RESEARCH ARTICLE

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BRIEF COMMUNICATION

Intraspecific polyploidy correlates with colonization by arbuscular mycorrhizal fungi in *Heuchera cylindrica*

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PREMISE: Polyploidy is known to cause physiological changes in plants which, in turn, can affect species interactions. One major physiological change predicted in polyploid plants is a heightened demand for growth-limiting nutrients. Consequently, we expect polyploidy to cause an increased reliance on the belowground mutualists that supply these growth-limiting nutrients. An important first step in investigating how polyploidy affects nutritional mutualisms in plants, then, is to characterize differences in the rate at which diploids and polyploids interact with belowground mutualists.

METHODS: We used *Heuchera cylindrica* (Saxifragaceae) to test how polyploidy influences interactions with arbuscular mycorrhizal fungi (AMF). Here we first confirmed the presence of AMF in *H. cylindrica*, and then we used field-collected specimens to quantify and compare the presence of AMF structures while controlling for site-specific variation.

RESULTS: Tetraploids had higher colonization rates as measured by total, hyphal, and nutritional-exchange structures; however, we found that diploids and tetraploids did not differ in vesicle colonization rates.

CONCLUSIONS: The results suggest that polyploidy may alter belowground nutritional mutualisms with plants. Because colonization by nutritional-exchange structures was higher in polyploids but vesicle colonization was not, polyploids might form stronger associations with their AMF partners. Controlled experiments are necessary to test whether this pattern is driven by the direct effect of polyploidy on AMF colonization.

KEY WORDS arbuscular mycorrhizae; arbuscules; belowground species interactions; coils; hyphae; mutualism; polyploidy; Saxifragaceae; vesicles.

Polyploidy, or whole-genome duplication (WGD), is exceptionally common in the plant kingdom, with an estimated 35% of vascular plant species being polyploid (Wood et al., 2009). Although polyploids are clearly abundant among plants, theory predicts that polyploids should often go locally extinct shortly after they arise due to competition with their diploid ancestors (Fowler and Levin, 2016). Indeed, there is evidence that although polyploidy occurs frequently within plant lineages, they suffer from higher extinction rates than their diploid ancestors (Levin, 2019). Reconciling this apparent contradiction has thus been a major focus of contemporary research on polyploidy.

A key mechanism that can explain why polyploids are so common despite theoretical predictions is that WGD often induces changes in plant physiology that can promote persistence by shifting ecological interactions (Madlung, 2013). Although we have accumulated evidence that WGD-induced shifts in physiology can greatly affect the way that plants interact with their abiotic environment (Maherali et al., 2009; Ramsey, 2011), we have only recently begun to appreciate the consequences for the biotic interactions of plants, and most of these studies have focused on aboveground interactions (Ramsey and Ramsey, 2014; Segraves and Anneberg, 2016). This is surprising, given that many belowground interactions

involve nutritional mutualisms that are crucial to the growth of the host plant (Vandenkoornhuyse et al., 2015), and one of the major expected physiological consequences of polyploidy is an increased need for nutrients from the soil (Leitch and Bennett, 2004; Guignard et al., 2016; Segraves and Anneberg, 2016). Consequently, we expect that polyploids will be more dependent on the belowground mutualists that provision plants with limiting nutrients. Despite these proposed linkages between polyploidy and belowground mutualisms, it remains unclear how polyploidy affects these interactions.

An attractive model for testing how polyploidy affects belowground species interactions is the association between plants and arbuscular mycorrhizal fungi (AMF). AMF are a group of near-ubiquitous belowground mutualists that provision their host plant with growth-limiting nutrients in exchange for carbohydrates (Smith and Read, 2008). Because colonization rates of AMF on their host are primarily driven by the nutritional needs of the plant and the nutritional quality of the soil (Smith and Read, 2008), we expect that polyploids will have increased colonization rates by AMF. From a plant-centric perspective, we can examine how polyploidy influences the quality of the mutualism by quantifying the percentage of colonization by arbuscules, the nutrient-exchange structures formed by AMF. Greater colonization rates by arbuscules implies that the plants experience a greater benefit from their AMF symbionts (Johnson et al., 2003). From a myco-centric point of view, fungi that have increased colonization by vesicles, the structures that AMF form to store a large proportion of the carbon acquired from their hosts, implies that the fungi receive more benefits from the mycorrhizal interaction (Johnson, 1993; Smith and Read, 2008). As a result, simultaneous assessment of colonization by arbuscules and vesicles can offer a way to examine how polyploidy impacts the placement of the plant–AMF interaction along the mutualism–antagonism continuum (Johnson and Graham, 2013). For example, if more arbuscules are observed in polyploids than in their diploid ancestors while vesicle formation by AMF is unaffected, it would imply that polyploids have a lower cost to benefit ratio than diploids since maintenance costs of the interaction (i.e., vesicle formation rates) are static while benefits (i.e., arbuscules) are greater.

One of the first steps in determining how WGD affects the plant interaction with AMF is to compare colonization rates by AMF on diploid and polyploid host plants. To date, only five studies have investigated how within-lineage WGD affects the interaction with AMF, and none have found differences in total AMF colonization on host roots (Jun and Allen, 1991; Sudová et al., 2010, 2018; Doubková et al., 2012). To better understand the contextual nature of how polyploidy influences plant–AMF interactions, we need studies that separately consider colonization by arbuscules, vesicles, and hyphae. By comparing the colonization rates of these structures individually, rather than reporting on total colonization alone, we may be better able to frame the consequences of polyploidy on this belowground interaction.

A good system for investigating how polyploidy affects the mycorrhizal interaction is *Heuchera cylindrica*. *Heuchera* has been used extensively to test ideas about how species interactions differ between diploids and polyploids (e.g., Thompson et al., 2004). This species has naturally occurring diploid and tetraploid populations that grow in single cytotype populations (Godsoe et al., 2013). The tetraploids likely formed via autopolyploidy (i.e., the union of unreduced, intraspecific gametes; Godsoe et al., 2013). In contrast with autopolyploidy, allopolyploidy is characterized by WGD that follows an interspecific hybridization event. As a result, *H. cylindrica* is a good model for studying the effect of polyploidy on species interactions because it allows us to focus on the effects of WGD without the complexity of interspecific hybridization. Furthermore, sampling efforts have shown that diploid and tetraploid populations grow adjacent to one another in overlapping climatic environments (Godsoe et al., 2013), mitigating the possibility that climate will confound differences between cytotypes. Although the Saxifragaceae are thought to be a non-mycorrhizal family (Maherali et al., 2016; Werner et al., 2018), there is evidence showing that some members of this group can form mycorrhizal associations (Read and Haselwandter, 1981; Zubek and Blaszkowski, 2009; Zubek et al., 2009; Peters et al., 2011; Oehl and Körner, 2014). Here we present evidence that *Heuchera cylindrica* is indeed able to form mycorrhizal associations.

In the present study, we first confirmed that *H. cylindrica* engages in the mycorrhizal interaction by quantifying the percentage AMF colonization on field-collected plants. Then we tested whether the rate of AMF colonization on *H. cylindrica* differed between ploidy levels. Specifically, we examined whether the total percentage colonization by AMF, colonization by nutrient-exchange structures, vesicle colonization, or hyphal colonization rates differed between cytotypes, as a proxy for the quality of the mutualism.

MATERIALS AND METHODS

Study organism and field sampling

Heuchera cylindrica is a semi-evergreen perennial herb that occurs in the western United States. To quantify root colonization by AMF in a natural setting, we collected roots from three diploid and three tetraploid field sites in May 2008, and then from six diploid and six tetraploid populations in early June 2018 (Fig. 1; Table 1). Only two of the sites that were sampled in 2008 were resampled in 2018 because we wanted to increase the geographic scale of our sampling design and obtain additional samples at sites near the zone of parapatry where the geographic ranges of diploid and polyploid populations abut. By doing so, we were more confidently able to demonstrate that the observed patterns of colonization were driven by the ploidy level of the host plant, regardless of site-specific differences. In our 2018 collection, all populations were sampled at the time of peak flowering, since the reproductive period is when plants are the most phosphorus limited (Gusewell, 2004) and would thus be more likely reliant on their AMF partners. Since natural populations of diploids and polyploids of *H. cylindrica* are morphologically indistinguishable, ploidy level was characterized using flow cytometry. Because ploidy levels of plants from nine of the 12 populations had previously been determined (Godsoe et al., 2013), we used flow cytometry to assess ploidy levels of the remaining three uncharacterized populations. These methods were identical to those presented by Godsoe et al. (2013).

Because diploids and tetraploids grow allopatrically, we wanted to control for potential confounding geographic effects. Previous climate modeling of diploid and tetraploid populations of *H. cylindrica* show that these plants grow in overlapping climatic environments (Godsoe et al., 2013). As a consequence, we did not include any climate-related site data in our analysis. We did, however, control for site-specific differences in soil quality by characterizing five edaphic factors (pH, electrical conductivity, Kjeldahl N, Olsen P, and molar N:P) and AMF spore densities, all of which are known to affect AMF colonization rates and are likely to differ among the study sites. At each site, we collected soil from directly beneath three to four *H. cylindrica* individuals

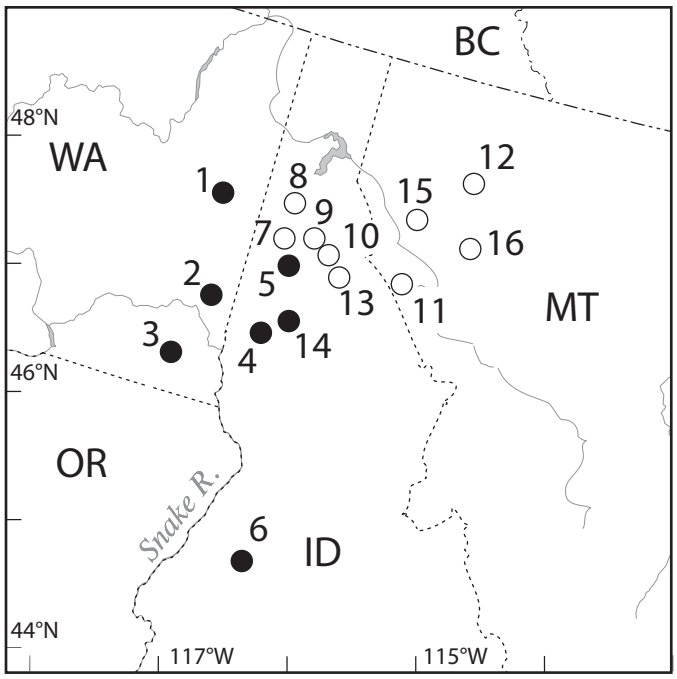

FIGURE 1. *Heuchera cylindrica* field sites. Diploid sites are filled circles; tetraploid sites are open circles. Numbers correspond to site numbers in Table 1.

at depths of 0–20 cm. These samples were homogenized and combined into a single bulk sample for each site and then stored in plastic bags until they had been transported to Syracuse University where we assessed their chemical profile. We first air-dried a half-liter subsample of each soil sample and then passed the dried soil samples through a 2-mm sieve. We determined the pH and electrical conductivity of soils in a 1:1 soil–deionized water mixture. We measured soil pH with a Mettler Toledo SevenGo SG2 pH meter (Columbus, OH, USA), and the electrical conductivity of soils was measured with a Thermo Fisher Scientific Orion conductivity meter (model 122; Waltham, MA, USA). We estimated the quantity of plant-available nitrogen with a 1 M KCl extraction and subsequent colorimetric analysis (EPA, 1978) at the Cornell Nutrient Analysis Laboratory (Ithaca, NY, USA). We then extracted plant-available phosphorus as Olsen phosphorus (Olsen, 1954) and stained with malachite green to estimate the concentration of plant-available phosphorus from each site (Rao et al., 1997). From the estimated nitrogen and phosphorus data, we calculated the molar nitrogen to phosphorus ratio available in each soil sample. We also controlled for potential differences in AMF propagule density across sites by estimating AMF spore densities via the sucrose flotation method (Allen et al., 1979). The total number of AMF spores per site was estimated by counting the number of spores on one-quarter of a petri dish and multiplying by four. We expressed our site-specific estimates as the total number of spores per 5 g of soil.

Confirmation and quantification of AMF colonization

We confirmed *H. cylindrica* is a mycorrhizal species and quantified differences in colonization rates of AMF on roots from our pilot data from 2008 and our 2018 data set (Table 1). To do so, we took subsamples of fine roots from an average of four individuals per site (range: 3–7 individuals). We preserved roots in 70% ethanol and kept them at 4°C. Roots were cleared and stained for fungal structures within 14 d after collection. Roots were cut into 2-cm segments and placed in 10% KOH (w/v) and cleared of cellular content via autoclaving at 121°C for 15 min. Roots were then washed with deionized water and stained with 0.03% chlorazol black E (w/v) during a second autoclave cycle at 121°C for 15 min. We then mounted the stained roots on glass slides with a 1:1:1 lactic acid–glycerol–deionized water solution (v/v/v) (Brundrett et al., 1984). We first confirmed that *H. cylindrica* is a mycorrhizal species by identifying AMF structures (i.e., aseptate hyphae, arbuscules, hyphal coils, and vesicles) colonized on stained roots of diploids and tetraploids. We then observed individual root samples with a Nikon E600 differential interference contrast microscope (Tokyo, Japan) set to 200× total magnification and counted the number of times that arbuscules, vesicles, hyphal coils, and hyphae intersected with the graticule of the ocular. We scored 50 views per root sample, for a total of 1500 views distributed equally across 30 samples in our 2008 data set and a total of 2650 views distributed across 53 root samples from our 2018 sampling (Appendix S1). We then calculated total percentage AMF colonization as the proportion of views containing at least one AMF structure to the total number of views for that root sample (Brundrett et al., 1984; McGonigle et al., 1990). Our root sampling and staining procedure from 2008 was carried out according to the methods described above except that only total colonization was determined and the colonization data were collected by another individual using a compound light microscope.

Statistical analyses

We used our measures of the percentage colonization by arbuscules as an estimator of the benefit received by the plant, and we used vesicle colonization as an estimator of the benefit the fungus received from the plant (Johnson, 1993; Johnson et al., 2003). We partitioned our 2018 data set to separately quantify the percentage colonization by nutrient-exchange structures (arbuscules), vesicles, and hyphae. The colonization rates of individual structures will not necessarily sum to the total colonization rate because multiple structures were often found together in the same view.

To address the question of how polyploidy in *H. cylindrica* affects colonization rates by AMF, we controlled for geographic variation in edaphic factors and AMF spore density by first summarizing the site-specific data using a principal component analysis of the five edaphic factors and AMF spore density. We saved the loadings of the first three axes, which explained >90% of the variation, and used them as covariates in a subsequent ANCOVA, which allowed us to compare the main effect of host plant cytotype while accounting for site-specific soil quality covariation. Our observed rates of hyphal-coil colonization were so low that we chose to exclude these structures from our analysis of nutrient-exchange-structure colonization. We calculated a separate ANCOVA for total AMF colonization, arbuscule colonization, vesicle colonization, and hyphal colonization rates, respectively. For our 2008 data set, we calculated the total percentage AMF colonization and used an ANOVA to compare the main effect of host plant ploidy level on total AMF colonization rates.

RESULTS

The 2008 data set revealed that diploid and tetraploid *H. cylindrica* significantly differed in total colonization by AMF

TABLE 1. Sampling locations and their site-specific soil qualities. Map numbers correspond to location numbers listed on the map in Fig. 1.

Map Number	Site	Coordinates	2008 *N*	2018 *N*	Ploidy	pH	Electrical conductivity	Olsen P (mg/kg)	Kjeldahl N (mg/kg)	Molar N:P	Spore count/5 g
1	Cheney, WA	47.481677 N, 117.567133 W	–	5	Diploid	6.51	20.3	2.27	4.74	4.63	1035
2	Albion, WA	46.839574 N, 117.280947 W	5	3	Diploid	6.35	20.85	2.08	4.01	4.28	457
3	Dayton, WA	46.199712 N, 117.766992 W	–	7	Diploid	6.19	18.7	1.44	3.97	6.09	1641
4	Orofino, ID	46.489156 N, 116.732209 W	5	6	Diploid	7.48	42.1	0.73	3.19	9.63	766
5	Santa, ID	47.166822 N, 116.483605 W	–	3	Diploid	6.57	21.7	1.80	3.03	3.73	1004
6	Boise, ID	44.314285 N, 116.070428 W	–	4	Diploid	6.39	27.7	4.67	4.35	2.06	1186
7	Benewah, ID	47.337876 N, 116.827284 W	–	5	Tetraploid	6.43	16.1	0.91	4.08	9.86	520
8	Coeur d'Alene, ID	47.618344 N, 116.662353 W	–	6	Tetraploid	5.95	18.2	1.91	4.51	5.22	1242
9	St. Joe, ID	47.315246 N, 116.270866 W	–	3	Tetraploid	6.91	52.8	0.93	9.31	22.13	1121
10	Calder, ID	47.279133 N, 116.215752 W	–	3	Tetraploid	6.05	12.6	1.21	2.84	5.21	410
11	St. Regis, MT	47.229085 N, 115.255366 W	–	4	Tetraploid	6.89	43.8	0.47	6.62	31.30	1857
12	Ashley, MT	48.120512 N, 114.576281 W	–	4	Tetraploid	7.21	36.9	3.05	7.38	5.34	757
13	Calder (Big Creek), ID	47.48111 N, 116.2231 W	5	–	Tetraploid	–	–	–	–	–	–
14	Spalding, ID	46.57972 N, 116.7847 W	5	–	Diploid	–	–	–	–	–	–
15	Beaver Creek, MT	47.71667 N, 115.6925 W	5	–	Tetraploid	–	–	–	–	–	–
16	Thompson River, MT	47.601239 N, 115.21943 W	5	–	Tetraploid	–	–	–	–	–	–

($F_{1, 4} = 8.63$, $P < 0.05$), with diploids hosting a mean of 28.0% total colonization as compared to 43.4% in tetraploids. In our 2018 data set, all root segments of *H. cylindrica* had greater than 50% colonization by AMF. After controlling for site-specific edaphic factors and AMF spore density, the ANCOVA comparing total colonization rates of plants showed that diploids and tetraploids were significantly different ($F_{1, 11} = 6.67$, $P < 0.04$), with diploids hosting an average of 58.9% total AMF colonization and tetraploids hosting 69.3% (Fig. 2).

To separately quantify the percentage colonization of nutrient-exchange structures, vesicles, and hyphae, we partitioned the total colonization data of each individual root sample into three categories: nutrient-exchange structures (arbuscules), vesicles, and hyphae. After controlling for site-specific differences in the soil, we found that colonization by nutrient-exchange structures significantly differed between diploids and tetraploids ($F_{1, 11} = 64.74$, $P < 0.0001$). Diploids hosted an average of 21.4% colonization by nutrient-exchange structures as compared to 36.0% in tetraploids (Fig. 2). We also re-analyzed the colonization data for nutrient-exchange structures with hyphal coils included and arrived at the same result ($F_{1, 11} = 69.63$, $P < 0.0001$). We found that vesicle colonization rates did not statistically differ between diploids and tetraploids ($F_{1, 11} = 0.87$, $P > 0.38$); however, hyphal colonization rates differed significantly between diploids and tetraploids ($F_{1, 11} = 5.96$, $P < 0.05$), with diploids hosting an average of 54.9% hyphal colonization compared to an average of 67.2% in tetraploids.

DISCUSSION

Polyploidy can cause strongly divergent physiologies in plants and, in turn, is expected to result in altered species interactions between diploids and polyploids in their belowground nutritional mutualisms. The handful of studies that have investigated the effect of polyploidy on plant–AMF interactions, however, have found no differences in total colonization by AMF. We argue that working at a finer scale by comparing the colonization rates by arbuscules, coils, vesicles, and hyphae separately may reveal differences in the way that diploids and polyploids interact with their AMF mutualists. We were thus interested in characterizing how polyploidy affects the belowground nutritional mutualism with AMF by comparing the rates of colonization by different AMF structures on roots of diploid and polyploid plants from the field.

In contrast with previous work in other systems, we found that polyploidy strongly correlates with the rate of AMF colonization. After controlling for site-specific differences, we found that diploid and tetraploid *H. cylindrica* differed in the total rate of AMF colonization, and this result was consistent for the two sampling periods 10 years apart. We found a similar, but stronger pattern in colonization by nutrient-exchange structures, specifically that tetraploids were colonized by more arbuscules than diploids. This finding is intriguing since we observed that vesicle colonization did not differ between diploid and tetraploid *H. cylindrica*. Thus, our results suggest that polyploids of *H. cylindrica* benefit more

from the interaction with AMF, because colonization by nutrient-exchange structures increased but carbon-storage structures did not. We are cautious in our interpretation of these results, however, since we did not profile the AMF communities colonized on plants. Some AMF species do not form vesicles, so it is possible that the patterns may have been driven by differences in the taxonomic profile of the AMF communities on diploids and tetraploids. Furthermore, we likely underestimated the carbon drain by AMF on their host plants since we did not quantify the number of AMF spores in the rhizosphere of each plant sampled in our study. AMF spores are another carbon-rich structure produced to varying degrees by different AMF species that can be a significant cost to host plants (Smith and Read, 2008). Nevertheless, observing differences in colonization between diploids and tetraploids suggests that the dynamics of belowground mutualisms may shift.

Nutrient-exchange-structure colonization by AMF may have been greater on tetraploids because polyploidy is expected to increase the nutritional needs of plants (Leitch and Bennett, 2004; Segraves and Anneberg, 2016; Guignard et al., 2017). Indeed, comparative studies suggest that polyploids are more nutrient-limited than diploids. For example, polyploids have been shown to produce less biomass and are less abundant than phylogenetically unrelated diploids in experimental plots with low nutrients as compared to nutrient enrichment plots (Šmarda et al., 2013; Guignard et al., 2016). By participating in nutrient-exchange mutualisms, perhaps polyploids are able to overcome an increased need for growth-limiting nutrients.

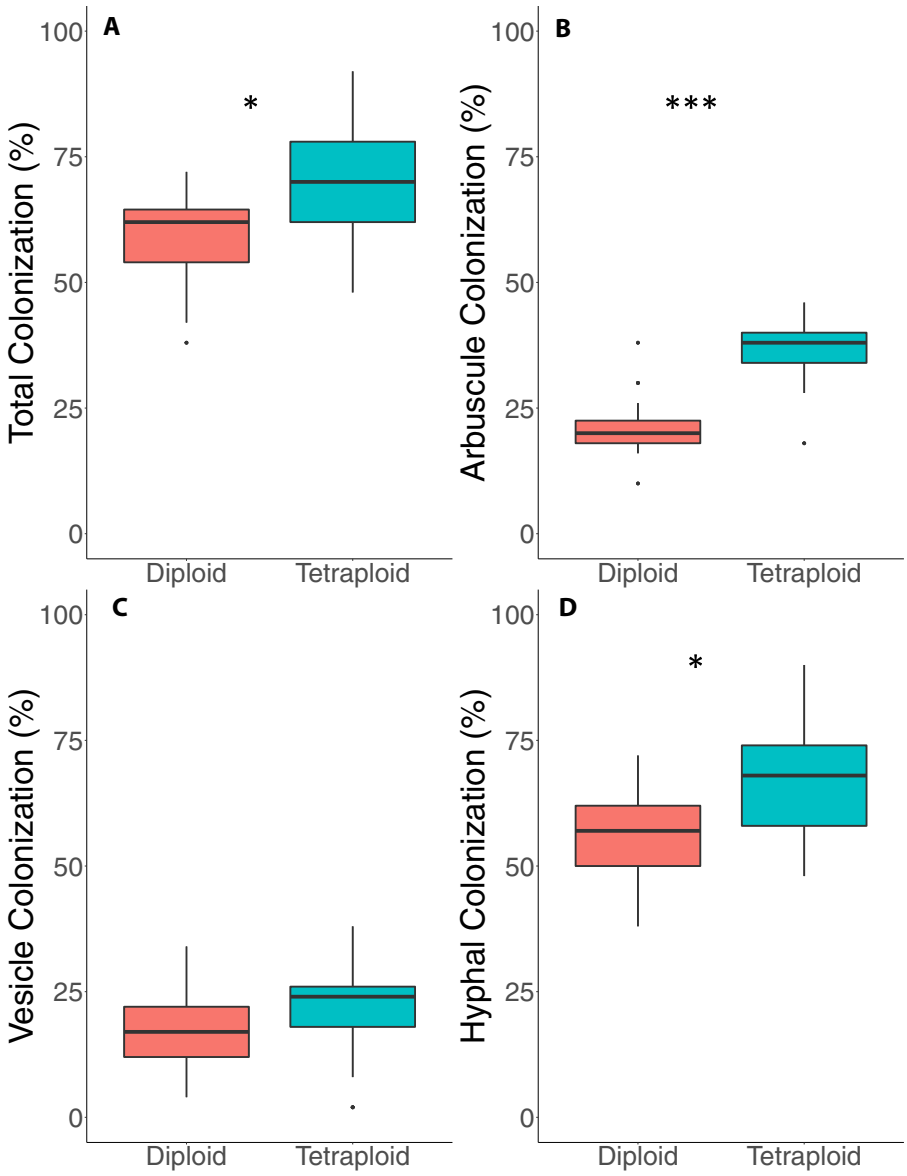

FIGURE 2. Colonization by arbuscular mycorrhizal fungi on *Heuchera cylindrica* roots collected in 2018. (A) Total colonization. (B) Nutritional-exchange structures (arbuscules). (C) Vesicles. (D) Hyphae. *$P < 0.05$, ***$P < 0.0001$.

Previous studies have suggested that AMF mutualists may not influence polyploid acquisition of limiting nutrients. For example, Sudová (2014) conducted a pot study in which they inoculated diploids and polyploids of *Aster amellus* with single isolates of AMF from field sites and found no differences in total AMF colonization or in extra-radical hyphal lengths, AMF structures that forage for mineralized nutrients in the soil. Interestingly, they also included a non-sterile soil treatment in their inoculation experiment and found that the AMF that colonized hexaploids of *A. amellus* produced significantly longer extra-radical hyphae than diploids, suggesting that taxonomic differences in AMF may influence polyploid–AMF interactions. Similarly, Sudová et al. (2018) found no difference in AMF colonization between field-collected cytotypes of *Centaurea stroebe* and were also unable to detect differences in mycorrhizal growth response in a controlled greenhouse study. In contrast, we found that polyploids of *H. cylindrica* had significantly greater AMF colonization via nutrient-exchange structures than diploids did. Although AMF taxonomy might explain the differences between the present study and these studies in Asteraceae, we also propose that these differences could be caused by contrasting selective pressures following whole-genome duplication. Extant polyploids such as *C. stroebe* and *H. cylindrica* grow in different soil environments and climates and have likely experienced different selective pressures that could impact interactions with belowground mutualists. To better understand how WGD affects the interaction with AMF, we not only require inoculation experiments that provide AMF partner options to their host plants, but we also need to use first generation polyploids as a point of comparison to their diploid parents. By doing so, we may be able to explain the variability among polyploid plant–AMF studies.

The fact that colonization rates by nutrient-exchange structures increased while those by vesicles were static between tetraploids and their diploid ancestors raises the question of how polyploidy affects carbon allocation strategies of plants. That is, we do not know if

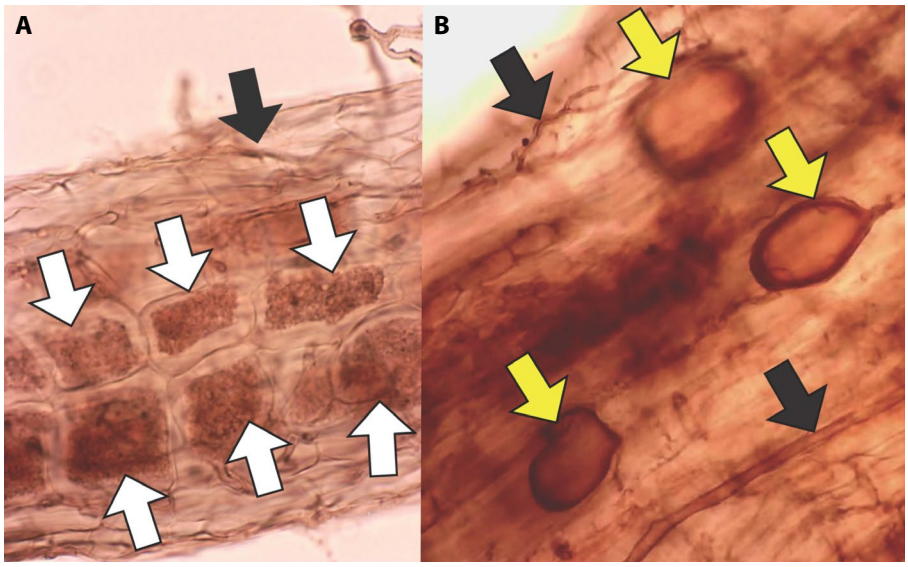

FIGURE 3. Photographs of *Heuchera cylindrica* roots colonized by (A) arbuscules and hyphae and (B) vesicles and hyphae. 200× magnification. Black arrows: hyphae, white arrows: arbuscules, yellow arrows: vesicles.

polyploids invest carbon into the plant–AMF interaction differently than their diploid progenitors. For example, we know from other studies that AMF can act as sinks for a significant amount of plant carbon (Kaschuk et al., 2009), so the question remains whether polyploids exchange more carbon for growth-limiting nutrients. The answer may be complex, since there is evidence of a negative correlation between polyploidy and maximum photosynthetic rates (Knight et al., 2005). Since polyploids may have a diminished capacity to photosynthesize as compared to their diploid ancestors, they may have limited ability to invest as much carbon into the interaction with AMF. If polyploidy does result in reduced pools of carbon for plants to allocate toward belowground interactions, then polyploids may preferentially associate with AMF partners that offer the highest rate of carbon to nutrient exchange. The next step in addressing this problem is to quantify and compare the rates at which diploids and their polyploid offspring acquire carbon and how much of that carbon is allocated to AMF partners in exchange for growth-limiting nutrients.

Our results suggest that polyploids may interact with their AMF partners differently than their diploid ancestors; however, this result may be confounded by the evolutionary history of the polyploid plants used in this study. Specifically, naturally occurring polyploids of *H. cylindrica* likely underwent WGD hundreds, if not thousands, of generations ago during the last glaciation period in the Pacific Northwest of the United States (Godsoe et al., 2013). The observed differences in colonization rates, then, may have been caused either by polyploidy, drift, selection that favored polyploid lineages to form stronger mycorrhizal associations or by a product of any of these processes. Consequently, the phenotypes of the extant polyploids of today may not necessarily match those of first-generation polyploids, which may explain why our observed difference in AMF colonization between diploids and polyploids contrast with those of previous efforts (Jun and Allen, 1991; Sudová et al., 2010, 2014, 2018; Doubková et al., 2012). In our future efforts, we will make use of neopolyploids to understand the direct effect of polyploidy on nutritional limitation, and ultimately, on species interactions.

In addition to examining AMF colonization patterns between diploids and polyploids, we also confirmed the mycorrhizal status of *H. cylindrical* (Fig. 3). Historically, the Saxifragaceae have been considered non-mycorrhizal or only weakly mycorrhizal (Maherali et al., 2016; Werner et al., 2018), and up to this point, efforts have solely focused on the genus *Saxifraga*. For example, three studies have shown that species of *Saxifraga* are not colonized by AMF in the field (Treu et al., 1996; Ruotsalainen et al., 2004; Brown and Jumpponen, 2014); in contrast, five studies have found colonization in *Saxifraga* species (Read and Haselwandter, 1981; Zubek and Blaszkowski, 2009; Zubek et al., 2009; Peters et al., 2011; Oehl and Körner, 2014). For example, Peters et al. (2011) sampled four species of *Saxifraga* and found an average total rate of AMF colonization upward of 39%. Oehl and Körner (2014) sampled roots of *Saxifraga oppositifolia* from the Swiss Alps and found that plants hosted five morphotypes of AMF, and although colonization rates were not quantified, they reported on the presence of intraradical AMF structures. Although the genus *Saxifraga* clearly includes species that can host AMF, until the present study, we were missing colonization information from the second major lineage within the Saxifragaceae.

Together, our results show that diploids and polyploids differ in how they interact with their AMF mutualists. We hypothesize that this pattern is driven by increased nutritional needs of plants as a result of polyploidy; however, we require further studies that control for the identity of the AMF partners to advance our understanding of how polyploidy affects the plant–AMF association. Doing so will help us discern whether polyploidy affects host plant preference for AMF partners. Furthermore, we must make use of early-generation polyploids in future efforts, which will allow us to disentangle the effects of selection and drift acting on polyploids following WGD and thereby understand the immediate effect of WGD on species interactions.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Site information with averages of the number of respective AMF structures observed.

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