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Multiple origins of polyploidy and the geographic structure of *Heuchera grossulariifolia*

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Abstract

Multiple origins of polyploidy from an ancestral diploid plant species were investigated using restriction site polymorphism and sequence variation in the chloroplast DNA (cpDNA) of *Heuchera grossulariifolia* (Saxifragaceae). Phylogenetic analysis indicated that autopolyploidy has arisen at least twice in the evolutionary history of this species and potentially up to as many as seven times. These results suggest a greater range of independent polyploid origins as compared to a previous study of *H. grossulariifolia* using cpDNA restriction sites that indicated a minimum of three independent origins. Moreover, most polyploid populations did not contain cpDNA haplotypes from a single origin, but rather combined haplotypes from at least two polyploid origins. Past migration among polyploid populations of independent origin or localized polyploid formation may explain the distribution of polyploid haplotypes within and among populations. The analysis also revealed a discrepancy between relatedness and geographical location. In nearly all sympatric populations of diploids and polyploids, polyploids had the same cpDNA haplotypes as diploids from a geographically remote population. This geographical discordance has several possible explanations, including small sample sizes, extinction of parental diploid haplotypes, chloroplast introgression, and homoplasy in the cpDNA sequence data. We conclude that the recurrent formation of polyploids is an important evolutionary mechanism in the diversification of *H. grossulariifolia*.

Keywords: chloroplast DNA, DNA sequence, multiple origins, phylogeography, polyploidy, RFLP

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Introduction

About 50% of angiosperm species are of polyploid origin (Stebbins 1971; Averett 1980; DeWet 1980; Levin 1983; Masterson 1994), and in many cases polyploidy may occur multiple times within a lineage (Soltis *et al*. 1992; Soltis & Soltis 1993). Studies of polyploid taxa have documented multiple origins of polyploidy in at least 40 species, including both autopolyploids and allopolyploids (reviewed by Soltis & Soltis 1993). However, the goal of many of these studies was not to document multiple origins of polyploidy *per se*, but rather to investigate the parentage of a polyploid species. As a consequence, Soltis & Soltis (1993) have suggested that our present estimates of the frequency of multiple origins are low and that multiple origins of polyploidy are the rule. The recurrent formation of polyploids within a species can create a mosaic of pre-existing diploid populations and newly established polyploid populations across a species' range. The evolutionary success of polyploids has often been attributed to the consequences of having multiple genomic copies. Three such consequences are increased heterozygosity, allelic diversity, and enzyme multiplicity that may provide polyploids with a genetic advantage that facilitates their establishment and persistence (Roose & Gottlieb 1976; Levin 1983; Stebbins 1985; Soltis & Rieseberg 1986; Samuel *et al*. 1990; Novak *et al*. 1991; Soltis & Soltis 1993, 1995). Rapid genomic changes can occur quickly following the formation of polyploids, potentially producing genotypes different from either of the diploid progenitor genotypes (Song *et al*. 1995) or causing

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chromosomal rearrangements (Bennett *et al*. 1992; Bailey *et al*. 1993; Mukai *et al*. 1993; D'Hont *et al*. 1996; Aggerwal *et al*. 1997; Friesen *et al*. 1997). Once several polyploid populations are established, mating among polyploids from independent origins can bring together novel genetic combinations that may subsequently enrich the polyploid gene pool (Soltis & Soltis 1993; Soltis *et al*. 1995). With these novel genetic combinations, polyploids may be able to adapt to a new environment or be competitively superior to the parental diploids (Roose & Gottlieb 1976; Hancock & Bringhurst 1981; Levin 1983; Thompson & Lumaret 1992; Soltis & Soltis 1995; Soltis *et al*. 1995; Song *et al*. 1995; Ehrendorfer *et al*. 1996).

Several mechanisms have been proposed to explain the distribution of polyploids relative to their diploid progenitors. Because newly formed polyploids are low in frequency, their successful establishment may be difficult due to a high rate of inviable matings with diploids (Levin 1975). However, this 'minority cytotype exclusion' may be overcome if there are large ecological differences between the cytotypes (Fowler & Levin 1984; Rodriguez 1996). Polyploids are often better able to withstand either broader environmental conditions or occupy new environments than their diploid parents (Lewis 1980; Levin 1983; Lumaret 1988; Lumaret & Barrientos 1990; Van Dijk *et al*. 1992). Therefore, mixed cytotype populations should occur in areas of environmental heterogeneity or under circumstances where only a limited number of crosses occur between ploidal levels.

Studies of *Heuchera grossulariifolia* Rydb. (Saxifragaceae), a rhizomatous perennial, have documented the distribution of diploids relative to polyploids (Wolf *et al*. 1990; Thompson *et al*. 1997). Diploids are widespread along most of the major rivers in Idaho and western Montana, USA whereas polyploids have a more limited distribution across north-central Idaho and western Montana. In several river systems, diploids and tetraploids occur sympatrically (Fig. 1). A broad geographical survey of populations indicated that triploids occur in at least one of these areas of overlap and account for about 1.4% of plants surveyed (Thompson *et al*. 1997). Diploid *H. grossulariifolia* also occurs along the Columbia River Gorge in Washington and Oregon (Wolf *et al*. 1990), but these populations have been relatively unstudied.

Two earlier studies have evaluated the geographical distribution of diploid and polyploid *H. grossulariifolia* in Idaho and Montana. Wolf *et al*. (1989, 1990) suggested that this species is a complex of diploid and tetraploid populations with little overlap among populations differing in ploidy. Tetraploid *H. grossulariifolia* demonstrated tetrasomic inheritance at four allozyme loci, indicating an autotetraploid origin (Wolf *et al*. 1989). Wolf *et al*. (1990) also provided evidence suggesting that there are at least three independent autotetraploid origins: one in the Seven Devils mountain range in Idaho, one along the Salmon River in Idaho, and one along the upper Selway River in Idaho (Fig. 1). Subsequently, Thompson *et al*. (1997) found increased evidence for overlap among populations differing in ploidy and also reported evidence that the evolution of polyploidy has influenced the geographical structure of interactions with a major floral parasite, the prodoxid moth *Greya politella*.

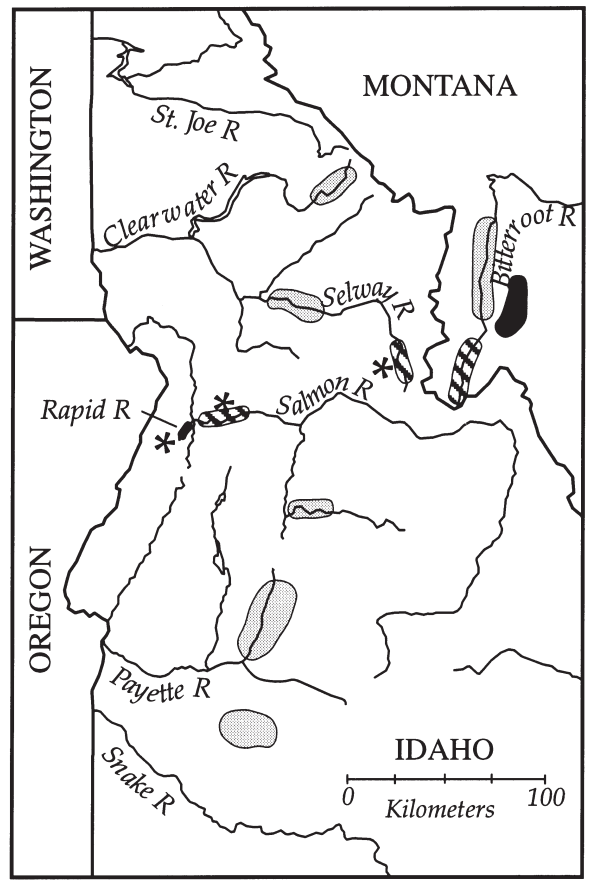

Fig. 1 Distribution of *Heuchera grossulariifolia* collection sites. Light-grey areas are diploid sites, black areas are tetraploid sites, and hatched areas are mixed cytotype sites. Asterisks show the three origins of autopolyploidy proposed by Wolf *et al*. (1990). From left to right, the three Wolf *et al*. (1990) origins are: Seven Devils mountain range, Main Fork Salmon River, and upper Selway River near Magruder Crossing Ranger Station. The cytotype distribution is according to Thompson *et al*. (1997).

In this study we determine the number of independent polyploid origins using restriction site polymorphisms and nucleotide sequence variation in the chloroplast DNA (cpDNA) to resolve further the phylogenetic hypothesis of Wolf *et al*. (1990) and the geographical structure of polyploidy in this species complex. This survey of *H. grossulariifolia* spans the majority of its geographical range, including populations not previously sampled and

populations of mixed cytotypes. Based on the results of the Wolf *et al*. (1990) study, we predicted that there would be a maximum of one polyploid origin along each river drainage that contains polyploids and, in areas of sympatry, we expected polyploids to be most closely related to diploids from the same area.

Materials and methods

Four plants from each site and ploidal level were collected and grown in a common garden on the Washington State University campus (Table 1). Voucher specimens were deposited at the Marion Ownbey Herbarium (Washington State). Ploidal level for these plants was determined previously using flow cytometry (Thompson *et al*. 1997).

Total genomic DNA was extracted following Doyle & Doyle (1987) with two modifications: 4% PVP was used and tissue samples were ground in liquid nitrogen. DNA from four individuals was combined in equal portions for each diploid and autotetraploid site. Combined DNA (0.5 μg) for each diploid and tetraploid site was digested with one of 12 restriction endonucleases (*Apa*I, *Bst*EII, *Bst*XI, *Cfo*I, *Eco*RI, *Eco*RV, *Hae*II, *Hin*dIII, *Pvu*II, *Sac*I, *Sal*I, and *Xho*I) following the specifications of the manufacturers. DNA fragments were separated on 1% agarose gels, denatured, and then transferred to nylon membranes (MagnaGraph, Micron Separations, Inc.). We used the Multiprime DNA Labelling System (Amersham) to radioactively label *Lactuca* and *Petunia* cpDNA probes (Jansen & Palmer 1987) to probe the entire chloroplast genome. Hybridizations followed the procedures of Palmer (1986).

Restriction site variation was also evaluated from two cpDNA intergenic spacer regions (*trnK*1/2 and *rbcL* T1/*orf* 512). DNA from each individual was amplified separately in 25 μl reaction volumes (1× PCR buffer (Gibco), 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 unit *Taq* polymerase (Gibco), 2.5 pmol of each primer, and 10 ng of DNA for 35 cycles (1 min at 94 °C, 1 min at 50 °C, 2 min at 72 °C). Primer sequences were provided by S. Brunsfeld. PCR products were digested overnight with two units of one of 10 restriction endonucleases (*Acc*I, *Ava*I, *Ava*II, *Ban*I, *Ban*II, *Bst*NI, *Hha*I, *Hin*cII, *Hpa*II, and *Nci*I). DNA fragments were separated on 1% agarose gels and visualized using ethidium bromide staining illuminated with UV light.

In addition, nucleotide sequence variation in two cpDNA intergenic spacer regions (*trnL* (UAA) and *trnF* (GAA) spacer; *trnL* (UAA) intron) was examined. These regions were initially amplified in 50 μl reaction volumes (1× PCR buffer (Gibco), 3 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit *Taq* polymerase (Gibco), 5 pmol of each primer, and 20 ng of DNA for 35 cycles (1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C), using the primers from Taberlet *et al*. (1991). Cycle sequencing products were amplified from polyethylene glycol (PEG)-cleaned initial product (50 ng

Table 1 List of site designations for 10 *Heuchera grossulariifolia* populations, their ploidal level, and their locations

Designation	River	Location
Idaho		
Boise	South Fork Boise R. (2X)	23.3–28.7 km along FR 189, Boise National Forest
NF Clearwater	North Fork Clearwater R. (2X)	6.4–22.9 km from junction of FR 247 and 250 on FR 250, Clearwater National Forest
Payette	Middle Fork Payette R. (2X)	10.0 km along FR 698, 3.7 km Harris Cr., 10.8 km FR 678, Boise National Forest
Rapid	Rapid R. (4X)	0.4–6.4 km on Rapid R. Tr. on FR 2114, Nez Perce National Forest
Salmon	Main Fork Salmon R. (2X/4X)	3.7–30.3 km E Riggins, ID, Payette National Forest
EFSF Salmon	East Fork South Fork Salmon R. (2X)	3.9–13.7 km along FH 48, Payette National Forest
Lower Selway	Selway R. (2X)	11.3–30.8 km along FR 223, 0.5 km FR 443, Nez Perce National Forest
Upper Selway	Selway R. (2X/4X)	15.5–33.7 km W of Nez Perce Pass on FR 468, 10.1 km along FR 285, Nez Perce National Forest
Montana		
WF Bitterroot	West Fork Bitterroot R. (2X/4X)	45.1–67.1 km along SR 473, 0.0 km FR 5669, 3.9 km FR 104D, 3.2 km FR 5703, 0.3 km FR 49, Bitterroot National Forest
MF Bitterroot	Main Fork Bitterroot R. (2X/4X)	0.8 km FR 701, 1.0 km FR 1327, 11.8–12.3 km FR 502, 1.6 km FR 711, 4.3 km FR 374, Lolo and Bitterroot National Forests

of initial product, 2.5 pmol primer, and PRISM dye primer labelling kit (Perkin-Elmer), using one-half of the manufacturer's recommendations for dye labelling chemistry) for 25 cycles (30 s at 96 °C, 30 s at 45 °C, 4 min at 60 °C). Both the forward and reverse strands were sequenced using an ABI Prism 377 automated DNA sequencer. Sequences were aligned by sight and gaps were scored as missing data. Insertions and deletions were not included in the analyses because many consisted of direct duplications of 1–16 bp. Golenberg *et al*. (1993) demonstrated that indels located in noncoding regions of the cpDNA consisting of direct duplications were homoplasious. Analyses including indels resulted in trees 23 steps longer and an increased homoplasy index. Sequences were deposited in GenBank under Accession nos AF038668–AF038779.

We used both a closely related species, *Heuchera micrantha*, and a closely allied genus, *Tolmiea menziesii*, to polarize mutations. These outgroups were chosen based on the cpDNA results of Soltis & Kuzoff (1995). We used a heuristic search and parsimony (PAUP* 4.0d56, courtesy of D. Swofford) with MULPARS (saves all shortest trees), TBR (tree bisection-reconnection) branch swapping, and both simple and random taxon addition with 100 replicates to find the shortest trees. The restriction site mutations, length mutations, and sequence data were combined. Characters were unordered. Genetically identical taxa within a site were excluded to simplify the resulting trees. Support for each node was evaluated with 100 bootstrap replicates.

We estimated the number of independent polyploid origins by assuming that tetraploids arose from diploids having the same cpDNA haplotype. Independent origins were inferred by examining the strict consensus tree for the number of branches in which diploids and tetraploids co-occurred. The maximum number of origins was determined by the number of unique tetraploid cpDNA haplotypes.

Results

Four restriction site mutations and two length mutations were found using the cpDNA probes (Table 2). The length mutations (5 and 6) were identified by finding identical length differences with several restriction enzymes. However, none of the restriction site mutations or length mutations corresponded to those found in Wolf *et al*. (1990), although the same restriction enzymes were used. There were no restriction site mutations in the *rbcL* T1/*orf* 512 spacer and two restriction site mutations in the *trnK* spacer. Neither of these mutations were parsimony informative.

Approximately 937 bp were sequenced from the *trnL* intron and *trnL/F* intergenic spacer combined, and sequence alignment required nine insertion/deletions. With the restriction site and sequence results combined, there were 26 parsimony-informative characters.

There were 13 cpDNA haplotypes, with all but three populations (Main Fork Bitterroot River tetraploid population, Boise River diploid population, and Payette River diploid population) having multiple haplotypes. In one population (Salmon River tetraploids) we found four different cpDNA haplotypes in a sample size of four (Figs 2 and 3).

We found 36 most parsimonious trees, each of 39 steps. The strict consensus tree showed five instances in which both diploids and tetraploids occurred on the same branch, and one additional clade in which diploid and tetraploid plants were also found together (Fig. 2). With the exception of tetraploid plants along Rapid River, all tetraploid cpDNA haplotypes had a corresponding diploid with an identical cpDNA haplotype. If we assume that tetraploids arose from diploids with the same cpDNA haplotype, then there are several discrepancies between the phylogeny and the geographical distribution of tetraploids and their parental diploids. For example, the phylogeny shows that a West Fork Bitterroot River tetraploid plant and a Salmon River tetraploid plant share common ancestry with diploid plants on the Selway River or Main Fork Bitterroot River rather than with diploid plants near their respective sites (Fig. 2, origin no. 4). This discrepancy between geography and relatedness was true for most origins, with the only exceptions being two of the Salmon River tetraploid plants that probably arose from Salmon River diploid plants and one of the upper Selway River tetraploid plants that had the same haplotype as an upper Selway River diploid plant.

No.	Enzyme	Mutation	Sites with mutations
1	*Apa*I	3.4 + 2.4 → 5.8	Rapid R. (4X)
2	*Cfo*I	1.4 → 0.6 + 0.8	Rapid R. (4X)
3	*Apa*I	14.8 → 8.9 + 5.9	MF Bitterroot (2X)
4	*Eco*RV	4.1 → 2.1 + 2.0	Payette R. (2X)
5		– 1.0 kb	Boise R., Payette R., EFSF Salmon R., WF Bitterroot R. (2X)
6		– 0.4 kb	Boise R., Payette R., EFSF Salmon R., WF Bitterroot R. (2X)

Table 2 List of cpDNA restriction site and length mutations for *Heuchera grossulariifolia*. For the restriction site mutations, the ancestral states are given first. *Tolmiea menziesii* and *Heuchera micrantha* were used as outgroups to polarize mutations. Ploidal levels for sites are provided in parentheses following the population designation(s): 4X = autotetraploid, 2X = diploid

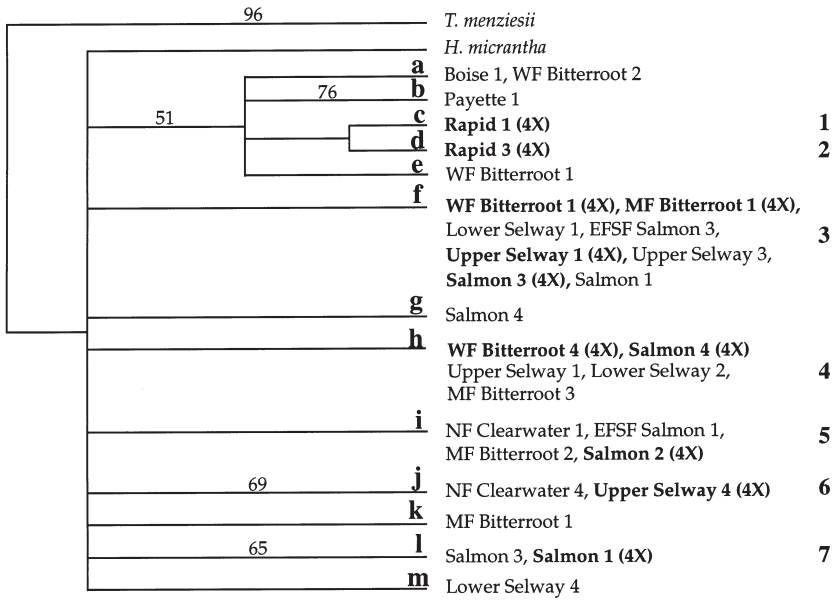

Fig. 2 Rooted strict consensus tree of diploid and autotetraploid *Heuchera grossulariifolia*. Tetraploid sites (4X) are indicated in bold and normal type indicates diploid sites. *Tolmiea menziesii* and *Heuchera micrantha* are included as outgroups. Numbers immediately following the site designation are collection numbers. Plants from the same site with identical haplotypes were excluded to simplify the tree. Bold letters above the branches indicate unique chloroplast DNA haplotypes. Bold numbers at the right of the figure indicate the number of potential polyploid origins. Numbers above the branches are bootstrap confidences. Only bootstrap values greater than 50% are shown.

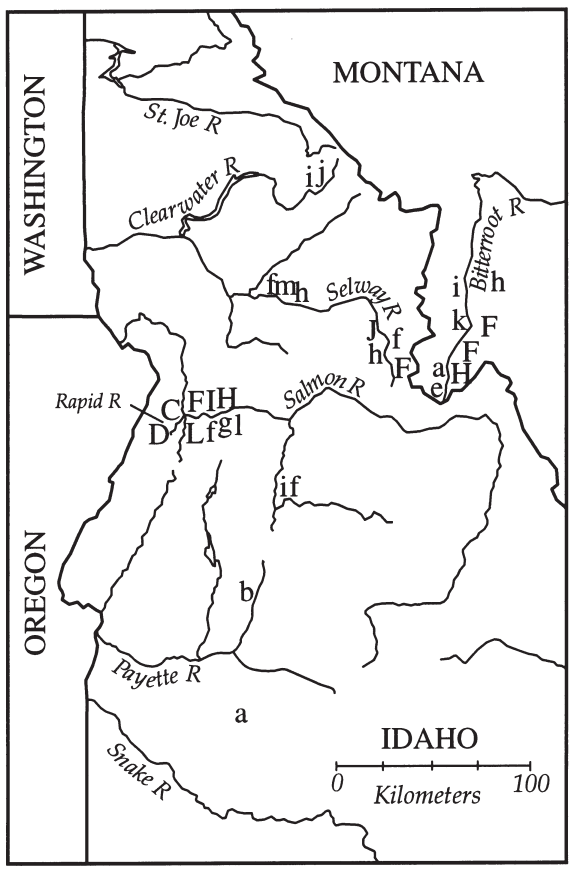

Fig. 3 Distribution of unique chloroplast DNA haplotypes of diploid and autotetraploid *Heuchera grossulariifolia*. Chloroplast DNA haplotypes correspond to the haplotypes labelled in Fig. 2. Upper-case letters are haplotypes from tetraploid individuals and lower-case letters are haplotypes from diploid individuals.

We assessed the strength of the discordance between geography and relatedness by examining the lengths of trees that were topologically constrained by geographical location. We used the most conservative approach by forcing diploids and autotetraploids from the same sites to group as polytomies. Constrained trees were considerably longer, having 55 steps whereas unconstrained trees were only 39 steps.

The Rapid River tetraploid cpDNA haplotypes differed from the other tetraploid haplotypes by four to seven mutations (Table 3). Pairwise comparisons of the other tetraploid haplotypes indicated that they differed from each other by one to three mutations. The differences in haplotypes between Rapid River tetraploids and tetraploid haplotypes from other populations suggest that there are at least two independent polyploid lineages. If each tetraploid haplotype is considered as a separate polyploid lineage then there may be up to seven independent origins of polyploidy.

With the exception of the Main Fork Bitterroot River tetraploid population, all tetraploid populations were a mixture of haplotypes representing at least two polyploid origins (Fig. 3). The West Fork Bitterroot River, Rapid River, and upper Selway River tetraploids were divided into two origins, and there were haplotypes reflecting at least four tetraploid origins on the Salmon River (Fig. 4). Two of these Salmon River origins probably arose from Salmon River diploids with the same haplotype whereas the other two appeared most closely related to a geographically remote diploid. Although several of the Salmon River samples were collected from areas where diploids and tetraploids grow in close proximity, these

Table 3 Pairwise comparisons of the combined number of nucleotide substitutions, restriction site mutations, and length mutations between tetraploid haplotypes of *Heuchera grossulariifolia*. Tetraploid haplotype numbers correspond to the seven potential tetraploid origins as shown in Fig. 2

	Tetraploid haplotype number						
	1	2	3	4	5	6	7
1	–						
2	2	–					
3	5	7	–				
4	6	4	3	–			
5	4	6	1	2	–		
6	5	7	2	3	1	–	
7	5	7	2	3	1	2	–

diploid and tetraploid plants did not have identical haplotypes. Instead, tetraploids from these areas were most closely related to other diploid populations 60–180 km away. In the two cases where Salmon River diploid and tetraploid plants had the same haplotypes, they were separated by about 22 km. The same pattern was also apparent for the Main Fork Bitterroot River tetraploid population. These plants were not most closely related to either of the diploid populations in Montana, but rather had the same haplotypes as several of the diploid populations in Idaho.

Discussion

Multiple origins of autopolyploidy

The cpDNA phylogeny indicates there are at least two origins of autopolyploidy in *Heuchera grossulariifolia* and potentially up to seven independent origins (Fig. 4). The differences observed between the Rapid River tetraploid haplotypes and the other tetraploid haplotypes suggest that Rapid River is a distinct origin, although we did not sample its diploid parents. This population is one of the few exclusively tetraploid sites and may have originated from a population in the Seven Devils Mountain range. Although the Rapid River tetraploids occur within 20 km of the lower Salmon River tetraploids, they appear to be of separate origin, differing by at least four restriction site changes. We found no haplotypes that were common to both of these tetraploid populations, suggesting that there is little or no gene flow between them. Instead, the Boise River, Payette River, and West Fork Bitterroot River diploids are the most closely related populations to the Rapid River tetraploid population (Fig. 2). Wolf *et al.* (1990) found a similar clade linking a Seven Devils tetraploid population to the Boise River and Payette River diploid populations, suggesting that Rapid River may be related to the tetraploid population in the Seven Devils mountains. Alternatively, the parental diploid of Rapid River may have been overlooked or has gone extinct. Several very small, disjunct diploid populations along the upper reaches of Rapid River are known (Thompson *et al.* 1997) and may be the progenitor of these tetraploids. (We did not evaluate these populations because of mortality of Rapid River diploids kept in the common garden.)

Not only may polyploids evolve repeatedly from a diploid progenitor species, but more than one haplotype may also occur within a single polyploid population. All tetraploid populations except for the Main Fork Bitterroot River tetraploid population consisted of combinations of haplotypes that reflect at least two different polyploid origins. For example, along the Salmon River alone, there were haplotypes that represent at least four independent

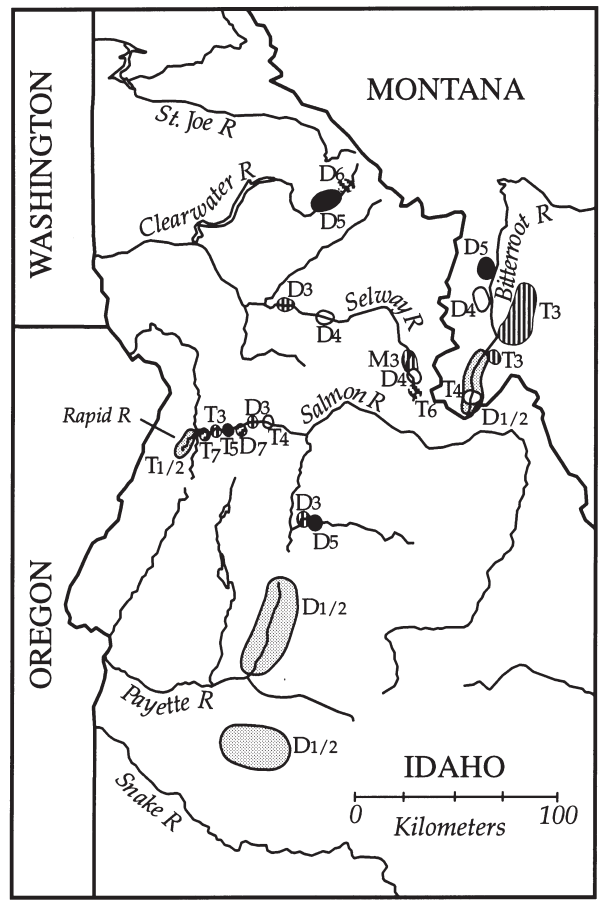

Fig. 4 Distribution of the seven proposed origins of autopolyploidy in *Heuchera grossulariifolia*. Each origin is designated with a different colour or pattern. Other sites are excluded. D = diploid, T = tetraploid, M = both cytotypes are within an origin from that site. The numbers following D, T, and M correspond to the polyploid origins as labelled in Fig. 2.

origins of polyploidy (Fig. 4). Although there is evidence for at least three origins of autopolyploidy in *Heuchera micrantha* (Ness *et al*. 1989; Soltis *et al*. 1989) and *Plantago media* (Van Dijk & Bakx-Schotman 1997) and evidence for two autopolyploid origins in *Musa acuminata* (Lanaud *et al*. 1992) and *Turnera ulmifolia* var. *elegans* (Shore 1991), to the best of our knowledge this is the first study to document that single autopolyploid populations may contain genotypes reflecting different origins. Recurrent polyploid formation on a local scale has been reported in both the *Tragopogon* (L. M. Cook *et al*., unpublished data) and *Draba* (Brochmann *et al*. 1992) allopolyploid complexes, indicating that there is potential for localized polyploid formation in at least some *H. grossulariifolia* populations. Alternatively, the present distribution of polyploid haplotypes in *H. grossulariifolia* may reflect past migration events among polyploid populations of separate origin, or chance mutational events may have resulted in divergence between tetraploids within a population. In the present study, we cannot distinguish between these hypotheses.

Haplotype and cytotype distributions

The results do not support our prediction that existing tetraploid populations originated from the geographically closest diploids. We propose four possible explanations for this lack of congruence between the geographical distribution of tetraploid origins and their diploid parents. First, there is a strong possibility that we have not sampled all of the haplotypes within each river drainage. In a sample size of four individuals from each site and ploidal level, we frequently found two to four haplotypes. Larger sample sizes are needed to investigate further the parentage of tetraploids and the frequency with which polyploidy occurs within a local area. The high degree of haplotype diversity may in part explain why the restriction site mutations used in this study do not correspond to the restriction sites in the Wolf *et al*. (1990) study. If there is considerable variation among individuals within and between populations, then sampling error could explain the mismatch of cpDNA restriction sites. Different sites were sampled along the river drainages in the two studies, which may also explain the lack of correspondence in restriction sites. The differences observed in restriction sites between these studies suggest that there is considerable cpDNA variation in *H. grossulariifolia*.

Second, some parental diploid haplotypes may be extinct. If the diploid parents are extinct, then tetraploids from that population may appear most closely related to a geographically distant population. Third, chloroplast introgression may have occurred through a triploid intermediate, creating a diversity of tetraploid haplotypes within a single origin. Triploids were found in about 1.4% (12/855) of *H. grossulariifolia* sampled throughout its range (Thompson *et al*. 1997). Triploids were confined to the Salmon and East Fork South Fork Salmon River populations, and six of 156 plants sampled on the Salmon River were triploid (Thompson *et al*. 1997), suggesting that chloroplast introgression through triploid intermediates is possible in at least the Salmon River population. There is also the potential for chloroplast introgression through hybridization with other species of *Heuchera*. In particular, *H. cylindrica* may hybridize with *H. grossulariifolia* in areas where the ranges of the species overlap. However, preliminary nucleotide sequence data of the cpDNA *trnL* intron provide no evidence for introgression between these species (K. A. Segraves, D. M. Althoff, and J. N. Thompson, unpublished data).

Fourth, the subsequent divergence of tetraploids after their formation may also alter the relationship between geographical location and relatedness. If the divergence of tetraploids involved homoplasious mutations, inferring patterns of relatedness would be difficult. In *H. grossulariifolia*, there may be a high level of homoplasy in the intergenic spacer sequences. High levels of homoplasy have been reported in length mutations of intergenic spacer regions (Golenberg *et al*. 1993; Van Ham *et al*. 1994), but these regions were not included in the analyses of *H. grossulariifolia*. Studies of other species have shown that the *trnL* intron and the intergenic spacer between the *trnL* exon and the *trnF* gene may be useful for inferring relationships at lower taxonomic levels (e.g. Taberlet *et al*. 1991; Gielly & Taberlet 1994, 1996; Van Ham *et al*. 1994). Moreover, comparisons of trees based on noncoding spacer sequences have demonstrated a high degree of congruence with previous phylogenetic hypotheses among genera within the Crassulaceae (Van Ham *et al*. 1994), indicating that levels of homoplasy in at least some intergenic cpDNA spacers may be low.

The present distribution of diploid and polyploid *H. grossulariifolia* may not reflect patterns of relatedness. Similar geographical discordance has been shown for populations of *Tragopogon mirus* based on rDNA restriction site variation and random amplified polymorphic DNA (RAPDs) and may be a result of extensive seed dispersal (Soltis & Soltis 1991; Soltis *et al*. 1995; Cook *et al*. 1998). Van Dijk & Bakx-Schotman (1997) also found geographical discordance in the distribution of cytotypes in several populations of the autopolyploid *Plantago media* and proposed that polyploidy had not occurred recently in this species. Similarly, the disparity between relatedness and geographical location in *H. grossulariifolia* suggests that polyploidization probably occurred a sufficiently long time ago to allow for substantial geographical movement of diploids and polyploids. We are unaware of any seed dispersal mechanisms that would account for such long-distance migration and dispersal of seeds by hydrochory seems unlikely because the direction

of flow of river drainages does not correspond to the distribution of cpDNA haplotypes between diploids and tetraploids. The present distribution of polyploid populations may reflect the remnants of a once-larger population or group of populations in which past gene flow among polyploids resulted in the displacement of the original cpDNA haplotype. Thus, the sampled tetraploid haplotypes would not match the resident diploid haplotypes.

The cpDNA phylogeny suggests that some tetraploids have arisen from a distant diploid population and migrated, invading a new site. In many areas, this would require that the tetraploid population displace or coexist with the pre-existing diploid population. Such geographical redistribution could result from chance dispersal or from differences in the ecological attributes of diploids and polyploids, and plants of different polyploid origin. Diploid and polyploid *H. grossulariifolia* differ in some life-history traits, but whether these differences occur with polyploid formation or by selection afterwards is unclear (K. A. Segraves & J. N. Thompson, unpublished). Thus, we cannot determine whether tetraploids were pre-adapted to invade some diploid populations or whether subsequent selection after polyploidization altered life-history strategies to minimize mating between the ploidal levels.

The geographical distribution of *H. grossulariifolia* polyploids does not reflect the common expectation that polyploidy allows for expansion of a species' geographical range. The evolutionary significance of polyploidy has often been attributed to their increased ability to invade new habitats and to fill new niches (e.g. Roose & Gottlieb 1976; Hancock & Bringhurst 1981; Lumaret 1984; Brammall & Semple 1990). However, polyploidy has not significantly expanded the range of *H. grossulariifolia*. Tetraploids are restricted to a narrow region across north-central Idaho and western Montana and are nearly always in close association with diploids (Fig. 1). One exception is the Rapid River tetraploid population, which may be an example of a tetraploid range expansion or an example of the exclusion of diploids through competition.

Phylogeography

The geographical pattern of genetic variation among populations of *H. grossulariifolia* fits the overall patterns found among other taxa across the same geographical range. Using mitochondrial DNA sequence data, D. M. Althoff & J. N. Thompson (unpublished) compared the patterns of geographical structure for two pairs of parasitoid–host species (*Agathis thompsoni–Greya subalba* and *Agathis n.* sp.–*Greya enchrysa*) in the inland Pacific Northwest and found little geographical structuring among populations. In many instances, individuals from more geographically distant populations appeared more closely related than individuals from the same population. Moreover, more than 50% of the individuals for each of the four insect species had unique mtDNA haplotypes. As a result, D. M. Althoff & J. N. Thompson (unpublished) suggest that, historically, there has been a high degree of gene flow among these populations. Another study of mtDNA variation in the prodoxid moth *Greya politella* across a broader geographical range has shown some structuring among populations that corresponds to host plant use and geographical location, but there is little structuring in Idaho and adjacent Washington (Brown *et al*. 1997).

Similarly, in *H. grossulariifolia*, there was little geographical structuring among populations. Many cpDNA haplotypes were spread across populations, creating a discordance between relatedness and geographical location. There are several explanations for the lack of phylogeographic pattern found in *H. grossulariifolia*. This species may have originally been one large population that has been reduced to its present distribution. There is some evidence for this in the distribution of haplotypes. In particular, the clade uniting the Boise River, Payette River, and West Fork Bitterroot River diploids suggests that the distributions of these haplotypes were previously more widespread or there was gene flow among these populations. More samples are needed from the wilderness areas of central Idaho along the upper portion of the Salmon River to determine whether there is a more continuous distribution of the same haplotypes across this broad geographical range. If a more continuous haplotype distribution is discovered, then at least some of these populations may have been recently colonized from a common source. Another possible explanation is that Pleistocene glaciation in the Selway and Bitterroot mountain ranges has influenced the phylogeographic pattern in this species. The extent of glaciation was primarily confined to the higher peaks in northern Idaho in the Selway and Bitterroot mountains (Dingler & Breckenridge 1982; Alt & Hyndman 1989). During these glacial periods, *H. grossulariifolia* may have migrated out of the Selway and Bitterroot River sites. Because there is a low degree of morphological differentiation in floral traits between diploid and tetraploid plants located on the Bitterroot and upper Selway Rivers (K. A. Segraves & J. N. Thompson, unpublished), the populations along these rivers may be the most recently colonized. If these populations are recent colonizations, there may not have been sufficient time for divergence of floral traits to occur.

Conclusions

The present study documents at least two and potentially seven independent origins of autopolyploidy in *Heuchera grossulariifolia*, in comparison to the three origins documented previously by Wolf *et al*. (1990). This propensity to form polyploids may enrich local polyploid populations

through the introduction of new combinations of alleles and gene flow among polyploid populations of independent origin. Moreover, with multiple origins of polyploidy present in a local region, there is even greater potential for genetic recombinations among polyploids. The results also indicate that the relationship between the present geographical distributions of diploid and polyploid populations may not reflect patterns of relatedness among those populations. However, there are important caveats to these interpretations. Extinction or loss of haplotypes, chloroplast introgression, gene flow, or homoplasy may have obscured relationships among populations and between ploidal levels. These results taken together demonstrate that repeated polyploidization in *H. grossulariifolia* has been important in structuring the geographical distribution of this autopolyploid and its diploid parent.

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