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SELECTION FOR PHENOTYPIC DIVERGENCE BETWEEN DIPLOID AND AUTOTETRAPLOID *HEUCHERA GROSSULARIIFOLIA*

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Abstract.—Much of the diversity of flowering plants is associated with genomic duplication through polyploidy. Little is known, however, about the evolutionary mechanisms responsible for the diversification of novel polyploid lineages. We evaluated the possibility that divergence is driven by natural selection by estimating the strength of phenotypic selection acting on three floral traits in sympatric populations of diploid and autotetraploid *Heuchera grossulariifolia* over three years. Our results demonstrate consistent directional selection for increasing scape length and floral display in both diploid and tetraploid populations. In contrast, selection acting on flowering phenology varied across year and ploidy. Specifically, selection was found to favor late-flowering diploids in 2001 and 2002 but early-flowering tetraploids in 2003. We investigated the mechanistic basis of divergent selection for flowering phenology in 2003 by estimating the relationship between plant flowering phenology and the probability of intercytotype pollinator movement. The results demonstrated that less divergent tetraploids were significantly more likely to experience intercytotype flights than were more divergent tetraploids. This result is consistent with the pattern of phenotypic selection observed. Taken together, our results suggest that divergence of polyploids and their diploid progenitors may be driven by a process analogous to reinforcement whereby selection favors phenotypes that reduce the probability of intercytotype matings with reduced fertility.

Key words.—Diversification, phenotypic selection, polyploidy, reinforcement, speciation.

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Much of the diversity of flowering plants is associated with genomic duplication through polyploidy, with up to 80% of angiosperm species undergoing genome duplication sometime in their evolutionary history (Levin 1983; Masterson 1994; Otto and Whitton 2000; Soltis et al. 2004). The importance of polyploidy has been further demonstrated by recent studies showing that novel polyploid lineages may arise frequently, with the potential for multiple origins within single populations (Wolf et al. 1990; Brochmann et al. 1992; Ramsey and Schemske 1998; Segraves et al. 1999; Soltis and Soltis 2000). Together, these results suggest that polyploidy is a much more dynamic evolutionary process than has been appreciated historically.

Despite the growing realization that polyploidy has played a central role in plant evolution, the ecological and evolutionary mechanisms favoring the diversification of novel polyploid lineages remain largely unknown (Whitton 2004). Two observations suggest that such diversification may be critical to the success of polyploid lineages. First, in the absence of diversification, both the ancestral diploid population and the novel polyploid population should frequently suffer from competition for both pollinators and resources (Levin 1983). Second, in the absence of phenotypic divergence, ineffectual mating between cytotypes is likely to be common, presumably reducing the fitness of both cytotypes (Levin 1975; Husband 2000). Indeed, mathematical models of polyploid establishment have shown that in the absence of assortative mating within cytotypes or other mitigating factors, the initially less frequent cytotype is doomed to extinction (Fowler and Levin 1984; Felber 1991; Rodriguez 1996). Clearly, mechanisms must exist that promote the diversification of novel polyploid lineages.

One mechanism that may be responsible for initial phenotypic diversification is spontaneous and likely due to altered patterns of gene expression in polyploids. Evidence for this mechanism comes primarily from studies of synthetically produced polyploids, which have shown that polyploids can differ phenotypically from their parental diploids immediately after their formation (Ramsey and Schemske 2002). The generality of this spontaneous differentiation and whether it also occurs in naturally formed polyploids remains largely unknown. Another possibility is that phenotypic divergence is driven by natural selection acting on initially similar cytotypes (Van Dijk and Bijlsma 1994; Petit et al. 1999). This view is supported by studies demonstrating that phenotypic differentiation occurs between naturally occurring diploid and polyploid populations but not between diploids and synthetically produced neopolyploids (Bretagnolle and Lumaret 1995; Petit et al. 1999).

Natural selection for phenotypic divergence could arise in multiple ways. Phenotypic divergence may occur as a consequence of selection for increased assortative mating within cytotypes (Van Dijk and Bijlsma 1994; Petit and Thompson 1998; Petit et al. 1999; Husband and Sabara 2004; Whitton 2004). Another possibility is that competition between cytotypes for pollinators and other resources generates selection for divergent phenotypes that minimize intercytotype competition (Petit et al. 1999). Divergent selection might also arise, however, for reasons independent of the other cytotype. For instance, slight differences in the initial community of pollinators associated with each cytotype might favor different suites of floral traits in each (e.g., Segraves and Thompson 1999). A similar effect could be generated by slight differences in the initial community of floral parasites associated with each ploidy (Thompson et al. 1997, 2004; Nuismer and Thompson 2001). Although each of these potential mechanisms has been shown to be important for the process of phenotypic divergence in general (e.g., Kondrashov and Shpak 1998; Dieckmann and Doebeli 1999; Doebeli and Dieckmann 2000; Schluter 2001; Turelli et al. 2001; Via

2002; Kirkpatrick and Nuismer 2004), the extent to which each is pertinent to the evolutionary divergence of sympatric populations of diploid and polyploid plants is largely unknown.

The possibility that divergence between cytotypes could be driven by natural selection for increased assortative mating has been previously explored in a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Petit et al. 1997). That study used an analysis of phenotypic selection to determine whether significant selection could be detected for flowering time in the two cytotypes. Although divergent selection was not detected, this may have been due to the high degree of phenotypic differentiation that already exists between the cytotypes in the system (Petit et al. 1997). Alternatively, the small sample sizes used in that study may have provided insufficient power to detect existing patterns of selection (e.g., Kingsolver et al. 2001; Hersch and Phillips 2004).

Here we report results from a three-year study of phenotypic selection in a sympatric population of diploid and autotetraploid *Heuchera grossulariifolia* growing along Idaho's Salmon River. Our overall goal is to investigate whether natural selection currently acts to promote the phenotypic divergence of these populations and, if so, through what mechanism. We report results that address the following questions. Does natural selection act on any of three floral traits likely to play important roles in pollinator attraction? Is the selection detected of a form that favors the evolutionary divergence of diploids and autotetraploids? Are observed patterns of natural selection consistent across years? Finally, can observed patterns of selection be explained by the frequency of intercytotype pollinator visitation?

Materials and Methods

Natural-History Background

Heuchera grossulariifolia (Saxifragaceae) is a rhizotomous perennial that grows primarily along the major river valleys of eastern Washington, Idaho, and western Montana. Within this broad geographic region there have been at least two and potentially up to seven independent origins of autotetraploidy (Wolf et al. 1990; Segraves et al. 1999). In at least three locations, diploid and autotetraploid individuals occur in sympatry: the lower Salmon River east of Riggins, Idaho; the upper Selway River west of Darby, Montana; and the West Fork of the Bitterroot River south of Darby, Montana (Thompson et al. 1997). Each of these populations exhibits a different degree of differentiation between the cytotypes, ranging from virtually indistinguishable (Selway and West Fork of Bitterroot) to substantially morphologically and phenologically differentiated (Salmon; Segraves and Thompson 1999).

Previous studies have consistently demonstrated reduced fertility of crosses between diploid and autotetraploid *H. grossulariifolia* collected from the Salmon River (Wolf et al. 1990; C. C. Fernandez and J. N. Thompson, unpubl. data). Controlled greenhouse crosses conducted by Wolf et al. (1990), for instance, demonstrated that intercytotype matings set between zero and six seeds, whereas intracytotype matings resulted in approximately 300–600 seeds (Wolf et al. 1990). A much larger crossing experiment extended these results by demonstrating that intercyotype matings result in reduced seed set, as well as in significantly reduced germination rate (C. C. Fernandez and J. N. Thompson, unpubl. data). This latter study also demonstrated that even when intercyotype matings do result in viable offspring, the majority (≈88%) are triploid. Although it is not yet known whether these triploid offspring are fertile, it is clear that intercytotype matings are likely to result in reduced fitness. Thus, at least for the Salmon River populations, the raw material exists for natural selection to drive the divergence of traits that increase levels of assortative mating within cytotypes.

This study focused on populations of *H. grossulariifolia* growing along the main stem of the Salmon River approximately 27 km east of Riggins, Idaho (45°25.352′N; 116°02.158′W). The study site spans a distance of approximately 1.6 km and contains large numbers of diploid and autotetraploid *H. grossulariifolia*. Diploid and autotetraploid plants within this site are very well mixed spatially and show little evidence of microhabitat selection. In addition, although the two cytotypes are quite differentiated for some phenotypic traits, they do exhibit substantial overlap for several other potentially important traits, such as flowering phenology (Thompson et al. 1997; Segraves and Thompson 1999; Segraves et al. 1999; Nuismer and Thompson 2001). As a consequence, substantial scope exists for further phenotypic divergence to evolve.

Along the Salmon River, plants flower between April and May and produce between one and 50 inflorescences, each of which may have up to 250 flowers. Plants growing at this site are regularly attacked by several species of insect parasite (Thompson et al. 1997; Nuismer and Thompson 2001) and are pollinated by multiple species of insect pollinator (Segraves and Thompson 1999). More recent work has shown, however, that a single pollinator species, *Bombus centralis*, is responsible for the bulk of *H. grossulariifolia* pollination along the Salmon River (K. F. Merg and J. N. Thompson, unpubl. data).

General Methodological Approach

Our general approach was to measure the magnitude and sign of selection gradients acting on three potentially important phenotypic traits within each ploidy using the multiple regression techniques pioneered by Lande and Arnold (1983). We chose to measure selection gradients rather than selection differentials because our primary interest was to determine which of these three traits, if any, was the target of divergent selection. Selection differentials are inappropriate for addressing this issue because they are confounded by selection acting on other phenotypically correlated traits (Lande and Arnold 1983; Kingsolver et al. 2001).

At the beginning of the study in April 2001, 128 diploid and 151 tetraploid plants were marked with individually labeled tags. Ploidy was determined through visual inspection of several key morphological traits (flower color, flower size, scape length, scape pubescence) that allow ploidy to be assigned rapidly and with a very high degree of accuracy (Thompson et al. 1997; Segraves and Thompson 1999; Nuismer and Thompson 2001). Our initial plan was to use these

same individuals throughout the three years of the study. Because a substantial number of plants did not flower in the second year (2002), however, we were forced to mark additional plants to maintain our desired sample size. The same issue arose in the last year of the study (2003). As a consequence, the number of plants used in each year of the study fluctuated slightly (2001: $n_{2x} = 128$, $n_{4x} = 151$; 2002: $n_{2x} = 147$, $n_{4x} = 177$; 2003: $n_{2x} = 162$, $n_{4x} = 167$) and contained a different subset of individual plants.

For each year of the study we collected the following data for each plant: the length of the longest flowering scape, the maximum number of flowers open simultaneously, and the average flowering date. All data was collected over the course of the flowering period during daily observations of each marked plant. Observations were carried out on most days during the flowering period, for a total of 30 days in 2001 and 33 days in 2002 and 2003. On each observation day, every study plant was observed and the number of open flowers and any insect visitors were recorded. Maximum scape length for each plant was measured once, on or near the date of peak flowering. Average flowering date was calculated for each individual at the end of the flowering season as:

$$\text{AFD} = \sum_{i=1}^{N} \left(n_i x_i \Big/ \sum_{i=1}^{N} n_i \right), \quad (1)$$

where n_i is the number of flowers open on a particular plant on day i, x_i is the Julian date of day i, and N is the last day the plant had an open flower. Although our data allows calculation of various other phenological traits (e.g., date of first flower, date of last flower, and date of peak flower, among others), we chose to limit our consideration to a single biologically relevant phenological trait to limit potential problems with multicollinearity.

In addition to phenotypic traits, we collected data from each marked plant that allowed us to estimate total seed set, which we used as our measure of total fitness. Specifically, we estimated the total seed set of each plant by multiplying the total number of floral capsules setting seed by the average seed set per capsule. The number of floral capsules setting seed was determined for each plant during daily observations by counting the number of capsules reaching maturity. Average seed set per capsule was determined by dissection of five floral capsules that were haphazardly collected from each marked plant.

Comparison of Trait Means, Phenotypic Correlations, and Fitness

Before evaluating patterns of selection within ploidies, we compared means of phenotypic traits and total seed set across the two ploidies in each year of the study. Prior to analysis, total seed set data was cube-root transformed and maximum floral display was natural-log transformed to normalize the data. Differences in trait and fitness means between diploid and tetraploid populations were then evaluated using standard analysis of variance for each year of the study. In addition to these comparisons of trait and fitness means, we also estimated the phenotypic correlations between traits for each of the ploidies.

Selection Gradient Analysis

All phenotypic traits were converted to standard deviations and absolute fitness (total seed set) was converted to relative fitness (Lande and Arnold 1983). Conversions were performed within ploidy and year so that selection gradients could be analyzed independently for each ploidy and year of the study. Seed set data was highly skewed and, thus, standard multiple regression techniques could not be used to establish the significance of estimated selection gradients (Lande and Arnold 1983). Therefore, we placed 95% confidence intervals on our regression estimates by bootstrapping (C++ code available upon request). For each ploidy and year of the study, 10,000 replicate datasets were created by bootstrapping the original data. The selection gradient acting on each trait was estimated for each replicate dataset using standard multiple regression. The reported estimate of the selection gradient acting on trait i was calculated as its average value over all 10,000 bootstrap replicates. Ninety-five percent confidence intervals for selection gradients were estimated by throwing out the top and bottom 2.5% of estimated values (Lynch and Walsh 1998, p. 449). In addition to estimating linear selection gradients, we also estimated quadratic selection gradients. These nonlinear gradients were estimated for our data in the same way as linear gradients with the exception of being performed on squared values of phenotypic standard deviations (Lande and Arnold 1983).

Pollinator Visitation Analysis

We also evaluated whether these floral traits played a role in shaping patterns of pollinator visitation during the 2003 flowering season. Specifically, we evaluated whether the probability of intercytotype movement by the pollinating bee *B. centralis* depended upon floral phenotype or ploidy. Intercytotype visitation by *B. centralis* was quantified during daily observations of marked plants as follows. If an individual *B. centralis* was visiting a plant when the observer arrived, it was followed until it moved to another plant, at which point the ploidy of this recipient plant was recorded. This information allowed us to code each plant that was observed to be visited by *B. centralis* as either a 0 for a within-cytotype flight or as a 1 for a between-cytotype flight. Pollinator movement data was analyzed using logistic regression with plant phenotypic traits converted to standard deviations, as described for the selection gradient analysis.

RESULTS

Comparison of Trait Means, Phenotypic Correlations, and Fitness

We evaluated whether the mean values for the three phenotypic traits differed between the two ploidies in each year of the study. In all years, tetraploids flowered significantly earlier than diploids and had significantly shorter flowering scapes (Fig. 1A, B). In 2001 and 2002, tetraploids also had significantly larger maximum floral displays, although in 2003 there were no significant differences between the ploidies (Fig. 1C). With regard to differences in total seed set, a significant difference was observed only in 2001, when tetraploids set more seeds, on average, than did diploids. In

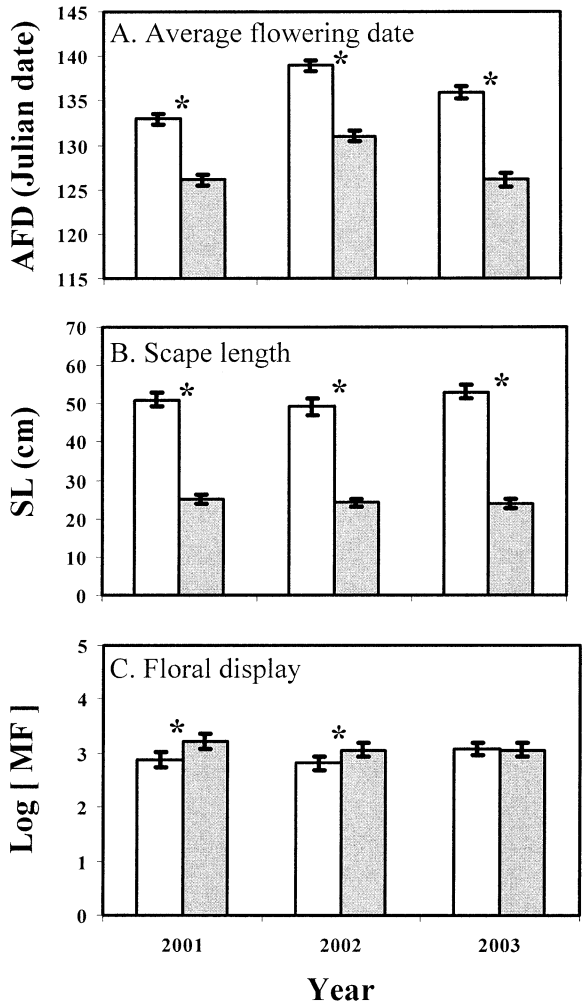

FIG. 1. Estimated means and 95% confidence intervals for the phenotypic traits (A) average flowering date, (B) maximum scape length, and (C) maximum floral display, in each year of the study. Diploids are represented by white bars and autotetraploids by gray bars, with asterisks indicating significant differences between ploidies at the $P < 0.05$ level. Data for maximum floral display was log transformed. Sample sizes for the three phenotypic traits respectively were $n_{2x} = 128$, 126, 128 and $n_{4x} = 151$, 150, 151 in 2001; $n_{2x} = 139$, 136, 147 and $n_{4x} = 176$, 175, 177 in 2002; and $n_{2x} = 161$, 154, 162 and $n_{4x} = 167$, 163, 167 in 2003.

the remaining years no significant effect of ploidy was observed on total seed set (Fig. 2). Finally, we estimated the phenotypic correlations between traits within each ploidy (Table 1).

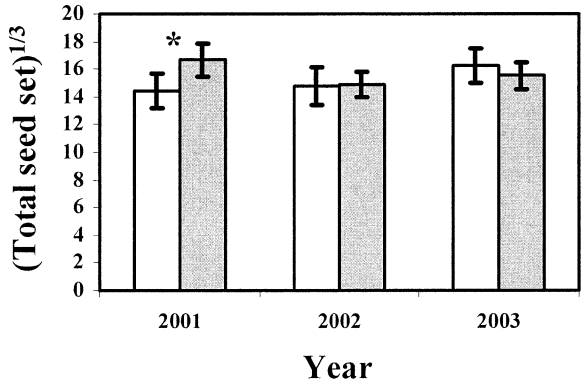

FIG. 2. Estimated means and 95% confidence intervals for total seed set in the three years of the study. Diploids are represented by white bars and autotetraploids by gray bars, with the asterisk indicating a significant difference between ploidies at the $P < 0.05$ level. Total seed set data was cube-root transformed. Sample sizes were $n_{2x} = 123$ and $n_{4x} = 148$ in 2001, $n_{2x} = 145$ and $n_{4x} = 176$ in 2002, and $n_{2x} = 158$ and $n_{4x} = 161$ in 2003.

Selection Gradient Analysis

We first tested whether statistically significant linear selection could be identified for either ploidy in any year of the study. Testing this hypothesis requires demonstrating that the confidence interval for the selection gradient in question does not overlap zero. Our analysis of phenotypic selection revealed that this condition was often met, with strong positive selection for increased scape length and increased maximum floral display acting on both ploidies and in all years (Table 2). Thus, to the extent that total seed set is an accurate predictor of individual fitness, natural selection consistently favors longer flowering scapes and increased maximum floral displays. In contrast, significant selection acting on average flowering date was detected for each ploidy in only a subset of years. Specifically, positive and statistically significant selection gradients were observed for diploids in 2001 and 2002, whereas a negative and statistically significant selection gradient was observed for tetraploids in 2003 (Table 2). Thus, unlike selection acting on maximum floral display and scape length, natural selection acting on average flowering date favored divergent phenotypes in the two ploidies. Specifically, over the three years of the study, natural selection generally favored earlier-flowering tetraploids but later-flowering diploids (Table 2).

In addition to demonstrating significant linear selection, our results revealed significant nonlinear selection acting on average flowering date and maximum flowering size in most years (Table 3). In all cases, the quadratic selection gradient

TABLE 1. Phenotypic correlations among three flowering traits (SL, maximum scape length; MF, maximum floral display; AFD, average flowering date) for each ploidy and year. Entries above the diagonal are for diploids, and entries below the diagonal are for tetraploids. Sample sizes were $n_{2x} = 108$ and $n_{4x} = 141$ in 2001, $n_{2x} = 127$ and $n_{4x} = 169$ in 2002, and $n_{2x} = 154$ and $n_{4x} = 141$ in 2003.

	2001			2002			2003		
Trait	SL	MF	AFD	SL	MF	AFD	SL	MF	AFD
SL		0.505	−0.219		0.483	−0.240		0.406	0.186
MF	0.439		−0.402	0.428		−0.203	0.493		0.021
AFD	−0.126	−0.153		−0.157	−0.071		−0.209	−0.028	

TABLE 2. Selection gradients acting on three flowering traits (SL, maximum scape length; MF, maximum floral display; AFD, average flowering date) for each ploidy and year. Sample sizes were $n_{2x} = 108$ and $n_{4x} = 141$ in 2001, $n_{2x} = 127$ and $n_{4x} = 169$ in 2002, and $n_{2x} = 154$ and $n_{4x} = 141$ in 2003. Asterisks indicate selection gradients significantly different from zero at the $P < 0.05$ level based upon 10,000 bootstraps.

Year	2x SL	4x SL	2x MF	4x MF	2x AFD	4x AFD
2001	0.261*	0.224*	0.458*	0.891*	0.244*	0.013
2002	0.307*	0.303*	0.707*	0.495*	0.130*	−0.060
2003	0.229*	0.267*	0.692*	0.665*	−0.005	−0.142*

TABLE 3. Quadratic selection gradients acting on three flowering traits (SL, maximum scape length; MF, maximum floral display; AFD, average flowering date) for each ploidy and year. Sample sizes were $n_{2x} = 108$ and $n_{4x} = 141$ in 2001, $n_{2x} = 127$ and $n_{4x} = 169$ in 2002, and $n_{2x} = 154$ and $n_{4x} = 141$ in 2003. Asterisks indicate selection gradients significantly different from zero at the $P < 0.05$ level based upon 10,000 bootstraps.

Year	2x SL	4x SL	2x MF	4x MF	2x AFD	4x AFD
2001	0.043	0.042	0.178*	0.275*	−0.113*	−0.138*
2002	0.086	0.087	0.248*	0.089*	−0.025	−0.072*
2003	0.042	0.135*	0.218*	0.229*	−0.124*	−0.087*

acting on average flowering date was negative, indicating selection with a curvature compatible with stabilizing selection. In contrast, the quadratic selection gradient acting on maximum floral display was always positive, indicating selection with a curvature compatible with disruptive selection. Only in a single year and ploidy was their significant quadratic selection gradient for scape length (Table 3). No further analysis was performed on nonlinear selection gradients.

The estimated values for linear selection gradients (Table 2) suggest that selection acted differently on average flowering date in the two ploidies. To more rigorously test this hypothesis, we evaluated whether the selection gradients acting on the two ploidies differed significantly for any year of the study by determining whether their confidence intervals overlapped. The selection gradients acting on the two ploidies were not significantly different for any year of study (Fig. 3). Thus, although selection acting on average flowering date appears to act differently in the two ploidies based upon the results of the simple selection gradient analysis (Table 2), this cannot be demonstrated conclusively for any individual year. Even so, the results presented in Figure 3 seem to suggest a temporally consistent pattern of divergent selection for average flowering date.

This observation led us to test whether the sign and magnitude of linear selection gradients for average flowering date were consistent across years. Selection gradients were considered to be temporally consistent if their 95% confidence intervals overlapped for all three years of the study. Significant temporal variation in selection occurred only for diploid average flowering date between 2001 and 2003 (Fig. 3). This difference is only marginally significant and does not show a reversal in the sign of the selection gradient, only a shift in its magnitude. In light of this general pattern of temporally consistent selection, we combined the data from all three years of the study into a single dataset. Our intent was to gain insight into the overall patterns of selection acting on the two ploidies. Combining the data in this way is not strictly valid statistically, as a subset of the same plants were used in multiple years of the study, and thus the data from each of the three years cannot be regarded as completely independent even though the trait and fitness measurements were conducted independently in each year. Therefore, we present this analysis primarily for heuristic value.

Our results from the analysis of the combined dataset confirm that divergent selection acted on average flowering date but not on maximum scape length or floral display. Specifically, the selection gradient acting on flowering time in the pooled dataset was significant and positive in diploids but significant and negative in tetraploids (Fig. 4). Thus, the selection gradients acting on average flowering date in the two ploidies were significantly different from one another. In contrast, estimates for selection acting on maximum scape length and maximum floral display were positive and significantly

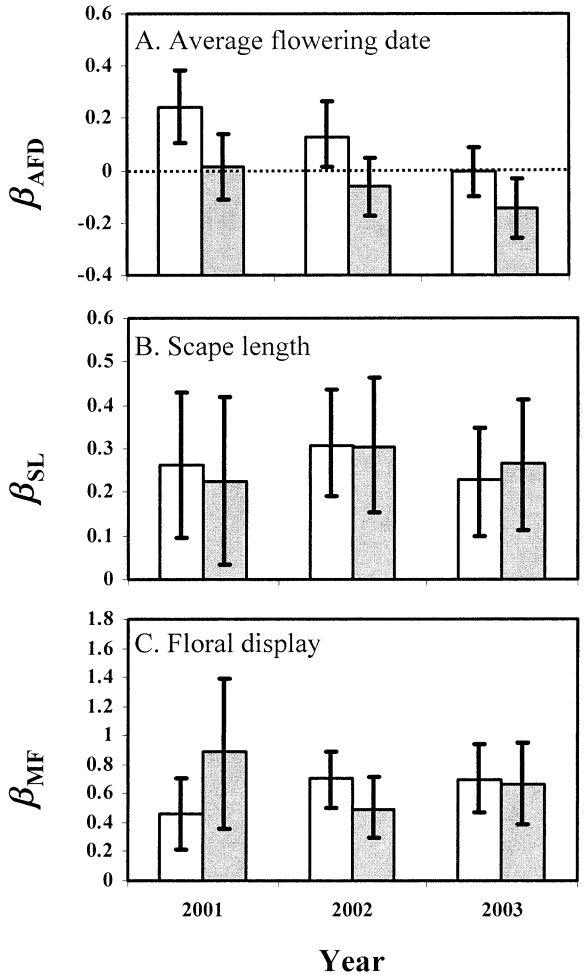

FIG. 3. Estimated selection gradients (β) and their 95% confidence intervals for the phenotypic traits (A) average flowering date, (B) maximum scape length, and (C) maximum floral display, in each year of the study. Diploids are represented by white bars and autotetraploids by gray bars. Sample sizes were $n_{2x} = 108$ and $n_{4x} = 141$ in 2001, $n_{2x} = 127$ and $n_{4x} = 169$ in 2002, and $n_{2x} = 154$ and $n_{4x} = 141$ in 2003. Estimates and their confidence intervals were determined from 10,000 bootstrap replicates.

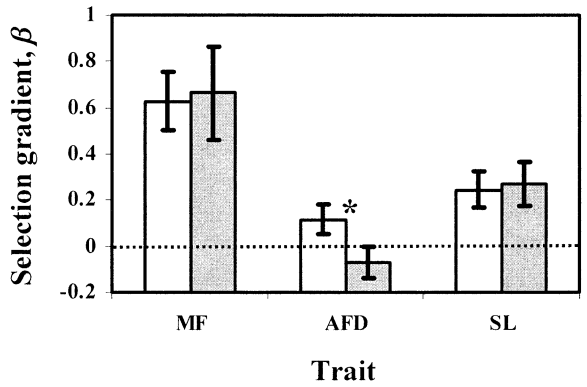

FIG. 4. Estimated selection gradients (β) and their 95% confidence intervals for the phenotypic traits maximum floral display (MF), average flowering date (AFD), and maximum scape length (SL) pooled over all three years of the study. Diploids are represented by white bars and autotetraploids by gray bars, with the asterisk indicating a significant difference between the ploidies. Sample sizes were $n_{2x} = 389$ and $n_{4x} = 451$. Estimates and their confidence intervals were determined from 10,000 bootstrap replicates.

TABLE 4. Coefficients for a logistic regression of intercytotype pollinator visits against three flowering traits (SL, maximum scape length; MF, maximum floral display; AFD, average flowering date). Asterisks indicate parameter estimates significantly different from zero at the $P < 0.05$ level based upon likelihood-ratio tests. Note that negative estimates indicate that the probability of intercytotype flights increases with increasing values of the phenotypic trait.

Trait	2x	4x
SL	0.324	−0.910
MF	0.194	2.307*
AFD	0.416	−0.965*

greater than zero in both ploidies (Fig. 4). More importantly, the confidence intervals for selection estimates on these latter traits overlapped substantially, indicating that selection was homogenous across ploidy.

Pollinator Visitation Analysis

Our analysis of selection demonstrated that selection favors divergent flowering times in the two ploidies. To investigate whether this pattern of selection could be the result of differential rates of inter-cytotype pollen transfer we analyzed the impact of ploidy and flowering phenotype on the frequency of inter-cytotype flights by the primary pollinator of *H. grossulariifolia*, *B. centralis*. Over the course of the 2003 flowering seasons, we recorded 47 visits to marked diploid plants and 56 visits to marked tetraploid plants. For diploid plants, 31.9% of visits were followed by movement to a tetraploid plant, whereas for tetraploid plants only 14.3% of visits were followed by movement to a diploid plant. A chi-squared test demonstrated that these results were significant ($\chi^2 = 4.60$, $P = 0.032$). To further investigate the factors that determine the probability of intercytotype movement of pollinators, we performed a logistic regression of pollinator movement (0, moves to same ploidy; 1, moves to different ploidy) on plant phenotypic traits. Late-flowering tetraploid plants were significantly more likely to have their pollinators move to diploids than were early-flowering tetraploids (Table 4). In contrast, no significant effect of average flowering date was found for diploid plants (Table 4). These results are compatible with estimated selection gradients in this year of the study, suggesting that the frequency of intercytotype visits may at least partially explain observed patterns of phenotypic selection.

DISCUSSION

Our results demonstrate that diploid and autotetraploid *H. grossulariifolia* differ consistently for several important phenotypic traits across years. Maximum scape length and average flowering date are particularly divergent. The same results were found in studies conducted in the field and in a common garden (Segraves and Thompson 1999). Our results strengthen these previous results by showing that the observed phenotypic differences are consistent across years. More importantly, however, our demonstration that tetraploids flower earlier than diploids forms a critical backdrop for our results on the strength and direction of phenotypic selection within ploidies. Specifically, natural selection currently acts to increase existing differences in the flowering times of the two ploidies, favoring tetraploids that flower earlier but diploids that flower later.

In addition, our results represent a first step toward demonstrating a potential underlying mechanism. Specifically, our analysis of visitation patterns by the primary pollinator of *H. grossulariifolia*, *B. centralis*, demonstrates that early-flowering (more divergent) tetraploids were significantly less likely to experience intercytotype visits during the 2003 flowering season. If the frequency of intercytotype pollen transfer at least partially determines seed set, this result could explain the increased fitness of early-flowering tetraploids that we observed in this year of the study (cf. Tables 2 and 4). In a broader sense, this result suggests that a process similar to reinforcement may be operating in this system whereby cytotype divergence is driven by selection for increased assortative mating (Petit et al. 1999).

Our results do not allow us to rule out many other biologically plausible explanations for the observed pattern of divergent selection. It is possible, for instance, that diploids and autotetraploids occupy subtly different microhabitats, each characterized by selection toward a different optimum trait value. Although possible, this explanation seems very unlikely given the high degree of cytotype sympatry observed in this region and the lack of any obvious differences in the microhabitat occupied by the two ploidies. Perhaps more likely is the possibility that slight differences in the pollinator communities of the two ploidies observed at this site (Segraves and Thompson 1999) might exert different patterns of phenotypic selection that have little or nothing to do with the presence or absence of the other cytotype. Similarly, large differences in the community of floral parasites associated with each ploidy at this site (Thompson et al. 1997; Nuismer and Thompson 2001) might also lead to differing patterns of phenotypic selection that, again, have nothing to do with the other cytotype. Distinguishing between these various possible sources of divergent selection will be an important focus of future work.

In contrast to the divergent selection for flowering time, selection favors increasing maximum scape length and maximum floral display in both ploidies. Given the tight correlation between maximum floral display and the total number of flowers produced by a plant, it is not surprising that we found selection for increasing values of this trait in both diploids and tetraploids. Somewhat more surprising, however, is that selection apparently favors increased scape lengths in both cytotypes, even though the cytotypes are quite divergent for this trait. There are at least three possible explanations for this discrepancy. First, divergence may have resulted from historical selection that no longer operates in these populations. Second, observed differences in scape length between the two ploidies may simply be a genetic consequence of polyploidization per se and have little to do with current or past selection (Ramsey and Schemske 2002; Pires et al. 2004). Third, our study of phenotypic selection may have failed to accurately capture the full scope of potential fitness trade-offs. For instance, increasing floral displays and scape lengths may increase seed set at the expense of vegetative reproduction. Similarly, increasing floral displays and scape lengths in one year may increase immediate seed set at the expense of future survival or reproduction. Neither of these possibilities would have been accurately captured in our study.

Our results provide the first empirical evidence that natural selection drives the divergence of sympatrically occurring diploid and polyploid plants. This result suggests an important role for natural selection in the diversification of polyploid lineages. Interpreting our data must be done in light of the following caveats. First, molecular data suggested that the autotetraploids used in this study may not be derived from the diploids with which they are currently sympatric (Segraves et al. 1999). Second, the age of the autotetraploids used in this study is not known. Finally, our proxy for lifetime fitness—total seed set—is only one component of fitness in *H. grossulariifolia* and may be confounded by the potential for vegetative reproduction and multiple episodes of reproduction spread over a perennial life history. Our results could be strengthened by additional information on seed viability, frequency of triploid embryos, and patterns of selection on the two cytotype in allopatry. Each of these will be an important focus for future work.

In a broader sense, our results further implicate natural selection as an important driving force in speciation (Dieckmann and Doebeli 1999; Schluter 2001, 2003; Via 2001; Doebeli and Dieckmann 2003). Although novel polyploid lineages are immediately isolated to some degree by postzygotic barriers, our results suggest that natural selection may be important for the evolution of prezygotic barriers. In this sense, speciation through polyploidy shows striking parallels with the process of reinforcement (Servedio and Kirkpatrick 1997; Kirkpatrick 2001; Kirkpatrick and Ravigne 2002). A fusion of these two areas of research should provide unique insights into both processes and further our general understanding of speciation.

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