiotum type on *F. arizonica* panicle number (bars, left y-axis; $p=0.04$) and seed mass:panicle (line, right y-axis; $p=0.01$). Overall seed mass production was not significantly affected ($p=0.14$). All error bars are ± 1 standard error

experience pollen limitation or incompatibility, given that they produced more panicles than nonhybrid infected plants but lowered seed mass per panicle (Fig. 3). The Flagstaff site is composed of only plants infected with a single nonhybrid *Neotyphodium* strain. Because *Neotyphodium* is strictly maternally transmitted, this may indicate that the Flagstaff population of *F. arizonica* is relatively narrow genetically (at least for maternal host plant genotype), and cross-pollination with other *F. arizonica* genotypes is less likely. If so, then pollen limitation or incompatibility may limit success of invading symbiote with hybrid *Neotyphodium*, providing another mechanism that maintains the high genetic structuring of *Neotyphodium* populations [47].

Coevolutionary theory predicts that interspecific interactions should vary between populations because of specific genotypes interacting with varying environments creating local adaptations. In this study, we find that there is variation in *F. arizonica*/*Neotyphodium* symbiote based on their native population, the environment in which they grew, and the interaction between these factors with symbiote from a marginal habitat having an advantage in a marginal habitat. We suggest that these differences may be related to the hybrid origin of the *Neotyphodium* genotype commonly found at Clint's Well.

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