POLYPLOIDY, INBREEDING DEPRESSION, AND THE EVOLUTION OF MATING SYSTEMS IN FLOWERING PLANTS

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POLYPLOIDY, INBREEDING DEPRESSION, AND THE EVOLUTION OF MATING SYSTEMS IN FLOWERING PLANTS

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Mating systems control the movement of genes through time and space, making the evolution of mating systems a central question in evolutionary biology. Some of the most persistent questions surrounding the evolution of mating systems in plants are those that address the evolution of self-fertilization. A selfing individual passes on three copies of its genome to offspring for every two copies passed on by an outcrossing individual. This "cost of outcrossing" provides a 50% fitness advantage to selfing variants, and, unless counteracted by some other selective force, results in increased population selfing rates. Several non-mutually exclusive phenomena can select against the evolution of selfing, including pollen discounting, temporal and spatial variation in environmental conditions, gender specialization, and inbreeding depression.

An interesting pattern noted by early plant biologists is the association between genome duplication (polyploidy) and self-fertilization, with polyploids exhibiting higher levels of self-fertilization than diploids. Although several phenomena might influence the evolution of this pattern, reduced inbreeding depression among polyploids relative to diploids could play a pivotal role. In this dissertation I (1) evaluate the validity and strength of the association between polyploidy and self-fertilization in flowering plants, (2) develop simulation-based models to explore the relationship between polyploidy and inbreeding depression, and (3) conduct experiments to compare levels of inbreeding depression in four species of annual

plants that vary in both mating system and ploidy.

I demonstrate that, on average, polyploid angiosperms exhibit higher levels of self-fertilization than their diploid relatives. I then show that polyploid and diploid populations differ in their response to selection and levels of inbreeding depression. Although younger polyploids should exhibit less inbreeding depression than diploids, older polyploids might exhibit the opposite pattern. Finally, I demonstrate that both mating system and ploidy influence levels of inbreeding depression in the genus *Clarkia* (Onagraceae). Selfing taxa exhibit less inbreeding depression than outcrossing taxa, and polyploid taxa exhibit less inbreeding depression than diploid taxa.

BIOGRAPHICAL SKETCH

The author was born on November 25th, 1969, in Placerville; a relatively small town located in the foothills of the Sierra Mountains of Northern California, U. S. A. When he was nine years old he moved to Sacramento, a much larger city located in the central valley of Northern California. His path to academics was unorthodox. After leaving high school he worked in restaurants for two years before beginning work as a laborer and carpenter's apprentice, working for small construction companies on projects throughout Northern California. He joined the United Brotherhood of Carpenters when he was 20 years old, and worked full-time as a union carpenter for five years, completing a four-year apprenticeship and earning his journeyman carpenter credentials. Although he enjoyed his profession in many ways, the relative lack of intellectual stimulation and job security in the construction industry caused him to question his career choice. He returned to school when he was 26 years old, attending Sacramento City College for two years before transferring to the University of California at Davis. He graduated from UC Davis in 2000 with a Bachelor of Science degree in Biology (Evolution and Ecology). He then moved to Ithaca, NY, where he earned his Ph.D. in the Department of Ecology and Evolutionary Biology at Cornell University. He leaves Cornell in January 2008 to begin postdoctoral research in the Department of Biology at The University of Virginia.

To my Father and Sara, my Mother and Mariano, and my brother Scott.

I did it. Thank you for always believing I could.

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I also owe a special thanks to my minor committee members, Jeff Doyle, Irby Lovette, and Rick Harrison. Jeff welcomed me into his lab early on during my graduate tenure, allowed me to play with his chemicals and micropipettes, and always encouraged me to stop by his office to chat about my interests and my progress. I only regret that I did not take advantage of his offers nearly enough. Irby Lovette helped me with my research in several ways, though it was his contribution to my teaching experience that I will remember and value the most. Irby acknowledged and nurtured my passion for teaching by inviting me to lecture in his Evolutionary Biology course and by requesting that I serve as his head teaching assistant during multiple semesters. These experiences were invaluable to me, as they reinforced my growing desire to make teaching a central part of my life, and gave me the confidence that I needed to make that happen. Finally, Rick Harrison contributed to my education and growth as a scientist in numerous ways. Rick is among the most brilliant men I've met, and his

ability to think critically and clearly about complex problems is both astonishing and inspiring. Perhaps more importantly, his office door was always open when I needed to converse about my dissertation or my personal life. I will miss those conversations greatly.

Several people outside of Cornell University also influenced my dissertation research, and I thank them for their time, their encouragement, and their interest in my work. Brian Husband hosted my visit to the University of Guelph, where he and his lab group provided valuable feedback regarding my research plans and interests, and Spencer Barrett (University of Toronto) and Chris Eckert (Queen's University) generously shared their plant selfing rate data with me, and offered much in the way of meaningful advice.

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The Department of Ecology and Evolutionary Biology has provided an unparalleled environment for me to grow as a scientist and a teacher, and, in addition to its outstanding faculty and students, my success as a graduate student was augmented by the wonderful atmosphere created by its caring and attentive staff. In

particular I thank John Howell, Patty Jordan, Brian Mlodzinski, Janeen Orr, and Sandy Smith for their invaluable help in all things. Whether finding me the perfect office space, preparing student evaluations of my teaching, fixing my computer, helping me to make photocopies, or engaging me in hundreds of fun and down to earth conversations during the early hours of the morning, they made my sometimes tedious life in Corson Hall somewhat more bearable.

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TABLE OF CONTENTS

LIST OF FIGURES

Page

CHAPTER 3

LIST OF TABLES

CHAPTER 1

POLYPLOIDY AND SELF-FERTILIZATION IN FLOWERING PLANTS *

Abstract

Mating systems directly control the transmission of genes across generations, and understanding the diversity and distribution of mating systems is central to understanding the evolution of any group of organisms. This basic idea has been the motivation for many studies that have explored the relationships between plant mating systems and other biological and/or ecological phenomena, including a variety of floral and environmental characteristics, conspecific and pollinator densities, growth form, parity, and genetic architecture. In addition to these examples, a potentially important but poorly understood association is the relationship between plant mating systems and genome duplication, i.e., polyploidy. It is widely held that polyploid plants self-fertilize more than their diploid relatives, yet a formal analysis of this pattern does not exist. Data from 235 species of flowering plants were used to analyze the association between self-fertilization and ploidy. Phylogenetically-independent contrasts and cross-species analyses both lend support to the hypothesis that polyploids self-fertilize more than diploids. Because polyploidy and self-fertilization are so common among angiosperms, these results contribute not only to our understanding of the relationship between mating systems and polyploidy in particular, but more generally, to our understanding of the evolution of flowering plants.

* Barringer, B. C. (2007). Polyploidy and self-fertilization in flowering plants. American Journal of Botany 94:1527-1533.

Introduction

The diversity and distribution of mating systems in plants have been of long-standing interest, and it is widely acknowledged that mating systems can profoundly influence the evolutionary trajectories and long-term success of taxa (Darwin 1859, 1876; Stebbins, 1950, 1957; Grant, 1981, Lande and Schemske 1985; Barrett and Eckert, 1990; Barrett et al., 1996; Barrett, 2003). Because mating systems are influenced and molded by environmental and genetic factors (Barrett and Eckert, 1990; Barrett, 2003) they are not simply static descriptors of a taxon's life-history, but are, rather, dynamic and evolving traits in and of themselves (Barrett and Eckert, 1990). For these reasons, a thorough understanding of the evolution of plant mating systems and the patterns of their distributions is clearly fundamental to an understanding of the evolution of flowering plants.

Some of the most persistent questions surrounding the evolution of plant mating systems are those that address the evolution of self-fertilization. In the absence of pollen discounting (the reduction due to selfing in the number of pollen grains available for outcrossing (Harder and Wilson, 1998)), selfing is advantageous because a selfing individual will pass on two copies of its genome for every copy passed on by an outcrossing individual (Fisher, 1941). This "cost of outcrossing" provides a 50% fitness advantage to selfing variants in otherwise outcrossing populations, and, unless counteracted by some other selective force, will translate to increased population selfing rates (Fisher, 1941; Charlesworth and Charlesworth, 1979). In addition, self-fertilization may be advantageous as a reproductive assurance mechanism (Stebbins, 1950, 1957; Grant, 1981; Pannell and Barrett, 1998; Morgan and Wilson, 2005) or as a means of fixing co-adapted gene complexes (Lande and Schemske, 1985).

In contrast to phenomena that might favor the evolution of selfing are a variety of factors that may select for outcrossing, such as pollen discounting (Harder and

Wilson, 1998), temporal and spatial variation in environmental conditions (Maynard-Smith, 1978; Lande and Schemske, 1985), and tradeoffs in the allocation of energy to male and female functions resulting in gender specialization (Charnov et al., 1976; Brunet, 1992; Thomson, 2006). In addition, inbreeding depression (the reduction in fitness of inbred relative to non-inbred individuals) is often hypothesized to be strong enough to overcome the selective cost to outcrossing (Charlesworth and Charlesworth, 1979, 1987; Lande and Schemske, 1985; Husband and Schemske, 1996, 1997). If inbreeding depression leads to a 50% fitness reduction in selfed progeny relative to outcrossed siblings, selfing may no longer be advantageous (Charlesworth and Charlesworth, 1979, 1987; Lande and Schemske, 1985; but see Holsinger, 1988). Despite the detrimental effects of inbreeding and the potential benefits of outcrossing, however, high levels of self-fertilization have evolved repeatedly in many groups (Stebbins, 1950; Johnston and Schoen, 1996).

The transition from outcrossing to selfing in plants is correlated with many biological and ecological phenomena, including a variety of floral and environmental characteristics, conspecific and pollinator densities, growth form, parity, and genetic architecture (Darwin, 1876; Stebbins, 1950, 1957; Grant 1956, 1981; Charlesworth and Charlesworth, 1979; Wyatt, 1984; Lloyd, 1992; Barrett et al., 1996; Pannell and Barrett, 1998; Morgan, 2001; Barrett, 2003; Morgan and Wilson, 2005; Scofield and Schultz, 2006). In addition to these examples, a potentially important though not well-understood association is the relationship between self-fertilization and genome duplication, i.e., polyploidy. Polyploids are organisms with more than two sets of chromosomes (Grant, 1981; Soltis et al., 2004), and, although relatively rare in most groups of animals (Otto and Whitton, 2000; Mable, 2004a; but see Legatt and Iwama, 2003), polyploidy is common in flowering plants (Soltis et al., 2004). The relationship between polyploidy and self-fertilization in plants has been of interest for

many years, because it is widely held that polyploids have higher selfing rates than their diploid relatives (Stebbins, 1950; Mable, 2004b). There are several non-mutually exclusive reasons for why polyploidy might be associated with increased levels of selfing in plants: First, polyploidy may facilitate the evolution of self-fertilization because it results in a breakdown of self-incompatibility (SI) systems in many groups of plants, especially those whose SI systems are gametophytic (Bateman, 1952; Barrett, 1987; Mable, 2004b). Since SI systems reduce or eliminate the ability to self-fertilize, it follows that some polyploids may exhibit increased levels of selfing compared to their diploid relatives whose SI systems remain intact. Second, the ability to self-fertilize may facilitate the evolution of polyploidy. Newly-arisen polyploids (i.e., *neo*polyploids) are likely to co-occur with their diploid progenitors (Levin, 1975; Jackson, 1976; Ramsey and Schemske, 1998), and since inter-cytotype crosses often result in offspring with low fitness (Levin, 1975; Ramsey and Schemske, 1998), minority cytotypes are expected to experience negative frequency-dependent selection, a phenomenon referred to as *minority cytotype disadvantage* (Levin, 1975). The ability to self-fertilize should reduce the effects of minority cytotype disadvantage by eliminating the need for a cytoplasmically-compatible mate, and for this reason it may be that selfing taxa, on average, successfully produce more polyploids than do outcrossing taxa (Grant, 1956, 1981; Stebbins, 1957; Levin, 1975; Ramsey and Schemske, 1998). Finally, some theoretical work predicts that polyploids may exhibit less inbreeding depression than diploids, owing to the presence of multiple gene copies and the associated reduction in the rate of formation of homozygotes (Lande and Schemske, 1985). The relationship between polyploidy and inbreeding depression is complex, however, and levels of inbreeding depression in polyploids have been shown to depend on many additional factors, including the level of dominance of deleterious alleles, the number and lethality of genes involved, and the age of the

polyploid in question (Ronfort, 1999; Pannell et al., 2004; Rausch and Morgan, 2005). Indeed, some studies predict that, under some circumstances, polyploids might exhibit greater inbreeding depression than their diploid relatives (Busbice and Wilsie, 1966; Bennett, 1976; Ronfort, 1999). In addition, polyploids differ in regard to the behavior of their chromosomes during cell division, and although *auto*polyploids (polyploids that possess only homologous chromosomes) may effectively mask deleterious alleles better than diploids, *allo*polyploids (polyploids that possess homeologous chromosomes) are expected to exhibit chromosomal behavior similar to that of diploids, and may not exhibit increased tolerance to inbreeding (Bever and Felber, 1992; Ronfort, 1999; Soltis and Soltis, 2000; Comai, 2005). To date, theoretical work has concentrated on autopolyploids, however, and no formal theoretical explorations of inbreeding depression in allopolyploids exist (Pannell et al., 2004; but see Lande and Schemske, 1985). Comparative data on inbreeding depression in closely-related polyploid and diploid taxa are few (Ramsey and Schemske, 2002; Pannell et al., 2004), however at least two studies indicate lower inbreeding depression in autopolyploids relative to diploids (Husband and Schemske, 1997; Rosquist, 2001). The ability to increase levels of selfing without suffering the detrimental consequences of inbreeding depression should select for increased selfing rates among polyploids.

Despite increasing interest in both polyploid and plant mating system evolution, there are surprisingly few studies that have carefully evaluated whether polyploids do, in fact, self-fertilize more than diploids (Ramsey and Schemske, 1998; Mable, 2004b). Among homosporous ferns, polyploids do tend to self-fertilize more than diploids (Soltis and Soltis, 1987; Masuyama and Watano, 1990; Soltis and Soltis, 1990), and very limited support for this trend in gymnosperms also exists (Barringer, B. C., unpublished data). Whether this pattern holds across the angiosperms remains unknown, however, despite many anecdotal examples (e.g., Stebbins, 1950; Grant,

1956, 1981). One way to answer this question would be to compare the mating systems of polyploid angiosperms and their immediate progenitor taxa (i.e., sister-taxon comparisons), however the relationships between polyploids and their progenitors are often unknown, and selfing rate estimates for those groups in which such relationships are known do not generally exist (Husband and Schemske, 1997). Both Stebbins and Grant describe several genera (e.g., *Amsinckia, Bromus, Clarkia, Gilia, Microseris,* etc.) wherein polyploids self more than diploids (Stebbins, 1950; Grant, 1956, 1981), and others have since documented this trend within specific groups (Ross, 1981; Husband and Schemske, 1997; Cook and Soltis, 2000; Quarin et al., 2001; Tate and Simpson, 2004; Guggisberg et al., 2006). The opposite pattern occurs among diploid and polyploid species of *Tragopogon* (Asteraceae) (Cook and Soltis, 1999), however, and although polyploidy might generally be associated with a loss or breakdown of gametophytic SI systems, a recent review failed to find a widespread association between polyploidy and self-compatibility, especially among taxa that exhibit sporophytic or heteromorphic SI (Mable, 2004b). Finally, although polyploidy has resulted in a breakdown of SI in the genus *Lycium* (Solanaceae), this may have led to an increase in inbreeding depression as rates of self-fertilization increased, which facilitated selection for higher outcrossing rates among polyploids via the evolution of gender dimorphism (Miller and Venable, 2000).

Here I examine the association between ploidy and self-fertilization using data from 235 species of flowering plants for which levels of self-fertilization have been estimated. I report results from two separate analyses of these data: (1) phylogenetically-independent contrasts (PICs), which control for phylogenetic relationships among taxa, and (2) an analysis that does not control for phylogeny, but instead treats each species as an independent data point (i.e., cross-species analysis). In each analysis I ask whether polyploids exhibit higher levels of self-fertilization than

diploids. Annuals, herbaceous perennials, and woody perennials differ in their average rates of self-fertilization (Barrett and Eckert, 1990; Barrett et al., 1996). Accordingly, these three life-history categories are represented by the inclusion of an additional independent variable (along with ploidy) in the second analysis (cross-species analysis). In addition, the relationship between polyploidy and self-fertilization might differ among major groups of angiosperms; therefore, in both analyses the Monocotyledons, Rosids, and Asterids were analyzed on their own in addition to the analysis of the entire dataset. Although within-family comparisons might be a more informative and/or biologically meaningful way to analyze angiosperm life history data, most families included in this study lack variation in ploidy (among those species represented in the dataset), making within-family comparative analysis impossible.

Methods

Selfing rate and ploidy database

Selfing rate estimates for angiosperm taxa were compiled from the primary literature. S. C. H. Barrett provided a database of selfing rates from studies published through 1995 (S. C. H. Barrett, personal communication). I gathered additional data on selfing rates published since 1995 (through March, 2006) using the *Science Citation Index Expanded* (SCI Expanded – Web of Science) online science literature database. Only levels of selfing measured in natural populations (i.e., occurring in their natural habitat and range) were included. Because most studies report population outcrossing rates, the selfing rate for a given study population is equal to 1-t, where t is the outcrossing rate. For studies reporting estimates of $t > 1$ (which may occur if multiple loci are used and one or more assumptions of the model used to estimate outcrossing rates are violated (Ritland and Jain 1981)), the selfing rate was set to zero.

For some taxa, levels of self-fertilization have been estimated for multiple populations and/or for the same population during multiple years. If multiple estimates of t were available for a species, I used the mean value of the estimates in my analyses.

Chromosome numbers for most taxa were obtained from the Missouri Botanical Garden's Index to Plant Chromosome Numbers (http://mobot.mobot.org/W3T/Search/ipcn.html) or the Chromosome Atlas of Flowering Plants (Darlington and Wylie, 1955). For a few species (6%), taxon-specific literature was consulted for chromosome counts not reported in these sources. As information on ploidy does not exist for most species, I inferred relative ploidy levels for individual taxa by comparing basal chromosome numbers for a given genus (Darlington and Wylie, 1955) to those reported for the species in question (sensu Mable, 2004b). Darlington and Wylie (1955) defined basal chromosome numbers as the largest common denominator of all published chromosome counts for a given genus. Taxa with chromosome numbers that are two times the basal number are treated as diploids while those with more than two times the basal number are treated as polyploids.

The complete dataset contains 235 species of flowering plants from 126 genera and 58 families (Cronquist 1981, 1988; Angiosperm Phylogeny Group, 2003), and includes 170 diploids and 65 polyploids. There are 74 annuals, 82 herbaceous perennials, and 79 woody perennials in the dataset. The numbers of diploids and polyploids in each of the three life history categories are shown in figure 1.1

Statistical analyses

To better meet assumptions of normality/equal variance, selfing rate data were arcsine ($\sqrt{y}$) transformed prior to analysis. Normality was assessed using Minitab (Version 13.1, Minitab Inc.) and a Ryan-Joiner normality test ($r = 0.9819$, $p = 0.0710$).

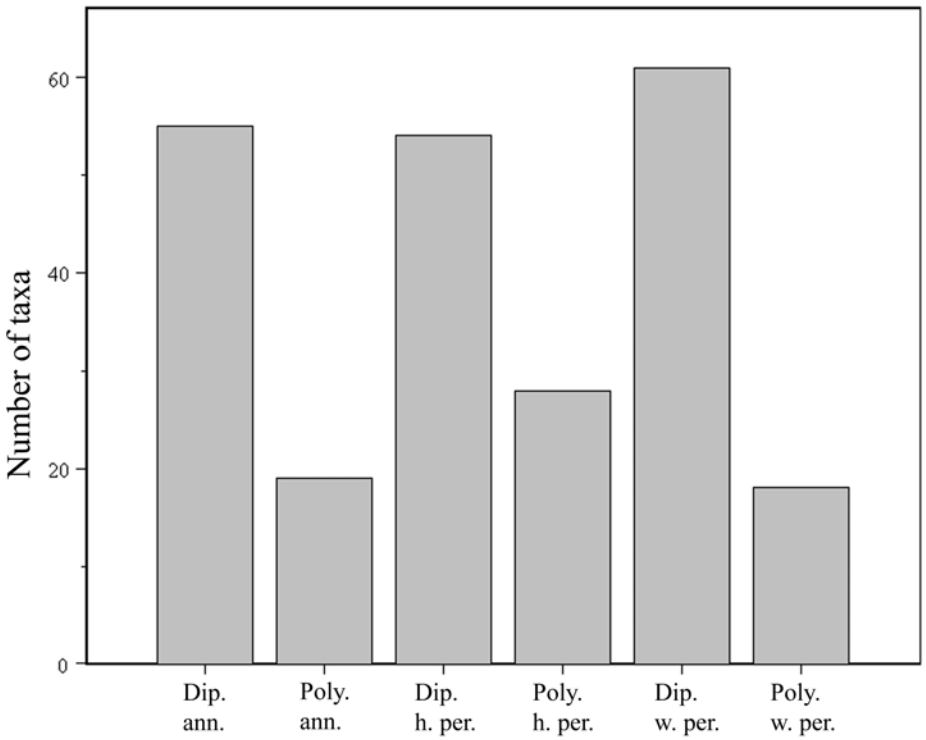

Figure 1.1. Numbers of taxa represented in the dataset by diploid annuals (Dip. ann.), polyploid annuals (Poly. ann.), diploid herbaceous perennials (Dip. h. per.), polyploid herbaceous perennials (Poly. h. per.), diploid woody perennials (Dip. w. per.), and polyploid woody perennials (Poly. w. per.).

Homogeneity of variance was assessed using Minitab and a Levene test ($L = 1.127$, $p = 0.341$). For all analyses, results from the analysis of non-transformed data were qualitatively the same.

Analysis 1 (phylogenetically-independent contrasts): Because plant mating systems are distributed non-randomly with respect to phylogeny (Barrett and Eckert, 1990), PICs were constructed to control for phylogenetic relations among taxa (Felsenstein, 1985). The MacClade software package (Maddison and Maddison, 1992) was used to build a phylogenetic tree containing all of the 235 species in the dataset, based on Davies et al.'s (2004) phylogeny of flowering plants. Taxon-specific literature was used to resolve relationships within families, however several genus-

and species-level polytomies remain in the finished tree, owing to a lack of available data for some groups. The Comparative Analysis by Independent Contrasts (CAIC) software package (Purvis and Rambaut, 1995) was then used to identify and calculate PICs for four different trees: (1) the entire tree containing all 235 species, (2) a tree that included Monocotyledons only (43 species), (3) a tree that included Rosids only (92 species), and (4) a tree that included Asterids only (77 species). All branch lengths were set equal; results from the analysis of trees for which branch lengths had been estimated using the algorithm described by Grafen (1989) were qualitatively the same. CAIC uses one of two different models to calculate PICs, CRUNCH (if all variables in the analysis are continuous) or BRUNCH (if the analysis includes one or more categorical variables) (Purvis and Rambaut, 1995). As ploidy is categorical the BRUNCH model was used. When performing PICs, CAIC automatically investigates potential violations in the assumptions of regression analysis (http://www.bio.ic.ac.uk/evolve/software/caic/assumptions.html); no violations were found.

Analysis 2 (cross-species analysis): Shifts between outcrossing and selfing as well as changes in ploidy are very common among angiosperms (Barrett et al., 1996; Soltis et al., 2004), and because mating systems and ploidy are evolutionarily labile (relative to rates of speciation), phylogenetic correction may not be necessary (Felsenstein, 1985; Westoby et al., 1995; Barrett et al., 1996; Ricklefs and Starck, 1996; Price, 1997; Larson and Barrett, 2000; Rheindt et al., 2004) (see discussion). In addition, levels of self-fertilization among flowering plants correlate strongly with life history; annuals tend to self more than herbaceous perennials, which in turn have higher selfing rates than woody perennials (Barrett and Eckert, 1990; Barrett et al., 1996). Therefore, for the cross-species analysis I included both ploidy and life history (and their interaction) as independent variables in a two-way analysis of variance

(ANOVA) using PROC GLM in SAS (SAS Institute, 1999 – 2001). As in analysis 1, the Monocotyledons, Rosids, and Asterids were analyzed separately in addition to the overall analysis, which included all 235 species in the dataset.

Results

Analysis 1 (phylogenetically-independent contrasts): The CAIC program identified 32 PICs from among the 235 species represented in the complete phylogeny, and the mean contrast value is significantly greater than zero, indicating that polyploids tend to have higher levels of selfing than their diploid relatives (n = 32 contrasts, P = 0.0011) (Table 1.1 and Figure 1.2). When analyzed on their own, Monocotyledons (n = 9 contrasts, P = 0.0274) and Rosids (n = 11 contrasts, P = 0.0013) also exhibit higher levels of selfing among polyploids relative to diploids. In contrast, the Asterids provide no support for the hypothesis that polyploidy is associated with increased levels of self-fertilization (n = 7 contrasts, P = 0.6255).

Table 1.1 Phylogenetically-independent contrasts. Positive contrast values indicate higher selfing rates among polyploids relative to diploids.

	# Taxa	# Contrasts	# Positive	p
All species	235	32	22	0.0011
Monocotyledons only	43	9	7	0.0274
Rosids only	92	11	10	0.0013
Asterids only	77	7	3	0.6255

Analysis 2 (cross-species analysis): When all 235 species are treated as independent data points, polyploids have significantly higher levels of selfing than diploids (n = 235, P = 0.0001) (Table 1.2). Results from the other three cross-species analyses, Monocotyledons (n = 43, P = 0.0067), Rosids (n = 92, P = 0.0143), and Asterids (n = 77, P = 0.0112) are similar. In addition, life-history correlates strongly

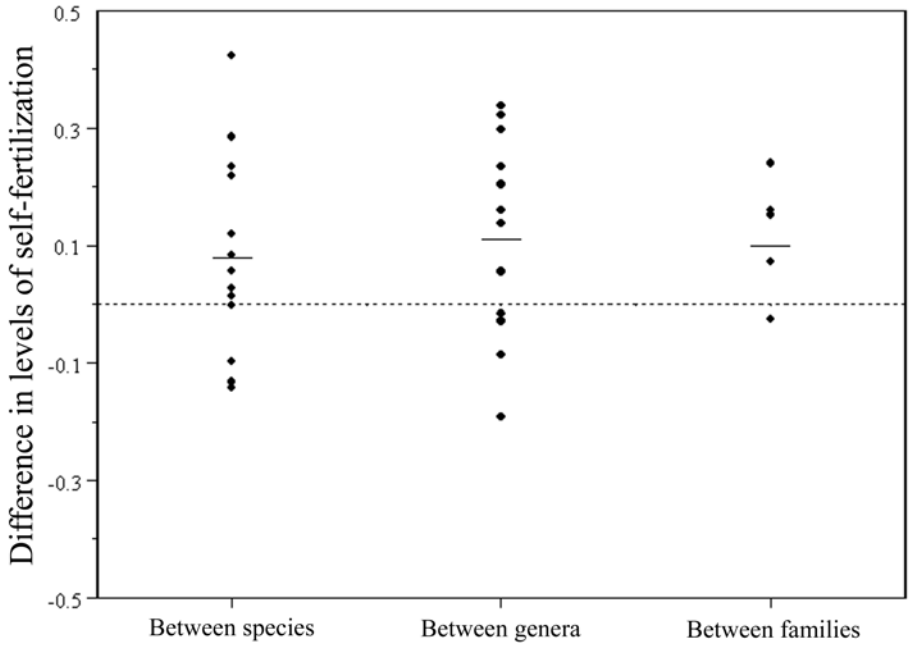

Figure 1.2. Differences in levels of self-fertilization between diploids and polyploids for each of the 32 phylogenetically-independent contrasts, arranged in three groups according to node depth (between species, between genera, and between families). For a given contrast, a positive value indicates higher levels of selfing in polyploids relative to diploids while a negative value indicates the reverse. For each group, a solid line indicates the mean contrast value.

with selfing rate in all four cross-species analyses, with annuals exhibiting higher levels of selfing than herbaceous perennials, which in turn exhibit higher levels of selfing than woody perennials. The interaction between ploidy and life history was non-significant for all four analyses. The distribution of selfing rates for all six possible combinations of ploidy and life history are shown in figure 1.3, and the least-squares mean selfing rates for diploids and polyploids and for annuals, herbaceous perennials, and woody perennials are shown in Tables 1.3a and 1.3b, respectively.

Table 1.2 Analysis of variance on the effects of ploidy, life history, and their interaction on selfing rate in the cross-species analysis.

Analysis	DF	Type III SS	F-value	Pr > F
All species				
Ploidy	1	1.25	15.30	0.0001
Life History	2	4.33	26.61	< 0.0001
Ploidy * Life History	2	0.22	1.35	0.2607
Error	229	18.65		
Monocotyledons Only				
Ploidy	1	0.98	8.21	0.0067
Life History	1	0.82	6.90	0.0122
Ploidy * Life History	1	0.11	0.96	0.3333
Error	39	4.64		
Rosids Only				
Ploidy	1	0.37	6.26	0.0143
Life History	2	0.88	7.43	0.0011
Ploidy * Life History	2	0.12	0.96	0.3855
Error	86	5.11		
Asterids Only				
Ploidy	1	0.60	6.78	0.0112
Life History	2	1.07	6.08	0.0037
Ploidy * Life History	2	0.06	0.34	0.7130
Error	71	6.27		

Discussion

This study is the first to analyze the relationship between polyploidy and self-fertilization in flowering plants in a phylogenetic context using quantitative estimates of selfing rates from natural populations. As indicated by phylogenetically-independent contrasts and cross-species analysis, polyploid angiosperms tend to have higher rates of self-fertilization than their diploid relatives. This trend is not apparent in the Asterids when PICs are used, however there are only 7 PICs in the Asterid phylogeny, and the lack of power in this analysis may be exacerbated by a relatively low level of phylogenetic resolution among the Asterids when compared to the other phylogenetic trees used in this study. Polytomies can reduce the validity and power of

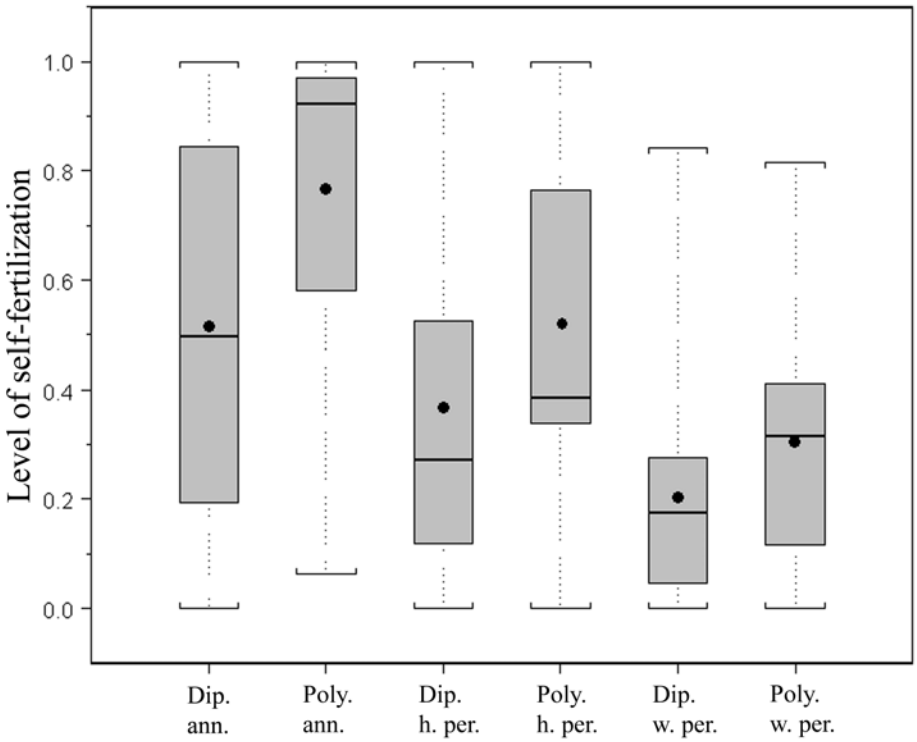

Figure 1.3 Levels of self-fertilization for diploid annuals (Dip. ann.), polyploid annuals (Poly. ann.), diploid herbaceous perennials (Dip. h. per.), polyploid herbaceous perennials (Poly. h. per.), diploid woody perennials (Dip. w. per.), and polyploid woody perennials (Poly. w. per.). For each group, the central bar indicates the median value, the filled circle indicates the mean value, the shaded box represents the interquartile range, and the dashed line indicates the total range.

phylogenetic tree is fairly unresolved (Purvis and Rambaut, 1995). In addition, although studies suggest that polyploidy is generally associated with a breakdown in gametophytic SI systems (Mable, 2004b), there is no evidence for a ploidy-dependent breakdown of SI in the Asteraceae (S. Good-Avila, personal communication). Of the 77 taxa analyzed in the Asterid clade, 29 (38%) belong to the Asteraceae, and this may further explain the lack of evidence for increased selfing rates among polyploid Asterids.

Selfing rates appear to evolve rapidly relative to rates of speciation in flowering plants (Barrett et al., 1996), and floral traits associated with selfing (e.g.,

Table 1.3. Least-squares means of selfing rates in the cross-species analysis for (A) diploids and polyploids and (B) annuals, herbaceous perennials, and woody perennials. Values in parentheses indicate standard errors. Different letters (superscript) within rows indicate means that differ significantly (Tukey-Kramer HSD, for $\alpha = 0.05$).

(a)

	Diploids	Polyploids
All Species	0.36 (.02)[a]	0.53 (.04)[b]
Monocotyledons Only	0.41 (.07)[a]	0.76 (.10)[b]
Rosids Only	0.43 (.03)[a]	0.60 (.06)[b]
Asterids Only	0.33 (.05)[a]	0.54 (.06)[b]

(b)

	Annuals	Herb. per.	Woody per.
All Species	0.64 (.04)[a]	0.43 (.03)[b]	0.25 (.04)[c]
Monocotyledons Only	0.74 (.10)[a]	0.43 (.07)[b]	na
Rosids Only	0.66 (.06)[a]	0.55 (.06)[a]	0.34 (.06)[b]
Asterids Only	0.61 (.06)[a]	0.41 (.07)[a,b]	0.27 (.08)[b]

small petal size, simultaneous maturation of pollen and receptivity of stigma, close spatial proximity of anther and stigma) often differ among subspecies and populations (Wyatt, 1984). For example, the two subspecies of *Clarkia xantiana* exhibit markedly different breeding systems (Runions and Geber, 2000), and both *C. exilis* and *C. tembloriensis* exhibit high variation in selfing rates among populations (Vasek and Harding, 1976; Holtsford and Ellstrand, 1989). Both genetic and environmental factors contribute to the evolution of mating systems in plants (Barrett and Eckert, 1990; Barrett, 2003), and rates of self-fertilization are known to change rapidly (relative to rates of speciation) in response to a changing environment (Stebbins, 1950, 1957; Grant, 1981; Schemske and Lande, 1985; Barrett and Eckert, 1990; Barrett et al., 1996; Barrett, 2003). Because plant mating systems are so evolutionarily labile, it could be argued that controlling for phylogenetic relations among taxa may be unduly conservative in this study. Indeed, a similar argument might be applied to changes in ploidy, as chromosome number varies among populations of many species (Grant,

1981; Otto and Whitton, 2000; Soltis et al., 2004), and changes in chromosome number are not necessarily related to speciation events. Because life history traits are often phylogenetically-constrained (Mazer, 1990; Peat and Fitter, 1994; Moles et al., 2005), however, it is possible that phylogenetic inertia of traits not included in this study could contribute to differences in selfing rates and/or differences in ploidy. This possibility justifies controlling for phylogenetic relationships among the taxa being analyzed here.

As shown in table 1.2, the effect of the interaction between ploidy and life history on levels of self-fertilization was non-significant in all four of the cross-species analyses, indicating that the relationship between ploidy and self-fertilization does not depend on life history. Annuals, herbaceous perennials, and woody perennials all tend to exhibit increased selfing rates among polyploids relative to diploids. As can be seen in figure 1.3, however, levels of self-fertilization range widely in all categories regardless of ploidy or life history, suggesting, though perhaps not surprisingly, that selective forces not considered in this study influence the evolution of self-fertilization. In agreement with previous studies (Barrett and Eckert, 1990; Barrett et al., 1996), selfing rates are negatively associated with life history, and this trend is apparent in both polyploids and diploids. Annual species exhibit higher selfing rates than perennials. Among perennials, herbaceous species self more than relatively long-lived woody species. This result is consistent with theoretical expectations that short-lived species should tend to self-fertilize more than long-lived species, owing to selection for reproductive assurance in the former (Stebbins 1950) and the cost of seed discounting (decreasing levels of outcrossed seed production due to increased levels of self-fertilization) in the latter (Lloyd, 1992; Morgan et al., 1997). There are other reasons why outcrossing rates might be higher in long-lived species as well. For example, long-lived species (e.g., trees and shrubs) are often larger than short-lived

species (e.g., herbs), and since plant cells are not differentiated into distinct somatic and germ cell lineages, mutations that occur during mitosis can contribute to the genetic load carried by gametes. Because larger plants are expected to experience a greater number of mitotic cell divisions between germination and the production of gametes (relative to smaller plants), this may lead to profound inbreeding depression and strong selection against selfing (Morgan, 2001; Scofield and Schultz, 2006).

Polyploids are known to experience *diploidization*, a process by which their chromosomal behavior reverts back to that of a diploid, owing to a variety of phenomena such as large-scale genomic rearrangements, gene silencing and/or loss, and one or more copies of duplicated genes evolving novel functions (Wendel, 2000; Wolfe, 2001; Soltis et al., 2004). Though some taxa that are treated as polyploids in this study may behave cytogenetically as diploids, the methods used ensure that such taxa have undergone a relatively recent polyploidization event because they were compared to congeners that possess at least half as many chromosomes. The assumption being made then is that the evolution of self-fertilization might be associated with polyploidization regardless of the current cytogenetic behavior of a given taxon.

Allopolyploids may not differ from diploids in terms of inbreeding depression (Bever and Felber, 1992; Ronfort, 1999; Soltis and Soltis, 2000; Comai, 2005), and if decreased inbreeding depression contributes to the evolution of higher selfing rates among polyploids, it would be of interest to know whether this pattern occurs in both allo- and autopolyploids, or is more prevalent in one group relative to the other. Unfortunately, the category of ploidy is not known for the majority of polyploids included in this study, and whether allo- and autopolyploids differ in terms of their relationship with self-fertilization remains unknown. Autopolyploids were once thought to be quite rare (Grant, 1981), however there is growing evidence that they are

much more common in nature than initially believed (Soltis et al., 2004). Since most theoretical and empirical work has focused on autopolyploidy and it's effects on the evolution of self-fertlization (e.g., Husband and Schemske, 1997; Ronfort, 1999; Rausch and Morgan, 2005), the results presented here suggest that either autopolyploids are indeed more common (at least among those polyploids represented in the dataset), or that the association between self-fertilization and polyploidy does not differ between allo- and autopolyploids.

Conclusions

In agreement with other studies (e.g., Cook and Soltis, 2000; Quarin et al., 2001; Tate and Simpson, 2004; Guggisberg et al., 2006), the data lend support to the hypothesis that polyploid angiosperms have, on average, higher rates of self-fertilization than their diploid relatives. Of continued interest are the evolutionary and ecological phenomena that underlie this pattern, and they are likely both numerous and varied. The ability to self-fertilize may increase the likelihood that newly-arisen polyploids can establish successful populations, and it may be that selfing taxa give rise to successful polyploid lineages more often than do outcrossing taxa. Decreased levels of inbreeding depression in polyploids might also help to explain why polyploids exhibit higher rates of self-fertilization than diploids. The relationship between ploidy and inbreeding depression is complex, however, and has been shown to depend on many factors, including the number and lethality of deleterious alleles, the degree of dominance among alleles and epistasis among loci, and the age of the polyploid in question (Ronfort, 1999; Pannell et al., 2004). More empirical studies that compare mating systems and inbreeding depression in closely-related polyploids and diploids are needed to further address these issues. In addition, auto- and allopolyploids may differ in their response to inbreeding. Accordingly, models that

compare and explore further the evolution of inbreeding depression in polyploids and diploids – especially those that differentiate between neopolyploids vs. older polyploids and autopolyploids vs. allopolyploids – will be of value.

LITERATURE CITED

Angiosperm Phylogeny Group (APG II). 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society 141: 399-436.

Barrett, S. C. H. 1987. The evolution, maintenance, and loss of self-incompatibility systems. In J. Lovett-Doust and L. Lovett-Doust [eds.], Reproductive Strategies of Plants. Oxford University Press, Oxford, UK.

Barrett, S. C. H. 2003. Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. Philosophical Transactions of the Royal Society of London B Biological Sciences 358:991-1004.

Barrett, S. C. H., and C. G. Eckert. 1990. Variation and Evolution of Mating Systems in Seed Plants. In S. Kawano (ed.) Biological Approaches and Evolutionary Trends in Plants. pp. 229-254. Academic Press, London, U.K.

Barrett, S. C. H., L. D. Harder, and A. C. Worley. 1996. The comparative biology of pollination and mating in flowering plants. Philosophical Transactions of the Royal Society of London B Biological Sciences 351:1271-1280.

Bateman, A. J. 1952. Self-incompatibility systems in angiosperms. I. Theory. Heredity 6:285-310.

Bennett, J. H. 1976. Expectations for inbreeding depression on self-fertilization of tetraploids. Biometrics 32:449-452.

Bever, J. D., and F. Felber. 1992. The theoretical population genetics of autopolyploidy. Pp. 185-217 in J. Antonovics and D. Futuyma, eds. Oxford Surveys in Evolutionary Biology. Oxford Univ. Press, New York.

Brunet, J. 1992. Sex allocation in hermaphroditic plants. Tremds in Ecology and Evolution 7:79-84.

Busbice, T. H., and C. P. Wilsie. 1966. Inbreeding depression and heterosis in autotetraploids with application to Medicago sativa L. Euphytica 15:52-67.

Charlesworth, D., and B. Charlesworth. 1979. The evolutionary genetics of sexual systems in flowering plants. Proceedings of the Royal Society of London B Biological Sciences 205:513-530.

Charlesworth, D. and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237-268.

Charnov, E. L., J. M. Smith, and J. J. Bull. 1976. Why be an hermaphrodite. Nature 263:125-126.

Comai, L. 2005. The advantages and disadvantages of being polyploid. Nature Reviews Genetics 6:836-846.

Cook, L. M., and P. S. Soltis. 1999. Mating systems of diploid and allotetraploid populations of Tragopogon (Asteraceae). I. Natural populations. Heredity 82:237-244.

Cook, L. M., and P. S. Soltis. 2000. Mating systems of diploid and allotetraploid populations of Tragopogon (Asteraceae). II. Artificial populations. Heredity 84:410-415.

Cronquist, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, USA.

Cronquist, A. 1988. The evolution and classification of flowering plants, 2nd ed. New York Botanical Garden, Bronx, New York, USA.

Darlington, C. D., and A. P. Wylie. 1955. Chromosome Atlas of Flowering Plants. George Allen and Unwin Ltd, London.

Darwin, C. D. 1859. The Origin of Species. Penguin Classics, London.

Darwin, C. D. 1876. The Effects of Cross- and Self-Fertilization in the Vegetable Kingdom. John Murray, London.

Davies, T. J., T. G. Barraclough, M. W. Chase, P. S. Soltis, D. E. Soltis, and V. Savolainen. 2004. Darwin's abominable mystery: Insights from a supertree of the angiosperms. Proceedings of the National Academy of Science 101:1904-1909.

Felsenstein, J. 1985. Phylogenies and the comparative method. American Naturalist 125:1-15.

Fisher, R. A. 1941. Average excess and average effect of a gene substitution. Annals of Eugenics 11:53-63.

Grafen, A. 1989. The phylogenetic regression. Philosophical Transactions of the Royal Society of London B Biological Sciences 326:119-157.

Grant, V. 1956. The influence of breeding habit on the outcome of natural hybridization in plants. American Naturalist 90:319-322.

Grant, V. 1981. Plant Speciation. Columbia University Press, New York.

Guggisberg, A., G. Mansion, S. Kelso, and E. Conti. 2006. Evolution of biogeographic patterns, ploidy levels, and breeding systems in a diploid-polyploid species complex of Primula. New Phytologist 171:617-632.

Harder, L. D., and W. G. Wilson. 1998. A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. American Naturalist 152:684-695.

Holsinger, K. E. 1988. Inbreeding depression doesn't matter: the genetic basis of mating system evolution. Evolution 42:1235-1244.

Holtsford, T. P., and N. C. Ellstrand. 1989. Variation in outcrossing rate and population genetic structure of Clarkia tembloriensis (Onagraceae). Theoretical and Applied Genetics 78:480-488.

Husband, B. C., and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54-70.

Husband, B. C., and D. W. Schemske. 1997. The effect of inbreeding in diploid and tetraploid populations of Epilobium angustifolium (Onagraceae): implications for the genetic basis of inbreeding depression. Evolution 51:737-746.

Jackson, R. C. 1976. Evolution and systematic significance of polyploidy. Annual Review of Ecology and Systematics 7:209-234.

Johnston, M. O., and D. J. Schoen. 1996. Correlated evolution of self-fertilization and inbreeding depression: An experimental study of nine populations of Amsinckia (Boraginaceae). Evolution 5:1478-1491.

Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. genetic models. Evolution 39:24-40.

Larson, B. M. H., and S. C. H. Barrett. 2000. A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society 69:503-520.

Legatt, R. A. and G. K. Iwama. 2003. Occurrence of polyploidy in the fishes. Reviews in Fish Biology and the Fisheries 13:237-246.

Levin, D. A. 1975. Minority cytotype exclusion in local plant populations. Taxon 24:35-43.

Lloyd, D. G. 1992. Self- and cross-fertilization in plants. I. The selection of self-fertilization. International Journal of Plant Sciences 153:370-380.

Mable, B. K. 2004a. Why polyploidy is rarer in animals than in plants: myths and mechanisms. Biological Journal of the Linnean Society 82:453-466.

Mable, B. K. 2004b. Polyploidy and self-compatibility: Is there an association? New Phytologist 162:803-811.

Mazer, S. J. 1990. Seed mass of Indiana Dune genera and families: taxonomic and ecological correlates. Evolutionary Ecology 4:326-357.

Maddison, W. P., and D. R. Maddison. 1992. MacClade: analysis of phylogeny and character evolution, version 3.0. Sinauer, Sunderland, Massachusetts.

Masuyama, S., and Y. Watano. 1990. Trends for inbreeding in polyploid pteridophytes. Plant Species Biology 5:13-17.

Maynard-Smith, J. 1978. The Evolution of Sex. Cambridge University Press, Cambridge.

Miller, J. S., and D. L. Venable. 2000. Polyploidy and the evolution of gender dimorphism in plants. Science 289:2335-2338.

Moles, A. T., D. D. Ackerly, C. O. Webb, J. C. Tweddle, J. B. Dickie, and M. Westoby. 2005. A brief history of seed size. Science 307:576-580.

Morgan, M. T. 2001. Consequences of life history for inbreeding depression and mating system evolution in plants. Proceedings of the Royal Society of London B 268:1817-1824.

Morgan, M. T., D. J. Schoen, and T. M. Bataillon. 1997. The evolution of self-fertilization in perennials. The American Naturalist 150:618-638.

Morgan, M. T., and W. G. Wilson. 2005. Self-fertilization and the escape from pollen limitation in variable pollination environments. Evolution 59:1143-1148.

Otto, S. P., and J. Whitton. 2000. Polyploid incidence and evolution. Annual Review of Genetics 34:401-437.

Pannell, J. R. and S. C. H. Barrett. 1998. Baker's law revisited: reproductive assurance in a metapopulation. Evolution 52:657-668.

Pannell, J. R., D. J. Obbard, and R. J. A. Buggs. 2004. Polyploidy and the sexual system: what can we learn from Mercurialis annua? Biological Journal of the Linnean Society 82:547-560.

Peat, H. J. and A. H. Fritter. 1994. Comparative analysis of ecological characteristics of British angiosperms. Biological Reviews 69:95-115.

Price, T. 1997. Correlated evolution and independent contrasts. Philosophical Transactions of the Royal Society of London B Biological Sciences 352:519-529.

Purvis, A., and A. Rambaut. 1995. Comparative analysis by independent contrasts (caic): an Apple Macintosh application for analyzing comparative data. Computer Applications in the Biological Sciences 11:247-251.

Quarin, C. L., F. Espinoza, E. J. Martinez, S. C. Pessino, and O. A. Bovo. 2001. A rise of ploidy level induces the expression of apomixis in Paspalum notatum. Sexual Plant Reproduction 13:243-249.

Ramsey, J., and D. W. Schemske. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annual Review of Ecology and Systematics 29:467-501.

Ramsey, J., and D. W. Schemske. 2002. Neopolyploidy in flowering plants. Annual Review of Ecology and Systematics 33:589-639.

Rausch, J. H. and M. T. Morgan. 2005. The effect of self-fertilization, inbreeding depression, and population size on autotetraploid establishment. Evolution 59:1867-1875.

Rheindt, F. E., T. U. Grafe, and E. Abouheif. 2004. Rapidly evolving traits and the comparative method: how important is testing for phylogenetic signal? Evolutionary Ecology Research 6:377-396.

Ricklefs, R. E., and J. M. Starck. 1996. Applications of phylogenetically independent contrasts: a mixed progress report. Oikos 77:167-172.

Ritland, K. and S. Jain. 1981. A model for the estimation of outcrossing rate and gene-frequencies using n independent loci. Heredity 47:35-52.

Ronfort, J. 1999. The mutation load under tetrasomic inheritance and its consequences for the evolution of the selfing rate in autotetraploid species. Genetical Research 74:31-42.

Rosquist, G. 2001. Reproductive biology in diploid Anthericum ramosum and tetraploid A. liliago (Anthericaceae). Oikos 92:143-152.

Ross, R. 1981. Chromosome counts, cytology, and reproduction in the Cactaceae. American Journal of Botany 68:463-470.

Runions, C. J., and M. A. Geber. 2000. Evolution of the self-pollinating flower in Clarkia xantiana (Onagraceae). I. Size and development of floral organs. American Journal of Botany 87:1439-1451.

Scofield, D. G. and S. T. Schultz. 2006. Mitosis, stature and evolution of mating systems: low-ϕ and high-ϕ plants. Proceedings of the Royal Society B: Biological Sciences 273:275-282.

Soltis, D. E., and P. S. Soltis. 1987. Polyploidy and breeding systems in homosporous pteridophyta: A reevaluation. American Naturalist 130:219-232.

Soltis, D. E., P. S. Soltis, and J. A. Tate. 2004. Advances in the study of polyploidy since Plant Speciation. New Phytologist 161:173-191.

Soltis, P. S., and D. E. Soltis. 1990. Evolution of inbreeding and outcrossing in ferns and fern-allies. Plant Species Biology 5:1-11.

Soltis, P. S., and D. E. Soltis. 2000. The role of genetic and genomic attributes in the success of polyploids. Proceedings of the National Academy of Sciences of the United States of America 97:7051-7057.

Stebbins, G. L. 1950. Variation and Evolution in Plants. Columbia University Press, New York.

Stebbins, G. L. 1957. Self-fertilization and population variability in higher plants. American Naturalist 41:337-354.

Tate, J. A., and B. B. Simpson. 2004. Breeding system evolution in Tarasa (Malvaceae) and selection for reduced pollen grain size in the polyploid species. American Journal of Botany 91:207-213.

Thomson, J. D. 2006. Tactics for male reproductive success in plants: contrasting insights of sex allocation theory and pollen presentation theory. Integrative and Comparative Biology 46:390-397.

Vasek, F. C., and J. Harding. 1976. Outcrossing in natural-populations. V. Analysis of outcrossing, inbreeding, and selection in Clarkia exilis and Clarkia tembloriensis. Evolution 30:403-411.

Wendel, J. F. 2000. Genome evolution in polyploids. Plant Molecular Biology 42:225-249.

Westoby, M., M. R. Leishman, and J. M. Lord. 1995. On misinterpreting the "phylogenetic correction". Journal of Ecology 83:531-534.

Wolfe, K. H. 2001. Yesterday's polyploids and the mystery of diploidization. Nature Reviews Genetics 2:333-341.

Wyatt, R. 1984. The evolution of self-pollination in granite outcrop species of Arenaria (Caryophyllaceae). I. Morphological correlates. Evolution 38:804-816.

CHAPTER 2

INBREEDING DEPRESSION AND THE RESPONSE TO SELECTION IN POLYPLOID AND DIPLOID PLANTS

Abstract

Population-level responses to natural selection depend on the strength of selection, population size, and breeding system. In addition, for a given level of polymorphism, the response to selective pressure depends on the effect of alternate alleles on fitness, which is determined in part by the level of dominance of deleterious mutations. Further, genome duplication (i.e., polyploidy) can affect the response to selection because polyploids have more than two copies of each gene. For this reason, polyploids may not respond to evolutionary pressures in the same manner as diploids. We simulated the effects of selection on deleterious alleles segregating at a single locus in diploid and autotetraploid, and two non-recombining loci in allotetraploid populations of hermaphroditic plants with non-overlapping generations (e.g., annuals). Model parameters included ploidy, population size, selfing rate, dominance, and strength of selection. Our model differs from previous models in that small populations of neopolyploids (rather than infinite populations of established polyploids in mutation-selection balance) are considered. Further, our model is the first to explore these issues in allopolyploids.

Our results suggest fundamental differences among all parameters in their effects on responses to selection, including the likelihood of, and time to, fixation of beneficial alleles. In particular, the diploid was more likely to fix the beneficial allele and did so faster than polyploids, indicating that adaptive evolution is more efficient in the former than the latter. Adaptive evolution was least effective in the allopolyploid

because it often fixes the beneficial allele at one locus and the deleterious allele at the other locus (permanent heterozygosity) – an option not available to the other cytotypes. As expected, the probability of fixing the beneficial allele increased in larger populations and under stronger selection and dominance. Likewise the time to fixation was reduced with stronger selection and dominance but increased with population size.

Many two-way interactions among ploidy, population size, selfing, dominance and selection had significant effects on one or more of the outcome variables. For example, the effects of population size and dominance in increasing the probability of fixing the beneficial allele were most pronounced in the autopolyploid, as were the effect of dominance and selection in reducing the time to fixation. These responses may derive from the fact that the genetic effective population size at the locus common to all cytotypes is largest in the autopolyploid.

In sum, the results of our simulations indicate complex, though generally intuitive, effects of model parameters on adaptive evolution, and that responses to natural selection differ with ploidy.

Introduction

Inbreeding depression is the reduction in fitness associated with inbreeding and is thought to be one of the most important phenomena influencing and shaping the evolution of mating systems, especially among flowering plants (Charlesworth and Charlesworth, 1979, 1987; Lande and Schemske, 1985; Husband and Schemske, 1996, 1997; Barrett 2002). The genetic basis of inbreeding depression is complex. Studies suggest, however, that most inbreeding depression is caused by the expression of recessive or partially recessive deleterious alleles (the *partial dominance* model of inbreeding depression) (Charlesworth and Charlesworth 1999; Carr and Dudash

2003). Other phenomena that have been shown to influence levels of inbreeding depression include a reduction in levels of heterozygosity (i.e., the *overdominance* model of inbreeding depression), reduced numbers of interactions among loci (i.e., epistasis), and the number and relative proportions of deleterious alleles with small vs. large effects on fitness (Charlesworth and Charlesworth 1987, Charlesworth and Charlesworth 1999; Carr and Dudash 2003). These phenomena are not mutually exclusive and may act in concert to affect the fitness of inbred individuals and populations.

Closely related to inbreeding depression is a population's ability to purge deleterious alleles that are exposed to selection as genome-wide levels of homozygosity increase with inbreeding, with the result that inbreeding depression is highest immediately following an increase in selfing and very low many generations later (Charlesworth and Charlesworth 1990; Crnokrak and Barrett 2002; but see Charlesworth et al. 1990; Byers and Waller 1999). Indeed, predominantly selfing taxa often show relatively little inbreeding depression (Husband and Schemske 1996). The ability to purge maladapted alleles may be crucial in population persistence, especially when populations are small or selection is strong (Gilpin and Soule 1986).

Polyploid angiosperms often exhibit higher rates of self-fertilization than their diploid relatives, an interesting pattern noted by early plant biologists and more recently explored in detail (Stebbins 1950, 1957, 1980; Grant 1956, 1981; Otto and Whitton 2000; Mable 2004; Barringer 2007). Several ecological and evolutionary forces are likely to contribute to the evolution and maintenance of this pattern, including selection for reproductive assurance (Stebbins 1950; Grant 1956, 1981; Levin 1975; Fowler and Levin 1984; Felber 1991; Rodriguez 1996; Ramsey and Schemske 1998), a breakdown of self-incompatibility systems (Bateman 1952; Miller and Venable 2000; Mable 2004), and reduced inbreeding depression (Lande and

Schemske 1985; Hedrick 1987; Ronfort 1999 but see Busbice and Wilsie 1966; Bennett 1976).

The relationship between polyploidy and inbreeding depression has received relatively little attention. The dearth of studies is surprising given that 30-80% of all flowering plants are of polyploid origin (Stebbins 1938; Grant 1963; Goldblatt 1980; Masterson 1994; Otto and Whitton 2000), changes in ploidy are responsible for 2-4% of all speciation events among angiosperms (Otto and Whitton 2000), and the successful establishment of polyploid populations can be negatively influenced by inbreeding depression (Baack 2005; Rausch and Morgan 2005). Early theoretical work suggested that autopolyploids might exhibit more inbreeding depression than diploids (Busbice and Wilsie 1966; Bennett 1976); however, this conclusion was based on the overdominance model, and very limited support for this hypothesis exists (Charlesworth and Charlesworth 1999; Dudash and Carr 1998; Carr and Dudash 2003). In contrast, more recent theoretical explorations based on the partial dominance model have suggested that autopolyploids should exhibit less inbreeding depression than diploids (Lande and Schemske 1985). The reverse can be true, however, depending on levels of dominance and the strength of selection (Ronfort 1999).

In empirical studies of agricultural species, inbreeding depression has been found to be higher (Kalton et al. 1952; Busbice and Wilsie 1966; Dewey 1966; Bingham and Groose 1994; Johnston and Schoen 1996; Auger et al. 2005) or lower (Alexander 1960; Davies 1961; Townsend and Remmenga 1968; Dewey 1969) in polyploids than diploids. Interpretation of these conflicting results is difficult given the history of artificial breeding and manipulation in crops. At the same time, no consistent outcome has emerged from the few studies of wild species; inbreeding depression was lower in polyploids than diploids in three studies (Husband and

Schemske 1997; Rosquist 2001; Barringer 2008) but higher in a fourth study (Johnston and Schoen 1996). While polyploids may benefit from reduced inbreeding depression via the masking of deleterious alleles, the masking may also slow the purging of deleterious alleles and contribute to higher genetic loads over the long term as populations reach mutation-selection balance (Otto and Whitton 2000).

Clearly, the relationships among ploidy, inbreeding depression, and the response to selection are complex, and although recent studies have shed light on this issue (e.g., Lande and Schemske 1985; Ronfort 1999; Otto and Whitton 2000; Rausch and Morgan 2005), all existing theoretical studies have addressed levels of inbreeding depression in populations that are large and at (or near) mutation-selection equilibrium. However, inbreeding depression in neopolyploids is also of interest because high levels of self-fertilization might facilitate the initial establishment of polyploid populations (Rausch and Morgan 2005), and levels of inbreeding depression suffered by neopolyploids will influence the evolution of mating systems immediately following genome duplication. Further, neopolyploids are likely to exist in small populations where stochastic forces (i.e., genetic drift) may play a relatively large role in shaping their genetic architecture. Yet, population size has rarely been considered in studies of inbreeding depression in polyploids (but see Otto and Whitton 2000; Rausch and Morgan 2005). Finally, the relationship between inbreeding depression and the response to selection in allopolyploids has not been examined (Pannell et al. 2004; but see Lande and Schemske 1985), even though allopolyploid taxa are common in nature (Grant 1981; Soltis et al. 2004). The successful establishment of allopolyploid taxa might be influenced by their ability to combine the ecological traits of two parental species in a state of permanent genomic heterozygosity (Stebbins 1984). For this reason, a better understanding of the relationships among allopolyploidy, the response to selection, and the formation of permanent genomic

heterozygotes would be of value.

To further explore the relationships between cytotype (diploid, auto- and allopolyploid) and inbreeding depression we simulated the effects of selection on deleterious alleles segregating at a single locus in diploid and autotetraploid, and at two non-recombining loci in allotetraploid populations of hermaphroditic plants with non-overlapping generations (e.g., annuals). Model parameters included cytotype, population size, selfing rate, dominance, and strength of selection (Table 2.1). For

Table 2.1. Model parameters and values used in simulations.

Parameter	Values
Ploidy	diploid / autotetraploid / allotetraploid
Population size	for diploids: 8 / 48 / 100
	for polyploids: 10 / 50 / 100
Selfing rate	0.00 / 0.25 / 0.50 / 0.75 / 1.00
Dominance	for diploids: 0.02 / 0.10 / 0.50
	for polyploids: $h_1 = 0.01$, $h_2 = 0.02$, $h_3 = 0.03$ $h_1 = 0.05$, $h_2 = 0.10$, $h_3 = 0.15$ $h_1 = 0.25$, $h_2 = 0.50$, $h_3 = 0.75$
Selection coefficient	0.02 / 0.10 / 0.50 / 1.00

each set of parameter values we compared the probability of, and time to, fixation of the beneficial allele among populations of diploids, autotetraploids and allotetraploids. For allotetraploids only, we also examined the effects of parameter values on the probability and number of generations required for the beneficial allele to fix in one genome and the deleterious allele in the other (i.e., permanent genomic heterozygosity).

Methods

General model

The Matlab software package (The Mathworks Inc., Version 6.5 release 13, 2002) was used to simulate the effects of selection on deleterious alleles segregating at a single locus in diploid and autotetraploid, and at two non-recombining loci in allotetraploid populations of hermaphroditic plants with non-overlapping generations (e.g., annuals). Source code is available from the authors on request. Model parameters included ploidy (3 levels), selfing rate (5 levels), population size (3 levels), dominance (3 levels), and strength of selection against deleterious alleles (4 levels) (Table 1). Dominance was defined as the relative effect of deleterious alleles in heterozygotes, such that completely recessive deleterious alleles would have a dominance of 0. Fifty replicate simulations were performed for each unique combination of parameter values. Simulations began with populations in Hardy-Weinberg equilibrium (Haldane 1927; 1930). During each generation a number of mating events (hereafter, "events") equal to 10 times the initial population size were conducted (i.e., each individual contributed genetic material to 10 zygotes, on average). The probability that a given event was a selfing event was equal to the selfing rate. Conversely, the probability that an event was an outcrossing event was equal to 1 – (selfing rate). For each event, one randomly chosen gamete was taken from each of two randomly chosen parents (if a selfing event, the same parent was sampled twice, with replacement) to form a zygote. For simplicity we assumed that the locus of interest was near the centromere and that no double reduction occurred (Haldane 1930; Mather 1936; Parsons 1959; Crow and Kimura 1970; Bever and Felber 1992) (see discussion). Ten percent of all zygotes were then randomly sampled to create the next generation. This process resulted in the population size at the beginning of each generation (i.e., before selection) being fixed and equal to the initial

population size, and also allowed for the incorporation of stochasticity (i.e., genetic drift) into our simulations. Offspring were subjected to selection before reproducing, with fitness dependent on genotype (Table 2.2).

Table 2.2. Fitness functions for each genotype. Levels of dominance among alleles are represented by *h*. The intensity of selection against recessive homozygotes is represented by the selection coefficient, *s*.

Ploidy	Genotype	Fitness
Diploid	AA	1
	Aa	1 - hs
	aa	1 – s
Autotetraploid	AAAA	1
	AAAa	$1 - h_1s$
	AAaa	$1 - h_2s$
	Aaaa	$1 - h_3s$
	aaaa	1 – s
Allotetraploid	AAAA	1
	AAAa	$1 - h_1s$
	AAaa	$1 - h_2s$
	AaAa	$1 - h_2s$
	Aaaa	$1 - h_3s$
	aaaa	1 – s

For all three cytotypes, a simulation trial could end in the fixation of the beneficial allele (mean fitness of the population equaled one) or the fixation of the deleterious allele. A third outcome was possible for allopolyploids, namely the fixation of the beneficial allele in one genome and the deleterious allele in the other genome (i.e., the population being composed entirely of permanent genomic heterozygotes). For each trial, we recorded the number of generations required to reach completion. These data were then used to compute (1) the probability of fixation of the beneficial allele, (2) the mean number of generations required to fix the beneficial allele, and, (3) for allotetraploids only, the probability of permanent genomic heterozygosity.

Statistical analysis

Analysis of variance (Proc mixed, SAS 2003) was used to test the effects of cytotype, selfing rate, population size, dominance, selection, and all two-way interactions on the (1) probability of, and (2) time to, fixation of the beneficial allele, and, (3) for allotetraploids, the probability of permanent genomic heterozygosity.

Results

The probability of fixation of the beneficial allele – With the exception of selfing rate, all model parameters had significant effects on the fixation of the beneficial allele (Tables 2.3 – 2.4). Diploids fixed beneficial alleles more often than polyploids, and the autopolyploid, in turn, was more likely to fix beneficial alleles than the allopolyploid (Figure 2.1). The probability of fixation of the beneficial allele was low in allopolyploids because a substantial proportion of trials ended in permanent genomic heterozygosity (Figure 2.1, see below). Population size, dominance, and selection were all positively correlated with the likelihood that the beneficial allele was fixed.

The interactions between ploidy and all other parameters significantly influenced the probability of fixing the beneficial allele (Tables 2.3 – 2.4 and Figure 2.1). For example, while the selfing rate did not affect the probability of fixation in diploid and autotetraploid populations, the beneficial allele was less likely to be fixed as selfing increased in the allopolyploid.

Population size was positively correlated with the fixation of the beneficial allele in all cytotypes, but the effect was strongest in the allotetraploid. The level of dominance was positively correlated with the probability of fixing the beneficial allele in the allotetraploid but had no significant effect in the diploid and autotetraploid.

Table 2.3. P-values from analysis of variance on the effects of different parameters and their two-way interactions on response variables.

Predictor Variables	Proportion of trials ending with mean population fitness of one	Number of generations required to reach mean population fitness of one	Proportion of trials ending in permanent genomic heterozygosity
Ploidy	< 0.0001	< 0.0001	na
Selfing rate	0.3124	< 0.0001	0.0018
Population size	< 0.0001	< 0.0001	< 0.0001
Dominance	< 0.0001	< 0.0001	< 0.0001
Selection	< 0.0001	< 0.0001	< 0.0001
Ploidy*Self. rate	0.0498	< 0.0001	na
Ploidy*Pop. size	< 0.0001	< 0.0001	na
Ploidy*Dominance	< 0.0001	< 0.0001	na
Ploidy*Selection	< 0.0001	< 0.0001	na
Self. rate*Pop. size	0.5944	< 0.0001	0.3500
Self. rate*Dominance	0.6498	< 0.0001	0.7525
Self. rate*Selection	0.9182	< 0.0001	0.6813
Pop. size*Dominance	0.3292	< 0.0001	0.3308
Pop. size*Selection	< 0.0001	< 0.0001	< 0.0001
Dominance*Selection	0.0024	0.3055	< 0.0001

Table 2.4. LS means from analysis of variance of response variables for all model parameters and two-way interactions. For a given parameter and response variable, different letters (superscripts) indicate means that differ significantly (Tukey-Kramer honestly significant difference, for $\alpha = 0.05$).

Parameter	Proportion of trials ending with mean population fitness of one	Number of generations required to reach mean population fitness of one	Proportion of trials ending in permanent genomic heterozygosity
Ploidy			
Diploid	0.9052^{a}	40.96^{a}	na
Autotetraploid	0.8532^{b}	110.70^{b}	na
Allotetraploid	0.6259^{c}	93.45^{c}	na
Selfing Rate			
0.00	0.7961	128.93^{a}	0.3267^{a}
0.25	0.7974	101.24^{b}	0.3306^{a}
0.50	0.7987	80.39^{c}	0.3356^{a}
0.75	0.8022	59.42^{d}	0.3361^{a}
1.00	0.7794	38.53^{e}	0.4106^{b}
Population Size			
8/10	0.6648^{a}	23.05^{a}	0.4830^{a}
48/50	0.8279^{b}	85.14^{b}	0.3163^{b}
100	0.8917^{c}	136.91^{c}	0.2443^{c}
Dominance			
0.02	0.7543^{a}	102.44^{a}	0.4840^{a}
0.10	0.7932^{b}	84.11^{b}	0.3593^{b}
0.50	0.8368^{c}	58.55^{c}	0.2003^{c}
Selection			
0.02	0.5444^{a}	159.57^{a}	0.5867^{a}
0.10	0.7664^{b}	93.85^{b}	0.4280^{b}
0.50	0.9197^{c}	43.56^{c}	0.2258^{c}
1.00	0.9486^{d}	29.82^{d}	0.1511^{d}
Ploidy*Self. rate			
Diploid*0.00	0.9111	59.3130	na
Diploid*0.25	0.9039	51.6651	na
Diploid*0.50	0.8978	39.9906	na
Diploid*0.75	0.9061	30.9483	na
Diploid*1.00	0.9072	22.8674	na
Autotetraploid*0.00	0.8467	201.77	na
Autotetraploid*0.25	0.8478	142.25	na
Autotetraploid*0.50	0.8583	107.37	na
Autotetraploid*0.75	0.8539	67.6034	na
Autotetraploid*1.00	0.8594	34.4999	na
Allotetraploid*0.00	0.6306	125.71	na
Allotetraploid*0.25	0.6406	109.80	na
Allotetraploid*0.50	0.6400	93.8032	na
Allotetraploid*0.75	0.6467	79.7141	na
Allotetraploid*1.00	0.5717	58.2158	na

Table 2.4 (Continued).

Ploidy*Pop. Size			
Diploid*8	0.8070	10.7673	na
Diploid*48	0.9347	43.2621	na
Diploid*100	0.9740	68.8413	na
Autotetraploid*10	0.7280	34.6941	na
Autotetraploid*50	0.8830	117.76	na
Autotetraploid*100	0.9487	179.65	na
Allotetraploid*10	0.4593	23.6874	na
Allotetraploid*50	0.6660	94.4024	na
Allotetraploid*100	0.7523	162.26	na
Ploidy*Dominance			
Diploid*0.02	0.9070	46.3542	na
Diploid*0.10	0.9037	41.6887	na
Diploid*0.50	0.9050	34.8278	na
Autotetraploid*0.02	0.8633	138.95	na
Autotetraploid*0.10	0.8577	116.14	na
Autotetraploid*0.50	0.8387	77.0099	na
Allotetraploid*0.02	0.4927	122.03	na
Allotetraploid*0.10	0.6183	94.4903	na
Allotetraploid*0.50	0.7667	63.8268	na
Ploidy*Selection			
Diploid*0.02	0.7347	98.4623	na
Diploid*0.10	0.8933	43.6522	na
Diploid*0.50	0.9929	13.8603	na
Diploid*1.00	1.0000	7.8529	na
Autotetraploid*0.02	0.5618	237.22	na
Autotetraploid*0.10	0.8604	122.11	na
Autotetraploid*0.50	0.9933	50.8347	na
Autotetraploid*1.00	0.9973	32.6345	na
Allotetraploid*0.02	0.3369	143.04	na
Allotetraploid*0.10	0.5453	115.78	na
Allotetraploid*0.50	0.7729	65.9962	na
Allotetraploid*1.00	0.8484	48.9852	na
Self. Rate*Pop. Size			
0.00*8/10	0.6789	35.3274	0.4383
0.00*48/50	0.8156	134.03	0.3083
0.00*100	0.8939	217.43	0.2333
0.25*8/10	0.6572	28.0972	0.4600
0.25*48/50	0.8361	105.13	0.3017
0.25*100	0.8989	170.49	0.2300
0.50*8/10	0.6656	23.6855	0.4700
0.50*48/50	0.8361	84.2029	0.2983
0.50*100	0.8944	133.28	0.2383
0.75*8/10	0.6589	16.6612	0.5267

Table 2.4 (Continued).

0.75*48/50	0.8506	61.6400	0.2650
0.75*100	0.8972	99.9646	0.2167
1.00*8/10	0.6633	11.4766	0.5200
1.00*48/50	0.8011	40.6948	0.4083
1.00*100	0.8739	63.4118	0.3033
Self. Rate*Dominance			
0.00*0.02	0.7611	170.73	0.4733
0.00*0.10	0.7994	130.12	0.3333
0.00*0.50	0.8278	85.9519	0.1733
0.25*0.02	0.7489	126.98	0.4617
0.25*0.10	0.8094	106.64	0.3350
0.25*0.50	0.8339	70.1010	0.1950
0.50*0.02	0.7589	95.6670	0.4617
0.50*0.10	0.7928	84.1325	0.3533
0.50*0.50	0.8444	61.3645	0.1917
0.75*0.02	0.7744	72.6733	0.4417
0.75*0.10	0.7933	60.7711	0.3583
0.75*0.50	0.8389	44.8213	0.2083
1.00*0.02	0.7283	46.1678	0.5817
1.00*0.10	0.7711	38.8801	0.4167
1.00*0.50	0.8389	30.5354	0.2333
Self. Rate*Selection			
0.00*0.02	0.5430	246.14	0.5156
0.00*0.10	0.7585	149.75	0.4533
0.00*0.50	0.9304	73.6409	0.2022
0.00*1.00	0.9526	46.1956	0.1356
0.25*0.02	0.5319	198.03	0.6067
0.25*0.10	0.7719	119.25	0.4000
0.25*0.50	0.9230	53.0622	0.2089
0.25*1.00	0.9630	34.6120	0.1067
0.50*0.02	0.5496	161.58	0.5933
0.50*0.10	0.7600	92.1568	0.4156
0.50*0.50	0.9267	39.6265	0.2111
0.50*1.00	0.9585	28.1920	0.1222
0.75*0.02	0.5578	118.72	0.5689
0.75*0.10	0.7741	66.5650	0.4178
0.75*0.50	0.9281	30.4047	0.2044
0.75*1.00	0.9489	21.9931	0.1533
1.00*0.02	0.5400	73.3827	0.6489
1.00*0.10	0.7674	41.5157	0.4533
1.00*0.50	0.8904	21.0843	0.3022
1.00*1.00	0.9200	18.1283	0.2378
Pop. Size*Dominance			
8/10*0.02	0.6360	27.5080	0.6100
8/10*0.10	0.6503	23.4828	0.5220

Table 2.4 (Continued).

8/10*0.50	0.7080	18.1580	0.3170
48/50*0.02	0.7773	103.87	0.4700
48/50*0.10	0.8380	89.4257	0.3070
48/50*0.50	0.8683	62.1302	0.1720
100*0.02	0.8497	175.95	0.3720
100*0.10	0.8913	139.41	0.2490
100*0.50	0.9340	95.3763	0.1120
Pop. size*Selection			
8/10*0.02	0.3862	28.9021	0.5960
8/10*0.10	0.5436	29.5743	0.5787
8/10*0.50	0.8351	19.7027	0.4493
8/10*1.00	0.8942	14.0192	0.3080
48/50*0.02	0.5436	158.78	0.6000
48/50*0.10	0.8533	103.60	0.4093
48/50*0.50	0.9471	45.7225	0.1587
48/50*1.00	0.9676	32.4619	0.0973
100*0.02	0.7036	291.04	0.5640
100*0.10	0.9022	148.36	0.2960
100*0.50	0.9769	65.2660	0.0693
100*1.00	0.9840	42.9915	0.0480
Dominance*Selection			
0.02*0.02	0.5404	172.54	0.6240
0.02*0.10	0.7218	113.85	0.5813
0.02*0.50	0.8653	69.1244	0.4000
0.02*1.00	0.8898	54.2592	0.3307
0.10*0.02	0.5324	162.67	0.6427
0.10*0.10	0.7551	102.39	0.4600
0.10*0.50	0.9240	43.9765	0.2187
0.10*1.00	0.9613	27.3919	0.1160
0.50*0.02	0.5604	143.50	0.4933
0.50*0.10	0.8222	65.3026	0.2427
0.50*0.50	0.9698	17.5903	0.0587
0.50*1.00	0.9947	7.8215	0.0067

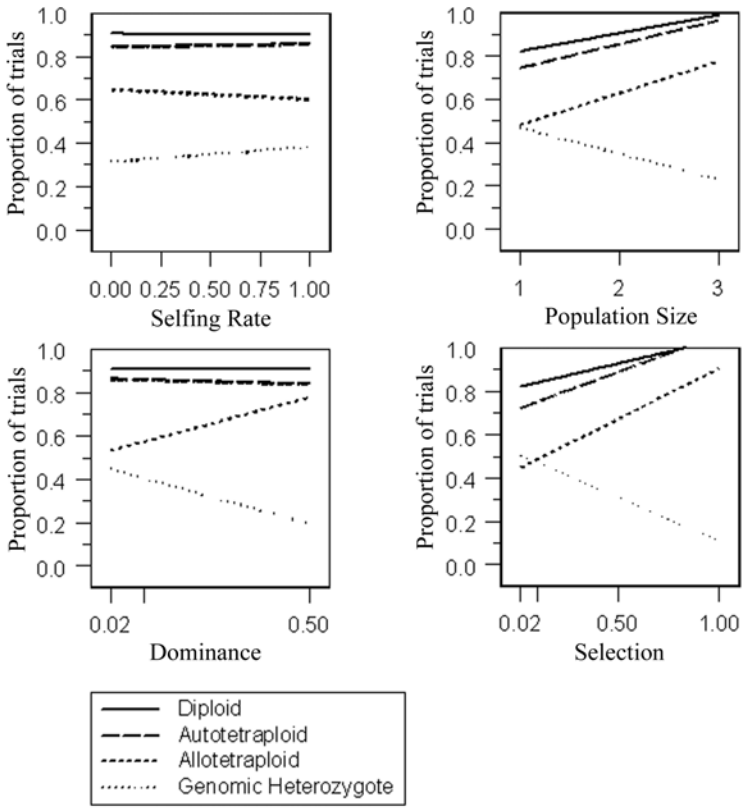

Figure 2.1. The effects of selfing rate, population size, dominance, and selection on the proportion of trials ending with fixation of the beneficial allele for diploids, autotetraploids, and allotetraploids, and the proportion of trials ending in permanent genomic heterozygosity in allotetraploids. Regardless of the values for other model parameters, diploid populations were more likely than polyploid populations to fix beneficial alleles. See text for details.

Finally, the level of selection was positively correlated with fixation of the beneficial allele in all cytotypes but its effect was greater in the two polyploids than in the diploid.

The probability of fixing the beneficial allele was also significantly influenced by the interactions between selection and both population size and dominance (Figure 2.2). While stronger selection always increased the probability of fixing the beneficial

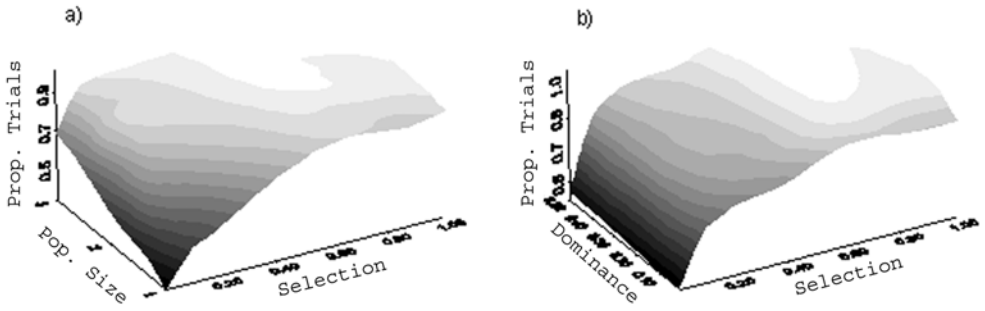

Figure 2.2. The influence of the interactions between (a) population size and selection and (b) dominance and selection on the proportion of trials ending with fixation of the beneficial allele. Increasing selection had a reduced effect in large populations, though it was augmented when levels of dominance were high. See text for details.

allele, the effect of increasing selection was weaker in large relative to small populations. In addition, the effect of selection on allele fixation was stronger when dominance was high.

The time to fixation of the beneficial allele – All model parameters affected the time to fixation of the beneficial allele (Tables 2.3 – 2.4 and Figure 2.3). Fixation occurred more rapidly in diploids than in polyploids, and faster in allopolyploids than in autopolyploids. In addition, population size was positively correlated with the time to fixation. Finally, selfing rate, dominance, and selection were all negatively correlated

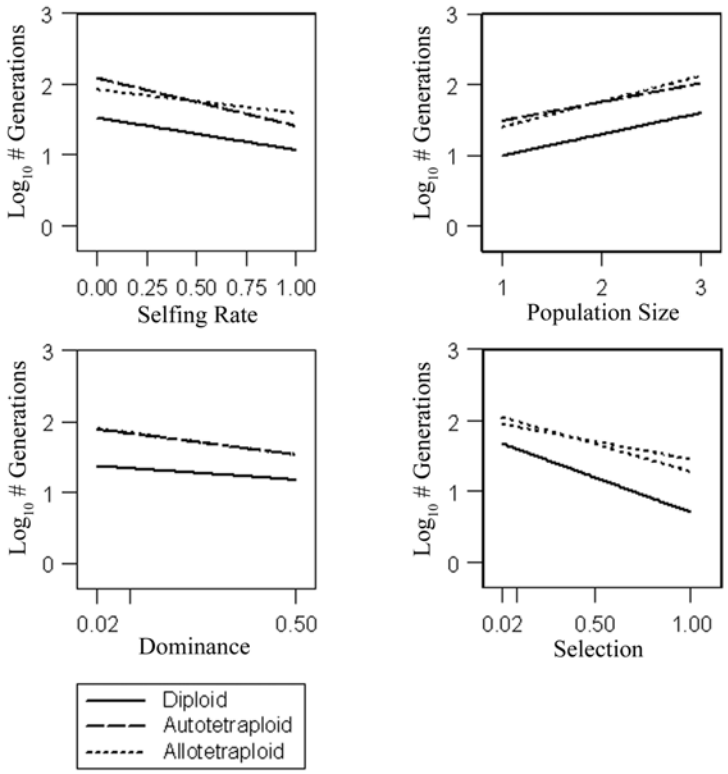

Figure 2.3. The effects of selfing rate, population size, dominance, and selection on the number of generations required to fix beneficial alleles in populations of diploids, autotetraploids, and allotetraploids. Regardless of the values for other model parameters, the rate of fixation was higher in diploid relative to polyploid populations. See text for details.

with the time to fixation in that allele fixation was faster when selfing rates, levels of dominance, and levels of selection were high.

The interactions between ploidy and all other parameters significantly influenced the time to fixation of the beneficial allele (Tables 2.3 – 2.4 and Figure 2.3). For example, while the time to fixation was lower at high selfing rates in all cytotypes, the effect of selfing was most pronounced in the autotetraploid. In addition, the time to fixation was always longer in larger populations, but the effect of population size was greater in the two polyploids than in the diploid. In like manner, while the time to fixation always decreased with increasing dominance, the effect was

most pronounced in the polyploids. Finally, the time to fixation always decreased as the strength of selection increased, but the effect of selection was greater in the autotetraploid than in the allotetraploid and diploid.

The interactions between selfing rate and population size, dominance, and selection also had significant effects on the time to fixation of the beneficial allele (Tables 2.3 – 2.4 and Figure 2.4). The beneficial allele always took longer to fix in large populations but the time difference between small and large populations was significantly reduced when selfing was high (Figure 2.4a). The effect of dominance was similar, in that higher levels of dominance always reduced the time to fixation, but the difference between dominance levels in time to fixation was significantly reduced at high selfing rates (Figure 2.4b). Finally, while the strength of selection was always negatively correlated with the time to fixation, the effect of selection was weaker at high relative to low selfing rates (Figure 2.4c).

The time to fixation of the beneficial allele was also significantly influenced by the interactions between population size and both dominance and selection (Tables 2.3 – 2.4 and Figure 2.5). Although dominance was negatively correlated with the time to fixation, the reduction was greater in large relative to small populations. Likewise, while selection always reduced the time to fixation, the effect was greater in large relative to small populations.

The probability of permanent genomic heterozygosity – Permanent genomic heterozygosity was the outcome of a large proportion of trials in the allopolyploid, and was significantly influenced by all model parameters (Tables 2.3 – 2.4 and Figure 2.1). The proportion of trials ending in permanent genomic heterozygosity increased with selfing rate and decreased with both dominance and selection.

In addition, the interaction between population size and selection significantly

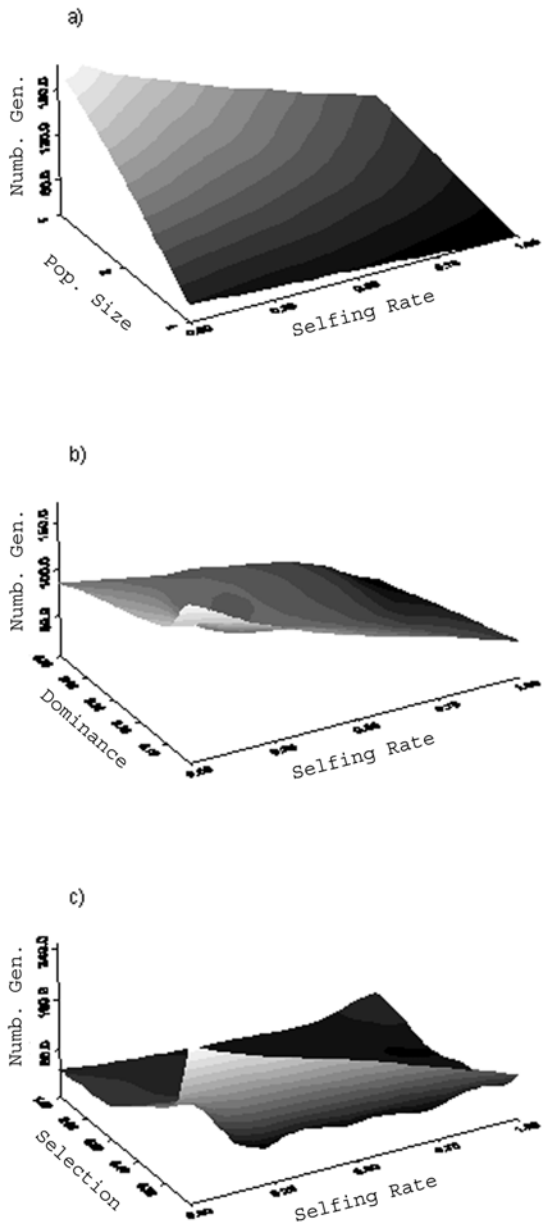

Figure 2.4. The effect of the interaction between selfing rate and (a) population size, (b) dominance, and (c) selection on the number of generations required for populations to fix beneficial alleles. The differences between small and large populations, low and high levels of dominance, and weak and strong selection in the number of generations required to fix beneficial alleles was significantly reduced among populations with high levels of self-fertilization. See text for details.

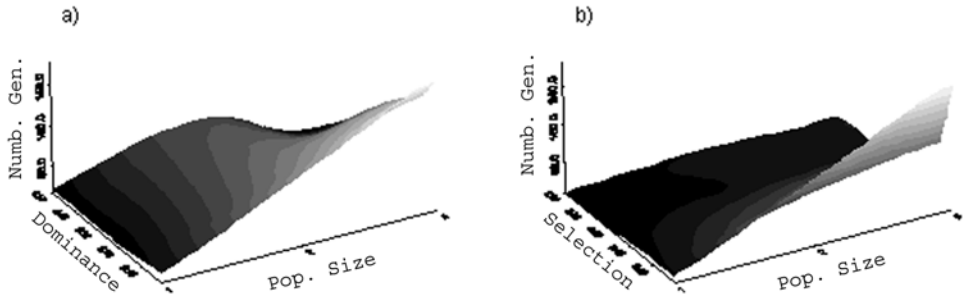

Figure 2.5. The effect of the interaction between population size and (a) dominance and (b) selection on the number of generations required for populations to fix beneficial alleles. The effects of increasing levels of dominance and selection were greater in large relative to small populations. See text for details.

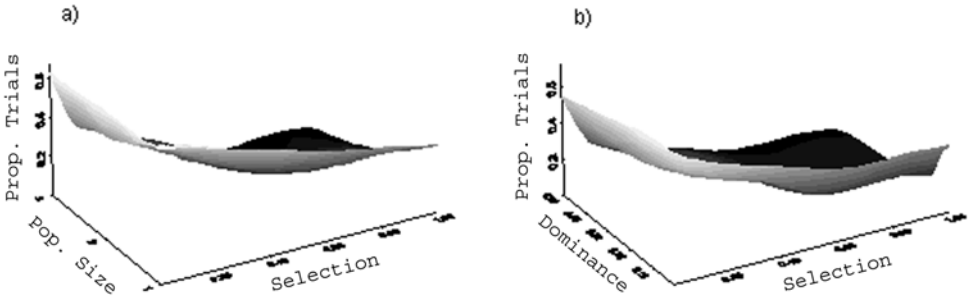

Figure 2.6. The effect of the interaction between selection and (a) population size and (b) dominance on the proportion of trials ending in permanent genomic heterozygosity in allotetraploids. The effects of increasing levels of selection were greater in large relative to small populations and for high relative to low levels of dominance. See text for details.

influenced the formation of genomic heterozygotes (Figure 2.6a). Higher levels of selection reduced the proportion of genomic heterozygotes across all population sizes, but did so more effectively as population size increased. Finally, the interaction between dominance and selection significantly influenced the formation of genomic heterozygotes (Figure 2.6b). Again, higher levels of selection reduced the proportion of genomic heterozygotes across all levels of dominance, though it did so more effectively as dominance increased.

Discussion

Increasing the level of selection always increased the probability of fixation of beneficial alleles, however the effect was not as strong in small relative to large populations. This result suggests that the ability of selection to shape allele frequencies may be reduced in populations of neopolyploids, which are likely to occur in relatively small populations where stochastic forces (e.g., genetic drift) can be profound and may be strong enough to overcome the role of selection (Fisher 1922, 1930; Wright 1931; Kimura 1983; Gillespie 1998). Selection was also more efficient when levels of dominance were high, as high levels of dominance allow for the selective elimination of deleterious alleles, even when such alleles are in the heterozygous state.

It has been suggested that whether diploids evolve faster than polyploids depends on the degree to which deleterious alleles are masked, and that under some conditions polyploids can evolve faster than diploids (Otto 2007; and see Orr and Otto 1994). However, for a given set of parameter values, we found that diploids always evolve faster than polyploids, as indicated by the mean number of generations required to fix beneficial alleles. Allopolyploids tended to be intermediate in this regard, while autopolyploids required the largest number of generations to fix beneficial alleles. This result coincides directly with the efficiency of masking in our model, in that deleterious alleles are exposed to selection more efficiently in diploids relative to polyploids. A similar process occurs among polyploids, in that allopolyploids are more efficient than autopolyploids at purging deleterious alleles.

The production of permanent genomic heterozygotes was influenced by all model parameters. Self-fertilization increased their production because selfing leads to increased homozygosity, and increased homozygosity, in turn, can promote the fixation of alleles, even when such alleles are deleterious. In contrast, their production

was reduced in larger populations (where stochastic forces are weaker) and in response to increases in levels of dominance or selection (when the selective elimination of the deleterious allele is more efficient). Selection was negatively correlated with the production of genomic heterozygotes; however, this effect was more pronounced in small populations, where genetic drift augments the fixation of deleterious alleles. Similarly, the effect of selection was more pronounced when dominance was high, allowing for the selective elimination of deleterious alleles in the heterozygous state.

The level of dominance among deleterious alleles varied in our model from $h = 0.02$ to $h = 0.5$. Relatively little is known about the levels of dominance exhibited by deleterious alleles segregating in natural populations, though studies of *Drosophila melanogaster* suggest extremely low levels of dominance (e.g., $0.00 < h < 0.05$) for lethals (Crow and Simmons 1983; Lynch et al. 1999), probably due to stronger selection against more dominant mutations (Lande and Schemske 1985). Interestingly, however, most lethal alleles are not completely recessive (Simmons and Crow 1977; Lande and Schemske 1985). In contrast to levels of dominance for lethal alleles, levels of dominance for mildly deleterious alleles can be relatively high (Lynch et al. 1999). Therefore, for deleterious alleles with high selection coefficients (i.e., $s \geq 0.5$) our results may be most relevant to natural populations when levels of dominance are low (i.e., $h \leq 0.1$). However, for deleterious alleles with low selection coefficients (i.e., $s \leq 0.1$), any level of dominance might apply.

The selection coefficient represents the intensity of selection experienced by individuals that are homozygous for the deleterious allele, and varied in our model from $s = 0.02$ (mildly deleterious) to $s = 1.00$ (lethal). Most deleterious alleles segregating in natural populations are only mildly deleterious (i.e., $s \leq 0.1$) (Crow and Simmons 1983; Lynch and Gabriel 1990; Lynch 1995), though studies suggest that

100 or more such alleles might be present within individuals (Lynch and Gabriel 1990; Lynch 1995), such that their cumulative effects could be profound (Lynch 1995). In contrast, strong selection against lethal mutations should keep their frequencies relatively low in most populations. For this reason, the frequency of lethal alleles is expected to be extremely low in populations of neopolyploids, which are likely to be founded by one or a few individuals.

Conclusions and Future Directions

The results presented here suggest several lines of inquiry that merit further study. First, populations of neopolyploids may be extremely small (i.e., only one or a few individuals) and may not be in Hardy-Weinberg equilibrium. Small population sizes can lead to decreased performance (e.g., Fischer and Matthies 1998) and eventual extinction (Gilpin and Soule 1986; Matthies et al. 2004). All of the populations we simulated were relatively small ($\leq$ 100 individuals), however it would be of interest to simulate the response to selection for populations composed of only one (or a few) heterozygous individual(s). The purging of deleterious alleles should occur extremely rapidly in such populations, though drift might cause the fixation of deleterious alleles before this could occur, even under conditions of strong selection. Second, the dominance coefficients and fitness functions used here represent only one of several possibilities. For simplicity it was assumed that dosage effects in polyploids were linear and additive (i.e., $h_1 < h_2 < h_3$). However, it could be that individuals with only one copy of a given deleterious allele are as (or nearly as) fit as those lacking them entirely, and that significant fitness effects occur only with two or more copies (i.e., $h_1 << h_2 < h_3$) (cf. Ronfort 1999). In addition, we assigned genotypic fitness as a function of the absolute number of deleterious alleles, regardless of how such alleles were distributed among chromosomes (e.g., for allotetraploids we

assumed that AAaa individuals had the same fitness as AaAa individuals). However, other possibilities exist. For example, a scenario worthy of exploration would be one in which the fitness of an allotetraploid is determined by finding the average fitness of the two homeologous genomes. In the specific case of h = 0.5 and linear dosage effects this would yield results identical to those presented here. However, for other levels of dominance and/or alternative dosage models the results could differ significantly. Third, we assumed that the locus of interest was close to the centromere and that double reduction in autotetraploids did not occur. However, double reduction might occur in relatively high frequencies (as high as ~14% for loci located at distal ends of the chromosome (Mather 1936; Parsons 1959; Bever and Felber 1992)) and would cause autopolyploid populations to behave more like diploids by increasing the proportion of homozygous gametes produced, a process that should lead to more effective purging. Finally, polyploids are often categorized as autopolyploids or allopolyploids, suggesting they contain either multiple sets of homologous chromosomes (leading to multisomic inheritance patterns), or multiple sets of nonhomologous chromosomes (leading to disomic inheritance patterns), respectively. However, these conditions represent the extremes of what is likely a continuum, and recombination between genomes can cause allopolyploids to exhibit multisomic inheritance at some loci and disomic inheritance at others (Sybenga 1969; Grant 1981; Jackson 1982; Jenkins and Rees 1991; Comai 2000; but see Sybenga 1996); so-called *segmental* allopolyploids (Stebbins 1950). Indeed, it might be that *genomic* allopolyploids (with no recombination between genomes) are relatively rare in nature (Sybenga 1996). To the extent that this is true, studies that explore the effects of intergenomic recombination in allopolyploids will be of value.

LITERATURE CITED

Alexander, D. E. 1960. Performance of genetically induced corn tetraploids. Am. Seed Trade Assoc. 15:68-74.

Auger, D. L., A. D. Gray, T. S. Ream, A. Kato, E. H. Coe, Jr., and J. A. Birchler. 2005. Nonadditive gene expression in diploid and triploid hybrids of maize. Genetics 169:389-397.

Baack, E. J. 2005. To succeed globally, disperse locally: effects of local pollen and seed dispersal on tetraploid establishment. Heredity 94:538-546.

Barrett, S. C. H. 2002. The evolution of plant sexual diversity. Nature Reviews Genetics 3:274-284.

Barringer, B. C. 2007. Polyploidy and self-fertilization in flowering plants. American Journal of Botany 94:1527-1533.

Barringer, B. C. 2008. Chapter 3: Mating System and Ploidy Influence Levels of Inbreeding Depression in Clarkia (Onagraceae). In Polyploidy, inbreeding depression, and the evolution of mating systems in flowering plants. Ph.D. Dissertation. Cornell University. Ithaca, NY. USA.

Bateman, A. J. 1952. Self-incompatibility systems in angiosperms. I. Theory. Heredity 6:285-310.

Bennett, J. H. 1976. Expectations for Inbreeding Depression on Self-fertilization of Tetraploids. Biometrics 32:449-452.

Bever, J. D. and F. Felber. 1992. The theoretical population genetics of autopolyploidy. Oxford Surveys in Evolutionary Biology 8:185-217.

Bingham, E. T., and R. W. Groose. 1994. Complementary gene interactions in alfalfa are greater in autotetraploids than diploids. Crop Sci. 34:823-829.

Busbice, T. H., and C. P. Wilsie. 1966. Inbreeding Depression and Heterosis in Autotetraploids With Application to Medicago Sativa L. Euphytica 15:52-67.

Byers, D. L. and D. M. Waller. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Ann. Rev. Ecol. Syst. 30:479-513.

Carr, D. E., and M. R. Dudash. 2003. Recent approaches into the genetic basis of inbreeding depression in plants. Phil. Trans. R. Soc. Lond. B. 358:1071-1084.

Charlesworth, B., M. T. Morgan, and D. Charlesworth. 1990. Genetic loads and estimates of mutation rates in highly inbred plant populations. Nature 347:380-382.

Charlesworth, B., and D. Charlesworth. 1999. The genetic basis of inbreeding depression. Genet. Res. 74:329-340.

Charlesworth, D., and B. Charlesworth. 1979. The evolutionary genetics of sexual systems in flowering plants. Proceedings of the Royal Society of London B Biological Sciences 205:513-530.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237-268.

Charlesworth, D. and B. Charlesworth. 1990. Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. Evolution 44:870-888.

Comai, L. 2000. Genetic and epigenetic interactions in allopolyploid plants. Plant Molecular Biology 43:387-399.

Crnokrak, P. and S. C. H. Barrett. 2002. Perspective: Purging the genetic load: A review of the experimental evidence. Evolution 56:2347-2358.

Crow, J. F. and M. Kimura. 1970. An introduction to population genetics theory. Harper and Row. New York, NY.

Crow, J. F. and M. J. Simmons. 1983. The mutation load in Drosophila. In M. Ashburner et al. (eds.) The genetics and biology of Drosophila, Vol 3c. Academic Press, New York, NY.

Davies, W. E. 1961. Herbage legume breeding: investigations on polyploid clovers. Report for the Welsh Plant Breeding Station, pp. 40-44.

Dewey, D. R. 1966. Inbreeding depression in diploid, tetraploid, and hexaploid crested wheatgrass. Crop Sci. 6:144-147.

Dewey, D. R. 1969. Inbreeding depression in diploid and induced-autotetraploid crested wheatgrass. Crop Sci. 9:592-595.

Dudash, M. R. and D. E. Carr. 1998. Genetics underlying inbreeding depression in Mimulus with contrasting mating systems. Nature 393:682-684.

Felber, F. 1991. Establishment of a tetraploid cytotype in a diploid population: effect of relative fitness of cytotypes. Journal of Evolutionary Biology 4:194-207.

Fischer, M. and D. Matthies. 1998. Effects of population size on performance in the rare plant Gentianella germanica. Journal of Ecology 86:195-204.

Fisher, R. A. 1922. On the dominance ratio. Proceedings of the Royal Society of Edinburgh 42:321-341.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press. Oxford, U. K.

Fowler, N. L., and D. A. Levin. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. American Naturalist 124:705-711.

Gillespie, J. H. 1998. Population genetics: a concise guide. The John Hopkins University Press. Baltimore, MD.

Gilpin, M. E. and M. E. Soule. 1986. Minimum viable populations: the processes of species extinctions. In Soule, M. E. (ed.) Conservation biology: the science of scarcity and diversity. Sinauer Associates Inc., Sunderland, MA.

Goldblatt, P. 1980. Polyploidy in angiosperms: Monocotyledons. In Lewis, W. H. (ed.) Polyploidy: Biological relevance. Plenum Press, New York.

Grant, V. 1956. The influence of breeding habit on the outcome of natural hybridization in plants. American Naturalist 90:319-322.

Grant, V. 1963. The origin of adaptations. Columbia University Press, New York.

Grant, V. 1981. Plant Speciation. Columbia University Press, New York.

Haldane, J. B. S. 1927. A mathematical theory of natural selection and artificial selection. Part 3. Proceedings of the Cambridge Philosophical Society 23:363-372.

Haldane, J. B. S. 1930. Theoretical genetics of autopolyploids. Journal of Genetics 22:359-372.

Hedrick, P. W. 1987. Genetic load and the mating system in homosporous ferns. Evolution 41:1282-1289.

Husband, B. C. and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54-70.

Husband, B. C., and D. W. Schemske. 1997. The effect of inbreeding in diploid and tetraploid populations of Epilobium angustifolium (Onagraceae): implications for the genetic basis of inbreeding depression. Evolution 51:737-746.

Jackson, R. C. 1982. Polyploidy and diploidy: new perspectives on chromosome pairing and its evolutionary implications. American Journal of Botany 69:1512-1523.

Jenkins, G. and H. Rees. 1991. Strategies of bivalent formation in allopolyploid plants. Proc. R. Soc. Lond. B. 243:209-214.

Johnston, M. O., and D. J. Schoen. 1996. Correlated evolution of self-fertilization and inbreeding depression: an experimental study of nine populations of Amsinckia (Boraginaceae). Evolution 50:1478-1491.

Kalton, R. R., A. G. Smit, and R. C. Leffel. 1952. Parent-inbred progeny relationships of selected orchardgrass clones. Agron. J. 44:481-486.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge University Press. Cambridge, U. K.

Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. genetic models. Evolution 39:24-40.

Levin, D. A. 1975. Minority cytotype exclusion in local plant populations. Taxon 24:35-43.

Lynch, M. 1995. Inbreeding depression and outbreeding depression. NOAA Tech Memo NMFS NWFSC-30.

Lynch, M. and W. Gabriel. 1990. Mutation load and the survival of small populations. Evolution 44:1725-1737.

Lynch, M., J. Blanchard, D. Houle, T. Kibota, S. Schultz, L. Vassilieva, and J. Willis. 1999. Perspective: spontaneous deleterious mutation. Evolution 53:645-663.

Mable, B. K. 2004. Polyploidy and self-compatibility: is there an association? New Phytologist 162:803-811.

Masterson, J. 1994. Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. Science 264:421-423.

Mather, K. 1936. Segregation and linkage in autotetraploids. Journal of Genetics 32:287-314.

Matthies, D., I. Brauer, W. Maibom, and T. Tscharntke. 2004. Population size and the risk of local extinction: empirical evidence from rare plants. Oikos 105:481-488.

Miller, J. S., and D. L. Venable. 2000. Polyploidy and the evolution of gender dimorphism in plants. Science 289:2335-2338.

Nagylaki, T. 1977. Selection in one- and two-locus systems. Springer-Verlag. New York, NY.

Orr, H. A. and S. P. Otto. 1994. Does diploidy increase the rate of adaptation? Genetics 136:1475-1480.

Otto, S. P. 2007. The evolutionary consequences of polyploidy. Cell 131:452-462.

Otto, S. P., and J. Whitton. 2000. Polyploid Incidence and Evolution. Annual Review of Genetics 34:401-437.

Pannell, J. R., D. J. Obbard, and R. J. A. Buggs. 2004. Polyploidy and the sexual system: What can we learn from Mercurialis annua? Biol. J. Linn. Soc. 82:547-560.

Parsons, P. A. 1959. Some problems in inbreeding and random mating in tetrasomics. Agron. J. 51:465-467.

Ramsey, J., and D. W. Schemske. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annu. Rev. Ecol. Syst. 29:467-501.

Rausch, J. H. and M. T. Morgan. 2005. The effect of self-fertilization, inbreeding depression, and population size on autopolyploid establishment. Evolution 59:1867-1875.

Rodriguez, D. J. 1996. A model for the establishment of polyploidy in plants. American Naturalist 147:33-46.

Ronfort, J. 1999. The mutation load under tetrasomic inheritance and its consequences for the evolution of the selfing rate in autotetraploid species. Genetical Research 74:31-42.

Rosquist, G. 2001. Reproductive biology in diploid Anthericum ramosum and tetraploid A. liliago (Anthericaceae). Oikos 92:143-152.

SAS Institute. 2003. Statistical analysis software, version 9.1. Cary, NC, USA.

Simmons, M. J. and J. F. Crow. 1977. Mutations affecting fitness in Drosophila populations. Annual Review of Genetics 11:49-78.

Soltis, D. E., P. S. Soltis, and J. A. Tate. 2004. Advances in the study of polyploidy since Plant Speciation. New Phytologist 161:173-191.

Stebbins, G. L. 1938. Cytological characteristics associated with the different growth habits in the dicotyledons. American Journal of Botany 25:189-198.

Stebbins, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York, NY, USA.

Stebbins, G. L. 1957. Self-fertilization and population variability in higher plants. American Naturalist 41:337-354.

Stebbins, G. L. 1980. Polyploidy in plants: unsolved problems and prospects in W. H. Lewis (ed.) Polyploidy: Biological Relevance. Plenum Press, New York.

Stebbins, G. L. 1984. Polyploidy and the distribution of the arctic alpine flora: new evidence and a new approach. Botanica Helvetica 94:1-13.

Sybenga, J. 1969. Allopolyploidization of autopolyploids I. possibilities and limitations. Euphytica 18:355-371.

Sybenga, J. 1996. Chromosome pairing affinity and quadrivalent formation in polyploids: do segmental allopolyploids exist? Genome 39:1176-1184.

Townsend, C. E., and E. E. Remmenga. 1968. Inbreeding in tetraploid alsike clover, Trifolium hybridum L. Crop Sci. 8:213-217.

Wright, S. 1931. Evolution in mendelian populations. Genetics 16:97-159.

CHAPTER 3

MATING SYSTEM AND PLOIDY INFLUENCE LEVELS OF INBREEDING DEPRESSION IN *CLARKIA* (ONAGRACEAE)

Abstract

Inbreeding depression is the reduction in offspring fitness associated with inbreeding and is one of the primary forces selecting against the evolution of self-fertilization. Studies suggest that most inbreeding depression is caused by the expression of recessive deleterious alleles in homozygotes whose frequency increases as a result of self-fertilization or mating among close relatives. This process leads to the selective elimination of deleterious alleles such that highly selfing species may show remarkably little inbreeding depression. Genome duplication (polyploidy) has been hypothesized to influence levels of inbreeding depression, with polyploids expected to exhibit less inbreeding depression than diploids. We studied levels of inbreeding depression in allotetraploid and diploid species of *Clarkia* (Onagraceae) that vary in mating system (each cytotype was represented by an outcrossing and a selfing species). The outcrossing species exhibited more inbreeding depression than the selfing species for most fitness components and for two different measures of cumulative fitness. In contrast, though inbreeding depression was generally lower for the polyploid species than for the diploid species, the difference was statistically significant only for flower number and one of the two measures of cumulative fitness. Further, we detected no significant interaction between mating system and ploidy in determining inbreeding depression. In sum, our results suggest that a taxon's current mating system is more important than ploidy in influencing levels of inbreeding depression in natural populations of these annual plants.

Introduction

Inbreeding depression is the reduction in fitness of inbred relative to non-inbred individuals and is thought to be one of the primary forces selecting against the evolution of self-fertilization (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Charlesworth and Charlesworth 1999; Husband and Schemske 1996, 1997; Carr and Dudash 2003). Although the genetic basis of inbreeding depression is complex, studies suggest that most inbreeding depression is caused by the unmasking of recessive (or partially recessive) deleterious alleles in homozygotes, whose frequency increases upon selfing or mating between relatives (i.e., the *partial dominance* model of inbreeding depression) (Charlesworth and Charlesworth 1987, Charlesworth and Charlesworth 1999; Carr and Dudash 2003). Other phenomena have been shown to influence levels of inbreeding depression, however, including a reduction in levels of heterozygosity (i.e., the *overdominance* model of inbreeding depression), reduced numbers of interactions among loci (i.e., epistasis), and the number and relative proportions of deleterious alleles with small vs. large effects on fitness (Charlesworth and Charlesworth 1987, Charlesworth and Charlesworth 1999; Carr and Dudash 2003). These sources of inbreeding depression are not mutually exclusive.

Interestingly, many plants that regularly self-fertilize exhibit very little inbreeding depression (Husband and Schemske 1996 and references therein). This seemingly paradoxical observation can be explained in the partial dominance model because the unmasking of recessive deleterious alleles leads to their selective elimination (purging). For this reason, inbreeding depression may be most severe during the initial generations that follow an increase in self-fertilization and decline over time (Darwin 1876; Lande and Schemske 1985; Husband and Schemske 1996; Charlesworth and Charlesworth 1990; Crnokrak and Barrett 2002; but see

Charlesworth et al. 1990; Byers and Waller 1999. Further, inbreeding depression may vary among different life stages, and the total amount of inbreeding depression suffered by an inbred individual is a product of reduced fitness at all stages of life. Therefore, in studies of inbreeding depression it is important to take into account the current mating system (e.g., Carr and Dudash 1996) and to compare the performance of inbred and outbred individuals throughout ontogeny (e.g., Husband and Schemske 1996).

It has been hypothesized, but rarely tested, that genome-wide changes in ploidy can also affect levels of inbreeding depression, with polyploids expected to exhibit less inbreeding depression than their diploid relatives (Lande and Schemske 1985; Ronfort 1999). The paucity of empirical tests of this hypothesis is surprising because predictions regarding inbreeding depression in diploids may not be applicable to polyploids, which have more than two copies of each gene. Furthermore, 30 to 80% of all flowering plants are thought to be polyploid (Stebbins 1938; Grant 1963; Goldblatt 1980; Masterson 1994; Otto and Whitton 2000), and polyploids often exhibit higher levels of self-fertilization than their diploid relatives (Stebbins 1950; Grant 1956, 1981; Soltis and Soltis 1987; Masuyama and Watano 1990; Soltis and Soltis 1990; Barringer 2007). Though several phenomena could contribute to the association between polyploidy and selfing (e.g., the breakdown of self-incompatibility systems [Mable 2004] and/or reproductive assurance [Rausch and Morgan 2005]), reduced inbreeding depression in polyploids relative to diploids may play a pivotal role. Finally, it is important in agricultural contexts to better understand the relationship between inbreeding depression and polyploidy because most crop species are polyploid (Stebbins 1950; Harlan and deWet 1975; Soltis and Soltis 2000), and the development of pure-breeding lineages frequently involves self-fertilization (Allard 1999).

Theoretical studies of the relationship between polyploidy and inbreeding depression have produced conflicting results. Bennett (1976) argued that a proportionally larger reduction in the number of positive interactions among alleles (i.e., heterosis) should lead to greater inbreeding depression in polyploids than in diploids. This conclusion, however, was based on the assumption that inbreeding depression is largely due to the genome-wide loss of heterozygosity that accompanies inbreeding (i.e., the *overdominance* model), and limited empirical support for this model exists (Dudash and Carr 1998; Charlesworth and Charlesworth 1999; Carr and Dudash 2003). In contrast, Lande and Schemske (1985) assumed that inbreeding depression is largely due to increased homozygosity and the associated expression of recessive and partially recessive deleterious alleles (i.e., the *partial dominance* model), and concluded that polyploids should generally exhibit less inbreeding depression than diploids. This conclusion was based on the prediction that, for a given selfing rate, levels of homozygosity in polyploids should increase more slowly than in diploids. For example, in each generation, homozygosity increases by 50% in a selfing diploid population compared to 17-21% in a selfing autotetraploid population (Haldane 1930; Wright 1938; Parsons 1959; Husband and Schemske 1997). Similarly, Ronfort (1999) assumed that overdominance plays a minor role in determining levels of inbreeding depression, however she found that the relationship between polyploidy and inbreeding depression is complex. Specifically, the severity of inbreeding depression depends strongly on the level of dominance of and on the strength of selection against deleterious alleles. If deleterious alleles are completely recessive, inbreeding depression in polyploids and diploids is not expected to differ. In contrast, if deleterious alleles are only partially recessive, polyploids tend to exhibit less inbreeding depression than diploids, though the reverse can be true depending on the strength of selection and the level of dominance among alleles (Ronfort 1999).

Finally, Otto and Whitton (2000) showed that, over time, polyploids can harbor greater mutational loads than diploids because a lower frequency of homozygosity leads to less effective purging in the former than the latter. Higher genetic loads should result in greater levels of inbreeding depression in polyploids relative to diploids.

Although theoretical models differ in several respects, there are at least two important characteristics shared by all of them. First, the models all assume that populations are at (or near) mutation-selection equilibrium and therefore of ancient derivation, and might not apply to newly formed polyploids (i.e., *neo*polyploids) (Pannell et al. 2004). Indeed, as Pannell and colleagues (2004) suggest, if the process of diploidization (wherein the cytogenetic behavior of a polyploid reverts back to that of a diploid owing to chromosomal rearrangements and gene silencing and/or loss) is relatively rapid (e.g., Song et al. 1995; Kashkush et al. 2002), polyploids may never reach mutation-selection equilibrium. Second, all models focus on *auto*polyploids that have homologous chromosomes and generally exhibit polysomic inheritance. To date, theoretical explorations of inbreeding depression in *allo*polyploids with homeologous chromosomes and disomic inheritance do not exist (Pannell et al. 2004; but see Lande and Schemske 1985), despite the fact that allopolyploids are common in nature (Grant 1981; Soltis et al. 2004).

The few empirical studies that have measured and compared levels of inbreeding depression in natural populations of polyploids and closely related diploids have produced discordant results. Husband and Schemske (1997) found lower levels of inbreeding depression in autotetraploid compared to diploid populations of *Chamerion angustifolium* (Onagraceae), as did Rosquist (2001) in allotetraploid vs. diploid species of *Anthericum* (Anthericaceae). In contrast, Johnston and Schoen (1996) found higher levels of inbreeding depression in allotetraploid than diploid

populations of *Amsinckia* (Boraginaceae). Accounting for these disparate results is complicated by the fact that the three taxa differ in mating system. The populations of *C. angustifolium* had a mixed mating system of outcrossing and selfing (Husband and Schemske 1997), the populations of *Anthericum* appeared to be largely outcrossing (Rosquist 2001), and the populations of *Amsinckia* ranged from highly outcrossing to highly selfing (Johnston and Schoen 1996). To date, no study has compared inbreeding depression in natural populations of diploids and allopolyploids of both mating systems.

We studied inbreeding depression in closely related diploid and allotetraploid species of *Clarkia* (Onagraceae), with each cytotype (diploid, allotetraploid) represented by a selfing and an outcrossing species. Using seed stock harvested from natural populations we generated self-fertilized and outcrossed half-siblings whose performance was then compared at multiple life stages to address the following questions: (1) Do outcrossing species exhibit higher levels of inbreeding depression than their selfing relatives? (2) Do diploid taxa exhibit higher levels of inbreeding depression than polyploid taxa? (3) Is there an interaction between ploidy and mating system in determining levels of inbreeding depression? In particular, do outcrossing diploids exhibit more inbreeding depression than outcrossing polyploids, and relative to outcrossers, is the difference between cytotypes in levels of inbreeding depression reduced among selfing taxa?

Methods

Study system

The genus *Clarkia* (Onagraceae) includes ~ 42 species and numerous subspecies of self-compatible winter annuals with a center of distribution in California, U.S.A. (Lewis and Lewis 1955). Although most species in the genus are

associated with well-drained soils in oak woodland and adjacent habitats, some taxa occur in chaparral or lower montane forest communities, and two species occur primarily on coastal dunes or bluffs (Lewis and Lewis 1955). Population sizes vary greatly depending on species and locality, but often contain many hundred to several thousand individuals (Lewis and Lewis 1955; Barringer, B. C. and M. A. Geber pers. obs.). Mating systems within the genus range from highly selfing to highly outcrossing, and can vary among subspecies and/or populations (Lewis and Lewis 1955; Vasek and Harding 1976; Holtsford and Ellstrand 1989, 1990; Runions and Geber 2000). Although all *Clarkia* are self-compatible, many taxa are protandrous and herkogamous, both of which facilitate outcrossing (Lewis and Lewis 1955; Runions and Geber 2000). Among outcrossers, pollination is effected primarily by specialist bees, though other insects, including generalist bees, butterflies, moths, and cyrtid flies serve as primary or secondary pollinators in some taxa (MacSwain et al. 1973; Moeller 2005). Selfing taxa, in contrast, generally lack significant protandry and herkogamy, and self-fertilization often occurs autogamously (Lewis and Lewis 1955). Finally, the genus is cytogenetically variable, and includes diploid, allotetraploid, and allohexaploid taxa. Although most of the polyploid species are highly selfing, several are outcrossing (Lewis and Lewis 1955).

We used two pairs of species of *Clarkia*, belonging to two separate sections of the genus (Lewis and Lewis 1955; Gottlieb and Ford 1996). Each pair consisted of an allotetraploid and a diploid. In the first pair (section Godetia), the tetraploid *C. davyi* is selfing and the diploid *C. williamsonii* is outcrossing; the diploid parental species of *C. davyi* within section Godetia have not been identified (Small et al. 1971). In the second pair (section Rhodanthus), the tetraploid *C. gracilis* ssp. *sonomensis* is outcrossing and the diploid *C. lassenensis* is selfing; *C. lassenensis* and *C. amoena* (a species not included in this study) are the diploid parental species of *C. gracilis* (Small

et al. 1971). Appendix Table 3.1 includes section affiliations, population locations, population sizes, base chromosome numbers, and mating systems for each focal species.

The selfing species are smaller flowered than the outcrossing species (petal size in *C. davyi*, 5-11 mm; *C. lassenensis*, 8-16 mm; *C. williamsonii*, 10-30 mm; *C. gracilis* ssp. *sonomensis*, 8-40 mm [Lewis and Lewis 1955; Hickman 1993]). *Clarkia davyi* is found on coastal dunes and sea bluffs in Northern California. *Clarkia gracilis* ssp. *sonomensis* is found in open sites in the Coastal Mountain Range of Northern California and Southern Oregon. *Clarkia lassenensis* is found in open sites in oak woodland and coniferous forest in the interior of Northern California and in parts of Nevada and Oregon. Finally, *C. williamsonii* is found in open sites in oak and coniferous forests in the foothills of the Sierra Nevada Mountain Range of Northern and Central California.

Seed collection

Seeds were harvested from two natural populations of each species in August 2005, however one population of *C. williamsonii* was misidentified in the field and was subsequently dropped from the study. In each population, two mature fruits were collected from each of ~ 50 maternal plants located along transects in the center and along the periphery of the site. With this collection method we were able to obtain a representative sample of genetic variation from a population and avoid collecting from neighboring plants that could be close relatives. Fruits were placed in coin envelopes (one maternal family per envelope) and stored with desiccant at 5 °C until used.

Production of selfed and outcrossed half-siblings

Inbreeding depression is estimated by comparing fitness components and

cumulative fitness between self-fertilized and outcrossed half-siblings (Charlesworth and Charlesworth 1987; Johnston and Schoen 1994). To this end, we first generated outbred families for each population. One adult plant was raised from field-collected seed from each maternal plant, and randomly paired and reciprocally crossed to an adult plant from a different mother (generation one). In generation two, one adult from each of the outcrossed families produced in generation one was raised to flowering, and flowers on each plant were selfed and outcrossed to produce inbred and outbred half-siblings. We recorded the number and weight of seeds produced from all crosses. During the third and final generation, we recorded seed germination rate, survival of seedlings to flowering, flower number, and final plant biomass for inbred and outbred half-siblings. We also recorded three phenological traits: the number of days to germination, first flowering, and senescence. Finally, flowers on one inbred and one outbred individual in ~12 randomly selected families per population were selfed and outcrossed to provide estimates of seed set under both crossing regimes. The experimental design is illustrated in Figure 3.1.

Generation one: For each maternal field plant, three seeds were haphazardly selected and sown into a single 164 ml Ray Leach "Cone-tainer" (Stuewe and Sons, Inc., Corvallis, OR, U.S.A.) filled with a 1:1 ratio of a peat-based potting soil and fritted clay. The containers were arranged in racks in a growth chamber (12 hr days at 10 °C and 12 hr nights at 7 °C) and watered lightly from overhead twice/day. Germination was close to 100%. One seedling in each container was then randomly selected and the others were removed. Fourteen days after emergence the seedlings were moved to the greenhouse (12 hr days at 27 °C and 12 hr nights at 16 °C) for the duration of their life cycle. In the greenhouse, plants were watered from overhead

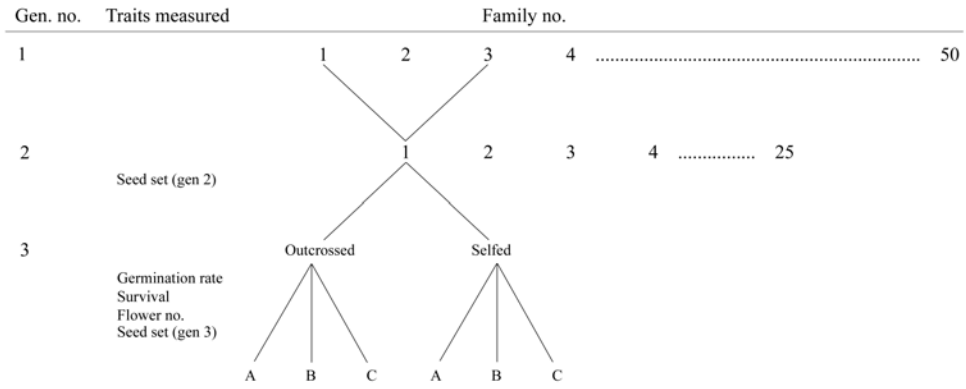

Figure 3.1. Experimental (crossing) design. Adult plants were raised from field-collected seed and randomly paired and crossed to create generation two. Plants in generation two were then used to generate inbred and outbred half-siblings (generation three), whose performance was compared to quantify inbreeding depression. See text for details.

once/day, with supplemental fertilizer (21:5:20 N:P:K @ 200 ppm) added to the water once every seven days. After flowering, adult plants were randomly paired to create families, and reciprocal crosses within pairs (two flowers per individual for a total of four flowers per pair) were performed to produce seed stock for generation two. All flowers were emasculated prior to stigma maturation to prevent self-fertilization. Forceps were used to remove two anthers from the pollen donor. The anthers were then applied directly to the stigma of a flower on the pollen recipient to saturate the stigmatic surface with pollen. Forceps were cleaned with ethanol between crosses to prevent contamination. Immediately following pollination a small amount of non-toxic fabric paint was applied to the pedicel of the flower to mark the resulting fruit. Plants were then allowed to mature and senesce naturally, and mature fruits were harvested, placed in coin envelopes (one family per envelope), and stored with desiccant at 5 °C until used.

Generation two: We used the same cultural practices to raise one adult plant per family in the second generation. Flowers on each adult plant were then selfed and

outcrossed. The third and fourth flowers to open on the main stem were used in crosses. The order of crosses (self vs. outcross) was randomly determined for each individual, and hand-pollinations were conducted as in generation one, except that selfed flowers were pollinated with pollen from the same flower or from another flower on the same individual. Mature fruits were harvested and their seeds were counted and weighed. All seeds were then placed in coin envelopes (one family per envelope), and stored with desiccant at 5 °C until used.

Generation three: For each family and cross-type (selfed vs. outcrossed), nine seeds were haphazardly selected, and three seeds were sown in each of three containers, and germinated as described for the previous two generations. Germination rate (% germination) and the number of days to germination were recorded. Containers were then thinned to one individual. Unless germination failed completely in a container, each family was represented by three selfed and three outcrossed half-siblings. Inbred and outbred half-siblings were placed together on racks (two families per rack) to minimize environmental differences experienced by selfed and outcrossed siblings. Cultural practices followed the same procedures as described for the previous two generations. Survival to flowering, the number of days from germination to flowering, and total flower number were recorded. To estimate differences between selfed and outcrossed half-siblings in their ability to produce seeds, we selfed and outcrossed flowers on one surviving inbred and outbred plant from 12 randomly selected families in each population. Outcrossed pollinations were conducted using pollen obtained from a randomly selected outcrossed individual from another family (i.e., inbred individuals did not serve as pollen donors). The date of senescence was recorded for all plants. We also counted and weighed seeds from hand-pollinated selfed and outcrossed fruits. Finally, we harvested the above-ground biomass of plants, including all fruits and seeds produced autonomously or from hand

pollinations, dried plants at 50 °C for 72 hours, and measured and recorded dry weights.

Estimation of inbreeding depression

Inbreeding depression, δ, is traditionally measured as $1 – (w_s/w_o)$, where w_s and w_o are the mean phenotypic values of inbred and outcrossed individuals, respectively (e.g., Johnston and Schoen 1996; Husband and Schemske 1997; Galloway et al. 2003; Galloway and Etterson 2007). This method, however, does not yield a symmetrical distribution of δ around zero when inbred individuals outperform their outcrossed relatives (i.e., outbreeding depression), as was observed in some cases in our experiment. Therefore, we compared the fitness of inbred and outcrossed individuals for each trait and family using a measure of relative performance (RP) defined as $RP = 1 – (w_s/w_o)$ when $w_s \leq w_o$, and $RP = (w_o/ w_s) – 1$ when $w_s > w_o$ (cf. Agren and Schemske 1993; Dudash et al. 1997). This method gives estimates of relative performance that are identical to traditional estimates of inbreeding depression when outcrossed progeny have higher fitness than their inbred relatives, however it applies equal weight to outcomes in which inbred individuals outperform their outcrossed relatives (Agren and Schemske 1993; Dudash et al. 1997).

We estimated RP for all fitness components: seed number (generation two), germination rate, survival to flowering, flower number, and seed number for hand-pollinated fruits (generation three). Because successful seed production through outcrossing vs. selfing can depend on the species' mating system (e.g., outcrossing taxa may set fewer seed from self- compared to outcross pollinations, whereas crosstype may have little effect in selfing taxa), we estimated the RP of seed set in generation three using three methods: (1) seed number from outcrossed flowers, (2) seed number from selfed flowers, and (3) seed number from outcross pollinations in

outcrossing taxa and seed number from self-pollinations in selfing taxa. We did not evaluate RP for seed weight in generation two because it was highly positively correlated with seed number across inbred and outbred families in all taxa (Pearson product moment correlation r = 0.76 – 0.93). Likewise, we did not evaluate the RP for plant biomass because it was highly positively correlated with flower number in generation three (Pearson product moment correlation r = 0.61 – 0.94).

Finally, we compared the mean number of days to germination, first flowering, and senescence between inbred and outbred half-siblings to determine whether inbreeding consistently advanced or delayed plant phenology.

Estimation of cumulative fitness

We estimated lifetime fitness in two ways (Figure 3.2). First, cumulative fitness was calculated as the product of germination rate * survival * flower number * seed set (generation 3, outcrosses) (method 1). However, because hand-pollinations were only performed during generation three on a subset of 12 families per population, fewer than half of all families (77 of 176) could be used in this estimate. We therefore calculated a second measure of cumulative fitness for each family as the product of seed set (generation 2) * germination rate * survival * flower number (method 2). Our second estimate of cumulative fitness included data for all 176 families.

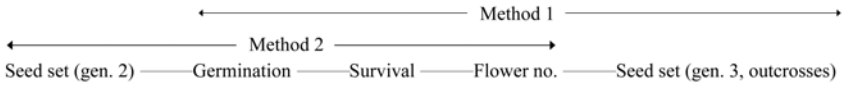

Figure 3.2. Components of fitness included in the two estimates of cumulative fitness.

Statistical analyses

For all fitness components except survival to flowering, and for both measures of cumulative fitness, the relative performance of inbred and outbred half-siblings was compared in a mixed-model ANOVA (Proc mixed in SAS, 2003). Ploidy (diploid or polyploid), mating system (selfing or outcrossing), and their interaction were treated as fixed effects and population as a random effect nested within ploidy and mating system. Denominator degrees of freedom were determined using the Kenward-Roger method. Because survivorship data were highly leptokurtic and did not meet assumptions of normality or equal variance, they were analyzed with a multinomial logistic regression (Proc glimmix in SAS, 2003) using three categorical levels: 1 if outcrossed individuals survived better than selfed individuals (i.e., inbreeding depression), -1 if selfed individuals survived better than outcrossed individuals (i.e., outbreeding depression), and zero if selfed and outcrossed individuals survived equally well.

Phenological data were analyzed by comparing the means for each species and trait (number of days to germination, first flowering, and senescence) using paired t-tests (Proc ttest in SAS, 2003).

Results

Levels of inbreeding depression varied among fitness components and taxa and depended on both mating system and ploidy (Figure 3.3 and Tables 3.1-3.2). Inbreeding depression was significantly lower in the selfing species (*C. lassenensis* and *C. davyi*) than in the outcrossing species (*C. williamsonii* and *C. gracilis* ssp. *sonomensis*) for all fitness components except survival to flowering, and was also lower in the selfers for the second measure of cumulative fitness. Inbreeding depression in the first measure of cumulative fitness was lower in the selfers than in

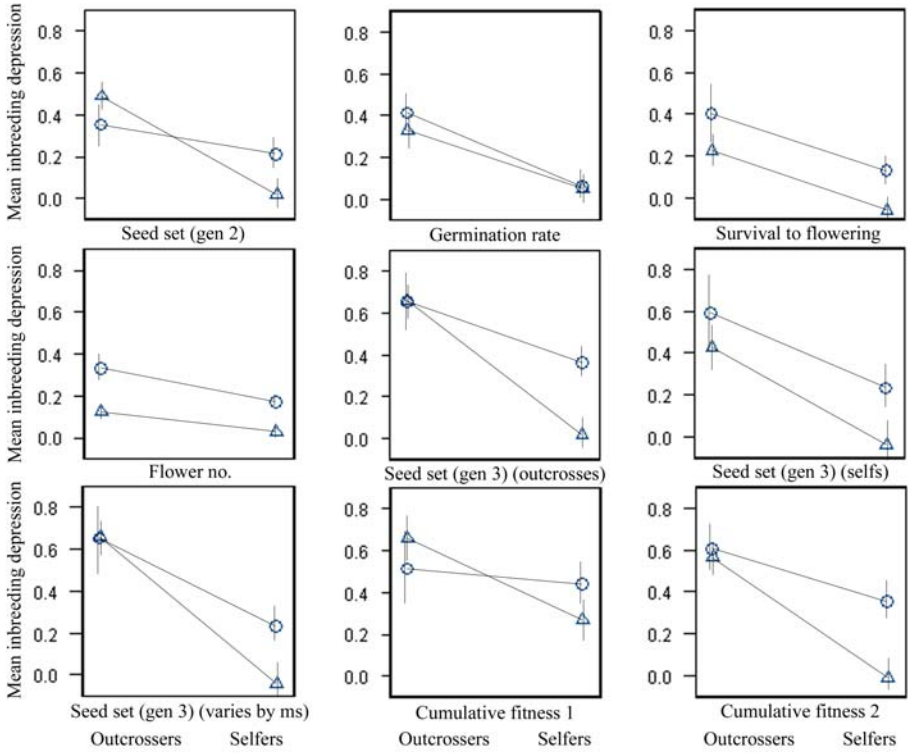

Figure 3.3. Estimated mean levels of inbreeding depression (+/- 1 standard error) for traits correlated with fitness in *Clarkia*. All values are least-squares means except those for survival to flower, which are arithmetic means. Circles represent diploids and triangles represent polyploids.

Table 3.1 Denominator degrees of freedom (d.f.) and F-values testing for the effects of ploidy, mating system (MS), and their interaction on levels of inbreeding depression for fitness components and cumulative fitness in *Clarkia*. Number superscripts indicate components that were used for estimating cumulative fitnesses 1 and 2, respectively. Mixed-model analysis of variance was used for all analyses except survival to flower, which was analyzed using a multinomial logistic regression. Values in bold are significant at $\alpha = 0.05$ and values in italics are significant at $\alpha = 0.08$.

Analysis	d.f.$^{+}_{den.}$	Ploidy	MS	Ploidy*MS
Seed set [2]	3.01	0.13	12.74	3.70
Germination rate [1,2]	143	0.33	16.29	0.20
Survival to flowering [1,2]	1	4.13	12.05	0.18
Flower no [1,2]	104	23.62	14.29	0.73
Seed set (outcrosses) [1]	73	*3.15*	26.54	*3.79*
Seed set (selfs)	5.27	2.90	10.97	0.25
Seed set (varies by ms)	4.11	1.48	27.20	1.85
Cumulative fitness 1	73	0.00	*3.31*	1.58
Cumulative fitness 2	172	4.72	21.75	*3.50*

+ num. d.f. equals 1 for all analyses.

Table 3.2 Estimated mean (standard error) levels of inbreeding depression for fitness components and cumulative fitness in the diploid outcrossing (D/O), diploid selfing (D/S), polyploid outcrossing (P/O), and polyploid selfing (P/S) species of *Clarkia*. All values are least-squares means except those for survival to flower, which are arithmetic means. Number superscripts indicate components that were used for estimating cumulative fitnesses 1 and 2, respectively. Different letter superscripts within rows indicate means that differ significantly (Tukey-Kramer honestly significant difference, at $\alpha = 0.05$).

Analysis	D/O	D/S	P/O	P/S
Seed set [2]	0.35 (0.10)[a]	0.21 (0.08)[a]	0.49 (0.07)[a]	0.02 (0.08)[a]
Germ. rate [1,2]	0.41 (0.09)[a]	0.06 (0.07)[b]	0.33 (0.08)[a]	0.05 (0.07)[b]
Surv. to flw. [1,2]	0.40 (0.13)[a]	0.13 (0.06)[a]	0.23 (0.07)[a]	-0.06 (0.04)[a]
Flower no. [1,2]	0.33 (0.05)[a]	0.17 (0.02)[b]	0.13 (0.03)[b]	0.03 (0.02)[c]
Seed set (outcrosses) [1]	0.65 (0.13)[a,b,c]	0.36 (0.07)[b]	0.66 (0.08)[c]	0.02 (0.07)[d]
Seed set (selfs)	0.59 (0.18)[a]	0.23 (0.10)[a]	0.43 (0.11)[a]	-0.04 (0.10)[a]
Seed set (varies by ms)	0.65 (0.15)[a]	0.23 (0.09)[a,b]	0.66 (0.09)[a]	-0.04 (0.09)[b]
Cumulative fitness 1	0.51 (0.18)[a,b]	0.44 (0.10)[a,b]	0.66 (0.11)[a]	0.27 (0.10)[b]
Cumulative fitness 2	0.60 (0.11)[a]	0.35 (0.09)[a]	0.57 (0.08)[a]	-0.01 (0.08)[b]

the outcrossers but the difference only approached statistical significance ($P < 0.08$, Table 3.1). Similarly, inbreeding depression was generally lower for the polyploid species (*C. davyi* and *C. gracilis* ssp. *sonomensis*) than for the diploid species (*C. lassenensis* and *C. williamsonii*), though the difference was statistically significant only for flower number and the second measure of cumulative fitness, and approached significance for the number of seeds produced by outcrossed hand-pollinated flowers ($P < 0.08$, Table 3.1).

There was no statistically significant interaction between mating system and ploidy for any fitness measure, although the interaction approached significance for the number of seeds produced by outcrossed hand-pollinated flowers and the second measure of cumulative fitness (Table 3.1). However, even in these two cases, the interaction was not the result of the diploid outcrosser having greater inbreeding depression than the polyploid outcrosser. Rather, the diploid selfer had greater inbreeding depression than the polyploid selfer (Figure 3.3).

The number of days to germination was not affected by inbreeding for any of the species included in this study (Appendix table 3.2). In contrast, inbreeding affected the timing of flowering and the timing of senescence for some taxa, depending on the level of ploidy and the mating system of the species in question. Inbred individuals flowered earlier than non-inbred individuals in the outcrossing species (*C. williamsonii* and *C. gracilis* ssp. s*onomensis*) but not in the selfing species (*C. lassenensis* and *C. davyi*). Similarly, inbred individuals senesced earlier than non-inbred individuals in both of the outcrossing species and the diploid selfing species (*C. lassenensis*). In contrast, inbred and non-inbred individuals did not differ in the number of days to senescence in the polyploid selfer (*C. davyi*).

Discussion

Relatively low levels of inbreeding depression are expected among taxa that regularly self-fertilize because self-fertilization increases genome-wide levels of homozygosity and results in the purging of deleterious alleles (Darwin 1876; Lande and Schemske 1985; Husband and Schemske 1996; Charlesworth and Charlesworth 1990; Crnokrak and Barrett 2002; but see Charlesworth et al. 1990; Byers and Waller 1999). Our results support this prediction, as the outcrossing species exhibited significantly more inbreeding depression than the selfing species for all fitness components except survival to flowering. Although the relationship between polyploidy and inbreeding depression is complex and may differ for neo- vs. ancient polyploids, most studies suggest that polyploids should exhibit less inbreeding depression than diploids (e.g., Lande and Schemske 1985; Ronfort 1999; but see Otto and Whitton 2000). Our results provide some limited support for this prediction: inbreeding depression was generally lower for the polyploid species than for the diploid species, although the difference was statistically significant only for flower number and the second measure of cumulative fitness, and approached significance for the number of seeds produced by outcrossed hand-pollinated flowers.

Self-compatible species that normally reproduce via outcrossing may have reduced seed set after artificial self-pollination because of cryptic self-incompatibility (e.g., Jones 1994; Eckert and Allen 1997) and/or early-acting inbreeding depression (Charlesworth and Charlesworth 1987). Similarly, species that normally reproduce via self-fertilization may have reduced seed set after artificial outcross pollination because of outbreeding depression (e.g. Parker 1992). To evaluate differences in seed production due to interactions between mating system and cross type, we quantified levels of inbreeding depression using three measures of seed set (seed number from outcrossed flowers on all taxa, seed number from selfed flowers on all taxa, and seed

number from outcross pollinations in outcrossing taxa and self-pollinations in selfing taxa). All three measures indicate significant effects of mating system on levels of inbreeding depression for the ability to set seed. In contrast, neither ploidy nor the interaction between ploidy and mating system are significant for any of the three analyses (though they both approach significance for the estimate of seed set based on outcrosses). These results suggest that inbreeding depression for seed set does not depend on the interaction of mating system and cross type, and that the ability to set seed is reduced among inbred individuals regardless of how seed set is measured.

Levels of inbreeding depression for survival to flowering were not affected by ploidy, mating system, or their interaction. Indeed, levels of inbreeding depression for this trait were not significantly different from zero for most families. It is worth noting that our measure of survival only includes that portion of ontogeny between germination and the onset of flowering. It could be that survival is determined earlier in life (i.e., before and/or during germination), and once an individual successfully germinates it is likely to survive to flower. To the extent that this is true, a better estimate of survival might be germination rate. Survival may also have been high in our study because of the benign (if not optimal) conditions in the greenhouse. Inbreeding depression can be more severe in natural settings (Jimenez et al. 1994; Crnokrak and Roff 1999; Armbruster and Reed 2005; but see Armbruster et al. 2000).

Interestingly, the interaction between mating system and ploidy in determining levels of inbreeding depression was not significant for any of the fitness traits measured. Further, though the effect of the interaction approached statistical significance for seed set (outcrossed) and the second measure of cumulative fitness, it was not because the outcrossing diploid exhibited more inbreeding depression than the outcrossing polyploid, as predicted by most models that compare inbreeding depression in diploids and polyploids (e.g., Lande and Schemske 1985; Ronfort 1999).

Rather, the selfing diploid exhibited more inbreeding depression than the selfing polyploid.

Because inbreeding depression may vary among different ontogenetic stages, (Schemske 1983; Schoen 1983; Charlesworth and Charlesworth 1987; Husband and Schemske 1995, 1996), the best metric with which to compare the effects of inbreeding is cumulative fitness. We found that neither ploidy nor mating system (nor their interaction) significantly affected levels of inbreeding depression for our first measure of cumulative fitness (though the effect of mating system approached statistical significance). However, both ploidy and mating system had significant effects on levels of inbreeding depression for our second measure of cumulative fitness (and the effect of the interaction between ploidy and mating system approached significance as well). The different results from the two analyses are probably due to differences in their sample sizes. Because we measured seed set for only a subset of all families, our first measure of cumulative fitness was based on a much smaller sample size than our second (77 families vs. 176 families, respectively).

Though phenology was not affected by inbreeding for any species during the earliest life stage (days to germination), inbreeding tended to advance the timing of phenological changes during later life stages (days to first flower and days to senescence), especially in the two outcrossing species (*C. williamsonii* and *C. gracilis* ssp. s*onomensis*). Interestingly, these results are generally in conflict with those found in other studies, where inbreeding resulted in delayed germination and/or flowering (e.g., Willis 1996; Shaw et al. 1998; Rao et al. 2002; Galloway et al. 2003; Ellmer and Andersson 2004; Galloway and Etterson 2007). Of the four taxa in this study, the polyploid selfer (*C. davyi*) showed no change in phenology in response to inbreeding.

Conclusions

Overall, the results presented here suggest that a taxon's mating system is more important than ploidy in terms of influencing levels of inbreeding depression. These results are consistent with expectations in several ways: first, a rich body of both theoretical and empirical work suggests that a species' mating system can have profound effects on levels of inbreeding depression (Lande and Schemske 1985; Campbell 1986; Charlesworth and Charlesworth 1987; Charlesworth and Charlesworth 1999; Charlesworth et al. 1990; Lande et al. 1994; Carr and Dudash 1996, 2003). Second, though some studies suggest that polyploids might exhibit less inbreeding depression than diploids, most empirical and all theoretical studies have concentrated on expectations of inbreeding depression in autopolyploids (Lande and Schemske 1985; Husband and Schemske 1997; Ronfort 1999). To date, formal theoretical explorations of inbreeding depression in allopolyploids do not exist (Pannell et al. 2004; but see Lande and Schemske 1985), and empirical explorations have produced conflicting results (e.g., Johnston and Schoen 1996; Rosquist 2001). It could be that diploids and allopolyploids do not generally differ in regard to inbreeding depression (Lande and Schemske 1985). In addition, although younger polyploid taxa may exhibit reduced inbreeding depression relative to diploids, the opposite may be true of older, more established polyploids (such as those included in this study). Older polyploids, whose populations are at (or near) mutation-selection equilibrium, are expected to harbor greater genetic loads than comparable diploids (Otto and Whitton 2000), and this may lead to higher levels of inbreeding depression in the former relative to the latter (Ronfort 1999; Pannell et al. 2004). Further, as diploidization takes place over time, polyploids begin to behave (cytogenetically) as diploids. The polyploids used in this study are relatively old and may harbor substantial genetic loads and/or be partially or completely diploidized.

Though several studies have demonstrated that both mating systems and ploidy can influence levels of inbreeding depression (e.g., Carr and Dudash 1996; Husband and Schemske 1997), many questions remain to be answered. Polyploids are often categorized as either auto- or allopolyploids, suggesting they contain either multiple sets of homologous chromosomes (leading to multisomic inheritance patterns), or multiple sets of nonhomologous chromosomes (leading to disomic inheritance patterns), respectively. Yet these conditions represent the extremes of what is likely a continuum, and many polyploids exhibit multisomic inheritance at some loci and disomic inheritance at others (Sybenga 1969; Grant 1981; Jackson 1982; Jenkins and Rees 1991; Comai 2000; but see Sybenga 1996); so-called segmental allopolyploids (Stebbins 1950). To the extent that this is true, models that incorporate variation among loci in terms of inheritance patterns will be of interest, and empirical studies that address levels of inbreeding depression in segmental allopolyploids will be of value. In addition, existing theoretical studies have modeled levels of inbreeding depression in populations that are at (or near) mutation-selection equilibrium. However, inbreeding depression in neopolyploids is also of interest because high levels of self-fertilization might facilitate the initial establishment of polyploid populations, and levels of inbreeding depression suffered by newly formed polyploids will influence the evolution of mating systems immediately following genome duplication. For this reason, models that explore and predict levels of inbreeding depression in neopolyploids are needed. Given that the majority of plant taxa are likely to have experienced one or more rounds of genome duplication at some point during their evolutionary history (Soltis et al. 2003), such studies will continue to contribute to our understanding of the evolution of plants and their mating systems.

APPENDIX

Appendix Table 3.1 *Clarkia* species and populations used in this study.

Species	Section	Population Number	Population Location	Approximate Population Size (# Individuals)	Base chromosome Number	Mating System
C. davyi	Godetia	1	Marin County; Point Reyes National Seashore; Sir Francis Drake Blvd at North Beach Road	1000	17	Selfing
C. davyi	Godetia	2	Marin County; Hwy 1 at Olema-Bolinas Road	900	17	Selfing
C. williamsonii	Godetia	1	Madera County; Hwy 41; 0.1 m N of mp 30.5	2200	9	Outcrossing
C. gracilis ssp. *sonomensis*	Rhodanthos	1	Mendocino County; Old River Road, 4.9 m N of Hwy 175	3100	14	Outcrossing
C. gracilis ssp. *sonomensis*	Rhodanthos	2	Sonoma County; Hwy 128; 0.4 m W of mp 18.17	2800	14	Outcrossing
C. lassenensis	Rhodanthos	1	Shasta County; Hwy 299; 0.5 m W of Hwy 89	2700	7	Selfing
C. lassenensis	Rhodanthos	2	Shasta County; Hwy 299; 1.7 m E of Hwy 89	1800	7	Selfing

Appendix Table 3.2 Mean numbers of days (standard deviation) to germination, first flowering, and senescence for each species. For a given trait, ploidy, and mating system (MS), the P-value refers to the probability that selfed (Self) and outcrossed (Out) individuals do not differ.

Trait	Ploidy	MS	Self	Out	P
# Days to germ.	Diploid	O	7.0 (2.6)	6.7 (2.6)	0.5204
	Diploid	S	8.4 (2.9)	8.3 (2.9)	0.6588
	Polyploid	O	5.8 (2.4)	5.8 (2.4)	0.8684
	Polyploid	S	7.2 (2.3)	7.2 (2.7)	0.7958
# Days to flow.	Diploid	O	92.9 (8.1)	98.5 (6.9)	0.0109
	Diploid	S	85.4 (6.4)	85.5 (5.7)	0.9144
	Polyploid	O	88.4 (5.5)	92.0 (6.1)	0.0007
	Polyploid	S	88.6 (4.5)	89.6 (4.1)	0.0703
# Days to senesce.	Diploid	O	143.6 (5.5)	149.8 (3.9)	1.0e-5
	Diploid	S	134.2 (3.3)	137.0 (2.9)	3.7e-9
	Polyploid	O	132.6 (3.7)	135.9 (3.4)	1.5e-7
	Polyploid	S	138.9 (5.7)	139.7 (5.3)	0.1972

LITERATURE CITED

Agren, J. and D. W. Schemske. 1993. Outcrossing rate and inbreeding depression in two annual monoecious herbs, Begonia hirsuta and B. semiovata. Evolution 47:125-135.

Allard, R. W. 1999. Principles of Plant Breeding. John Wiley and Sons, Inc., New York, NY.

Armbruster, P., R. A. Hutchinson, and T. Linvell. 2000. Equivalent inbreeding depression under laboratory and field conditions in a tree-hole-breeding mosquito. Proc. R. Soc. Lond. B Biol. Sci. 267:1939-1946.

Armbruster, P. and D. H. Reed. 2005. Inbreeding depression in benign and stressful environments. Heredity 95:235-242.

Barringer, B. C. 2007. Polyploidy and self-fertilization in flowering plants. Am. J. Bot. 94:1527-1533.

Bennett, J. H. 1976. Expectations for inbreeding depression on self-fertilization of tetraploids. Biometrics 32:449-452.

Byers, D. L. and D. M. Waller. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Ann. Rev. Ecol. Syst. 30:479-513.

Campbell, R. B. 1986. The interdependence of mating structure and inbreeding depression. Theor. Popul. Biol. 30:232-244.

Carr, D. E. and M. R. Dudash. 1996. Inbreeding depression in two species of Mimulus (Scrophulariaceae) with contrasting mating systems. Am. J. Bot. 83:586-593.

Carr, D. E. and M. R. Dudash. 2003. Recent approaches into the genetic basis of inbreeding depression in plants. Philos. Trans. R. Soc. Lond. B Biol. Sci. 58:1071-1084.

Charlesworth, B., M. T. Morgan, and D. Charlesworth. 1990. Genetic loads and estimates of mutation rates in highly inbred plant populations. Nature 347:380-382.

Charlesworth, B. and D. Charlesworth. 1999. The genetic basis of inbreeding depression. Genet. Res. 74:329-340.

Charlesworth, D. and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Ann. Rev. Ecol. Syst. 18:237-268.

Charlesworth, D. and B. Charlesworth. 1990. Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. Evolution 44:870-888.

Comai, L. 2000. Genetic and epigenetic interactions in allopolyploid plants. Plant Mol. Biol. 43:387-399.

Crnokrak, P. and S. C. H. Barrett. 2002. Perspective: Purging the genetic load: A review of the experimental evidence. Evolution 56:2347-2358.

Crnokrak, P. and D. A. Roff. 1999. Inbreeding depression in the wild. Heredity 83:260-270.

Darwin, C. 1876. The effects of cross and self-fertilization in the vegetable kingdom. John Murray, London.

Dudash, M. R. and D. E. Carr. 1998. Genetics underlying inbreeding depression in Mimulus with contrasting mating systems. Nature 393:682-684.

Dudash, M. R., D. E. Carr, and C. B. Fenster. 1997. Five generations of enforced selfing and outcrossing in Mimulus guttatus: inbreeding depression variation at the population and family level. Evolution 51:54-65.

Eckert, C. G. and M. Allen. 1997. Cryptic self-incompatibility in tristylous Decodon verticillatus (Lythraceae). Am. J. Bot. 84:1391-1397.

Ellmer, M. and S. Andersson. 2004. Inbreeding depression in Nigella degenii (Ranunculaceae): fitness components compared with morphological and phenological characters. Int. J. Plant Sci. 165:1055-1061.

Galloway, L. F. and J. R. Etterson. 2007. Inbreeding depression in an autotetraploid herb: a three generation field study. New Phytol. 173:383-392.

Galloway, L. F., J. R. Etterson, and J. L. Hamrick. 2003. Outcrossing rate and inbreeding depression in the herbaceous autotetraploid, Campanula Americana. Heredity 90:308-315.

Goldblatt, P. 1980. Polyploidy in angiosperms: Monocotyledons. In Lewis, W. H. (ed.) Polyploidy: Biological relevance. Plenum Press, New York.

Gottlieb, L. D. and V. S. Ford. 1996. Phylogenetic relationships among the sections of Clarkia (Onagraceae) inferred from the nucleotide sequences of PgiC. Syst. Bot. 21:45-62.

Grant, V. 1956. The influence of breeding habit on the outcome of natural hybridization in plants. Am. Nat. 90:319-322.

Grant, V. 1963. The origin of adaptations. Columbia University Press, New York.

Grant, V. 1981. Plant Speciation. Columbia University Press, New York, NY.

Haldane, J. B. S. 1930. Theoretical genetics of autopolyploids. J. Genet. 22:359-372.

Hamilton, W. D. 1966. The moulding of senescence by natural selection. J. Theor. Biol. 12:12-45.

Harlan, J. R. and J. M. J. deWet. 1975. On O. Winge and a prayer: the origins of polyploidy. Bot. Rev. 41:361-389.

Hickman, J. C. (ed.). 1993. The Jepson Manual: Higher plants of California. University of California Press. Berkeley, CA.

Holtsford, T. P. and N. C. Ellstrand. 1989. Variation in outcrossing rate and population genetic structure of Clarkia tembloriensis (Onagraceae). Theor. Appl. Genet. 78:480-488.

Holtsford, T. P. and N. C. Ellstrand. 1990. Inbreeding effects in Clarkia tembloriensis (Onagraceae) populations with different natural outcrossing rates. Evolution 44:2031-2046.

Husband, B. C. and D. W. Schemske. 1995. Magnitude and timing of inbreeding depression in a diploid population of Epilobium angustifolium (Onagraceae). Heredity 75:206-215.

Husband, B. C. and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54-70.

Husband, B. C. and D. W. Schemske. 1997. The effect of inbreeding in diploid and tetraploid populations of Epilobium angustifolium (Onagraceae): Implications for the genetic basis of inbreeding depression. Evolution 51:737-746.

Jackson, R. C. 1982. Polyploidy and diploidy: new perspectives on chromosome pairing and its evolutionary implications. Am. J. Bot 69:1512-1523.

Jenkins, G. and H. Rees. 1991. Strategies of bivalent formation in allopolyploid plants. Proc. R. Soc. Lond. B Biol. Sci. 243:209-214.

Jimenez, J. A., K. A. Hughes, G. Alaks, L. Graham, and R. C. Lacy. 1994. An experimental study of inbreeding depression in a natural habitat. Science 266:271-273.

Johnston, M. O. and D. J. Schoen. 1994. On the measurement of inbreeding depression. Evolution 48:1735-1741.

Johnston, M. O. and D. J. Schoen. 1996. Correlated Evolution of self-fertilization and inbreeding depression: An experimental study of nine populations of Amsinckia (Boraginaceae). Evolution 50:1478-1491.

Jones, K. N. 1994. Nonrandom mating in Clarkia gracilis (Onagraceae): a case of cryptic self-incompatibility. Am. J. Bot. 81:195-198.

Kashkush, K., M. Feldman, and A. A. Levy. 2002. Gene loss, silencing, and activation in a newly-synthesized wheat allotetraploid. Genetics 160:1651-1659.

Lande, R. and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39:24-40.

Lande, R., D. W. Schemske, and S. T. Schultz. 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. Evolution 48:965-978.

Lewis, H. and M. E. Lewis. 1955. The genus Clarkia. University of California Press, Berkeley, California.

Mable, B. K. 2004. Polyploidy and self-compatibility: Is there an association? New Phytol. 162:803-811.

MacSwain, J. W., P. H. Raven, and R. W. Thorp. 1973. Comparative behavior of bees and Onagraceae. IV. Clarkia bees of the western United States. Univ. Calif. Publ. Entomol. 70:1-80.

Masuyama, S. and Y. Watano. 1990. Trends for inbreeding in polyploid pteridophytes. Plant Species Biol. 5:13-18.

Masterson, J. 1994. Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. Science 264:421-423.

Moeller, D. A. 2005. Pollinator community structure and sources of spatial variation in plant-pollinator interactions in Clarkia xantiana ssp. xantiana. Oecologia 142:28-37.

Otto, S. P. and J. Whitton. 2000. Polyploid incidence and evolution. Annu. Rev. Genet. 34:401-437.

Pannell, J. R., D. J. Obbard, and R. J. A. Buggs. 2004. Polyploidy and the sexual system: What can we learn from Mercurialis annua? Biol. J. Linn. Soc. 82:547-560.

Parker, M. A. 1992. Outbreeding depression in a selfing annual. Evolution 46:837-841.

Parsons, P. A. 1959. Some problems in inbreeding and random mating in tetrasomics. Agron. J. 51:465-467.

Rao, G., B. Widen, and S. Andersson. 2002. Patterns of inbreeding depression in a population of Brassica cretica (Brassicaceae): evidence from family-level analysis. Biol. J. Linn. Soc. 76:317:325.

Rausch, J. H. and M. T. Morgan. 2005. The effect of self-fertilization, inbreeding depression, and population size on autopolyploid establishment. Evolution 59:1867-1875.

Ronfort, J. 1999. The mutation load under tetrasomic inheritance and its consequences for the evolution of the selfing rate in autotetraploid species. Genet. Res. 74:31-42.

Rosquist, G. 2001. Reproductive biology in diploid Anthericum ramosum and tetraploid A. liliago (Anthericaceae). Oikos 92:143-152.

Runions, C. J. and M. A. Geber. 2000. Evolution of the self-pollinating flower in Clarkia xantiana (Onagraceae). I. Size and development of floral organs. Am. J. Bot. 87:1439-1451.

SAS Institute. 2003. Statistical analysis software, version 9.1. Cary, NC, USA.

Schemske, D. W. 1983. Breeding system and habitat effects on fitness components in three neotropical Costus (Zingiberaceae). Evolution 37:523-539.

Schoen, D. J. 1983. Relative fitnesses of selfed and outcrossed progeny in Gilia achilleifolia (Polemoniaceae). Evolution 37:292-301.

Shaw, R. G., D. L. Byers, and F. H. Shaw. 1998. Genetic components of variation in Nemophila menziesii undergoing inbreeding: morphology and flowering time. Genetics 150:1649-1661.

Small, E., I. J. Bassett, C. W. Crompton, and H. Lewis. 1971. Pollen phylogeny in Clarkia. Taxon 20:739-746.

Soltis, D. E. and P. S. Soltis. 1987. Polyploidy and breeding systems in homosporous pteridophyta: A reevaluation. Am. Nat. 130:219-232.

Soltis, D. E., P. S. Soltis, and J. A. Tate. 2003. Advances in the study of polyploidy since Plant speciation. New Phytol. 161:173-191.

Soltis, P. S. and D. E. Soltis. 1990. Evolution of inbreeding and outcrossing in ferns and fern-allies. Plant Species Biol. 5:1-11.

Soltis, P. S. and D. E. Soltis. 2000. The role of genetic and genomic attributes in the success of polyploids. Proc. Natl. Acad. Sci. U. S. A. 97:7051-7057.

Song, K., P. Lu, K. Tang, and T. C. Osborn. 1995. Rapid genome change in synthetic polyploids of Brassica and its implications for polyploid evolution. Proc. Natl. Acad. Sci. U. S. A. 92:7719-7723.

Stebbins, G. L. 1938. Cytological characteristics associated with the different growth habits in the dicotyledons. Am. J. Bot. 25:189-198.

Stebbins, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York.

Sybenga, J. 1969. Allopolyploidization of autopolyploids I. possibilities and limitations. Euphytica 18:355-371.

Sybenga, J. 1996. Chromosome pairing affinity and quadrivalent formation in polyploids: do segmental allopolyploids exist? Genome 39:1176-1184.

Vasek, F. C. and J. Harding. 1976. Outcrossing in natural populations. V. Analysis of outcrossing, inbreeding depression, and selection in Clarkia exilis and Clarkia tembloriensis. Evolution 30:403-411.

Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398-411.

Willis, J. H. 1996. Measures of phenotypic selection are biased by partial inbreeding. Evolution 50:1501-1511.

Willis, J. H. 1999. The role of genes of large effect on inbreeding depression in Mimulus guttatus. Evolution 53:1678-1691.

Wolfe, K. H. 2001. Yesterday's polyploids and the mystery of diploidization. Nat. Rev. Genet. 2:333-341.

Wright, S. 1938. The distribution of gene frequencies in populations of polyploids. Proc. Natl. Acad. Sci. U. S. A. 24:372-377.