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THE GEOGRAPHIC DISTRIBUTION OF POLYPLOIDY IN A PACIFIC NORTHWEST PLANT

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ABSTRACT

Polyploidy is an important evolutionary mechanism of speciation in plants. Because polyploids may evolve cryptically, there can be several levels of ploidy in a species. The distribution of polyploid species is the first step in understanding the role of polyploidy in plant speciation. I examined the geographic distribution of polyploidy in *Heuchera cylindrica* (Saxifragaceae) by using flow cytometry to determine the ploidy level of 595 individuals from 39 populations spread across the geographic range of the species. Only single cytotype populations of diploids or tetraploids were found, and no triploids were observed. In contrast to other studies of polyploid distribution, diploids and tetraploids of *H. cylindrica* were allopatric. I conclude that the allopatric distribution of cytotypes may be a result of the historical biogeography of the area.

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ADVICE TO FUTURE HONORS STUDENTS

Over the next few years as an honors student, you will be given a lot of advice. People will tell you to do well in school, to follow your dreams, to get more sleep. Advisors will advise you on what classes to take and what internships to apply for. Your peers will advise to you take a break once in awhile and enjoy college life. However, people can only advise others based on personal experiences and biases, and so my advice for you is simple: be proud of your work. In an environment such as this, it is tempting to compare yourself to your fellow honors students. Not surprisingly, this would be similar to comparing apples to oranges. You, your advisor, and those that are close to you know how hard you have worked. I hope that when you complete this program, you will leave feeling proud and accomplished. After all, that is the spirit of the Renée Crown Honors Program.

INTRODUCTION

Polyploidy is thought to be an important mechanism of speciation in plants by generating instantaneous speciation through genome doubling (Stebbins, 1950; Grant, 1981; Soltis & Soltis, 1995). Polyploidy occurs more often in plants than in animals, and estimations posit 47-70% or more of angiosperms are of polyploid origin (Masterson, 1994; Soltis & Soltis, 1999). Recent studies have shown that an ancient polyploidization event may have occurred before the evolution of angiosperms, suggesting that all flowering plants are of polyploid descent (Bowers et al., 2003); thus, examining the evolution of polyploidy in plant species will increase our understanding of speciation and diversification of plant lineages.

Polyploids are defined as having a higher number of chromosome sets as compared to diploid cells. Polyploidy occurs as a result of unreduced gametes and can be a result of meiotic nondisjunction, in which the diploid germline cell produces diploid gametes. As the daughter cells divide, the number of chromosomes is doubled. Polyploidy can also occur as a result of mitotic nondisjunction, where diploid somatic cells generate a tetraploid cell. This tetraploid cell then produces diploid gametes, and again the number of chromosomes is doubled. Polyploidy can arise in two ways depending on whether the unreduced gametes that join are from the same or different species. Autopolyploids form as a result of the fusion of unreduced gametes from within a species. In contrast, allopolyploids are formed from unreduced gametes from two different species. This interspecific hybridization produces polyploid offspring.

Thus, autopolyploidy is a within species phenomenon whereas allopolyploidy arises through hybridization between species.

Irrespective of the mechanism of formation, a key first step in understanding polyploidization is to investigate the geographic distribution of ploidal levels. Examining these distributions are important because many polyploids are cryptic. In other words, polyploid lineages often go unnoticed because they are morphologically similar to their diploid progenitors and therefore share the same scientific name as opposed to having their own distinct species names (Soltis et al., 2007). This means that biologists have vastly underestimated the number of species from an evolutionary standpoint. Investigating the distributions of polyploids allows biologists to uncover any geographical or reproductive barriers that may be present and further cause a reason for distinguishing polyploids as a separate species.

Polyploidy may be advantageous for the polyploid species (Comai, 2005). As a result of multiple genomic copies, polyploids can have a higher evolutionary success due to increases in heterozygosity, greater diversity of alleles, and gene redundancy (Levin, 1983; Stebbins, 1985; Soltis & Soltis, 1993; Soltis & Soltis, 1995; Comai, 2005). Neopolyploids, individuals of the first generation of a polyploid species, may be pre-adapted to new ecological conditions and, thus, have a higher tolerance for broad ecological parameters and a higher evolutionary potential (Levin, 2002). Polyploids may be able to colonize new habitats as well as respond better to changes in the environment. In many cases polyploids expand past the ranges of their diploid ancestors (Comai, 2005).

Although polyploidy has obvious benefits, the formation of polyploid individuals may be problematic depending on the taxa. In their study of neopolyploids, Ramsey & Schemske, (2002) observed that pollen viability is reduced by an average of 20%, and seed production decreased by 50%. Moreover, polyploids must find other polyploid individuals for mating as crosses between polyploids and diploids result in the production of offspring with odd numbers of chromosome sets (e.g., triploids). Triploids are often sterile as a consequence of meiotic irregularities and a high frequency of aneuploid gametes (Marks, 1966; Ramsey & Schemske, 1998); thus, newly formed polyploids must contend with mates that do not produce sterile crosses. Triploid blocks have been observed in a number of plants including *Ipomoea*, *Oryza sativa, Costus speciousus, Triticum* and others (reviewed by Ramsey & Schemske, 1998), yet a number of species do make viable triploids which may create an important source of gene flow between ploidies. For instance, *Arabidopsis thaliana* triploids are fertile, but their degree of fertility is lower than that of their diploid progenitors or related tetraploids (Henry et al., 2005). Not only were the triploids fertile, but they also produced diploid, triploid, and tetraploid offspring. Triploids, then, could serve as a bridge or an intermediate event in the formation of tetraploids from diploids (Ramsey & Schemske, 1998).

Polyploidy has played an important role in the evolution of the plant genus *Heuchera*. Recent studies have shown that autopolyploidy was the most likely mechanism for the evolution of tetraploids in *Heuchera micrantha* and *H. grossulariifolia* (Ness, et al., 1989; Wolf et al., 1989), and polyploid cytotypes

have evolved multiple times for both species (Ness et al., 1989; Segraves et al., 1999; Wolf et al., 1989). Ness et al. (1989) found that diploid cytotypes of *H. micrantha* were mostly found in the north and south of the species' range, while tetraploids were observed in the center of the range. No triploid cytotypes were found, although the study's small sample size limits the amount of variation that could have been observed. In *H. grossulariifolia,* a large sample size of 855 individuals from 14 populations showed that the two ploidies were nearly completely reproductively isolated as very few seeds were produced when the two were crossed in the lab; thus, polyploidy caused sympatric speciation. Strictly diploid, tetraploid, and mixed ploidy populations of *H. grossulariifolia* were found throughout Idaho and western Montana, and triploid individuals have been documented in mixed ploidy populations (Thompson et al., 1997).

During a pilot study of two populations of *H. cylindrica,* diploids and tetraploids were observed (Segraves & Althoff, unpublished data). *Heuchera cylindrica* (Saxifragaceae) is an herbaceous perennial found on rocky outcrops and talus slopes across the Pacific Northwest east of the Cascade summits. The purpose of this study was to further investigate the geographic distribution of polyploidy in *H. cylindrica.* I hypothesized that, like *H. grossulariifolia*, *H. cylindrica* consists of diploid, tetraploid, and mixed ploidy populations and I expected to find triploid individuals within mixed populations.

METHODS

Sample collection

I collected a total of 621 plants from 39 field sites across the range of *H. cylindrica*, including Idaho, Washington, Oregon, Montana, and British Columbia (Fig. 1, Table 1). To avoid collecting clones, samples were taken from plants one meter apart. Each sample consisted of a three-centimeter segment of rhizome which was wrapped in a damp paper towel and shipped to Syracuse University. Rhizomes were potted in Metro-Mix 360 soil in 335cm^3 Panterra Pots and grown in a common garden until leaf buds were available to use for flow cytometry, an average of 28 days. Plants were fertilized weekly. Of the 621 plants collected, 597 individuals survived.

Determining ploidy level

Flow cytometry was used to determine ploidy level by measuring the DNA content of nuclei (Table 2) (Kron et al., 2007). Five leaf buds were finely chopped (Galbraith et al., 1983) for 20 s or less in a magnesium sulfate buffer (Arumuganathan et. al 1991), consisting of 10mM magnesium sulfate heptahydrate (Mallinckrodt, Hazelwood, Missouri, United States), 50mM potassium chloride (EMD Chemicals, Gibbstown, New Jersey, United States), 5mM hepes (EMD Chemicals, Gibbstown, New Jersey, United States), 6.8mM dithiothreitol (MP Biomedicals, Solon, Ohio, United States), 1mM polyvinyl pyrrolidone (PVP-40; MP Biomedicals, Solon, Ohio, United States), and a final concentration of 10% w/v Triton X-100 (Alfa Aesar, Ward Hill, Massachusetts,

United States). New razor blades (American Line Single Edge, American Safety Razor Company, Verona, VA) were used for each plant. Chopped tissue was filtered using 30μm nylon filter membranes (Cole-Parmer Instrument Co, Nile, Illinois, United States). Samples were then centrifuged at 13,200 rpm for 30 s and the supernatant was discarded. The nuclei were re-suspended in a solution of 5mg/ml propidium iodide stain, 10mg/ml RNase (Sigma, St. Louis, Missouri, United States), and fresh rainbow trout blood diluted 1:11 with Alsever's solution (MP Biomedicals, Solon, Ohio, United States). The samples were mixed by vortexing and immediately run on a LSR II flow cytometer (Becton Dickinson LSRII flow cytometer; San Jose, CA 2003). The parameters for the flow cytometer required slight adjustments among runs to obtain the best results (average voltage settings: Forward Scatter Fluorescence: 604, Side Scatter Fluorescence: 418, and Propidium Iodide Fluorescence: 638). Data were analyzed using the BD FACSDiVa 1.4 software (Hicks 2004) to view the average fluorescence of plant and trout nuclei. DNA content was calculated by comparing the mean peak fluorescence for plant nuclei with the mean peak fluorescence for the trout blood standard. Rainbow trout nuclei contain 5.05pg per diploid genome (Vindelov et al.,1983); thus, the mean plant peak/mean trout blood peak * 5.05pg yields the plant DNA content. This equation provides the 2C value, where C is the total amount of DNA per nucleus (Harbaugh, 2008). A Welch ANOVA correcting for heteroscedasticity, was used to determine whether the average 2C values differed between diploids and tetraploids. Statistical analyses were

implemented using JMP Version 5.0.1.2 (SAS Institute Inc. 1989-2003. Cary, NC).

RESULTS

Based on the results from flow cytometry, diploid plants had a mean of 1.07 $\pm$ 0.006 pg of DNA (mean $\pm$ standard deviation) and tetraploid plants had a mean of 2.04 $\pm$ 0.008 pg of DNA. Diploid and tetraploid plants had significantly different DNA contents ($t=85.99$, $p<0.0001$) and the frequency distributions did not overlap (Fig. 2).

A total of 39 sites were examined. Of these sites, 22 populations were exclusively diploid, while 13 were entirely comprised of tetraploid individuals. Only single cytotype populations were found, and no triploids were observed. There is an allopatric distribution dividing diploid and tetraploid populations in northern Idaho and southern Montana (Fig. 1).

A total of 597 individuals were run on the cytometer, but only 595 samples were successful. Compared to diploid samples, tetraploid nuclei were more likely to breakdown. This may be due to an increased amount of secondary compounds in tetraploids that caused the nuclei to break down. To obtain intact nuclei, leaf buds had to be young and healthy.

DISCUSSION

Biologists have historically studied the ecological, physiological, and genetic differences between ploidy levels to better understand the evolutionary significance of polyploids (Stebbins, 1950; Husband & Schemske, 1998).

Examining the geographic distribution of polyploid species and their diploid progenitors is the primary step to understanding the relationship between populations of different ploidy levels. Based on the findings in *H. grossulariifolia*, I hypothesized that *H. cylindrica* would consist of diploid, tetraploid, and mixed ploidy populations and I expected to find triploid individuals within mixed populations. In contrast, the results of this study suggest instead that diploid and tetraploid populations do not occur in sympatry (Fig. 1).

In comparison to other species that have been extensively sampled and studied, *H. cylindrica* appears to exhibit a distinctive geographic distribution of cytotypes. I was surprised to find that there were no mixed cytotype populations or triploids, especially because previous work on other plants has consistently found both pure and mixed cytotype sites (Thompson et al., 1997; Husband & Schemske, 1998; Burton & Husband, 1999; Hardy et al., 2000; Stuessy et al., 2004; Halverson et al., 2008). For example, Burton & Husband (1999) investigated the geographic distribution of ploidy levels of *Galax urceolata*, found in the Blue Ridge Mountains. They sampled 1570 individuals from 42 populations, and documented diploid, tetraploid, and mixed populations, and some mixed populations contained triploids. In another study, Hardy et al. (2000) investigated the distribution of diploid and tetraploid *Centaurea jacea* and found that the two cytotypes generally have a parapatric distribution based on elevation, but yet mixed populations were found at intermediate elevations.

Studies conducted with smaller sampling schemes have also detected mixed populations or minority cytotypes (Husband & Schemske, 1998; Stuessy et

al., 2004; Halverson et al., 2008). For example, Halverson et al. (2008) examined the distribution of diploid, tetraploid, and hexaploid cytotypes in *Solidago altissma*. In this study, 500 individuals were collected from 16 populations, and of these populations, 8 contained only diploid individuals, while the rest were mixed. The tetraploid cytotypes of S. altissma serve the role of a triploid, and the presence of tetraploids and mixed populations of *S. altissma* in the contact zone demonstrates a major difference between the geographic distributions of *H. cylindrica*, which has no contact zone, triploids or mixed populations. Similarly, the distribution of *Chamerion augustifolium* was found to have mixed populations and triploids (Husband & Schemske, 1998), and an additional study investigating the distribution of diploid and polyploid of *Melampodium cinereum* and *M. leucanthum* shows populations exist in diploid populations, tetraploid populations, and uncommonly in mixed populations. *Melampodium cinereum* contained mostly single cytotype populations and mixed populations and only one triploid was found (Stuessy et al., 2004). Regardless of the rarity of triploids and mixed populations, an extensive survey of *H. cylindrica* does not exemplify a pattern like *Melampodium sp.* with at least one or two mixed populations and at least one triploid. Even in instances where mixed populations are lacking, odd ploidal levels are usually found (Mandáková & Münzbergová, 2006). For instance, *Aster amellus* appears to have a distribution closer to that observed in *H. cylindrica*: only single diploid and hexaploid cytotype populations were observed, although triploids, a pentaploid and nonaploids were occasionally found (Mandáková & Münzbergová, 2006). Even in this case, however, *A. amellus* differs from *H.*

cylindrica in that the distribution is not allopatric. The populations are segregated into two mosaic populations of diploids and hexaploids, each dominated by one cytotype or the other.

The distribution of polyploidy in *H. cylindrica* contrasts sharply with patterns observed in the large sample size of *H. grossulariifolia*, even though their natural ranges overlap (Thompson et al. 1997). Ness et al. (1989) found that diploid cytotypes of *H. micrantha* were mostly found in the north and southwest of the species' range, while tetraploids were found in the center and southeast of the range. Only single cytotype populations were observed and no triploids were found. However, this species was under sampled; only one to four plants were collected from each site. This sample size severely limits the amount of variation that could be observed within the species. For *H. cylindrica*, additional sampling in the St. Joe River drainage may reveal mixed populations, although this seems unlikely given the large sample size examined in the present study.

Environmental factors can influence the geographic distribution of cytotypes, and this may be the reason for the distribution of *H. cylindrica* cytotypes. The last glacial maximum in North America occurred 18kya (Graham, 1999). During this time, glacial patches covered the valleys between the mountains of the Pacific Northwest. As the glaciers receded, the central panhandle of Idaho, parts of Washington, and the Snake River canyon were severely flooded by the expulsion of the Pleistocene Lake Missoula in Montana (Graham, 1999). As the ice age came to an end, new land was made available to species for colonization. Soltis et al. (1997) discuss two hypotheses regarding the

mechanisms responsible for genetic differentiation and geographic structuring of plant species in the Pacific Northwest. In the first hypothesis, glacial events may have influenced the discontinuities observed in the geographic distribution of plant populations due to the survival of well-isolated northern and southern populations. As the glaciers receded, populations that were once isolated migrated and overlapped, thus resulting in a continuous geographic distribution with a major genetic discontinuity. The other hypothesis suggests that plants moved northward from southern populations, and genotypes were established in one or a few populations on the boundaries of recolonization. I hypothesize that the biogeography of this region, especially in the St. Joe River system, influenced the dispersal and polyploid distribution of *H. cylindrica.* As the ice age ended, tetraploid *H. cylindrica* could have dispersed into the new habitats available for species to colonize. Perhaps due to higher tolerance for different environments (Levin, 1983, 2002) and/or limited competition from diploid populations, tetraploids have been able to maintain populations in the northeastern portion of the species' distribution.

CONCLUSIONS

The present study describes the geographic distribution of diploid and tetraploid populations of *H. cylindrica.* Surprisingly, I observed only single cytotype populations, and no triploid individuals were found. Tetraploids were isolated in the northeastern portion of the range. This allopatric distribution dividing the distribution of the range of *H. cylindrica* into diploid and tetraploid

regions may be a result of differences in ecological niches, including altitude, precipitation, soil, and/or temperature differences. The historical biogeography of the region could have resulted in the dispersal of tetraploid individuals to a region free from competition with diploids. Populations around the St. Joe River system may provide interesting insights into the relationship between ploidy levels of *H. cylindrica* due to the close proximity of diploid and tetraploid populations. The distribution of *H. cylindrica* found in this study suggests that polyploids may colonize and expand into new areas where diploid ancestors cannot.

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Table 1: Site information for *H. cylindrica* samples. Site numbers correspond to the numbers in Figure 1.

Site No.	Population	Latitude	Longitude	Elevation (m)
1	Spalding, ID	46º 27.467’	116º 47.005’	304.5
2	Hilgard, OR	45º 20.492’	118º 14.205’	1039.4
3	Red Bridge, OR	45º 16.069’	118º 21.937’	1000.7
4	Little Hay Creek, OR	44º 24.606’	120º 29.014’	1282.6
5	Ochoco, OR	44º 24.178’	120º 30.294’	1232.6
6	Moss Hill, OR	44º 25.697’	120º 21.618’	1421.6
7	Kimberly, OR	44º 31.389’	119º 37.662’	816.6
8	Durst Creek Road, WA	47º 19.403’	120º 40.621’	924.8
9	Alpine Lake, WA	47º 34.687’	120º 47.795’	709.3
10	Josaphine Crag, WA	47º 39.260’	120º 43.773’	534.0
11	Powerline, WA	47º 47.090’	120º 53.564’	847.9
12	Lake Kalamalka, BC	50º 09.628’	119º 22.251’	536.4
13	Marrow Road, BC	49º 22.125’	119º 40.816’	667.8
14	Blue Creek Road, WA	48º 17.944’	117º 54.246’	643.7
15	Sherman, WA	48º 36.217’	118º 30.304’	1598.7
16	Thirteen Mile, WA	48º 30.732’	118º 44.204’	830.6
17	Coeur d’Alene Lake, ID	47º 37.236’	116º 40.727’	653.8
18	Petty Creek, MT	46º 55.833’	114º 26.720’	1021.4
19	Blackfoot River, MT	46º 52.447’	113º 51.695’	1008.0
20	Ashley Lake, MT	48º 06.646’	114º 34.788’	1068.3
21	West Glacier, MT	48º 32.300’	113º 54.672’	972.6
22	East Glacier, MT	48º 45.145’	113º 26.763’	1369.2
23	Gould Creek, MT	46º 53.174’	112º 23.031’	1496.3
24	Flesher Pass, MT	46º 58.403’	112º 20.389’	1756.0

Table 1: Cont.

Site No.	Population	Latitude	Longitude	Elevation (m)
25	Salmon Lake, MT	47º 04.308’	113º 23.058’	1229.6
26	Lolo Creek, MT	46º 46.855’	114º 24.689’	1164.6
27	Benewah Lake, ID	47º 20.843’	116º 41.882’	653.8
28	Eleven, ID	46º 23.054’	116º 10.178’	491.6
29	Santa Creek, ID	47º 09.169’	116º 31.387’	842.2
30	Dent Bridge, ID	46º 35.956’	116º 10.053’	514.2
31	Calder (Big Creek), ID	47º 17.712’	116º 07.383’	707.4
32	Sula, MT	45º 94.000’	113º 73.000’	1659.6
33	Orofino, ID	46º 30.626’	116º 33.537’	279.2
34	Blue Mountains, WA	46º 13.000’	117º 46.000’	1241.0
35	Teal Springs, Blue Mtns, WA	46º 12.223’	117º 34.363’	683.4
36	Beaver Creek, MT	47º 42.060’	115º 33.513’	817.5
37	Calder, ID	47º 16.455’	116º 13.786’	696.2
38	Albion, WA	46º 47.244’	117º 15.147’	770.5
39	Lake Creek Rd, Salmon R, ID	45º 23.967’	116º 12.908’	543.2

Table 2: DNA content (picograms) for *H. cylindrica* populations. 2X refers to diploid populations and 4X refers to tetraploid populations.

Population	N	Mean (range) pg	Ploidy Level
Spalding, ID	20	1.08 (1.00-1.21)	2X
Hilgard, OR	16	1.12 (1.00-1.24)	2X
Red Bridge, OR	13	1.10 (0.97-1.30)	2X
Little Hay Creek, OR	16	1.12 (0.98-1.26)	2X
Ochoco, OR	17	1.06 (0.95-1.25)	2X
Moss Hill, OR	20	1.10 (0.92-1.22)	2X
Kimberly, OR	20	1.11 (0.91-1.23)	2X
Durst Creek Rd, WA	10	1.04 (0.75-1.22)	2X
Alpine Lake, WA	17	1.09 (0.96-1.18)	2X
Josephine Crag, WA	16	1.11 (1.04-1.22)	2X
Powerline, WA	12	1.15 (0.99-1.26)	2X
Lake Kalamalka, BC	17	1.05 (0.90-1.26)	2X
Marron Rd, BC	19	1.06 (0.96-1.14)	2X
Blue Creek Rd, WA	20	1.10 (0.92-1.34)	2X
Sherman, WA	10	1.08 (1.02-1.06)	2X
Thirteen Mile, WA	18	1.13 (1.02-1.22)	2X
Coeur d'Alene Lake, ID	19	1.98 (1.77-2.22)	4X
Blackfoot River, MT	17	2.01 (1.82-2.26)	4X
Petty Creek, MT	20	2.07 (1.81-2.31)	4X
Ashley Lake, MT	21	2.13 (1.86-2.34)	4X
East Glacier, MT	4	2.00 (1.85-2.20)	4X
West Glacier, MT	9	1.99 (1.82-2.17)	4X
Gould Creek, MT	20	2.10 (1.76-2.35)	4X
Flesher Pass, MT	20	2.02 (1.76-2.25)	4X
Lolo Creek, MT	20	2.02 (1.85-2.28)	4X
Salmon Lake, MT	20	2.09 (1.82-2.27)	4X
Benewah Lake, ID	20	2.03 (1.77-2.31)	4X
Eleven, ID	20	1.04 (0.90-1.23)	2X
Santa Creek, ID	12	1.03 (0.89-1.16)	2X
Dent Bridge, ID	17	1.05 (0.89-1.21)	2X
Calder, ID	20	2.00 (1.82-2.27)	4X
Sula, MT	19	1.01 (0.88-1.19)	2X
Orofino, ID	15	1.02 (0.92-1.26)	2X
Blue Mountain, WA	10	0.95 (0.86-1.07)	2X
Beaver Creek, MT	12	2.00 (1.79-2.21)	4X
Teal Springs, Blue Mtns, WA	12	1.04 (0.98-1.26)	2X
Albion, WA	3	1.12 (0.98-1.22)	2X
Lake Creek Rd, Salmon R, ID	4	0.96 (0.87-1.05)	2X

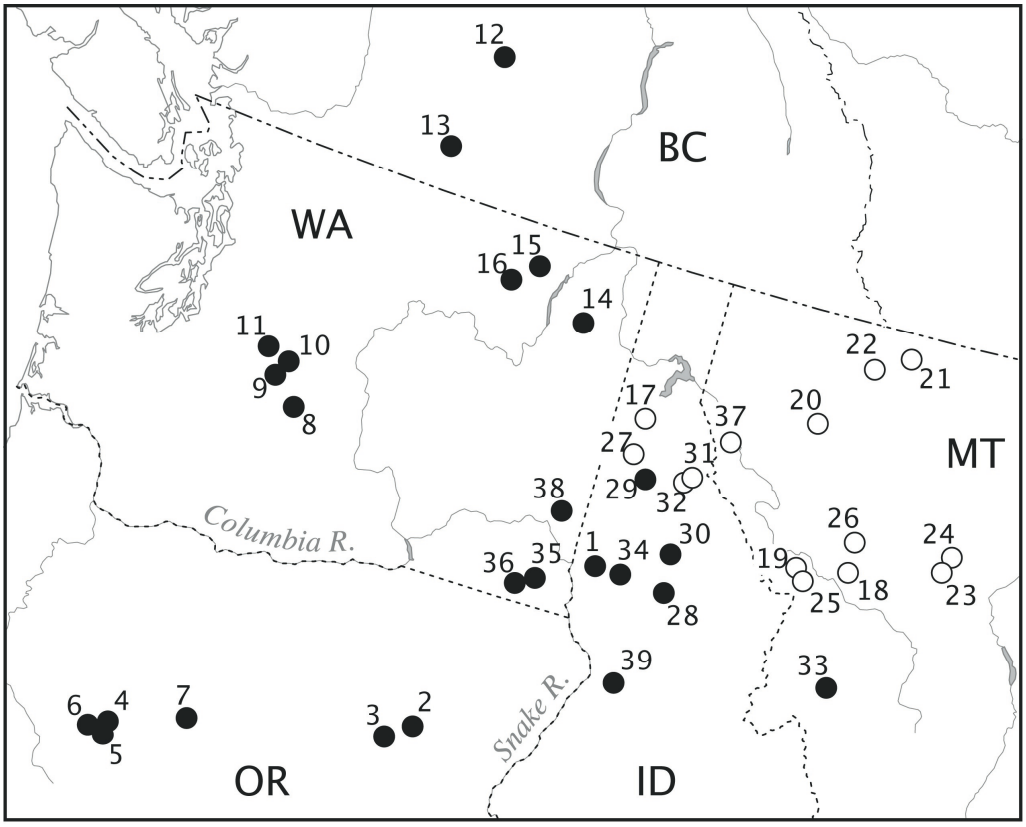

Figure 1: Map of the distribution of ploidy levels in populations of *Heuchera cylindrica.* Closed circles indicate diploid populations, open circles indicate tetraploid populations.

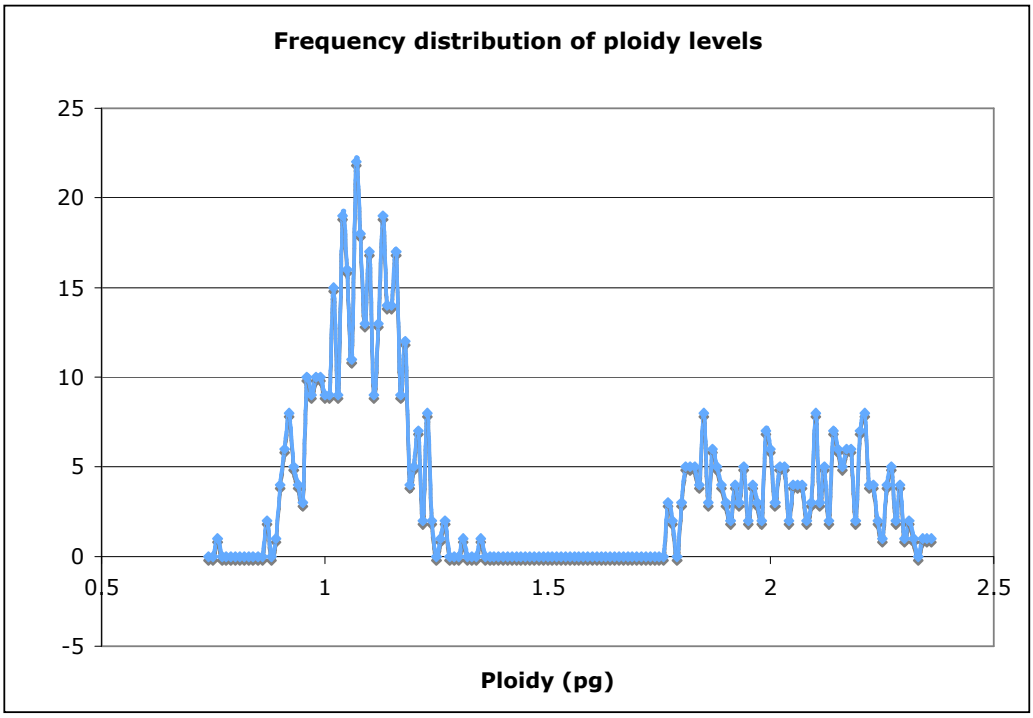

Figure 2: The frequency distribution of diploid and tetraploid individuals of *Heuchera cylindrica*. Diploids (mean=1.07 $\pm$ 0.006 pg of DNA) and tetraploid plants (mean= 2.04 $\pm$ 0.008 pg of DNA). Diploid and tetraploid plants had significantly different DNA contents ($t=85.99$, $p<0.0001$).

Written Capstone Summary

Megan A. Larson

One of the largest problems facing society is the shortage of food. Today, more than 963 million people are hungry, and nearly 16,000 children die every day from starvation, malnourishment, or other hunger-related causes. That means that every five seconds one child dies from hunger. Improving agriculture is one solution to this problem. By studying crops, or simply domesticated plants, the solutions to world hunger may be found. After all, plants are the basis of terrestrial ecosystems, and a greater understanding of these important species will increase the knowledge of the world we live in and provide us with insights on how to produce better crops.

Plants make up the 90% of the earth's terrestrial biomass and biologists estimate that over 260,000 plant species exist. Plants are incredibly diverse, ranging from tropical Costa Rica to frozen Antarctica. Some plants are microscopic while others, like the sequoia trees in California, are among the largest species on the planet. Some plants reproduce by flowering, others create cones.

This myriad of sizes, differences in distribution, and differences in reproduction leads to one of the greatest questions in biology: what generates plant diversity? Since the first plants evolved millions of years ago, they have speciated into the amazing diversity of plants we know today. A species is defined as a group of organisms that can breed and produce viable offspring and, thus, speciation is the evolutionary process by which new species arise. Although this

process is constantly studied, many mechanisms may be involved in driving speciation, thus complicating the issue. Genetics, or the study of inheritance and variation among organisms, is certainly a major factor in speciation. Every living organism has DNA, sometimes called the genetic blueprint of life. In many species, DNA is found coiled into structures called chromosomes. Chromosomes and the DNA within them help determine the characteristics of an individual, such as gender or eye color. Generally, each species has a certain number of chromosomes. Humans, for example, have 23 chromosomes per set, inheriting one set from the father and one set from the mother for a total of 46 chromosomes. These individuals are called diploids, and have a diploid cytotype.

Some species, however, have more than two chromosome sets. These species are called polyploids. Usually when egg and sperm are produced only one chromosome set is retained. Thus when they unite, the offspring has two sets. When the egg and sperm accidentally retain both chromosome sets and unite to form an offspring, that individual will have four sets of chromosomes. In other words, some individuals have twice the amount of DNA as diploids. Usually the differences between diploids and polyploids are noticeable. Due to the larger amount of chromosomes, polyploids generally have larger cells and hence larger fruit, bigger roots and leaves, and larger seeds. For example, the fruit of a diploid strawberry plant is much smaller than the fruit of an octoploid (eight chromosome sets) strawberry plant. Many organisms are polyploids, including goldfish, the red vizcacha rat, ferns, maple trees, and strawberries. In fact, many crops, such as corn, bananas, and wheat are polyploids. Polyploidy occurs far more often in

plants than in animals, although the reason is not known (probably related to sex chromosomes). Scientists estimate that 47-70% or more of flowering plants are of polyploid origin (Masterson, 1994; Soltis & Soltis, 1999).

In many ways, polyploidy is still a genetic mystery. Biologists understand that polyploidy can arise in two ways. The first is called autopolyploidy, where polyploidy forms within a species by spontaneously doubling the amount of chromosomes. The second is called allopolyploidy. Polyploids that form through allopolyploidy are hybrids because the parents are different species. Although the mechanism seems simple, the origin of polyploidy in species is often difficult to determine and requires molecular experiments. Every species is different, and it is possible for a polyploid species to be both allo- and autopolyploid. Biologists also want to understand why polyploids form and then how they persist. Are polyploids better adapted for certain environments than their diploid ancestors? Why does polyploidy occur so frequently in plants? Why is polyploidy so helpful for generating new crops? All these questions, if answered, would help scientists to understand the immense diversity of plants and improve agriculture.

For the past two years, I have been working with the roundleaf alumroot plant, *Heuchera cylindrica*, that grows on rocky outcrops and talus slopes across the Pacific Northwest east of the Cascade Mountains. This species is interesting for a scientific study because some individuals are diploids and others are tetraploids, or polyploid individuals that have four chromosome sets. To completely understand the role of polyploidy in *H. cylindrica*, we must first investigate the geographic distribution of polyploidy. In other words, what is the

distribution of polyploidy among *H. cylindrica* populations-- are some populations composed of diploids and tetraploids, are some populations only diploid or tetraploid, are there triploids (three chromosome sets) that suggest mating between diploids and tetraploids? In order to do this, we needed to collect plants from their native range and determine the amount of DNA in their cells.

We collected a total of 621 plants from 39 field sites across the range of *H. cylindrica*, including Idaho, Washington, Oregon, Montana, and British Columbia. To avoid collecting clones, samples were taken from plants one meter apart. Each sample consisted of a three-centimeter segment of root tissue which was wrapped in a damp paper towel and shipped to Syracuse University. Roots were potted in Metro-Mix 360 soil in 335cm^3 Panterra Pots and grown in a common garden until leaf buds were available to use in subsequent DNA analyses.

Flow cytometry is a method used to measure the amount of DNA in a given sample. From this measurement, the number of chromosomes of an organism can be calculated. In most cases, samples are prepared by releasing the cell nucleus where the chromosomes are housed. Once released, the nuclei are stained with a DNA specific dye that can be detected after excitation by a laser. Samples are placed in a flow cytometer and the machine uses a laser and camera to detect the fluorescence from the nuclei. The stained nuclei refract the light in different directions, either forward or to the side. The flow cytometer measures these refractions to calculate the fluorescence of the stain. With this measurement,

we can calculate the amount of DNA in a sample, and determine whether our sample is a diploid, tetraploid, or another ploidy level.

In our study, flow cytometry was used to determine ploidy level for 595 plants from 39 localities. Five leaf buds from each plant were finely chopped with razor blades for 20 seconds or less in a buffer (Arumuganathan, Slattery, Tanksley, & Earle, 1991). To avoid clogging the flow cytometer, chopped tissue was filtered using nylon filter membranes. Samples were then centrifuged at 13,200 rpm for 30 seconds to pellet the nuclei in the bottom of the tube and the remaining liquid was discarded. The nuclei were re-suspended in a solution of propidium iodide stain and a rainbow trout blood standard. The samples were mixed and immediately run on a LSR II flow cytometer at the SUNY Upstate Medical University. The parameters for the flow cytometer required slight adjustments among runs to obtain the best results. Data were analyzed using BD FACSDiVa 1.4 software to view the average fluorescence of plant and trout nuclei. DNA content was calculated by comparing the mean peak fluorescence for plant nuclei with the mean peak fluorescence for the trout blood standard. Rainbow trout nuclei contain 5.05 picograms (pg) of DNA per diploid genome; thus, the mean plant peak/mean trout blood peak * 5.05pg yields the plant DNA content. This equation provides the total amount of DNA per nucleus. Statistics were used to determine whether the DNA content differed between diploid and tetraploid plants.

Based on the results from flow cytometry, diploid plants were found to have a mean of 1.07 $\pm$ 0.006 pg of DNA (mean $\pm$ standard deviation). Tetraploid

plants were found to have a mean of 2.04 $\pm$ 0.008 pg of DNA. Thus, tetraploids had twice as much DNA as diploids. Diploid and tetraploid plants had significantly different DNA contents ($t=85.99$, $p<0.0001$) and the frequency distributions did not overlap (Fig. 2).

For the 39 sites examined, 22 sites contained exclusively diploids, and 13 were entirely comprised of tetraploid individuals. Only single cytotype populations were found, and no triploids were observed. Diploids and tetraploids are geographically distinct with the dividing line occurring in northern Idaho and southern Montana (Fig. 1). There are several reasons as to why the populations may be distributed in this fashion, but additional experiments need to be performed to determine which of these hypotheses is correct.

The dividing line between diploid and tetraploid regions may be a result of differences in the characteristics of the regions, including altitude, precipitation, soil, and/or temperature differences. The glacial history could also have resulted in the movement of tetraploid individuals into a region free from competition with diploids once the glaciers retreated. Populations around the St. Joe River system may provide interesting insights into the relationship between ploidy levels of *H. cylindrica* due to the close proximity of diploid and tetraploid populations. The distribution of *H. cylindrica* found in this study suggests that polyploids may colonize and expand into new areas where diploid ancestors cannot.

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