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PLANT POLYPLOIDY AND POLLINATION: FLORAL TRAITS AND INSECT VISITS TO DIPLOID AND TETRAPLOID *HEUCHERA GROSSULARIIFOLIA*

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Abstract.—In many polyploid species, polyploids often have different suites of floral traits and different flowering times than their diploid progenitor species. We hypothesized that such differences in floral traits in polyploids may subsequently affect their interactions with pollinating and other insect visitors. We measured floral morphology and flowering phenology in 14 populations of diploid and autotetraploid *Heuchera grossulariifolia* Rydb. (Saxifragaceae), determined if repeated evolution of independent polyploid lineages resulted in differentiation in floral morphology among those lineages, and ascertained if there was a consistent pattern of differentiation among genetically similar diploid and autotetraploid populations. In addition, we evaluated the differences in suites of floral visitors within a natural community where diploids and autotetraploids occur sympatrically. Overall, flowers of autotetraploid plants were larger and shaped differently than those of diploids, had a different flowering phenology than that of diploids, and attracted different suites of floral visitors. In comparison with flowers of diploids, tetraploid floral morphology varied widely from pronounced differences between cytotypes in some populations to similar flower shapes and sizes between ploidal levels in other populations. Observations of floral visitors to diploids and autotetraploids in a natural sympatric population demonstrated that the cytotypes had different suites of floral visitors and six of the 15 common visitors preferentially visited one ploidy more frequently. Moreover, we also found that floral morphology differed among independent autotetraploid origins, but there was no consistent pattern of differentiation between genetically similar diploid and autotetraploid populations. Hence, the results suggest that the process of polyploidization creates the potential for attraction of different suites of floral visitors. Multiple origins of polyploidy also presents the opportunity for new or different plant-insect interactions among independent polyploid lineages. These differences in turn may affect patterns of gene flow between diploids and polyploids and also among plants of independent polyploid origin. Polyploidy, therefore, may result in a geographic mosaic of interspecific interactions across a species' range, contributing to diversification in both plant and insect groups.

Key words.—Floral morphology, flowering phenology, *Heuchera*, insect visitation, plant-insect interactions, pollination, polyploidy.

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The evolution of polyploidy has played a major role in the creation and maintenance of plant biodiversity (e.g., Thompson and Lumaret 1992; Soltis and Soltis 1993, 1995; Song et al. 1995; Goldblatt and Takei 1997). About one-half of all angiosperm species are thought to have polyploids in their lineages (Stebbins 1971; Averett 1980; DeWet 1980; Levin 1983; Masterson 1994), and within polyploid species, multiple origins of polyploidy are considered the rule (Soltis et al. 1992; reviewed in Soltis and Soltis 1993). The success of many polyploid taxa has been associated with numerous changes that may differentiate them from their diploid ancestors. For example, polyploidy can influence genetic variation (e.g., Klekowski and Baker 1966; Roose and Gottlieb 1976; Soltis and Rieseberg 1986; Moody et al. 1993; Ainouche et al. 1995; Purdy and Bayer 1995; Soltis et al. 1995; Song et al. 1995; Ehrendorfer et al. 1996), life histories (e.g., Marks 1966; Smith and Phipps 1988; Barrett 1989; Husband and Schemske 1997), morphology (e.g., Khare and Kaur 1983; MacDonald and Chinnappa 1988; Patwary et al. 1989; Chmielewski 1994; Vandenhout et al. 1995), physiology (e.g., Tiwari et al. 1980; Levin 1983; Koul et al. 1985; Bhargava et al. 1988; Warner and Edwards 1989; Griesbach and Kamo 1996; Letchamo 1996), and geographic distributions (e.g., Tothill and Hacker 1976; Novak et al. 1991; Van Dijk et al. 1992; Lamade et al. 1994; Husband and Schemske 1998).

In angiosperms, two other common effects of polyploidy are shifts in the timing of flowering (Tothill and Hacker 1976; Garbutt and Bazzaz 1983; Lumaret et al. 1987; Lumaret 1988; Lumaret and Barrientos 1990; Van Dijk et al. 1992; Maceira et al. 1993; Bretagnolle and Lumaret 1995; Petit et al. 1997) and changes in floral morphology (Giles 1942; Smith 1946; Kliphuis 1972; Taylor and Smith 1979; Garbutt and Bazzaz 1983; MacDonald et al. 1988; Barrett 1989; Brochmann 1993). These differences in polyploids may be a direct result of polyploidization itself or may be caused by subsequent selection after the polyploid event (Bretagnolle and Lumaret 1995). Once changes in flower morphology and phenology have occurred, changes in the pollinator assemblage may follow. Polyploids can have pollinating visitors very different from their diploid ancestors (Taylor and Smith 1979), potentially forming new interactions with insects that may subsequently alter patterns of gene flow within and among populations. Differences in floral visitation to diploids and polyploids may facilitate polyploid establishment and persistence by reducing the number of backcross fertilization events with diploids. Moreover, these differences in floral traits may vary geographically, potentially creating unique assemblages of plant-insect interactions and a mosaic of genetic exchange between ploidal levels from population to population. Therefore, changes in floral morphology, phenology, and interac-

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tions with pollinators can move the polyploid species onto a different evolutionary trajectory than its diploid ancestor.

These morphological changes may not only occur between polyploids and their diploid parents, but they may also occur among independent polyploid lineages. Depending on the divergence and composition of the parental diploid genomes and the strength and direction of selection on polyploids after their formation, each polyploid origin has the potential to have a unique suite of floral traits and, subsequently, different assemblages of pollinators. Because both allopolyploids and autopolyploids commonly arise multiple times within a species (Soltis et al. 1992; Soltis and Soltis 1993), understanding how polyploidy has facilitated diversification through changes in floral traits and pollinator assemblages hinges on studies of multiple origins of polyploids. Hence, each origin of a given polyploid provides a natural experiment to test the effects of polyploidy under different ecological and genetic conditions.

Despite the number of studies that have documented shifts in floral traits in polyploids, there is only anecdotal evidence (Taylor and Smith 1979) that polyploids can have different suites of pollinating visitors than diploids. Moreover, no study has examined the effect of multiple polyploid origins and tested whether independent polyploid lineages can differ in floral characters within natural populations. In this study we assessed whether the evolution of autopolyploidy in *Heuchera grossulariifolia* Rydb. (Saxifragaceae) has influenced floral morphology, flowering phenology, and insect visits to diploids and autotetraploids. We hypothesized that if polyploidy resulted in a shift in floral traits, then polyploids would also harbor a different assemblage of floral visitors. In addition, we predicted that depending on the strength and direction of selection on polyploid morphology or the divergence of the diploid parental genomes, polyploid floral morphology may not always most closely resemble that of the diploid parents. We addressed four specific questions. Do autotetraploids differ from diploids in floral morphology and flowering phenology? Provided there are differences between the cytotypes in these floral traits, do these differences translate into a change in the assemblage of insect visitors to diploids and polyploids in a population where the cytotypes were sympatric? Is there differentiation in floral morphology among autotetraploids of independent origin? Is there a consistent pattern of differentiation in floral morphology between genetically similar diploid and autotetraploid populations?

Materials and Methods

Heuchera grossulariifolia Rydb. (Saxifragaceae) is a rhizomatous perennial that inhabits granitic and basaltic outcroppings along most of the major river drainages in the mountainous regions of Idaho and western Montana as well as sites along the Columbia River gorge in Washington and Oregon (Hitchcock and Cronquist 1973). Flowering stems of *H. grossulariifolia* are scapose with flowers arranged spirally on a panicle. There are typically 10 to 80 flowers per scape and approximately one to 50 scapes per plant. Flowers usually open consecutively beginning with the flowers lowest on the scape. *Heuchera grossulariifolia* is particularly amenable to studying the effects of polyploidy on floral morphology and phenology because polyploids of this species are autopolyploid (Wolf et al. 1989), which avoids the morphological and genetic complications involved with hybridization between species. Moreover, *H. grossulariifolia* also has multiple origins of polyploidy. Estimates range from at least three (Wolf et al. 1990) to possibly seven independent origins (Segraves et al. 1999) of autopolyploidy. Herein, we use the estimate of Segraves et al. (1999) because this is a more extensive survey of the range of genetic variation found in *H. grossulariifolia*.

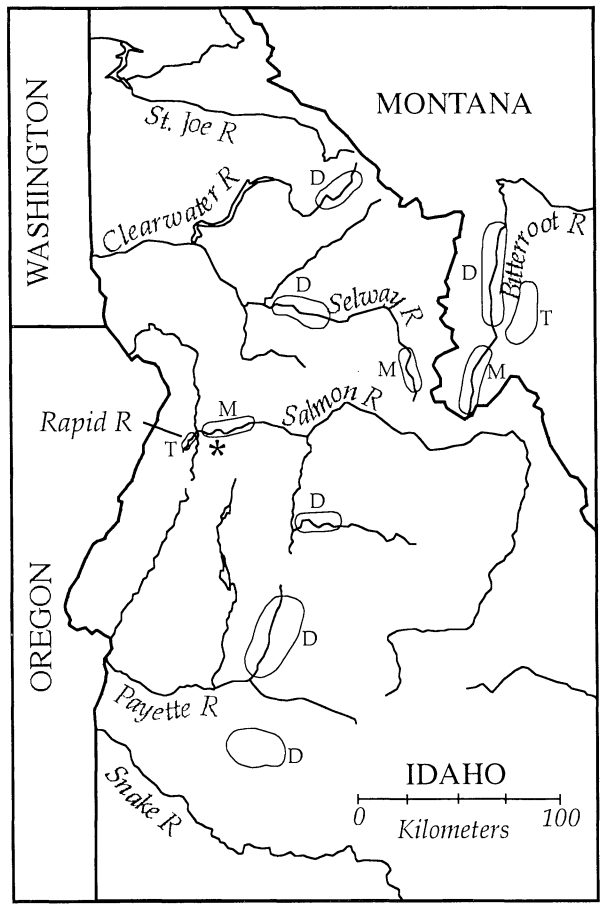

FIG. 1. Distribution of the 11 collection sites for *Heuchera grossulariifolia* common-garden plants and flowers collected from naturally occurring plants. D, diploid; T, autotetraploid; M, mixed cytotype site. The asterisk denotes the site along the Salmon River where observations of floral visitors were conducted. Samples were taken from the 14 populations shown. Two populations (one diploid and one tetraploid) are located in each of the mixed cytotype sites.

Fourteen populations of diploids and autotetraploids were used to investigate floral evolution in this study, and this sample includes most of the major river drainages in which this species complex occurs (Fig. 1). Of these populations, five are exclusively diploid populations located in Idaho: the Boise River, Payette River, North Fork Clearwater River, East Fork South Fork Salmon River, and the lower Selway River. Sympatric populations of diploids and autotetraploids are located along the Salmon and upper Selway River drainages in Idaho and along the West Fork Bitterroot River drainage in Montana (Thompson et al. 1997). In addition, diploids and

TABLE 1. *Heuchera grossulariifolia* collection sites for plants used in the common-garden study and for flowers from naturally occurring plants. Abbreviated river names are used to refer to populations. 2× and 4× refer to diploid and tetraploid populations, respectively. FR, forest road; FH, forest highway; SR, state route.

River	Location
Idaho	
South Fork Boise (2×)	9.4–28.7 km along FR 189, 32.8–45.2 km along FH 227, Boise National Forest
North Fork Clearwater (2×)	6.4–22.9 km along FR 250, 3.2–8.1 km along FR 247, Clearwater National Forest
Middle Fork Payette (2×)	10–23.5 km along FR 698, 17.5–19.3 km along FR 671, Boise National Forest
Rapid (4×)	0.5–9 km along Rapid River Trail from FR 2114, Nez Perce National Forest
Main Fork Salmon (2×/4×)	3.7–41.9 km E of Riggins, ID, Payette National Forest
East Fork South Fork Salmon (2×)	0–33.3 km along FH 48, Payette National Forest
Lower Selway (2×)	11.3–30.8 km along FR 223, Nez Perce National Forest
Upper Selway (2×/4×)	0–4.3 km FR 478, 15.5–33.7 km W of Nez Perce Pass on FR 468, Nez Perce National Forest
Montana	
Main Fork Bitterroot (2×/4×)	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
West Fork Bitterroot (2×/4×)	45.1–67.1 km along SR 473, 0.0 km FR 5669, 3.9 km R 104D, 3.2 km FR 5703, 0.3 km FR 049, Bitterroot National Forest

autotetraploids occur parapatrically along the Main Fork Bitterroot River in Montana and also along Rapid River in Idaho (Thompson et al. 1997). Sample sizes of the very small Rapid River diploid population that occurs in the upper reaches of this drainage were inadequate to include in the analyses. Voucher specimens for diploid and autotetraploid populations were deposited in the Marion Ownbey Herbarium, Washington State University.

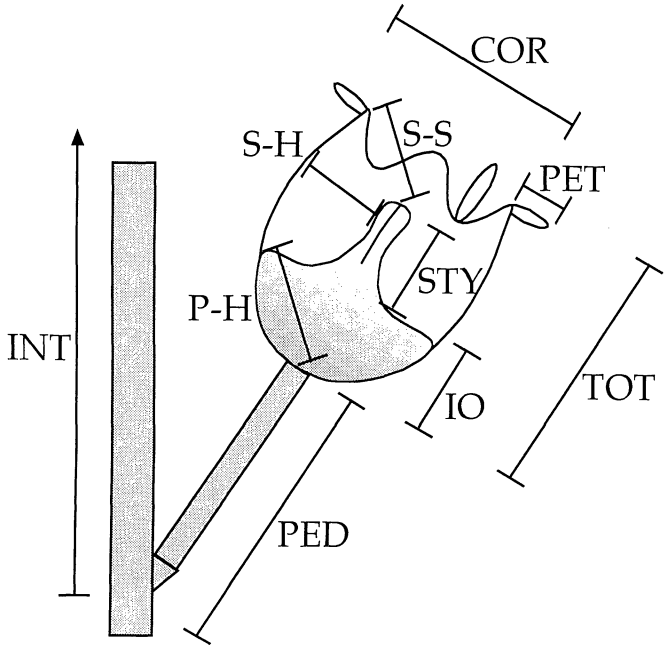

FIG. 2. Schematic of floral measurements for *Heuchera grossulariifolia*. INT, internode distance between the base of the pedicel shown and the next highest pedicel base; PED, pedicel length; P-H, distance from the base of the flower at the pedicel to hypanthium wall; S-H, distance from the stigma to the hypanthium wall; S-S, distance from the stigma to the sepal tip; COR, corolla width; PET, petal length; STY, style length including the stigma; IO, distance from the nectary to the base of the flower; TOT, total exterior length of the flower. All measurements are expressed in millimeters.

Flower Morphology and Phenology

Common-Garden

Eight to 23 (mean = 19.4) plants of *H. grossulariifolia* for each site and ploidal level were grown from field collected rhizomes in 3.8-L plastic pots in a common-garden on the Washington State University campus for at least one year before use (Table 1). Ploidal level of these plants had been previously determined using flow cytometry to assess nuclear DNA content (Thompson et al. 1997). Plants were randomized in the common-garden nine months prior to flowering to reduce any environmental effects. Flowers from positions 1, 5, and 10 on the first flowering scape were preserved in FAA (5% formaldehyde, 5% glacial acetic acid, 90% of 50% ethanol) on the first day of each flower's receptivity. Receptivity was determined by the presence of a shiny exudate on the stigmatic surface. Before removing each flower, the internode distance (INT) between the pedicel of that flower and the next highest pedicel was measured with a hand-held micrometer (e.g., distance between pedicels of flowers from positions five and six; Fig. 2). At this time sepal color was also estimated for each flower using a Royal Horticultural Society (1966) standard color chart. On completion of flowering, the length of the first scape and the total number of flowers produced on the first scape were recorded. The second scape was used in the few cases where the first scape was broken or wilted. Flowers collected from the second scape did not differ significantly from flowers collected from the first scape (MANOVA $F = 1.47$; df = 10; 187, $P = 0.15$ for flower 1; $F = 0.78$; df = 10, 194; $P = 0.65$ for flower 5; $F = 0.55$; df = 10, 186; $P = 0.85$ for flower 10). The timing of flowering was recorded as the dates of the first and last flowers opening on the first inflorescence. Plants were also checked daily throughout the flowering period for the number of flowers receptive on the first scape (30 April to 2 July 1996), which provided an estimate of the peak flowering date on the first inflorescence.

Natural Populations

For comparison to the common-garden plants, flowers from positions 1, 5, and 10 were also collected from naturally

occurring diploid and tetraploid plants during peak flowering for six sites (May to August 1996; North Fork Clearwater River, Main Fork Salmon River, Rapid River, upper Selway River, Main Fork Bitterroot River, and West Fork Bitterroot River). For each of these populations at least 20 flowers each of flower position 1, 5, and 10 were preserved in FAA from 60 plants separated by at least one meter. At the sites with mixed ploidal levels, flowers were collected from both the diploid and tetraploid populations. With the exception of the Salmon River site, the ploidy of plants in sympatric populations was determined using flow cytometry following the procedures of Thompson et al. (1997). At the Salmon River site, flow cytometry was used to assess ploidal level of about one half of the plants prior to flower collection. In this particular area, ploidy is easy to identify by sight because diploids and autotetraploids appear as phenotypically different as species.

The timing of flowering was recorded for one site along a 1.6-km portion of the Salmon River in Idaho where diploids and tetraploids co-occur (Fig. 1). Prior to flowering, 50 diploid and tetraploid pairs of plants were tagged by haphazardly selecting a diploid plant and then choosing the nearest tetraploid plant within three meters. Approximately half were assessed for ploidal level to make certain that visual determination of plant ploidy in the field was accurate. All visual assessments of ploidy matched the determinations using flow cytometry. Pairs of plants ranged in distance from growing immediately next to one another to three meters apart. These 50 pairs were observed daily; we recorded the date the first flower on the first scape opened, the date of maximum floral display (first day that at least 50% of flowers on a plant were open), and the date the last flower on the last scape opened.

Measurements

Preserved flowers from both the common-garden and natural plants were measured using a microscope (Wild M8 microscope) fitted with an ocular micrometer (Fig. 2). The following distances were measured: (1) pedicel length (PED); (2) total length (TOT); (3) distance from the nectary to base of the flower (IO); (4) corolla width (COR); (5) petal length (PET); (6) style length (STY); (7) distance from the base of flower at the pedicel to the hypanthium wall (P-H); (8) distance from the stigma to the hypanthium wall (S-H); and (9) distance from the stigma to the tip of sepal (S-S). Flowers were uniformly sectioned longitudinally through a bract on the pedicel to allow the same sides and petals to be measured on all flowers.

Flower Visitation

The same 50 pairs of diploid and tetraploid plants used to study flowering phenology along the Salmon River were also used to examine insect visitation. On each observation day a diploid-tetraploid pair of plants was observed for 30 min, after which another pair was chosen and observed. The pairs observed and the order in which they were observed were chosen at random each day by drawing numbers from a bag. The number of visits to flowers of both cytotypes by each insect species or guild was recorded during these half-hour intervals throughout the flowering period of both ploidal levels. We recorded only those visits where foraging or oviposition behavior on the flowers was observed. Observations were made between 1000 h and 1600 h from 2 May to 29 May 1997. A pilot study in 1996 suggested that there was little insect activity to the flowers before 1000 h or after 1600 h. Analyses were based on a total of 108.5 h of observation distributed over 17 days.

Statistical Analysis

A preliminary factor analysis on the floral measurements showed that the first three principle components explained about two-thirds of the variance in the data. The loadings on the eigenvectors were not consistent between the common-garden and natural plants, nor were the loadings consistent with floral position. Because of these inconsistencies, the factor analysis did not provide a means to simplify the analyses. Therefore, differences in floral morphology between diploids and tetraploids across the range of *H. grossulariifolia* were assessed using a MANOVA on all floral measurements (SAS Institute 1994). For this analysis, each flower position was treated separately. We used identity and sum response design matrices and found that the conclusions were the same for both designs. The sum transformation showed a consistent difference for each measurement between diploids and tetraploids for all flower positions from both field and garden collected flowers. Because of this consistency and the fact that sum and identity matrices provided the same results, we used a sum transformation to simplify the rest of the analyses. We determined whether there were differences among tetraploid origins using both ANOVA with repeated measures and MANOVA with repeated measures on the sum transformed measurements (repeated measure was flower position). In addition, differences in sepal color were assessed with an ANOVA on the distributions of the percent reflectance for each ploidy. In all, 747 flowers were measured from 10 populations collected from naturally occurring diploid and tetraploid *H. grossulariifolia*, and 641 flowers were measured from 14 populations from common-garden plants. From naturally occurring and common-garden plants, a total of 1388 flowers were measured.

Flowering phenology was assessed using univariate ANOVAs to detect differences in the first, peak, and last flowering dates between cytotypes (SAS Institute 1994). The common-garden flowering dates were treated separately from flowering phenology dates observed at the Salmon River. Peak flowering for the common-garden plants was determined by the date of maximum floral display on the first inflorescence.

Differences in floral morphology among independent tetraploid origins were similarly assessed using MANOVA on all floral measurements for each pairwise comparison between origins (SAS Institute 1994). The phylogenetic hypothesis of Segraves et al. (1999) suggests that there are up to seven independent origins of autotetraploids. However, only one of the sampled tetraploid populations (Main Fork Bitterroot River) comprised a single haplotype, reflecting one origin. Concomitant analyses showed that the other tetraploid populations such as the Salmon and upper Selway Rivers have at least two to four haplotypes, each potentially reflect-

ing a separate polyploid origin. We therefore evaluated differences in floral morphology only between the Rapid and Main Fork Bitterroot River populations because they were of known distinct origin. These two populations differed by at least four nucleotide substitutions, restriction site mutations, or length mutations (Segraves et al. 1999). The results for other comparisons are also shown, but interpretations may be confounded by having multiple haplotypes from independent origins within a population.

We determined whether there was a consistent pattern of differentiation in floral morphology between genetically similar diploid and autotetraploid populations by testing for a correlation between genetic distance and morphological distance for each flower position separately. Genetic distances were determined from the results of Segraves et al. (1999) and were calculated as the distance between the mean number of chloroplast DNA nucleotide substitutions, restriction site mutations, and length mutations for all pairwise comparisons of populations (Appendix). The genetic distances were based on a sample of four individuals per population and ploidal level. Morphological distances were determined by calculating the distance between the mean sum measurement for all pairwise combinations of populations. If there was a consistent pattern of differentiation, we expected to find a positive correlation between morphological and genetic distance: The more genetically similar two populations, the more morphologically similar they should be. Confidence limits for these correlations were determined using the bootstrap procedure of Efron and Tibshirani (1993). Two thousand bootstrap replicates were computed, and the 2.5th and 97.5th percentiles were used to construct a 95% confidence interval.

We ascertained whether the assemblage of floral visitors differed between diploids and tetraploids by comparing the frequencies of all visitors during three time periods (only diploids flowering, only tetraploids flowering, and both cytotypes flowering) using a G test (SAS Institute 1994). Univariate ANOVAs were used to examine differences in the number of visits by each insect species or guild to diploids and tetraploids when both cytotypes were flowering simultaneously. Species within several genera were not easily distinguished from one another when in flight (*Amecocerus, Bombus*, syrphid flies) and were combined into guilds for the analyses. Within three genera (*Dolichogenidea, Lasioglossum*, and *Nomada*), species were not separated because the taxonomy is poorly understood. In addition, we also tested for differences in the overall rate of visitation (number of visits per 30-min period) to diploids and tetraploids using univariate ANOVAs. Voucher specimens for insect visitors were deposited in the Washington State University entomology museum.

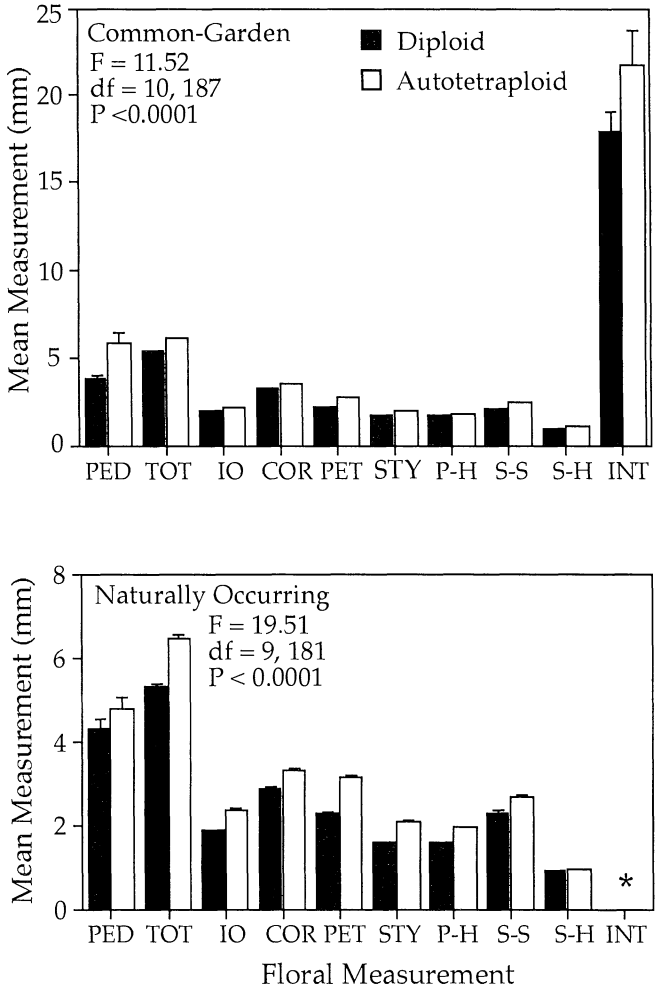

FIG. 3. Comparison of diploid (black bars) and autotetraploid (white bars) *Heuchera grossulariifolia* floral morphology for flowers collected from both common-garden plants (top) and from naturally occurring plants (bottom). All floral measurements are expressed in millimeters and are described in Figure 2. An asterisk indicates that no measurements were taken. Standard error bars are also included. Only the results for flower position 1 are shown. Measurements for flower positions 5 and 10 did not differ significantly from flower position 1 except for PED and INT; however, PED and INT measurements were consistent between ploidal levels. Statistical results refer to MANOVA on floral measurements.

RESULTS

Differences between Cytotypes

Overall, flowers of tetraploids were significantly larger than flowers of diploids (univariate repeated measures $F = 20.99$; df = 1, 378; $P < 0.0001$; multivariate repeated measures $F = 57.23$; df = 1, 174; $P < 0.0001$). Each of the nine floral characters measured was larger for flowers of tetraploids, regardless of floral position or whether flowers were collected from field or common-garden plants (Fig. 3). In general, flowers of tetraploids were longer, slightly wider, and had a considerably deeper ovary than flowers of diploids. Tetraploid petals were also longer than those of diploids and tended to have a wider opening into the flower, whereas flowers of diploids were typically pinched at the opening, creating a more spherical floral form.

Flowers of tetraploids also differed from flowers of diploids in sepal color ($F = 18.02$; df = 1, 218; $P < 0.0001$; Fig. 4B). Tetraploids ranged in color from a light yellow-green to white, and diploids were usually a dark green-yellow to yellow-green color. The West Fork Bitterroot and Main Fork Bitterroot River diploid populations were the nearest in color to flowers of tetraploids, with most sepals being light yellow to white.

Measurements made on the inflorescence design showed that tetraploid plants produced significantly fewer flowers than diploids (ANOVA $F = 5.25$; df = 1, 200; $P = 0.02$). Because tetraploid plants produce significantly larger and fewer flowers, resource allocation may play a role in determining inflorescence design. Overall, there was no difference between diploid and tetraploid plants in scape length (Fig. 4A). For the Salmon River diploid and tetraploid populations, however, scape length was markedly different. Inflorescences of tetraploids were shorter than those of diploids on the Salmon River (Fig. 4). For the West Fork Bitterroot River populations we found the opposite pattern where scapes of diploids were shorter than scapes of tetraploids. These results show that there is no clear pattern of differentiation in scape length between diploid and tetraploid populations.

The duration of the flowering season for diploid and tetraploid plants did not differ significantly for either the common-garden or the Salmon River studies (garden: ANOVA $F = 0.96$; df = 1, 172; $P = 0.33$; Salmon River: ANOVA $F = 1.33$; df = 1, 45; $P = 0.26$). Because tetraploid plants have fewer flowers than diploid plants, flowers of tetraploids must either last longer or the time interval between the opening of flowers is longer. An ANOVA on the mean number of days each flower remained receptive showed no difference in the length of time that flowers of diploids and tetraploids lasted ($F = 1.70$; df = 1, 169; $P = 0.19$), indicating that flowers of tetraploids probably have a greater time interval between the opening of flowers.

Although the duration of flowering for diploid and tetraploid plants did not differ, the timing of flowering was different between diploid and tetraploid plants. Common-garden tetraploid plants tended to flower later than diploid plants (Fig. 5A). Diploid plants flowered approximately five days before tetraploid plants and reached peak flowering about three days earlier than tetraploid plants. For the populations on the Salmon River, however, tetraploid plants flowered significantly earlier than diploid plants (Fig. 5B). Salmon River tetraploid plants flowered about eight days earlier than diploid plants and reached peak flowering five days before diploid plants. Similar trends in flowering times were also noted for the upper Selway River diploid and tetraploid populations where tetraploid plants appeared to flower earlier than diploid plants (K. Segraves, pers. obs.).

Differences among Tetraploid Origins

Floral morphology differed significantly between the Rapid River and Main Fork Bitterroot River tetraploid origins (MANOVA $F = 8.09$; df = 9, 81; $P < 0.0001$; Fig. 6). Flowers of Rapid River tetraploids had longer petals and styles and had considerably wider corollas than flowers of tetraploid plants from the Main Fork Bitterroot River population. Comparisons of the other tetraploid populations indicated that all five populations were significantly different from each other in floral morphology (MANOVAs $P < 0.0001$ for all comparisons except Rapid vs. Salmon River [$P = 0.02$] and upper Selway vs. Main Fork Bitterroot River [$P = 0.04$]; Fig. 6). Rapid River and Salmon River tetraploid populations had the largest and most cylindrical flowers of all the tetraploid plants sampled. The West Fork Bitterroot River tetraploid flowers were the smallest and most conical of all tetraploid populations.

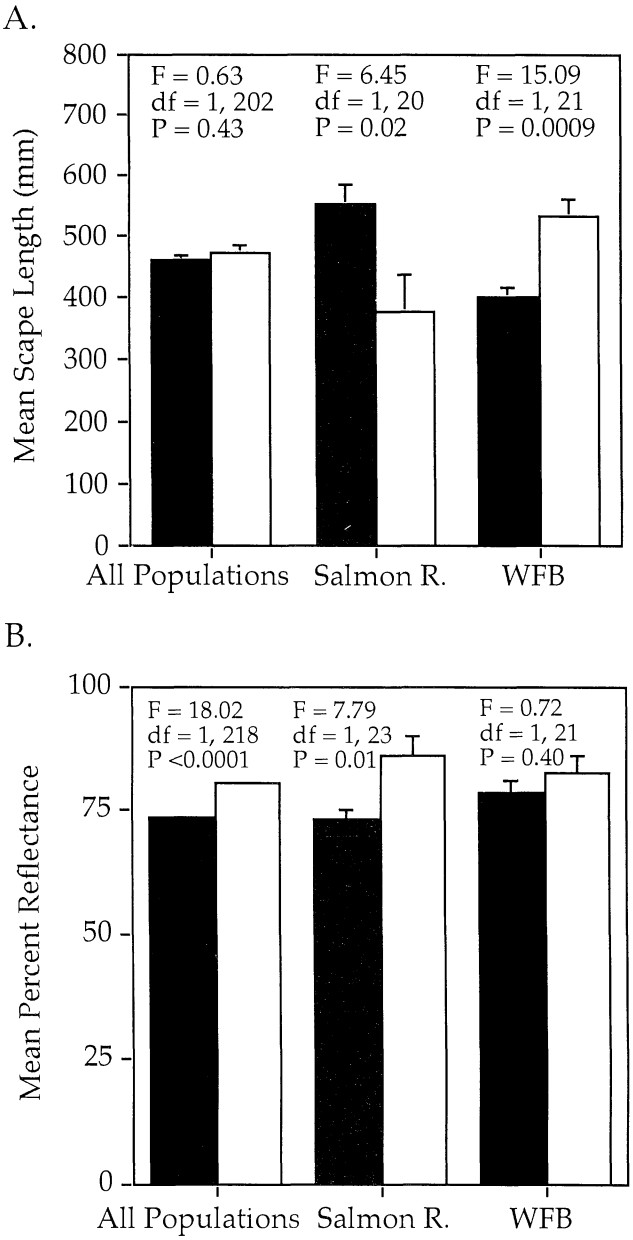

FIG. 4. (A) Mean scape length in millimeters for diploid (black bars) and autotetraploid (white bars) *Heuchera grossulariifolia* for all populations combined, for the Salmon River diploids and autotetraploids, and for the West Fork Bitterroot River (WFB) diploids and autotetraploids. (B) Mean percent reflectance for diploids (black bars) and autotetraploids (white bars). Percent reflectance of 100% is white and 0% is black. The results for each comparison are shown above each pair of bars. Statistical results refer to ANOVA on scape length or percent reflectance. Standard error bars are also included.

Scape length differed significantly among several tetraploid populations (Fig. 7). The Salmon River tetraploid plants had the shortest scapes, whereas plants from the Main Fork Bitterroot River population had the longest scapes. Several tetraploid populations also differed in the total number of

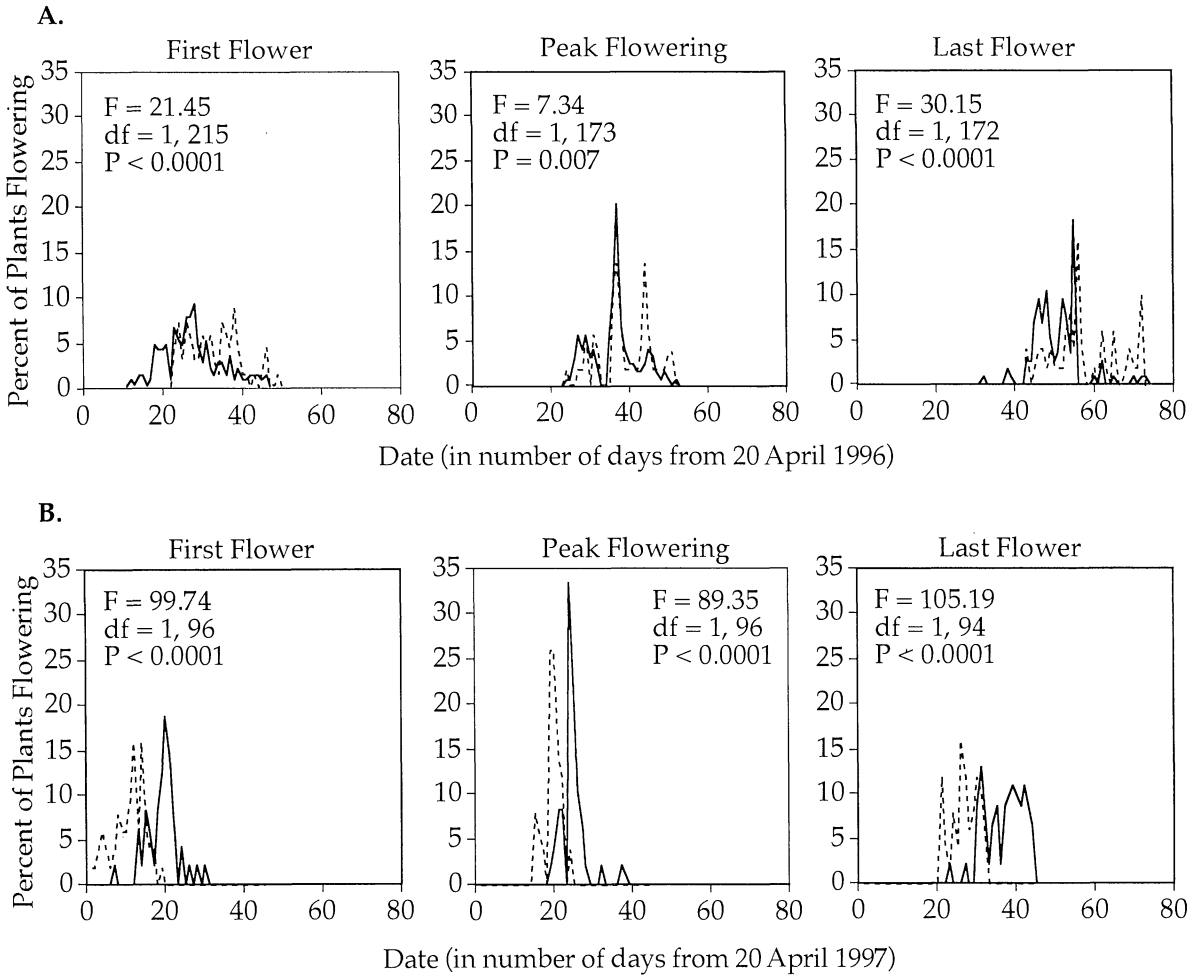

FIG. 5. Comparison of the percent of diploid (solid lines) and autotetraploid (dotted lines) *Heuchera grossulariifolia* plants in first, peak, or last flower through time. (A) The flowering phenology of plants in the common-garden study during 1996. Statistics refer to ANOVAs on the number of plants flowering through time. Significant values show that tetraploids flowered later than diploids. (B) Flowering phenology of plants at the Salmon River during 1997. For the Salmon River population, tetraploids flowered earlier than diploids.

flowers on the first inflorescence (Fig. 8). The Main Fork Bitterroot River tetraploid population had the highest number of flowers, and the Salmon River population had the fewest number of flowers. Because all tetraploid populations, with the exception of Rapid River and Main Fork Bitterroot River, were combinations of haplotypes reflecting at least two origins, interpretations of these comparisons may be confounded. The Rapid River and Main Fork Bitterroot River tetraploid populations are of known distinct origin (Segraves et al. 1999); hence, the comparisons for these populations are sound.

Comparison of Genetic Similarity and Morphology

There was no direct relationship between genetic similarity of populations and morphological similarity. Within floral positions, there were no significant correlations between genetic and morphological distance (flower 1: $r^2 = 0.01$, $P = 0.34$, CI = −0.26–0.17; flower 5: $r^2 = 0.02$, $P = 0.18$, CI = −0.35–0.07; flower 10: $r^2 = 0.008$, $P = 0.45$, CI = −0.27–0.11). However, because our realized sample size is relatively small ($n = 14$ populations), we may not be able to detect a correlation. But given that the confidence intervals included zero, we conclude that the likelihood of finding a correlation is small.

Flower Visitation

The overall rate of visitation (visits per half-hour observation period) to diploid and tetraploid plants on the Salmon River did not differ (ANOVA $F = 0.77$; df = 1, 168; $P = 0.38$). On average, both cytotypes were visited approximately 1.3 times (SE ± 0.15) per observation period throughout the flowering season. Although the rate of visitation did not differ between cytotypes, the assemblage of visitors differed significantly for diploid and tetraploid plants when they were flowering simultaneously ($G = 98.62$, df = 19, $P < 0.0001$; Fig. 9). The assemblage of visitors also changed over the flowering season ($G = 344.81$, df = 57, $P < 0.0001$; Fig. 9).

Heuchera grossulariifolia plants on the Salmon River were frequently visited by approximately 15 species or guilds of insects. Of these visitors, six foraged or oviposited on plants of one ploidy more frequently than the other during the time

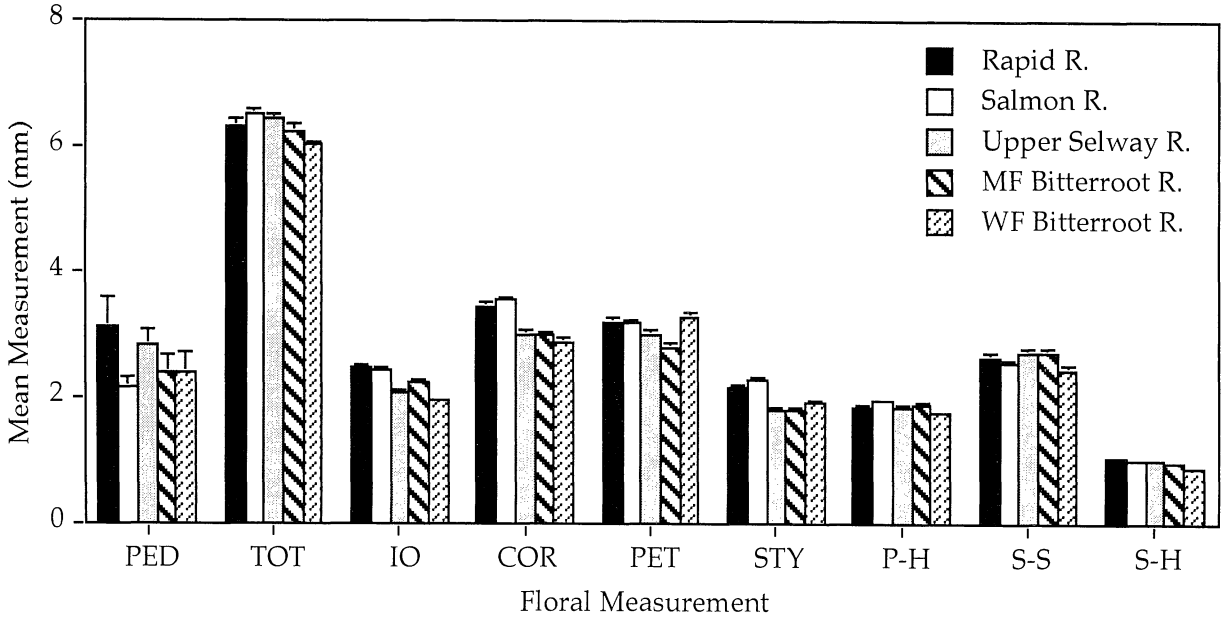

FIG. 6. Comparison of floral morphology among five autotetraploid populations of *Heuchera grossulariifolia*. All floral measurements are expressed in millimeters and are described in Figure 2. Standard error bars are also included.

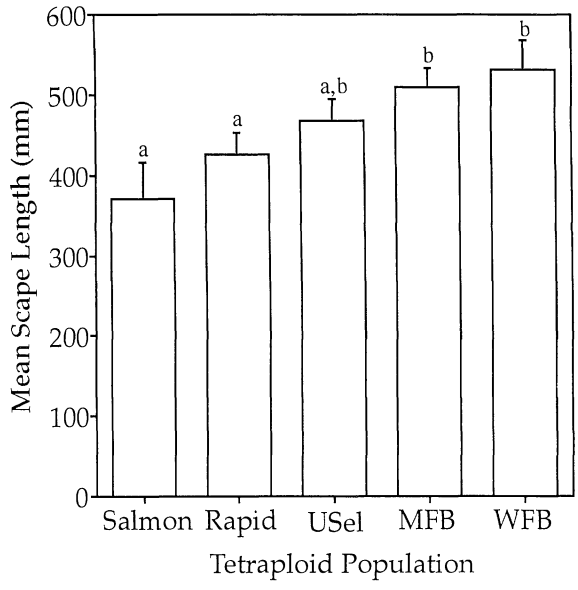

FIG. 7. Mean scape length in millimeters for five autotetraploid populations of *Heuchera grossulariifolia*. USel, upper Selway River; MFB, Main Fork Bitterroot River; WFB, West Fork Bitterroot River. Lower case letters above each bar indicate significance. Populations with different letters are significantly different in scape length (Salmon River vs. Main Fork Bitterroot River: $F = 5.55$; df = 1, 22; $P = 0.03$; Salmon River vs. West Fork Bitterroot River: $F = 5.07$; df = 1, 10; $P = 0.05$; Rapid River vs. Main Fork Bitterroot River: $F = 5.96$; df = 1, 31; $P = 0.02$; Rapid River vs. West Fork Bitterroot River: $F = 6.88$; df = 1, 19; $P = 0.02$). Standard error bars are also included.

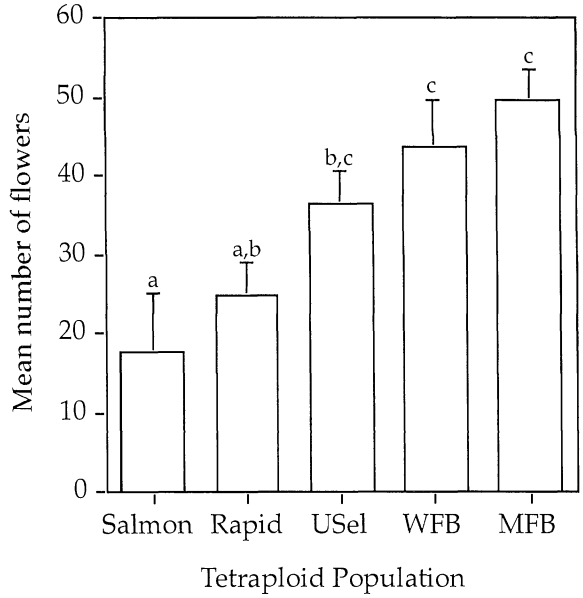

FIG. 8. Comparison of the mean number of flowers on the first inflorescence for five autotetraploid populations of *Heuchera grossulariifolia*. USel, upper Selway River; WFB, West Fork Bitterroot River; MFB, Main Fork Bitterroot River. Lower case letters above each bar indicate significance. Populations with different letters are significantly different in the total number of flowers on the first inflorescence (Salmon vs. upper Selway River: $F = 4.79$; df = 1, 17; $P = 0.04$; Salmon vs. West Fork Bitterroot River: $F = 7.21$; df = 1, 10; $P = 0.02$; Salmon vs. Main Fork Bitterroot River: $F = 14.95$; df = 1, 22; $P = 0.0008$; Rapid vs. West Fork Bitterroot River: $F = 6.86$; df = 1, 19; $P = 0.02$; Rapid vs. Main Fork Bitterroot River: $F = 19.89$; df = 1, 31; $P < 0.0001$; upper Selway vs. Main Fork Bitterroot River: $F = 4.56$; df = 1, 31; $P = 0.04$). Standard error bars are also included.

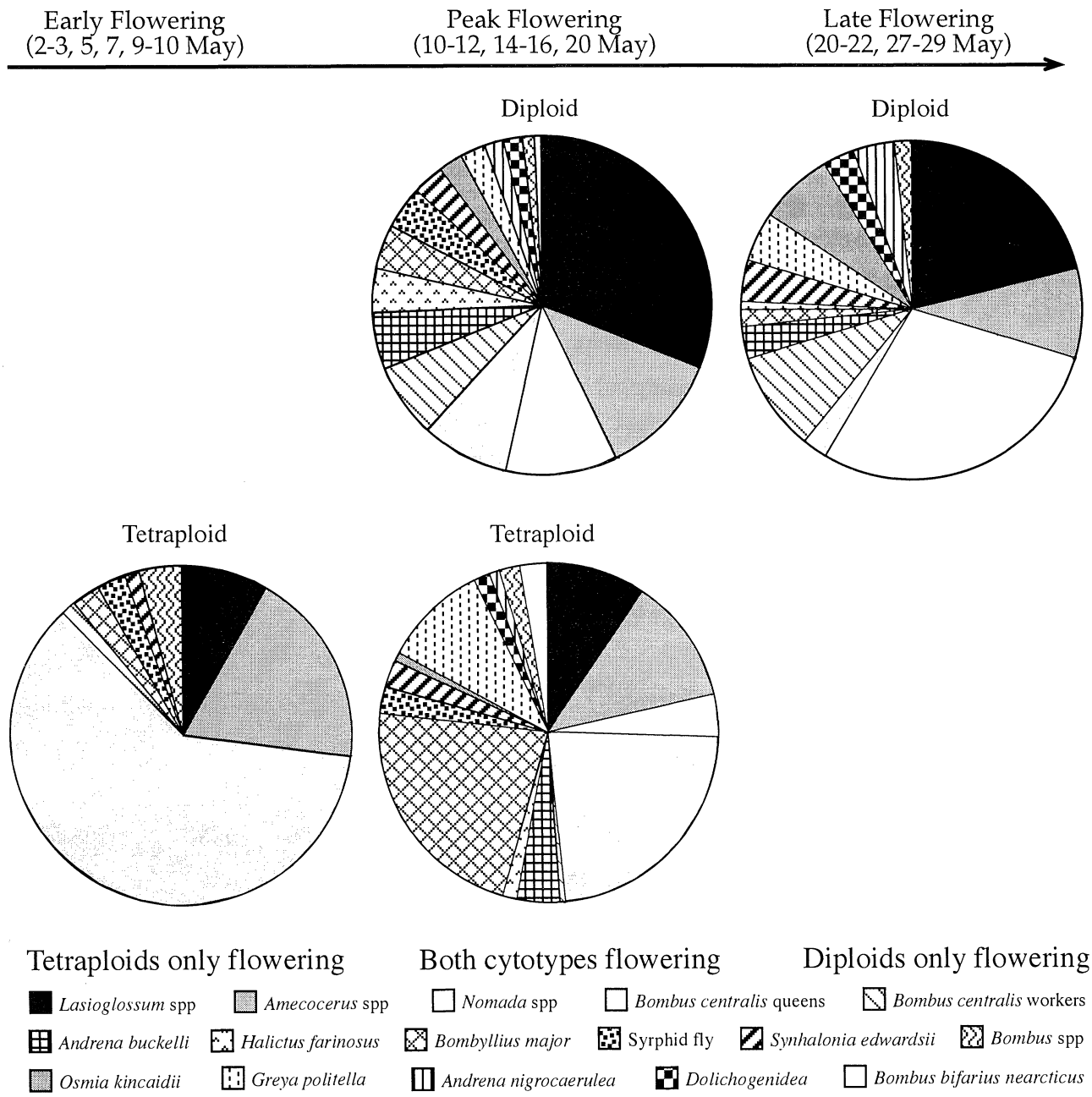

FIG. 9. Proportion of visits to diploid and autotetraploid *Heuchera grossulariifolia* by each insect guild or species when only diploids were flowering, only tetraploids were flowering, and when both diploids and tetraploids were flowering simultaneously.

when diploid and tetraploid plants were both flowering (Table 2). The most prominent insect guild was *Lasioglossum*, which represented about one-quarter of the total visits to flowers of diploids, but only about one-tenth of the total visits to flowers of tetraploids. Another frequent visitor was the bombyliid fly, *Bombyllius major*, that foraged for nectar on flowers of tetraploids 6.4 times more frequently than on flowers of diploids. The prodoxid *Greya politella*, a close relative of the yucca moths, oviposited or foraged on flowers of tetraploids about five times more frequently than on flowers of diploids. Lastly, the foraging behavior of *Bombus centralis* varied across ploidal levels, with the smaller worker *B. centralis* visiting flowers of diploids more frequently than flowers of tetraploids, whereas the larger queens visited flowers of tetraploids more frequently.

DISCUSSION

Differentiation between Cytotypes

Flowers of autotetraploid *H. grossulariifolia* are consistently larger, have a slightly different floral shape, have a different flowering phenology, and attract different suites of

TABLE 2. Means ± standard error for diploid and autotetraploid insect visits to *Heuchera grossulariifolia* on the Salmon River. Means are expressed as the mean number of visits by a species or guild per observation period when both cytotypes were flowering simultaneously.

Insect visitor	Diploid mean ± SE (*n*)	Tetraploid mean ± SE (*n*)	*F*	*P*
Coleoptera				
Amecocerus spp	1.11 ± 0.41 (20)	1.33 ± 0.43 (24)	0.13	0.72
Diptera				
Bombyllius major	0.27 ± 0.11 (7)	1.73 ± 0.26 (45)	31.95	< 0.0001*
Syrphid fly	1.40 ± 0.51 (7)	1.00 ± 0.63 (5)	0.23	0.65
Hymenoptera				
Andrena buckelli	0.90 ± 0.23 (9)	0.80 ± 0.33 (8)	0.04	0.84
Andrena nigrocaerulea	0.60 ± 0.25 (3)	0.40 ± 0.25 (2)	0.17	0.70
Bombus spp	0.40 ± 0.25 (2)	0.80 ± 0.20 (4)	0.37	0.37
Bombus bifarious nearcticus	0.25 ± 0.25 (1)	1.25 ± 0.25 (5)	8.00	< 0.0001*
Bombus centralis queens	0.44 ± 0.11 (14)	1.38 ± 0.17 (44)	18.44	< 0.0001*
Bombus centralis workers	1.20 ± 0.25 (12)	0.10 ± 0.10 (1)	14.96	0.003*
Dolichogenidea spp	1.50 ± 0.50 (3)	1.50 ± 0.50 (3)	0	1.0000
Halictus farinosus	1.17 ± 0.60 (7)	0.50 ± 0.34 (3)	0.69	0.44
Lasioglossum spp	1.68 ± 0.19 (52)	0.71 ± 0.22 (19)	11.59	0.001*
Nomada spp	1.39 ± 0.43 (18)	0.62 ± 0.21 (8)	2.39	0.14
Osmia kincaidii	0.67 ± 0.21 (4)	0.33 ± 0.21 (2)	0.63	0.47
Synhalonia edwardsii	0.56 ± 0.18 (5)	0.56 ± 0.24 (5)	0	1.00
Lepidoptera				
Greya politella	0.25 ± 0.12 (4)	1.25 ± 0.31 (20)	8.16	0.009*

* Asterisk indicates significance.

floral visitors than those of diploids. In comparison to diploids, tetraploid floral morphology varied from pronounced differences between diploid and tetraploid plants to similar floral shapes between the ploidal levels. Our results are consistent with previous studies of morphology in polyploids (e.g., Giles 1942; Kliphuis 1972; Taylor and Smith 1979; Garbutt and Bazzaz 1983; MacDonald and Chinnappa 1988; Chmielewski 1994; Bretagnolle and Lumaret 1995), suggesting that some changes in floral morphology between cytotypes may be due to an instantaneous effect of polyploidy. Typically, an increase in flower size is attributed to the "gigas effect," which is an increase in polyploid cell size that often results in increased organ size throughout the plant (Stebbins 1971). Nevertheless, the gigas effect may not shape tetraploid floral morphology in *H. grossulariifolia* because there are no differences in stomate or pollen size in other autotetraploid saxifrages (D. Soltis, pers. comm.). We have not, however, undertaken a quantitative assessment of cell size in *H. grossulariifolia*.

These changes in floral morphology do not in themselves explain how inviable mating between cytotypes is prevented in mixed populations of diploids and tetraploids. For example, in one sympatric population of diploid and tetraploid *H. grossulariifolia* (West Fork Bitterroot River), flowers of tetraploids closely resembled those of diploids. Morphologically cryptic polyploids are also present in many other species including *Dactylis glomerata* (Borrill and Lindner 1971; Lumaret 1988; Lumaret and Barrientos 1990), *Deschampsia cespitosa* (Rothera and Davy 1986), and *Tolmiea menziesii* (Soltis 1984; Soltis et al. 1989), indicating that in these examples either differences in flowering time or microhabitat differentiation may reduce the number of intercytotype crosses in sympatric populations. Although flowering phenology for plants in the common-garden study had a pattern opposite to plants growing at the Salmon River, the results indicate that diploids and tetraploids differ in floral phenology. The discrepancy in flowering phenology between the common-garden and Salmon River studies suggests that there may be a genotype-by-environment interaction influencing the timing of flowering in *H. grossulariifolia*. Hence, the environment at the Salmon River may cause tetraploids to flower earlier than diploids, whereas the opposite pattern was observed in the common-garden environment on the WSU campus.

Differentiation in flowering dates coupled with differences in floral morphology and insect visitation may decrease pollen movement between diploid and tetraploid plants. Differences in flowering time have been found in several polyploid taxa, especially when there is little spatial separation between diploid and polyploid populations (e.g., Kliphuis 1972; Clark 1975; Lumaret 1988; Lumaret and Barrientos 1990; Petit et al. 1997). Many of the polyploid species that exhibit differences in flowering time are wind pollinated, implying that changes in other floral traits would not be as effective in preventing intercytotype crosses. Because *H. grossulariifolia* is insect pollinated, the combination of changes in floral traits and flowering time increases the potential for isolation of the ploidal levels in sympatric populations. Moreover, our observations at the Salmon River demonstrated that six floral visitors differentially visited one cytotype more frequently than the other. Whether these differences in visitation correspond to a reduction in the quantity of pollen transferred between cytotypes remains untested, but the results suggest that changes in floral morphology can influence pollinator assemblages.

One of the insect visitors, *G. politella*, may cause differences in pollen transfer between the ploidal levels. This species oviposited into flowers of tetraploids more frequently than into flowers of diploids, which is consistent with pre-

vious records of attack rates by *G. politella* on diploid and polyploid *H. grossulariifolia* populations (Thompson et al. 1997). The pollination efficiency of *G. politella* on *H. grossulariifolia* remains untested, but it is a highly effective pollinator of one of its other saxifrage host plants, *Lithophragma parviflorum* (Thompson and Pellmyr 1992; Pellmyr and Thompson 1996). Moreover, observations of its sibling species, *G. enchrysa*, demonstrate that *G. enchrysa* is a highly effective pollinator of *H. cylindrica*, accounting for about 51.2% of seed set per oviposition (Pellmyr et al. 1996). We have frequently observed pollen adhering to the abdomens of *G. politella* females after oviposition, indicating that this species may be an important pollinator of tetraploid *H. grossulariifolia. Greya politella* is a specialist on *L. parviflorum* and *H. grossulariifolia*, but whether females are constant on one ploidy remains to be tested.

Another insect visitor, *B. centralis*, may also cause differential pollen movement between plants differing in ploidy. This species was unusual because queens tended to visit tetraploid plants more frequently, whereas the smaller workers visited diploid plants more frequently. *Bombus centralis* queens and workers are also common visitors of *H. cylindrica*, accounting for about 17.2% of seed set per visit (Pellmyr et al. 1996). Thus, differential visits by *G. politella* and *B. centralis* together may cause some partitioning between cytotypes at the Salmon River. Whether this insect discrimination eliminates gene flow between ploidies remains to be tested, especially because some backcrossing with parental diploids is possible during the overlap in flowering. About 1.4% of plants cytotyped in a large geographical survey were triploid (Thompson et al. 1997). One-half of these were located on the Salmon River and may have been created by intercytotype crosses. Alternatively, these triploids could have been produced by unreduced diploid gametes crossing with reduced diploid gametes (Ramsey and Schemske 1998). This alternative is equally likely because the remaining triploids in that survey were located in a pure diploid population. Further studies of floral visitors in combination with gene flow experiments in this and other sympatric populations of diploids and tetraploids would enhance our understanding of the evolution of these interactions under differing environmental and genetic conditions.

Differences among Plants from Independent Autotetraploid Origins

More detailed studies of pollinators and the effects of polyploidy on plant traits may aid in understanding the geographic differences in floral morphology in this and similar species. Part of the geographic variation in floral traits in *H. grossulariifolia* may be due to the recurrent formation of polyploidy. We found a significant difference in floral morphology between the Rapid and Main Fork Bitterroot River tetraploid origins, as well as differences between all other pairwise combinations of tetraploid populations. We cannot distinguish whether these differences among tetraploid origins and populations were a direct result of the polyploid event itself or whether they were created by selection under different ecological conditions (e.g., selection by pollinators). Novel genetic combinations can occur in newly formed polyploids (e.g., Bennett et al. 1992; Bailey et al. 1993; Mukai et al. 1993; Song et al. 1995; D'Hont et al. 1996; Aggerwal et al. 1997; Friesen et al. 1997), which may explain some of the differences we observed among tetraploid origins. Because these polyploids are autopolyploids, the production of new genetic combinations is probably not as likely an explanation as natural selection. The differences in floral traits among origins spanned a relatively large geographic region with presumable broad ecological differences, including possible changes in guilds of locally available pollinators. Selection, then, could be an important factor in shaping the degree of variation among independent polyploid lineages. The increase in heterozygosity, allelic diversity, and enzyme multiplicity often associated with polyploids (e.g., Roose and Gottlieb 1976; Levin 1983; Soltis and Rieseberg 1986; Wolf et al. 1989; Samuel et al. 1990; Ashton and Abbott 1992; Soltis and Soltis 1993, 1995) could accelerate the speed at which selection acts on floral traits in polyploids and among polyploid lineages.

At least in some instances, polyploidy in *H. grossulariifolia* may generate different floral morphologies among tetraploid origins. Some populations, however, have genotypes reflecting multiple polyploid origins (Salmon River, West Fork Bitterroot River, and upper Selway River). For example, along the Salmon River alone there are genotypes representing at least four independent origins of polyploidy (Segraves et al. 1999). Despite this, casual observation of tetraploid floral morphology appeared consistent throughout the population. If differences existed initially among tetraploid origins along this river, subsequent mating among origins may have homogenized any morphological differences. Alternatively, mating among independent polyploid lineages has the potential to yield new variants through recombination of polyploid genotypes (Soltis et al. 1995). Further investigations of floral traits in independent polyploid lineages are needed to quantify the effects of multiple origins within a localized area.

Parental diploid genomes may not constrain polyploid morphology. If this were the case, tetraploids would more closely resemble their diploid parents than they would more distantly related diploids; thus, differentiation among polyploid populations in floral traits may depend on the genetic parentage of each polyploid lineage (Soltis and Soltis 1989; Brochmann et al. 1992). However, we found no correlation between genetic and morphological similarity, indicating that polyploid morphology may rapidly diverge from diploid morphology. One possible explanation for this result is that rapid changes in the polyploid genome may alter the pattern between genetic similarity and morphological similarity. Although rapid genetic changes in newly formed polyploids have been demonstrated (Song et al. 1995), the most probable explanation is that selection for reproductive isolation has driven divergence between genetic and morphological similarity. This is further supported because triploid production is relatively low and confined to only a few populations (Thompson et al. 1997). Alternatively, low triploid production may be due to triploid block, where many fewer triploids are produced than expected in a mixed population. The effects of independent polyploid origins, then, may potentially drive newly

formed polyploid lineages along different evolutionary pathways than their diploid progenitors.

Conclusions

The results of this study demonstrate differentiation in floral morphology and flowering phenology between diploid and autotetraploid *H. grossulariifolia* that results in differential visitation by some insect species. Moreover, we found differentiation in these traits between closely related diploids and tetraploids as well as differences among plants of independent tetraploid origin. These results have implications for understanding the evolution of polyploidy in flowering plants. First, changes in polyploids may arise quickly, causing differences in floral traits between diploids and polyploids and also among polyploid origins. The evolution of interactions, then, may differ between insect visitors and polyploids and insect visitors and their diploid progenitors as well as among polyploid origins. Second, interactions with pollinators may isolate polyploid angiosperms from their diploid progenitors, potentially allowing them to coexist. Isolation through differential visitation by insect pollinators provides yet another mechanism by which polyploids can coexist with diploids. Subsequent evolution of pollinators to specialize on polyploids may therefore change the efficacy of establishment and persistence of polyploid populations, possibly leading to speciation in both plant and insect taxa. Third, gene flow among polyploid origins may bring together novel genetic combinations that could create new suites of floral traits and polyploid-insect interactions. Finally, polyploidization can result in a diversity of changes in floral traits, contributing to a geographic mosaic of interactions with other species across a species' range, augmenting the geographic dynamics, diversification of species, and their interactions (Thompson 1994).

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Appendix

Matrix of genetic and morphological similarities of all pairwise combinations of *Heuchera grossulariifolia* populations. Morphological distances are shown separately for the three flower positions. The bold numbers are genetic distances and the other numbers are morphological distances for flowers 1, 5, and 10, respectively. 2× indicates diploid populations and 4× indicates autotetraploid populations.

	Boise (2×)	Clear-water (2×)	Payette (2×)	Rapid (4×)	Salmon (2×)	Salmon (4×)	Lower Selway (2×)	Upper Selway (2×)	Upper Selway (4×)	WF Bitterroot (2×)	WF Bitterroot (4×)	MF Bitterroot (2×)	MF Bitterroot (4×)
Boise (2×)	—												
Clearwater (2×)	**3.33** 0.13 1.72 0.83	—											
Payette (2×)	**3.25** 0.84 0.55 0.06	**5.83** 0.97 1.18 0.89	—										
Rapid (4×)	**3.48** 2.38 0.43 0.36	**5.08** 2.51 2.15 1.19	**6.25** 1.55 0.98 0.30	—									
Salmon (2×)	**6.33** 1.24 2.94 1.69	**3.67** 1.11 1.22 0.86	**8.17** 2.08 2.39 1.76	**8.08** 3.62 3.37 2.06	—								
Salmon (4×)	**5.00** 1.42 0.73 0.56	**2.33** 1.55 0.99 1.39	**7.25** 0.59 0.18 0.50	**6.25** 0.96 1.16 0.20	**3.83** 2.66 2.21 2.26	—							
Lower Selway (2×)	**6.25** 0.77 0.73 0.53	**3.83** 0.64 0.99 0.30	**8.06** 1.60 0.81 0.59	**7.88** 3.15 1.16 0.89	**4.17** 0.47 2.21 1.17	**4.00** 2.19 0.00 1.09	—						
Upper Selway (2×)	**6.25** 0.94 2.03 2.24	**3.83** 0.81 0.31 1.41	**8.31** 1.77 1.48 2.30	**7.12** 3.32 2.46 2.60	**5.50** 0.30 0.91 0.54	**3.44** 2.36 1.30 2.80	**4.75** 0.17 1.30 1.71	—					
Upper Selway (4×)	**6.25** 3.27 0.80 2.01	**3.42** 3.40 2.52 2.84	**8.00** 2.43 1.34 1.95	**8.00** 0.89 0.37 1.65	**2.58** 4.50 3.74 3.70	**3.75** 1.85 1.53 1.45	**3.75** 4.04 1.53 2.54	**5.25** 4.21 2.83 4.25	—				
WF Bitterroot (2×)	**3.25** 1.49 2.56 2.65	**5.08** 1.36 0.84 1.82	**5.12** 2.33 2.01 2.71	**5.50** 3.87 2.99 3.01	**5.42** 0.25 0.38 0.96	**5.81** 2.91 1.83 3.21	**6.12** 0.72 1.83 2.12	**7.12** 0.55 0.53 0.41	**5.00** 4.76 3.36 4.66	—			
WF Bitterroot (4×)	**6.75** 5.57 4.77 3.88	**4.08** 5.70 6.49 4.71	**8.50** 4.74 5.32 3.81	**8.12** 3.19 4.34 3.51	**3.08** 6.81 7.71 5.57	**3.88** 4.15 5.50 3.31	**3.88** 6.34 5.50 4.40	**4.62** 6.51 6.80 6.11	**2.50** 2.30 3.98 1.87	**5.38** 7.06 7.33 6.53	—		
MF Bitterroot (2×)	**5.75** 2.15 0.38 1.16	**3.83** 2.28 2.10 1.99	**7.44** 1.31 0.93 1.10	**7.50** 0.23 0.05 0.80	**7.17** 3.39 3.32 2.85	**4.62** 0.73 1.11 0.60	**5.62** 2.92 1.11 1.69	**4.25** 3.09 2.41 3.40	**6.38** 1.12 0.41 0.85	**7.62** 3.64 2.94 3.81	**6.12** 3.42 4.39 2.72	—	
MF Bitterroot (4×)	**7.00** 2.28 2.12 1.93	**4.33** 2.41 3.85 2.76	**8.50** 1.45 2.67 1.86	**8.75** 0.10 1.69 1.56	**2.00** 3.52 5.06 3.62	**4.00** 0.86 2.85 1.36	**3.50** 3.05 2.85 2.45	**5.50** 3.22 4.15 4.16	**1.25** 0.99 1.33 0.08	**4.75** 3.77 4.68 4.58	**1.75** 3.29 2.65 1.95	**7.00** 2.51 1.74 0.77	—