

INVITED PAPER

For the Special Issue: The Evolutionary Importance of Polyploidy

Species interactions and plant polyploidy¹

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Polyploidy is a common mode of speciation that can have far-reaching consequences for plant ecology and evolution. Because polyploidy can induce an array of phenotypic changes, there can be cascading effects on interactions with other species. These interactions, in turn, can have reciprocal effects on polyploid plants, potentially impacting their establishment and persistence. Although there is a wealth of information on the genetic and phenotypic effects of polyploidy, the study of species interactions in polyploid plants remains a comparatively young field. Here we reviewed the available evidence for how polyploidy may impact many types of species interactions that range from mutualism to antagonism. Specifically, we focused on three main questions: (1) Does polyploidy directly cause the formation of novel interactions not experienced by diploids, or does it create an opportunity for natural selection to then form novel interactions? (2) Does polyploidy cause consistent, predictable changes in species interactions vs. the evolution of idiosyncratic differences? (3) Does polyploidy lead to greater evolvability in species interactions? From the scarce evidence available, we found that novel interactions are rare but that polyploidy can induce changes in pollinator, herbivore, and pathogen interactions. Although further tests are needed, it is likely that selection following whole-genome duplication is important in all types of species interaction and that there are circumstances in which polyploidy can enhance the evolvability of interactions with other species.

KEY WORDS coevolution; flower visitors; herbivory; pathogens; plant–fungal interactions; pollination; polyploidy; seed dispersal; soil microbes; tri-trophic interactions

The myriad ways that plants interact with other organisms have driven spectacular species radiations. Evolutionary divergence in floral form due to interactions with specialist pollinators, for instance, may have spurred speciation in angiosperms (e.g., Bradshaw and Schemske, 2003; Crepet and Niklas, 2009; Van der Niet et al., 2014). Coevolutionary arms races between plants and herbivorous insects have also been suggested as another potential mechanism of diversification (Ehrlich and Raven, 1964; Wiens et al., 2015), and specialization to these herbivores may have prompted subsequent speciation of natural enemies at the third trophic level (Abrahamson and Blair, 2008). The diverse interactions of plants with pollinators, herbivores, parasitoids, seed dispersers, microbes, and other organisms create rich opportunities for trait divergence that will be directed in part by the underlying genetic architecture of the plant (e.g., De Bodt et al., 2005). One important shift in genetic architecture is polyploidy, a phenomenon commonly observed in plant lineages. The many phenotypic and genotypic effects of whole-genome duplication make polyploidy an important force that can shape species interactions, and likewise, interactions with

other species are expected to play a role in shaping polyploid lineages.

Despite the importance of both species interactions and polyploidy in plant evolution, we have only just begun to disentangle how these factors together contribute to diversity. Part of the challenge in resolving these connections is to identify the changes that are directly caused by polyploidy vs. those that have evolved following whole-genome duplication. Each of these pathways is likely to have contrasting implications for whether there are predictable effects on species interactions. For example, polyploidy itself can cause predictable phenotypic changes that directly affect interactions with other organisms such as increases in flower size that lead to attraction of different pollinator species. However, there are also indirect effects of polyploidy that can alter species interactions. The formation of an instantaneous, reproductively isolated polyploid lineage, for instance, opens an opportunity for selection to create divergence in traits and species interactions. In this example, divergence is likely to be erratic, driven by the local selective environment. Consequently, a major aim of ecological research on polyploidy is to tease apart the direct vs. indirect effects of polyploidy on species interactions.

In this paper, we review the available evidence that connects species interactions and whole-genome duplication. More than 10 years have passed since the last survey of polyploid plant interactions

¹ Manuscript received 16 December 2015; revision accepted 21 April 2016.

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doi:10.3732/ajb.1500529

with pollinators and herbivores (Thompson et al., 2004), presenting an opportunity to evaluate not only new study systems but also new types of species interactions. Recent reviews have briefly highlighted key studies (Ramsey and Ramsey, 2014; Soltis et al., 2014a, b), and here we will expand on these discussions to examine the existing evidence. We organize this review around three nonmutually exclusive questions: (1) Does polyploidy directly cause the formation of novel interactions not experienced by diploids, or does it create an opportunity for natural selection to then form novel interactions? (2) Does polyploidy cause consistent, predictable changes in species interactions vs. the evolution of idiosyncratic differences? (3) Does polyploidy lead to greater evolvability in species interactions? Answers to these questions are essential if we are to develop a predictive framework linking polyploidy and species interactions. Because the genotypic and phenotypic consequences of polyploidy have been considered in depth elsewhere (e.g., Ramsey and Schemske, 2002; Ramsey and Ramsey, 2014), we will only briefly touch on this topic. Instead, we will focus on interactions of polyploid plants with other species and how these interactions might influence the evolutionary trajectory of both parties. To avoid overlap with recent reviews of plant invasions and competition (e.g., te Beest et al., 2012), we will concentrate on interactions involving nonplant species. We also restrict our discussion to wild plant populations to avoid any potential biases inherent in agricultural systems such as the effects of artificial selection and growth in monoculture.

Interactions with pollinators and floral visitors—A primary focus of research on polyploids has been to understand the factors that contribute to their reproductive isolation from diploid progenitors. Newly emerged polyploids may experience reduced fitness and extinction due to minority cytotype exclusion unless polyploids have a mechanism to selectively mate with plants of the same ploidy level (Levin, 1975). There is likely a diversity of mechanisms that interact to influence reproductive isolation between polyploids and their diploid progenitors (Husband and Sabara, 2003; Roccaforte et al., 2015). Assortative mating could be accomplished by immediate changes to phenotypes as a result of whole-genome duplication that places polyploid lineages on an evolutionary path leading to complete reproductive isolation (e.g., Segraves and Thompson, 1999; Husband and Schemske, 2000; Anssour et al., 2009; Balao et al., 2011). For taxa that rely on animal-mediated pollination, one instantaneous effect of whole-genome duplication could be shifts in the assemblage of pollinators. Differentiation in pollinator communities could isolate diploids and polyploids even within mixed populations and may facilitate polyploid establishment. If whole-genome duplication causes divergence in traits attractive to pollinators then polyploids may “automatically” have different pollinator communities, or alternatively, polyploidy might increase the variation upon which selection can act to change pollination traits.

Comparisons of naturally occurring diploids and polyploids suggest that polyploidy can have both direct and indirect impacts on pollination. For instance, we know that flower shape, size, and color as well as flowering phenology, nectar quantity, and scent chemistry can change in polyploids (e.g., Segraves and Thompson, 1999; Husband and Schemske, 2000; Jersáková et al., 2010; Balao et al., 2011; Gross and Schiestl, 2015; McCarthy et al., 2015), but for most species, we have yet to identify the cause of these trait shifts. Some of these differences are a direct result of whole-genome duplication as evidenced by immediate changes that are observed

in synthesized neopolyploids (reviewed by Ramsey and Schemske, 2002). For example, *Brassica* neopolyploids instantly differ in flowering phenology (Schranz and Osborn, 2000) and synthesized allotetraploid *Tragopogon* differ in flower morphology from their diploid counterparts (Tate et al., 2009) that is consistent with the variation observed in recently derived polyploid populations (Ownbey, 1950). From the standpoint of a newly formed polyploid lineage, these immediate phenotypic changes may be exceedingly important in shaping interactions with pollinators and could mean the difference between establishment and extinction.

Some of the observed phenotypic differences between diploids and polyploids, however, are likely caused by natural selection following polyploidization. For instance, in the saxifrage *Heuchera grossulariifolia*, populations of autotetraploids growing on the Salmon River in Idaho flower earlier than the co-occurring diploids (Segraves and Thompson, 1999), and phenotypic selection analyses suggest that pollinator-mediated selection favors earlier flowering of tetraploids (Nuismer and Cunningham, 2005). Yet, when neopolyploids were generated from plants collected on the Salmon River, they flowered later than diploids, and the same pattern was observed when neopolyploids were synthesized from a geographically distant population (Oswald and Nuismer, 2011). This example highlights that while polyploidy itself may create distinctive phenotypes, selection is also likely to play a role, particularly for pollination traits that can have direct effects on fitness. A remaining question, then, is what are the relative contributions of whole-genome duplication vs. subsequent selection in shaping pollination traits? If selection is the main contributor, does this mean that polyploidy is a mundane genetic change that simply creates distinct entities that are free to evolve independently as any reproductively isolated populations would? Although we are not yet able to fully answer these questions, there are multiple lines of evidence that suggest that polyploidy can actually increase the evolutionary potential of a population and that this increased evolvability might favor establishment and persistence of polyploids. For example, Martin and Husband (2012) examined the response of diploid, tetraploid, and neotetraploid *Chamerion angustifolium* to selection on flowering time. They showed that a strong response to selection in the neotetraploids could allow them to quickly adapt, suggesting that newly formed polyploids may have an adaptive advantage over their diploid progenitors (Martin and Husband, 2012). Although the mechanisms underlying this increase in evolvability are unknown, we do know that polyploidy can cause increased trait variation, heterozygosity, and capacity for neofunctionalization of redundant gene copies (Flagel and Wendel, 2009). Together, these factors suggest that even if selection is the main contributor to changes in pollination traits, there are qualities of polyploids that set them apart from other types of genetic changes that cause reproductive isolation.

Polyploids may be phenotypically differentiated from their diploid ancestors with respect to pollination traits, but the key question is whether these differences matter to pollinators. Pollinator-mediated reproductive isolation is an intriguing possibility, as there are a number of examples in which phenological and geographic separation are insufficient to prevent gene flow between cytotypes. Can shifts in plant–pollinator interactions cause assortative mating of plants differing in ploidy level? Although few studies have assessed this question, we have some evidence suggesting that pollen flow can be assortative even when diploids and polyploids co-occur within populations. However, whether assortative mating occurs because of

polyploid-induced changes in pollinator behavior is unclear. For instance, Gross and Schiestl (2015) found a pattern of asymmetry in pollen flow in *Gymnadenia conopsea* orchids where pollinators were more likely to be constant on tetraploids, thus increasing the movement of pollen among polyploid plants. Whether this constancy is caused by divergence in pollinator assemblage remains to be tested. Even so, an asymmetry in assortative mating could favor establishment of the minority cytotype and promote coexistence of diploids and polyploids (Husband and Sabara, 2003; Sabara et al., 2013). A similar pattern of constancy was observed in fireweed, *Chamerion angustifolium*. Three common *Bombus* species visited tetraploids more frequently than expected by chance; however, this floral constancy was likely caused by the spatial arrangement of plants within the population rather than a preference for tetraploid flowers per se. The idea that the spatial arrangement of plants caused this pattern is further supported by the finding that the flower visitor assemblages largely overlapped, despite differences in floral morphology between diploid and tetraploid fireweed (Husband and Schemske, 2000; Kennedy et al., 2006). In *Chamerion*, polyploidy does not appear to have an effect on interactions with pollinators.

If polyploidy has a predictable effect on pollinator behavior, then we would expect to observe repeated shifts in pollinator communities between cytotypes. The challenge of testing this prediction is that we currently lack data sets on pollinator assemblages. Most studies use indirect methods that describe the flower visitor community, but not the pollination efficacy of the flower visitors themselves. Although all pollinators are flower visitors, not all flower visitors are pollinators, and the inclusion of nonpollinating flower visitors in data sets could be misleading for conclusions on reproductive isolation of cytotypes. Keeping this caveat in mind, however, we can use information on flower visitor communities to test whether there is the potential for a predictable effect of polyploidy on pollinators and reproductive isolation.

Studies on flower visitor assemblages suggest an array of outcomes, from no divergence of communities to complete specialization of a flower visitor to one cytotype. For example, comparisons of diploid and hexaploid *Aster amellus* suggested that flower visitors did not differentiate between the cytotypes and that the communities of visiting insects were largely overlapping (Castro et al., 2011). Similarly, the visitors of diploid and tetraploid *Libidibia ferrea* overlapped in species composition, although there may be frequency-based differences in visitation rates (Borges et al., 2012). In a study of diploid and neotetraploid *Acacia mangium*, Nghiem et al. (2011) showed that while the community of flower visitors did not differ between cytotypes, a majority of the pollen carried by individual insects was from one cytotype. Together, these studies suggest that diploids and polyploids often have overlapping communities but that intercytotype visits might be reduced through frequency-based preference of visitors. For some plants, however, strong differentiation in insect visitor preference has been observed. For instance, in *Erythronium* lilies, Roccaforte et al. (2015) showed that *Andrena erythronii* bees exclusively visited diploids, whereas *Andrena carlini* more frequently visited tetraploids. If these species are effective pollinators, this level of specialization could isolate the cytotypes and shows that cytotype-specific specialization is possible. Thus far, the range of divergence in flower visitor communities suggests that polyploidy does not have a predictable effect on these interactions. Further exploration of this question will require assessment of pollinator visitation frequency and pollination efficacy on both diploids and polyploids.

We are aware of only one study that has examined divergence in pollinator communities between polyploids and their diploid progenitors. Thompson and Merg (2008) observed pollinator efficacy and visitation rates in two mixed populations of diploid and autotetraploid *Heuchera grossulariifolia* on the Salmon and Selway Rivers in Idaho. Diploid and tetraploid flowering times overlap substantially within these sites, thus allowing a test of the extent to which pollinators reinforce preexisting isolation. The sites also differ in floral morphology; diploids and tetraploids are highly divergent in floral form on the Salmon River, whereas the cytotypes are morphologically indistinguishable on the Selway River (Segraves and Thompson, 1999). Because autotetraploid *H. grossulariifolia* have arisen repeatedly from diploid ancestors (Wolf et al., 1989; Segraves et al., 1999), Thompson and Merg (2008) were also able to test whether pollinator communities were consistent between polyploid origins. This question is particularly interesting as it allows a test of whether polyploidy causes predictable changes in pollinator interactions. By using single visit trials in which each potential pollinator species was allowed to forage on a virgin flower, Thompson and Merg (2008) estimated mean seed set per visit for each insect species on both cytotypes. The main pollinators of tetraploids at both locations were *Bombus centralis* queen bumblebees. Queen *B. centralis* were the most efficient pollinators per visit and they contributed to ~65% of total seed set. In contrast, the main pollinators of diploids differed between the sites. Worker bees of *B. centralis* were efficient pollinators of diploids on the Salmon River, contributing ~55% of total seed set. In contrast, visits by *Lassioglossum* bees caused about 65% of seed set on the Selway River as *B. centralis* workers were uncommon on diploids. Although *Lassioglossum* was also highly abundant on tetraploids at the Selway River, this species only contributed ~19% of seed set due to swamping by the abundant and comparatively efficient bumblebee queens that produced almost seven times more seeds per visit. Interestingly, the results also suggested that pollination efficiency of some insects depended on the cytotype of the plant they visited. For example, *B. centralis* workers produced 13 times more seeds on tetraploid Selway River plants than on diploids at this site. On the Salmon River, *Andrena nigrocaerulea* bees visited both cytotypes at approximately equal frequency, but produced nearly five times more seeds on diploids than tetraploids.

These results suggest that insect pollinators can discriminate between cytotypes, although in the case of *H. grossulariifolia*, pollinators may be relying on cues other than flower shape (Thompson and Merg, 2008). Work on orchids has shown that cues such as floral scent volatiles can differ between cytotypes and are likely to influence pollinator decisions (Jersáková et al., 2010; Gross and Schiestl, 2015). If these cues are providing a reliable signal of ploidy level and pollinators are specializing to one cytotype, we expect pollinators to visit the same cytotype in subsequent visits. Indeed, there is some evidence for floral specialization on the Salmon River. Nuismer and Cunningham (2005) showed that 84% of visits to tetraploids were followed by a visit to another tetraploid, and 68% of visitors to diploids subsequently visited a diploid. Furthermore, the consistent preference of queen bumblebees for tetraploids between independent origins suggests that polyploidy may be driving the shifts in pollinators rather than this being a product of selection. A sample size of two origins is small, but this example illustrates the power of making observations across recurrently formed polyploid populations.

Studies of *H. grossulariifolia* also underscore that assessing pollinator efficiency is critical when making statements about

pollinator-mediated isolation. Pollination efficiency can change between cytotypes as a function of differences in the number of seeds produced per visit, visitation rate, or a combination of the two. Thus, common floral visitors can be ineffective pollinators and contribute little toward precluding isolation in polyploids, as highlighted by the work of one of the authors (K. Segraves) on the floral visitors of *H. grossulariifolia* at the Salmon River. In addition to the pollinators mentioned, two additional insect species strongly differentiated between the cytotypes: the prodoxid moth *Greya politella* and the bombyliid fly *Bombylius major* (Segraves and Thompson, 1999). Although both of these insects were common visitors, they contributed little to seed set or precluding isolation (Thompson and Merg, 2008).

At present, the paucity of studies on polyploid plant–pollinator interactions makes it difficult to assess the extent to which pollinators contribute to precluding isolation during polyploid establishment. There are currently two major gaps that we need to bridge. First, studies should test whether flower visitors differ in identity, visitation frequency, and pollination efficacy on diploids vs. polyploids. By doing so, we will be able to directly test how often polyploidy impacts pollinator behavior and the underlying mechanisms that shape patterns of pollen flow within mixed-cytotype populations. Second, if we wish to understand how polyploids actually become established, this work needs to be conducted in early-generation polyploids that have not had the opportunity to diverge and (co) evolve with their pollinators (Ramsey and Ramsey, 2014). Observations of pollinator-mediated reproductive isolation between diploids and neopolyploids would strongly support divergence in pollinator communities as a key mechanism of establishment.

Another avenue worth exploring is whether pollinators behave similarly on recurrently formed polyploid lineages. Underlying genetic differences among origins could create novel phenotypes that subsequently alter the behavior and/or composition of pollinator communities visiting polyploids. The benefits to this approach are 2-fold. First, independent origins could be compared to assess whether there is a consistent effect of polyploidy. This approach could be used as an alternative to synthesizing neopolyploids and would bolster arguments based on well-established polyploid populations. Second, examining a geographic mosaic in pollinator communities among independent origins could tell us a lot about macroevolutionary change in polyploid lineages (Thompson, 2005). A reasonable hypothesis is that divergence in pollinator communities among origins will contribute to reproductive isolation of the independent polyploid lineages, potentially leading to speciation.

Interactions with seed dispersers—Another way that polyploids might overcome minority cytotype exclusion is via spatial aggregation within populations. For instance, the clumped distribution of polyploid fireweed reduced pollinator movement between cytotypes, thus causing some degree of precluding isolation between ploidy levels (Husband and Schemske, 2000). Short-distance seed dispersal is one mechanism by which polyploids might become aggregated within populations. Models show that local seed dispersal can enhance polyploid establishment (e.g., Li et al., 2004; Baack, 2005); thus, the question is whether polyploidy alters interactions with seed dispersers that results in decreased dispersal distance. Surprisingly, this area of research remains largely unexplored even though polyploidy-induced morphological changes have the potential to strongly impact seed dispersal. For example, the gigas effect, or increases in cell size, can increase seed and fruit size,

potentially changing dispersal distance by altering the attractiveness of fruit to animals or attracting different disperser species. Other changes in seed morphology may also play a role. In *Artemisia*, for instance, achenes often have a slime coat that adhere fruit to animals during dispersal. Some polyploid populations lack or have a reduced slime coat (Kreitschitz and Vallès, 2007), suggesting that dispersal distance may be reduced in polyploids. Given the potential importance of spatial structure and seed dispersal in polyploid establishment, tests of whether polyploidy creates novel seed dispersal interactions or predictable changes in these interactions are critical.

Interactions with herbivores—Interactions between polyploid plants and herbivores present an opportunity for diversification in both plant and herbivore lineages (Thompson et al., 1997). One pathway is via classic escape-and-radiate coevolution (Ehrlich and Raven, 1964) where the evolution of polyploidy allows plants to escape their herbivores by creating novel defenses. The plants radiate in the absence of herbivory as they move into new adaptive zones until the herbivores evolve counterdefenses that allow them to subsequently radiate onto the new plant lineage. Alternatively, rather than creating a barrier to herbivory, polyploidy may instead increase ecological opportunity by expanding herbivore host ranges. Ongoing differentiation in polyploid lineages could lead to divergence in the herbivores feeding on different cytotypes. Each of these scenarios predicts diversification, highlighting the potential for polyploidy to drastically impact the evolutionary trajectory of plant and herbivore lineages.

Evidence suggests support for both scenarios of diversification. For instance, in a macroevolutionary analysis of escape and radiate coevolution, Edger et al. (2015) showed that whole-genome duplication events in Brassicales allowed the plants to create new glucosinolate defensive chemicals via neofunctionalization of regulatory and biosynthesis genes. By doing so, the plants likely escaped herbivory and increased in species number. Important herbivores of these plants are pierid butterflies that responded to these changes in chemical defense by evolving counterdefenses. In some cases, the plant chemicals were actually co-opted by the butterflies and are now used as attraction signals. Following polyploidy events in Brassicales, there were three major escalations in diversification rate of both the plants and insects. In this example, it appears that the enhanced evolvability of polyploid genomes via neofunctionalization promoted escape-and-radiate coevolutionary diversification between plants and herbivores.

There is also strong evidence that the evolution of polyploidy can lead to host range expansion. For example, the evolution of octoploids in *Cardamine pratensis* has expanded the host range of the bud gall midge *Dasineura cardaminis* (Arvanitis et al., 2010). These midges attack their alternate host *C. amara* in moist, shady habitats where octoploid *C. pratensis* simultaneously flower. Although this ecological opportunity offered the appropriate conditions for a host shift, tetraploid *C. pratensis* are also available at some of these sites, yet are not attacked. Even when both octoploid and tetraploid cytotypes were placed into a common garden, gall midges rarely attacked tetraploids, showing extreme specialization to octoploids. Thus, in this example, the evolution of higher-level polyploids appears to have facilitated a specialized host shift leading to the incorporation of a novel host.

To the best of our knowledge, the gall midge on *Cardamine* is the only example where an herbivore has exclusively specialized to

a single cytotype; however, there are several examples where herbivores show differential attack on cytotypes in mixed populations. For example, the specialist prodoxid moth *Greya politella* has expanded its host range from using the ancestral host *Lithophragma* to also including diploid and autotetraploid *Heuchera grossulariifolia* (Thompson et al., 1997). This parasitic moth feeds on seeds of both cytotypes, yet across its geographic range in Idaho and Montana, *G. politella* has consistently higher attack rates on tetraploid plants even when the cytotypes occur in sympatry. Moreover, because Thompson et al. (1997) surveyed across recurrently formed tetraploid populations, the consistency in tetraploid attack suggests that this pattern is the result of a general preference for polyploids (Janz and Thompson, 2002). In an expanded survey of herbivores on *H. grossulariifolia*, Nuismer and Thompson (2001) also showed a divergent pattern of attack for a close relative of *G. politella*. *Greya piperella*, an inflorescence stem-boring moth, attacked diploids more frequently than tetraploids. In contrast, the generalist geometrid moth *Eupithecia misturata* attacked tetraploids at a higher rate than diploids, suggesting that herbivores may have differential preferences (Nuismer and Thompson, 2001). In *Heuchera*, then, it appears that polyploidy has consistently impacted interactions with herbivores, but that herbivore responses to the cytotypes are species specific.

Variable patterns of attack have also been observed in other plant species with mixed cytotype populations. In mixed populations of triploid and tetraploid *Arnica cordifolia*, the tephritid fly *Campiglossa footeorum* attacked tetraploids more commonly than triploids, and these seed parasites significantly reduced fitness of tetraploids (Kao, 2008). Phloem-feeding aphids were more common on diploid *Gymnadenia conopsea* orchids than on tetraploids, but this effect varied annually and was nonsignificant the following year (Gross and Schiestl, 2015). Using experimental arrays of diploid and tetraploid spotted knapweed, *Centaurea stoebe*, Collins and Müller-Schärer (2012) showed that a root-feeding weevil attacked tetraploids more frequently than diploids and that a generalist slug caused more damage on tetraploids, but there was no cytotype-specific preference by a root-feeding moth. Variation in attack has also been observed for *Solidago altissima*. Halverson et al. (2008) examined herbivory at seven sites that varied in the proportion of diploid, tetraploid, and hexaploid plants. Across the geographic range, the parasitic stem-galling fly *Eurosta solidaginis* consistently attacked tetraploids more frequently than expected given their relative abundance in the population, yet four other herbivore species showed strong variation in attack from population to population. Together, these examples illustrate that variability is common among and within study systems. Understanding why will require studies that tease apart the contributions of polyploidy vs. selection on herbivore attack and determine the factors that contribute to herbivore preference.

One factor that might cause differentiation in herbivore attack is habitat selection. Studies of *C. pratensis*, for example, show that the predispersal seed-feeding butterfly *Anthocharis cardamines* attacks tetraploid plants more frequently than octoploids (Arvanitis et al., 2007). Butterfly preferences, however, are likely driven by differences in the habitats of the cytotypes such that butterflies prefer to forage in the habitats where tetraploids are common rather than having specific preferences for the tetraploid cytotype itself (König et al., 2014b). A similar situation might also arise in *Solidago altissima*. Richardson and Hanks (2011) showed greater numbers of aphids and leaf-galling insects on hexaploid *S. altissima* in the field,

but when diploids, tetraploids, and hexaploids were placed into a common garden array, herbivores visited the three cytotypes equally. They also showed subtle differences in spatial distribution and habitats of the cytotypes that may have contributed to preference. Finally, a study of *Aster amellus* showed that hexaploids had more seed predator moths and higher damage than diploids (Münzbergová, 2006). These ploidy level effects explained 8% of the variance in attack rate, whereas plant population size explained 21% of the variance. Clearly, there are characteristics of polyploids that influence herbivory in *A. amellus* and these other examples, but herbivore decisions will also be based on a suite of additional factors.

Collectively, the results from these different systems suggest that as polyploid lineages arise, they can become incorporated into an herbivore's diet, expanding the host range; thus, the evolution of polyploidy is not a barrier to herbivores. In fact, for many of these systems the higher-level cytotypes actually experience increased attack. At first glance, this pattern seems somewhat surprising given that herbivores should exploit the common resource and polyploids are initially less common than their diploid counterparts. Furthermore, we know that polyploidy can cause instantaneous changes in physiology, morphology, and gene expression that can impact resistance to herbivores (e.g., Lou and Baldwin, 2003; Hull-Sanders et al., 2009a; Augustine et al., 2013). If polyploidy confers resistance, we would expect to see lower attack rates on these plants. Work in *Nicotiana*, however, shows considerable variation in defense among synthesized allopolyploid lines such that some lines have similar defenses to the parents whereas other lines have intermediate defenses (e.g., Pearse et al., 2006; Anssour and Baldwin, 2010). If polyploids commonly exhibit defensive responses similar to their diploid parents, we would not predict polyploidy to be a barrier to herbivores. Higher herbivory in polyploids also suggests that there is likely to be selection on them to avoid the cost of attack. Indeed, Nuismer and Ridenhour (2008) showed modest divergent selection in diploid and tetraploid *H. grossulariifolia* in response to herbivory. This pattern of divergent selection between cytotypes underscores how herbivory could drive divergence between polyploids and their diploid parents and lead to diversification in both plant and insect lineages.

One consequence of whole-genome duplication may be an enhanced ability of plants to alter or upgrade their defensive machinery. For example, as mentioned above, the evolution of polyploidy in Brassicales allowed the plants to evolve novel glucosinolate defenses by neofunctionalization of biosynthesis genes (Edger et al., 2015). In a study of diploid, tetraploid, and hexaploid *Solidago gigantea*, Hull-Sanders et al. (2009a) showed an increase in sesquiterpene production in tetraploids and an increase in monoterpene and diterpene production in hexaploids. These differences in secondary chemistry may have impacted survival and growth of herbivores in a common garden experiment (Hull-Sanders et al., 2009b). For instance, a generalist moth performed best when feeding on tetraploids, whereas a specialist beetle performed equally well on all three cytotypes. Although the mechanism conferring improved growth and survival on tetraploid *S. gigantea* is unclear, one possibility is that the moths are better equipped to detoxify the secondary chemicals of tetraploids (Hull-Sanders et al., 2009b). Although it seems reasonable that polyploidy should confer enhanced evolution of antiherbivore defenses, not all studies have shown such changes. Secondary chemistry of *Centaurea phrygia* was quite similar between diploids and tetraploids, and for the compounds that

differed significantly in concentration, diploids had much higher levels, suggesting a reduction in polyploid defenses (Münzbergová et al., 2015). This idea is supported by the observation that tetraploids had higher levels of herbivore damage than diploids. If polyploids can be more poorly defended than diploids, what are the alternative strategies that polyploids use?

One possibility is that polyploids may be more tolerant to herbivory than diploids. Tolerance is a mechanism by which plants are able to ameliorate the negative effects of herbivory (e.g., Fornoni, 2011). For example, *Centaurea stoebe* has been shown to overcompensate in response to root herbivory (Hahn and Müller-Schärer, 2013). Roots of tetraploids grew more in response to herbivory than diploid plants, leading to increased plant productivity. Similarly, Boalt et al. (2010) showed that octoploid *Cardamine pratensis* had higher tolerance than tetraploid plants to herbivory by pierid butterflies. These butterflies impose strong selection because they can consume up to 95% of the aboveground tissue, favoring increased tolerance in populations with high herbivory (König et al., 2014a). It remains unclear whether tolerance in these species was caused by polyploidy or selection after whole-genome duplication; however, there is indirect evidence suggesting that polyploidy itself could confer enhanced tolerance to herbivory. Scholes and Paige (2011) showed that in *Arabidopsis thaliana*, loss of apical dominance due to herbivory caused increased endoreduplication (localized polyploidy within specific groups of cells), allowing the plants to overcompensate for herbivore damage. Plants with higher ploidy within endoreduplicated cells grew faster after herbivory, leading to increased fitness. Thus, although polyploids might not be able to escape their herbivores, whole-genome duplication may afford a mechanism that allows polyploids to compensate for herbivore damage.

Together, the results suggest that polyploid plant–herbivore interactions are diverse and are likely to affect the evolutionary trajectory of both plant and insect lineages. Yet, there remain several gaps in our understanding of these interactions. First, it is unclear whether polyploidy confers resistance to herbivory, and if so, whether resistance is a trait that evolves after polyploid establishment or is an instantaneous advantage of polyploidy. Addressing this question will require examining the resistance mechanisms in diploids, established polyploids, and neopolyploids in both auto- and allopolyploid systems. Second, purported resistance mechanisms should be confirmed using preference and performance assays with herbivores. Field tests of herbivore preference and performance are critical as they would allow us to assess whether resistance is a direct result of polyploidy or a consequence of subsequent selection. Third, we know very little about whether polyploids are generally tolerant to herbivory. It would be interesting to compare reaction norms of fitness under differing levels of damage for a range of cytotypes within species. If there is a connection between tolerance levels and ploidy of endoreduplicated cells, it is possible that higher-level polyploids will have enhanced tolerance. Finally, additional tests of whether herbivory causes divergent selection between diploids and polyploids would provide much needed information about how often host-associated divergence is driven by polyploidy.

Tritrophic interactions—Host-associated differentiation caused by the host plant cytotype is likely to be an important process creating divergence in herbivores; thus, it stands to reason that these processes might cascade up to the natural enemies feeding at the third

trophic level. At present, there are no studies that have examined whether polyploidy leads to changes in the behavior or genetic structure of natural enemies, so here we examine a data set on geographic variation in parasitoid searching behavior (Althoff and Thompson, 2001) in light of recent findings on the ploidy level of the plants that the hosts occupy (Godsoe et al., 2013). Althoff and Thompson (2001) used behavioral assays to assess whether populations of the braconid parasitoid *Agathis* varied in how they searched for their host *Greya enchrysa*. This species of *Greya* primarily uses the host plant *Heuchera cylindrica* that has recently been shown to have both diploid and tetraploid cytotypes. Godsoe et al. (2013) sampled plants from four of the same sites as Althoff and Thompson (2001) and found that *H. cylindrica* occurs in single-cytotype populations. Here we present a reanalysis of searching behavior, but emphasize that there are issues of pseudoreplication because there is only a single tetraploid site in the data set. We intend this to be a preliminary discussion of whether polyploidy might affect tri-trophic interactions.

Caveats aside, the results are consistent with the idea that polyploidy can have cascading effects beyond a single trophic level. Female wasps spent more time searching for *Greya* on tetraploid fruit capsules than on diploids ($2x = 26$ s; $4x = 41$ s). Capsule size was not different between cytotypes, but size-corrected ovipositor length differed between females collected from plants differing in ploidy. Parasitoids searching on diploids had shorter ovipositors than those on tetraploids despite no difference in body size ($2x = 6.5$ mm; $4x = 6.8$ mm). Furthermore, Althoff and Thompson (2001) previously noted that some *Agathis* females searched for larvae within the infructescence stem, and this behavior coincides with plant ploidy level and differences in stem pubescence. Females searching on diploids were less likely to search stems than those on tetraploids ($2x = 6\%$ females searched stems; $4x = 50\%$ females searched stems), suggesting a relationship between parasitoid behavior and the ploidy level of the population in which they live.

While these observations are interesting, the results of this reanalysis could also be explained by population-specific variation in parasitoid searching behavior. Formal analyses on replicate populations are critically needed to evaluate the role of polyploidy in altering interactions with natural enemies. We propose that an ideal study system would have an array of herbivores and associated parasitoids in which to test these ideas. *Solidago*, for example, would make an excellent model as there are many well-studied herbivores and parasitoids, and moreover, host-associated divergence has occurred in both of these groups (e.g., Stireman et al., 2005, 2006).

Interactions with mutualistic fungi—Polyploidy may also affect interactions with above- and belowground mutualistic fungi. Mutualistic fungi are exceptionally common plant associates that live within the roots, shoots, and leaves of most plant species. In exchange for plant carbohydrates, fungi can acquire plant-inaccessible nutrients and may confer resistance to stress and herbivory. Due to the genetic and phenotypic changes induced by polyploidy, whole-genome duplication may have significant effects on interactions with these mutualists. For example, increased DNA content and elevated transcriptional activity of polyploid cells may alter interactions with fungi by increasing the demand for nitrogen and phosphorus from belowground mutualistic fungi (Wildermuth, 2010; Šmarda et al., 2013). Thus, given these predicted differences between the cytotypes, we might expect a shift in the interactions between mutualistic fungi and polyploids.

Studies of aboveground leaf endophytes and their host plants have revealed that polyploids host significantly fewer endophytes as compared with their diploid progenitors, suggesting that polyploidy may have a predictable, direct effect on these associations. In a comparison of endophyte incidence within diploid and neotetraploid *Lolium multiflorum*, Franco et al. (2015) showed that tetraploids had approximately one-third the infection rate of diploids. A similar pattern was observed in natural populations of tetraploid *Festuca vivipara* that had an uncommonly low endophyte infection rate (Gundel et al., 2014). These results suggest that physiological changes in polyploids might reduce the need to form mutualisms with endophytes and that polyploids may experience reduced benefits from these interactions. Alternatively, physiological changes in polyploids might simply prevent endophyte establishment within the plant. For example, the decreased colonization rates of endophytes may be explained by heightened production of defensive plant hormones that are known to increase resistance to fungal infections (Schenk et al., 2000; van Loon et al., 2006). Additional studies are needed to fully assess whether suppression of endophyte infection is common and whether suppressed infection rates influence polyploid fitness.

In addition to aboveground fungal mutualisms, polyploidy could also impact species associations with belowground mycorrhizal fungi. In the only study to test this hypothesis, diploid and tetraploid *Gymnadenia conopsea* orchids were shown to have highly divergent mycorrhizal species assemblages especially when growing in mixed-ploidy populations (Těšitelová et al., 2013). This effect, however, is likely an outcome of selection after polyploid formation because comparisons across populations showed that species associations were not cytotype-specific. Rather, there appears to be local specialization of orchid fungal species to each cytotype that might lessen competition. Examining a diversity of systems will allow us to determine whether polyploidy causes divergence of mycorrhizal communities or creates novel associations with these mutualists.

Although we have yet to examine whether polyploidy causes shifts in the community of arbuscular mycorrhizal fungi (AMF), polyploidy might affect this interaction in other ways. For example, interactions between AMF and polyploids can yield greater plant benefits than diploid-AMF interactions. In *Knautia arvensis*, extraradical (soil) hyphal growth was greater for AMF that associated with tetraploids, and inoculated tetraploids had a higher growth response than diploids (Doubková et al., 2012). Most studies, however, have shown no effect or even a slightly negative effect of AMF inoculation of polyploids. Comparisons of diploid and polyploid *Campanula gentilis* and *Pimpinella saxifraga* showed no ploidy-specific growth response to inoculation with the AMF species *Glomus mosseae* and *Rhizophagus irregularis* (formerly *Glomus intraradices*); however, diploid and hexaploid *Aster amellus* had different growth responses when interacting with these AMF species (Sudová et al., 2010). Inoculated hexaploids grew less or showed no change in growth. In contrast, diploids were strongly dependent on AMF as indicated by high mortality of control plants. Together, the results show an array of outcomes, suggesting that polyploidy may not cause predictable effects on interactions with AMF. However, some of this variability may be an artifact of experimental design. In particular, the use of nonnative AMF species in experiments could cause species pairings that are misrepresentative of nature.

Although the paucity of data currently limits our ability to generalize, at the outset, we would expect that polyploids would often

benefit from interacting with AMF. Increased DNA content is associated with increased need for nitrogen and phosphorus (Leitch and Leitch, 2008), and this nutrient demand may impact polyploid persistence in populations (Šmarda et al., 2013). Because AMF are more efficient at foraging for limiting nutrients like phosphorus, polyploids should benefit from these associations. Furthermore, polyploidy may also affect nutrient exchange between the plant and AMF. Some AMF actually induce polyploidy in small populations of root cells (e.g., Tahiri-Alaoui et al., 2002; Fusconi et al., 2005), suggesting that nuclear ploidy level of the host plant plays a functional role in the mutualism and that higher ploidy levels of host plants may affect nutrient transfer from plant to fungus. For example, changes in metabolism of polyploids could increase the resources that can be traded with their fungal partners (e.g., Berta et al., 2000; Ilut et al., 2012; Ježilová et al., 2015). To understand this dynamic process, we will need studies of the exchange of resources between diploid and polyploid hosts and their native AMF partners. Moreover, it would also be interesting to know whether cytotypes specialize to specific fungal species and whether any specialization provides a general benefit. We predict that local specialization is more likely than global because specializing locally could alleviate competitive interactions between cytotypes in mixed populations.

Interactions with soil microbes—Along with the belowground community of fungi, other soil microorganisms such as bacteria play an important role in structuring aboveground community dynamics (Wardle et al., 2004). Moreover, there can also be feedbacks from the plants, where plant-induced changes in the soil alter the microbial community immediately surrounding the plant (Schweitzer et al., 2008). Together, these feedbacks contribute to plant and microorganism coexistence. Because polyploidy can cause changes in physiology such as secondary chemistry, nutrient use, or photosynthetic rates that could have a direct impact on soil microbes, we might expect polyploidy to induce large changes in the soil community (e.g., Knight et al., 2005; Thébault et al., 2010). In the only study comparing soil communities of diploids and tetraploids, Thébault et al. (2010) showed that soil bacterial diversity increased in pots containing tetraploid *Centaurea maculosa* and that the abundance of nonbacterial microbes decreased in soil containing tetraploid *Senecio inaequidens*. These results suggest a species-specific pattern that might be caused by selection following whole-genome duplication. The next step in assessing whether these changes in microbial community structure are a direct result of polyploidy will be to conduct experiments using neopolyploids.

Interactions with pathogens—A commonly cited advantage of polyploidy is escape from parasites and pathogens because polyploids are predicted to have increased or novel secondary chemistry that can confer resistance (Levin, 1983). This advantage could facilitate establishment of a newly formed polyploid lineage, providing a competitive edge over diploids. Indeed, in a model considering interactions between pathogens and neopolyploids, Oswald and Nuismer (2007) showed that polyploids are predicted to have greater resistance than their diploid parents. Although this idea has featured prominently in the literature, surprisingly few studies outside of agriculture have examined the effect of polyploidy on pathogens and parasites. Here we restrict our discussion to non-insect parasites (but see *Interactions with herbivores* and *Tri-trophic interactions* for discussion of insect parasites).

Studies of naturally occurring polyploids and their diploid progenitors suggest a range of outcomes for interactions between plants and pathogens. For instance, neotetraploid *Trifolium pratense* were more resistant to *Sclerotinia trifoliorum* clover rot than their diploid progenitors (Vestad, 1960; Vleugels et al., 2013), and tetraploid *Glycine tabacina* were more resistant than diploids to the leaf rust pathogen *Phakopsora pachyrhizi* (Burdon and Marshall, 1981). However, the expected pattern of increased resistance in polyploids is not always observed. Schoen et al. (1992) showed similar levels of resistance to *P. pachyrhizi* by diploid and tetraploid *G. tomentella*, whereas in synthesized allopolyploid *Nicotiana*, the pattern of resistance was additive (Gottula et al., 2014). *Nicotiana* allopolyploids generated from crosses between resistant and susceptible parents yielded allopolyploids with intermediate resistance to a nematode transmitted virus and crosses between two resistant or two susceptible parents produced allopolyploids with similar resistance to the parents (Gottula et al., 2014). Together, these results fit with recent opinions on polyploid–pathogen interactions that suggest the effect of polyploidy on resistance is complex (King et al., 2012).

Our ability to assess whether resistance is a general outcome of polyploidy is currently constrained by a lack of research on naturally occurring plants. So far, our understanding of polyploid plant–pathogen interactions is limited to one plant family (Fabaceae) for autopolyploid studies, and there is only a single study of allopolyploids. Furthermore, most of the above examples have focused on fungal pathogens; thus, increasing the taxonomic breadth of pathogens studied will be essential for developing general predictions on the evolution of resistance in polyploids. Comparisons of resistance of established polyploid populations with neopolyploids will be informative about the role of selection in polyploid resistance, and examining patterns of resistance in auto- vs. allopolyploids will allow us to test the effect of hybridization.

Prospective—Assessing whether polyploidy plays a significant role in the origins of biodiversity will only be accomplished by examining how and when whole-genome duplication impacts interactions with other species. Answering this question requires tests of whether polyploidy immediately changes interactions with other species or whether polyploidy makes plant lineages uniquely adaptable such that species interactions could quickly evolve. Support of either of these mechanisms would offer evidence that polyploidy is a multifaceted promoter of diversification. We also need to know how common is the alternate option: that polyploidy triggers reproductive isolation of lineages and permits selection to shape now distinct populations. If selection after whole-genome duplication is the primary mode of divergence in species interactions, then polyploidy might not be a distinctive mechanism of diversification. To make sense of the patterns observed in natural populations, efforts should focus on comparisons of diploids, polyploids, and neopolyploids. With rare exception, experiments have used polyploids that have had tens, if not thousands, of generations in which to evolve, and this bias makes it impossible to draw general conclusions about polyploid-driven diversification. Although creating synthetic polyploids may be challenging or impossible in some systems, there are alternative approaches that could be used. For species in which polyploidy has recurrently formed, independent polyploid origins could be compared. This type of analysis assumes that selection will be variable across populations; thus, observing a consistent pattern among origins suggests a predictable outcome of polyploidy on species interactions. Another alternative is to use high throughput

screening approaches such as flow cytometry to identify neopolyploids in the field or from controlled crosses of diploids (e.g., Ramsey, 2011).

In addition to teasing apart the relative contributions of polyploidy vs. selection, it would also be instructive to determine why and how novel species interactions occur. The formation of novel interactions increases the potential for specialization and diversification. If novel interactions are rare, however, then newly formed polyploids are phenotypically similar to diploids and are readily incorporated as hosts. Similarly, an exciting area would be to examine whether the degree of specialization of the interaction has an impact. We predict that highly specialized interactions with diploids would be more strongly affected by the evolution of polyploidy than generalized ones. Finally, if we are to paint a complete picture of how polyploidy and species interactions impact biodiversity, we will need studies that simultaneously examine multiple types of species interaction. Polyploids interact with many types of species, and the direct and indirect effects of these cumulative interactions will ultimately shape the process of diversification.

ACKNOWLEDGEMENTS

We thank D. Althoff for kindly providing the original data on parasitoid-searching behavior. We also thank D. Althoff, S. Heard, L. Porturas, S. Wang, and an anonymous reviewer for insightful comments on an earlier draft of the manuscript.

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