

Plant polyploidy and non-uniform effects on insect herbivores

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Genomic duplication through polyploidy has played a central role in generating the biodiversity of flowering plants. Nonetheless, how polyploidy shapes species interactions or the ecological dynamics of communities remains largely unknown. Here we provide evidence from a 4 year study demonstrating that the evolution of polyploidy has reshaped the interactions between a widespread plant and three species of phytophagous moths. Our results show that polyploidy has produced non-uniform effects, with polyploids less attacked by one insect species, but significantly more attacked by two other species. These results suggest that the evolution of plant polyploidy may not generally confer uniform resistance to multiple species of insect herbivores. In the absence of such a uniform release, the extreme evolutionary success of polyploid plants is probably due to factors other than escape from herbivory. Together, these results suggest that a primary consequence of plant polyploidy may be to shape the ecological structure of plant–insect interactions, thereby providing opportunities for diversification in both plant and insect taxa.

Keywords: polyploidy; herbivory; parasitism; evolution; coevolution; resistance

1. INTRODUCTION

Much of the diversity of flowering plants may result from genomic duplication through polyploidy, with anywhere from 4 to 80% of angiosperm species of polyploid origin (Levin 1983; Masterson 1994; Otto & Whitton 2000; Soltis & Soltis 2000). The importance of polyploidy has been further demonstrated by recent studies that have shown that novel polyploid lineages may arise frequently, with the potential for multiple origins within single populations (Brochmann *et al.* 1992; Ramsey & Schemske 1998; Segraves *et al.* 1999; Soltis & Soltis 2000). In addition to its central role in plant evolution, polyploidy has been widely used in agriculture to generate novel phenotypic qualities, including increased resistance to parasitism or herbivory (Alam & Gustafson 1988; Busey *et al.* 1993; B Rai & Rao 1994; Dhawan & Lavania 1996). Despite its demonstrated importance in plant biology, there has only been one previous study investigating the impact of polyploidy on interactions between plants and their insect herbivores within and across natural communities (Thompson *et al.* 1997). Furthermore, there have been no studies that have examined the effects of polyploidy across multiple herbivore taxa and multiple years. Consequently, plant polyploidy has not been integrated into our understanding of the ecology and evolution of species interactions and the structure of biological communities.

Heuchera grossulariifolia is a rhizotomous perennial that grows primarily along the major river valleys of eastern Washington, Idaho and western Montana. There have been at least two and potentially up to seven independent origins of autopolyploid populations within this geographic range (Wolf *et al.* 1990; Segraves *et al.* 1999). These autopolyploids are particularly useful for evaluating the impacts of plant polyploidy *per se* on interspecific

interactions. Unlike allopolyploids, which arise through hybridization between species, autopolyploids arise from the duplication of genomes within a single species. Thus, analysis of autopolyploids avoids the potentially confounding effects of interspecific hybridization and its well-documented effects on species interactions (Strauss 1994; Whitham *et al.* 1994; Thompson *et al.* 1997; Fritz 1999; Fritz *et al.* 1999).

Previous work using extensive flow cytometric analysis of the ploidy levels of plants along the Salmon River drainage of northern Idaho identified populations in which both diploid and autotetraploid individuals grow sympatrically (Thompson *et al.* 1997). This work also revealed very consistent and reliable morphological differentiation between the ploidy levels at some sites, thereby allowing the two cytotypes to be readily distinguished visually within these populations (Thompson *et al.* 1997; Segraves & Thompson 1999). This previous work allowed us to establish marked pairs of diploid and tetraploid individuals within each of the 4 years of this study from 1997 to 2000. The ability to pair plants of different ploidy levels is ideal for studying the effects of polyploidy on patterns of insect herbivory, because it minimizes the potential influence of microhabitat differentiation between the ploidy levels (Hancock & Bringham 1981; Lumaret *et al.* 1987; Hardy *et al.* 2000; Husband 2000).

We used the interactions between *H. grossulariifolia* and three species of phytophagous insects to investigate whether the evolution of plant polyploidy has had a significant effect on the pattern of insect attack within local populations. The insects included two closely related *Greya* species, *Greya politella* and *Greya piperella* in the family Prodoxidae and a more distantly related moth species, *Eupithecia misturata* in the family Geometridae. These three insect species differ in the plant tissues on which they feed, thereby allowing evaluation of the impacts of polyploidy on herbivory across a relatively broad range of

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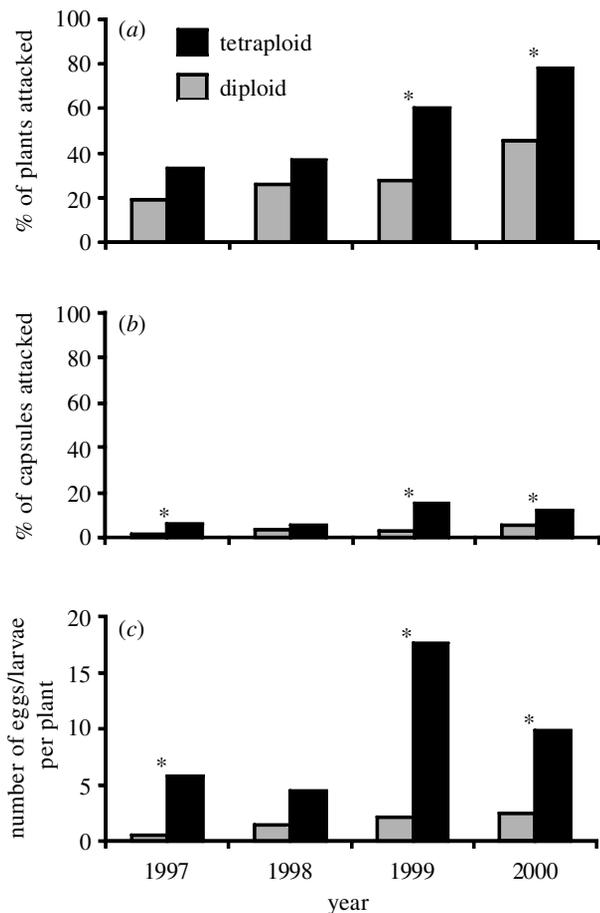


Figure 1. Patterns of attack by *G. politella* on diploid and tetraploid *H. grossulariifolia*. The asterisks indicate statistical significance at the $p < 0.05$ level. (a) The percentage of diploid and tetraploid plants attacked, with statistical significance determined using the McNemar test for paired binary data. (b) The mean percentage of capsules attacked for diploids and tetraploids, with statistical significance established using the Wilcoxon signed-rank test. (c) The mean number of eggs or larvae in diploid and tetraploid individuals, with statistical significance determined using the Wilcoxon signed-rank test. The sample sizes were $n = 36$ pairs in 1997, $n = 27$ pairs in 1998, $n = 40$ pairs in 1999 and $n = 37$ pairs in 2000.

feeding habits. The two *Greya* species, which are closely related to yucca moths, oviposit directly into plant tissues, where the larvae then develop internally. *Greya politella* oviposits into the flowers of *H. grossulariifolia* and, as the larvae develop, they consume some of the developing seeds (Thompson *et al.* 1997). In contrast, *G. piperella* oviposits into the flowering stems and the developing larvae feed internally by mining the stem tissue (Davis *et al.* 1992). Unlike the two *Greya* species, *E. misturata* oviposits directly onto the flowering scapes. The larvae develop externally, eating both flowers and flowering stems, frequently destroying all floral tissues and part of the stem on individual plants.

2. METHODS

At the beginning of the study we marked all available pairs of diploid and tetraploid *H. grossulariifolia* growing along a 12 km stretch of the Salmon River east of Riggins, Idaho. All marked

pairs consisted of a diploid individual and tetraploid individual of approximately equal size and separated by less than 2 m. Because all marked individuals did not flower in every year, the number of pairs with both diploid and tetraploid individuals flowering differed across years.

We quantified attack by *G. politella* in all 4 years by dissecting the floral capsules from a subset of the marked diploid/tetraploid pairs of *H. grossulariifolia*. This subset consisted of all pairs with at least 20 flower capsules remaining after all moth activity had ceased. At this time 20 capsules were collected from each plant and stored frozen in alcohol until laboratory dissections could be performed. Dissections were performed using a dissecting scope, thereby allowing us to determine the total number of eggs and larvae present within each floral capsule.

Attack by *G. piperella* was evaluated over 3 years by dissecting the floral scapes from a subset of the marked diploid/tetraploid pairs of *H. grossulariifolia*. Because *G. piperella* is active before *G. politella* and our sampling strategy required the removal of entire scapes, scapes were collected from a subset of the marked pairs not used for estimating the patterns of attack by *G. politella* or *E. misturata*. Two floral scapes were haphazardly collected from each plant for each diploid/tetraploid pair and stored frozen in alcohol until laboratory dissections could take place. These dissections allowed us to determine the number of eggs and larvae present within each scape.

We quantified attack by *E. misturata* for 3 years through observational surveys of the subset of marked diploid/tetraploid pairs with both individuals flowering. The numbers of larvae feeding on each plant were recorded for all of the marked pairs on multiple days interspersed throughout the period of larval activity. These surveys were conducted for the majority of the days that larvae were active in all years.

The percentage of attacked diploid and tetraploid individuals was analysed for all three species of phytophagous insect using the McNemar test for paired binary data. All other statistical comparisons used the Wilcoxon signed-rank test, which explicitly incorporates paired data. Differences were considered statistically significant in all analyses if $p < 0.05$.

3. RESULTS

Dissection of the floral capsules revealed that a significantly greater percentage of tetraploid plants was attacked by *G. politella* in 1999 and 2000. The percentage of tetraploid individuals attacked exceeded diploids by 32.50% in 1999 and 32.43% in 2000 (figure 1a). In addition, the mean percentage of attacked capsules was significantly greater for tetraploids in 1997, 1999 and 2000. In these years, the mean percentages of attacked tetraploid capsules exceeded diploids by 4.75, 12.63 and 6.76%, respectively (figure 1b). Furthermore, tetraploids harboured a significantly larger mean number of eggs or larvae per plant in 1997, 1999 and 2000, with the tetraploid means exceeding those of diploids by 5.28, 15.45 and 7.35, respectively (figure 1c). Thus, the data provide strong evidence that, within sympatric populations of diploid and tetraploid plants, tetraploids generally suffer much higher levels of attack by *G. politella*.

In direct contrast to the results for *G. politella*, the levels of attack by the closely related *G. piperella* were significantly higher on diploid *H. grossulariifolia*. A significantly higher percentage of diploid plants was attacked in all 3 years that attack by *G. piperella* was investigated. The

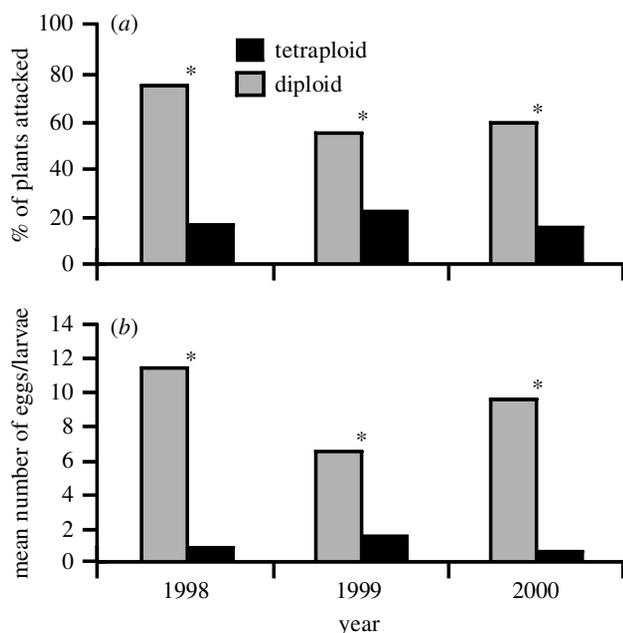


Figure 2. Patterns of attack by *G. piperella* on diploid and tetraploid *H. grossulariifolia*. The asterisks indicate statistical significance at the $p < 0.05$ level. (a) The percentage of diploid and tetraploid plants attacked, with statistical significance determined using the McNemar test for paired binary data. (b) The mean number of eggs or larvae in diploid and tetraploid individuals, with statistical significance determined using the Wilcoxon signed-rank test. The sample sizes were $n = 24$ pairs in 1998, $n = 31$ pairs in 1999 and $n = 32$ pairs in 2000.

percentage of attacked diploids exceeded tetraploids by 58.33% in 1998, 32.26% in 1999 and 43.75% in 2000 (figure 2a). Furthermore, diploid individuals harboured a higher mean number of eggs and larvae than did tetraploids across all 3 years, with diploids exceeding tetraploids by 10.63 in 1998, 5.03 in 1999 and 8.94 in 2000 (figure 2b). Thus, despite the close phylogenetic relationship between *G. politella* and *G. piperella*, the evolution of polyploidy within *H. grossulariifolia* has produced divergent effects on the patterns of attack by these species.

Much like the patterns observed for *G. politella*, attack by *E. misturata* was much greater on tetraploid *H. grossulariifolia*. A significantly higher percentage of tetraploid plants was attacked in both 1998 and 1999, with 20.0% more tetraploids attacked in 1998 and 21.06% more attacked in 1999 (figure 3a). Likewise, individual tetraploid plants sustained significantly higher mean daily larval loads than did diploids during 1998 and 1999 (figure 3b). There were no significant differences in either the percentage of plants attacked or the mean daily larval load between the ploidy levels during the 2000 field season.

4. DISCUSSION

Our results show that the evolution of polyploidy within *H. grossulariifolia* has had important consequences for interactions with insect herbivores. In particular, genomic duplication through polyploidy has produced effects that are strongly non-uniform across insect taxa. Autotetraploids within our study population of

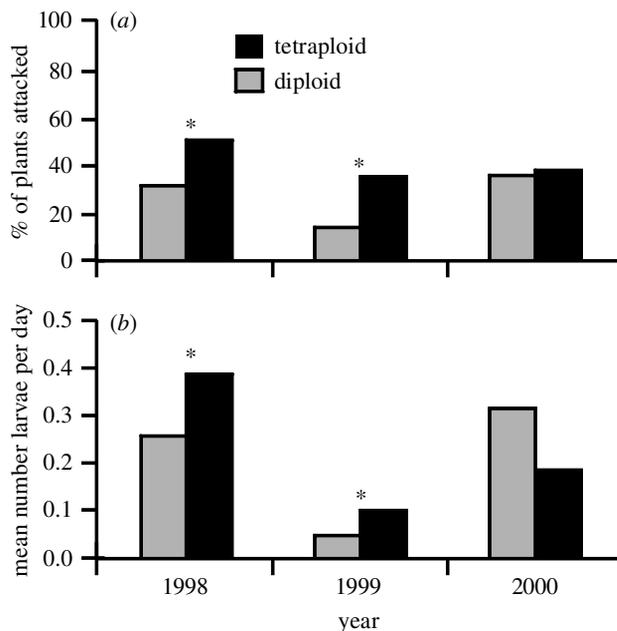


Figure 3. Patterns of attack by *E. misturata* on diploid and tetraploid *H. grossulariifolia*. The asterisks indicate statistical significance at the $p < 0.05$ level. (a) The percentage of diploid and tetraploid plants attacked over the entire season, with statistical significance determined using the McNemar test for paired binary data. (b) The mean daily abundance of *E. misturata* on diploid and tetraploid individuals, with statistical significance determined using the Wilcoxon signed-rank test. The sample sizes were $n = 80$ pairs in 1998, $n = 76$ pairs in 1999 and $n = 81$ pairs in 2000. Observations were carried out over 12 days in 1998, 26 days in 1999 and 20 days in 2000.

H. grossulariifolia growing along Idaho's Salmon River experienced greatly reduced attack by *G. piperella*, but increased levels of attack by *G. politella* and *E. misturata*. These results suggest that, at least for some plant taxa, the evolution of polyploidy may produce non-uniform effects on interactions with insect herbivores. Furthermore, the different impact of polyploidy on the two closely related species *G. politella* and *G. piperella* suggests that non-uniform impacts may occur across even very narrow phylogenetic scales.

An important consequence of our results is that novel polyploid lineages may not generally receive a uniform or consistent release from herbivory. In the absence of such a uniform release, it seems unlikely that the evolutionary diversification and success of polyploids has resulted from increased resistance to herbivory. This 'evolutionary barrier hypothesis' has been previously rejected based upon broad geographic surveys showing that *G. politella* has colonized both ploidies of *H. grossulariifolia* (Thompson *et al.* 1997). We cautiously interpret the current results as providing further evidence against the barrier hypothesis. Nevertheless, our results do not preclude the possibility that the non-uniform effects disappear under some ecological conditions (e.g. no significant effect of ploidy on attack by *E. misturata* in 2000). Tetraploids could have a fitness advantage over diploids under different sets of ecological conditions or in geographic areas where insect taxa favouring tetraploids are absent.

Overall, these results and the potential they suggest for geographic differences in selection on diploids and polyploids suggest that the evolution of polyploidy may be an important force in the diversification of both plant and insect lineages. Novel polyploid lineages may frequently be subjected to different suites of herbivores and different selection pressures. These different herbivore suites may promote evolutionary diversification between the ploidies as each adapts to the selective pressures imposed by its particular suite of insect herbivores. Similarly, as diversification between the ploidies proceeds, the potential exists for parallel radiation within the associated taxa of herbivorous insects. The potential importance of this mechanism in generating evolutionary diversification is bolstered by recent work demonstrating that polyploids may arise much more frequently than previously expected (Brochmann *et al.* 1992; Soltis & Soltis 1995, 1999; Ramsey & Schemske 1998; Segraves *et al.* 1999). As a result, plant polyploidy may play a fundamental role in both structuring the ecological dynamics of communities and in the evolutionary diversification of interactions between plants and insects.

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