doi:10.1111/j.1558-5646.2007.00234.x

EPISTATIC AND CYTONUCLEAR INTERACTIONS GOVERN OUTBREEDING DEPRESSION IN THE AUTOTETRAPLOID *CAMPANULASTRUM AMERICANUM*

Julie R. Etterson,[1,2] Stephen R. Keller,[3,4] and Laura F. Galloway[3,5]

[1] *Department of Biology, University of Minnesota, Minnesota 55812-3004*

[2] *E-Mail: jetterso@d.umn.edu*

[3] *Department of Biology, University of Virginia, Virginia, 22904-4328*

[4] *E-mail: srk3d@virginia.edu*

[5] *E-mail: lgalloway@virginia.edu*

Received January 30, 2007
Accepted July 23, 2007

The consequences of combining divergent genomes among populations of a diploid species often involve F1 hybrid vigor followed by hybrid breakdown in later recombinant generations. As many as 70% of plant species are thought to have polyploid origins; yet little is known about the genetic architecture of divergence in polyploids and how it may differ from diploid species. We investigated the genetic architecture of population divergence using controlled crosses among five populations of the autotetraploid herb, *Campanulastrum americanum*. Plants were reciprocally hybridized to produce F1, F2, and F1-backcross generations that were grown with parental types in a greenhouse and measured for performance. In contrast to diploid expectations, most F1 hybrids lacked heterosis and instead showed strong outbreeding depression for early life traits. Recombinant hybrid generations often showed a recovery of performance to levels approximating, or at times even exceeding, the parental values. This pattern was also evident for an index of cumulative fitness. Analyses of line means indicated nonadditive gene action, especially forms of digenic epistasis, often influenced hybrid performance. However, standard diploid genetic models were not adequate for describing the underlying genetic architecture in a number of cases. Differences between reciprocal hybrids indicated that cytoplasmic and/or cytonuclear interactions also contributed to divergence. An enhanced role of epistasis in population differentiation may be the norm in polyploids, which have more gene copies. This study, the first of its kind on a natural autotetraploid, suggests that gene duplication may cause polyploid populations to diverge in a fundamentally different way than diploids.

KEY WORDS: **Intraspecific hybridization, line-cross analysis, outbreeding depression, polyploid, population differentiation.**

Populations of a species with limited gene exchange will diverge genetically over time due to natural selection and genetic drift (Wright 1931). This basic premise of population genetics forms the foundation for our understanding of population divergence and ultimately the process of speciation (Coyne and Orr 1998; Turelli and Orr 2000). However as many as 70% of plant species are thought to have polyploid origins (Masterson 1994), and doubling the chromosome set, either within species (autopolyploid) or in interspecific hybrids (allopolyploid), may influence the patterns and process of future population divergence. For example, a larger number of allele copies per locus exist in polyploids relative to diploids, increasing the potential for allelic diversity, allelic interactions, and redundancy. Additionally, multiple polyploid lineages may arise independently within a species, creating a mosaic of populations with different diploid ancestry (Seagraves et al. 1999; Soltis and Soltis 2003). Although polyploidy is common

in plants, we know very little about patterns of population divergence and the consequences of hybridizing polyploid lineages with different evolutionary histories.

According to diploid genetic models and empirical work, admixture between lineages with different evolutionary histories may result in diverse outcomes. For example, hybridization may enhance the evolutionary potential of a species by bolstering genetic diversity and producing novel genotypes with unique genetic and ecological attributes (Rieseberg et al. 1999). This genetic exchange may also relieve inbreeding depression within populations if F1 hybrids exhibit partial dominance or overdominance at loci that influence fitness, causing heterosis or hybrid vigor (Lynch 1991). However, if populations are highly diverged, hybridization may disrupt positive allele combinations that enhance fitness and have been fixed by selection on different genetic backgrounds ("intrinsic" outbreeding depression; e.g., Whitlock et al. 1995). Reduced hybrid fitness may also arise due to Dobzhansky–Muller incompatibilities made prominent by drift or selection in isolated populations. The benefits of heterosis and the detriments of outbreeding depression can operate concurrently to determine the fitness of hybrids, although both are expected to change over successive generations (Lynch 1991). Heterozygosity and thus heterosis are greatest in the F1. In contrast, outbreeding depression is expected to be greatest in recombinant hybrid generations as positively interacting loci are broken up through the action of recombination and independent assortment. The timing of heterosis and outbreeding depression thus creates an often observed pattern of F1-hybrid vigor, followed by hybrid breakdown in the F2 and later generations (Lynch and Walsh 1998). Outbreeding depression may abate with successive generations if recombination generates genotypic novelty that is favored by selection (Stebbins 1969; Erickson and Fenster 2006).

The expectations for trait expression following hybridization may differ for polyploids compared to diploids (Bever and Felber 1992). There are several reasons why polyploidy may modulate the occurrence and magnitude of heterosis and outbreeding depression. First, autopolyploidy increases the effective population size of nuclear genes that may slow the erosion of allelic diversity among isolated lineages due to drift (Wright 1938; Moody et al. 1993) and decrease genetic load (Butruille and Boiteux 2000). However, this effect may be counteracted by the small effective population size early in the establishment of a new polyploid lineage. Genome duplication also increases the number of potential allelic interactions within loci. The greater number of allele copies provides greater opportunities for beneficial overdominance to be expressed (e.g., Bingham et al. 1994). High allelic diversity may also increase the sheltering of deleterious recessives within populations, reducing the heterotic effect typically observed in F1 hybrids of diploid populations.

Second, the increased number of gene copies enhances the potential for gene interactions to develop among loci of quantitative traits. Genes often function in concert with other loci in developmental pathways or expression networks. The greater number of alleles per locus in polyploids may facilitate the evolution of positive epistatic interactions (e.g., Blanc and Wolfe 2004). Thus admixture among polyploid populations may be more likely to disrupt coadapted gene complexes and lead to greater outbreeding depression. An alternative fate of duplicated genes is differential gene silencing, where alternate redundant copies are silenced independently among populations (Werth and Windham 1991; Lynch and Force 2000). Alternate gene silencing should occur frequently in nature, as populations of many polyploid species display independent origins (e.g., Seagraves et al. 1999; Soltis et al. 2004). Even populations sharing a common polyploid origin may display alternate gene silencing if they become isolated during the period of genome stabilization that follows the duplication event. Differential silencing of paralogues results in lost or reduced function when these alleles are brought back together in hybrids (Lynch and Force 2000; Soltis and Soltis 2003), again enhancing outbreeding depression relative to diploid expectations. Although the role of gene silencing has been more carefully studied in allopolyploids, older lineages with putative autopolyploid ancestry also exhibit extensive gene silencing (e.g., soybean, Zhu et al. 1994; Straub et al. 2006). Furthermore, genes expressed in diploid *Arabiodopsis thaliana* were silenced in a newly formed autotetraploid *A. thaliana* and then reactivated in a synthetic allotetraploid produced through hybridization with *Cardaminopsis arenosa* (Comai et al. 2000; Adams and Wendel 2005). This suggests that gene expression is dynamic during the formation of both auto- and allopolyploid lineages.

Finally, polyploidy creates opportunities for novel interactions between the nuclear and cytoplasmic genomes. Mitochondria and chloroplast genomes encode genes critical for respiration and photosynthesis, and a growing number of studies suggest that cytoplasmic interactions with the nucleus may impact the process of divergence (Galloway and Fenster 1999; Tiffin et al. 2001; Rawson and Burton 2002). Although polyploidy results in a chromosome doubling; the cytoplasmic genomes remain unchanged. Changes in ploidy are known to have consequences for the relative dosage of gene expression (Osborn et al. 2003). However, it is not known whether a change in the ratio of nuclear to cytoplasmic genes influences trait expression. Regardless, changes in gene copy number following a polyploid shift are likely to exert selection on cytoplasmic genes to ensure continued functional integration with the increased expression level of nuclear genes (Wendel 2000). This process is expected to lead to divergence among populations in cytonuclear interactions, however this area is just beginning to receive attention.

Studies that dissect the genetic architecture of population divergence in polyploids are needed to address the process of evolutionary diversification in plant species. This is particularly relevant as recent genomic work reveals evidence for ancient polyploidy in most plant taxa (Soltis and Soltis 2003), suggesting that genetic architecture during divergence may largely develop within the genomic context of duplicated genes. In this study, we evaluate the contribution of additive, nonadditive (dominance and epistasis), and cytoplasmic genetic effects to population divergence in the autopolyploid herb, *Campanulastrum americanum*. Earlier work found the performance of F1 hybrids in crosses between Indiana, North Carolina, and Virginia populations was high for proximate crossing distances, but dropped considerably for longer-distance crosses, frequently displaying nonadditive and cytoplasmic genetic effects (Galloway and Etterson 2005). Here, we report on three generations of hybrid offspring (F1, F2, and backcross F1) originating from the same parental populations to evaluate the performance of recombinant hybrid generations. We also fit models from an analysis of line means to determine the contribution of additivity, dominance, and different forms of epistasis to divergence.

Materials and Methods

STUDY ORGANISM

Campanulastrum americanum Small (= *Campanula americana* L., Campanulaceae) is a semelparous woodland herb that is distributed throughout the Eastern half of North America. *Campanulastrum americanum* is an autotetraploid ($2N = 58$; Galloway and Etterson 2005). Marker segregation in progeny arrays produced by controlled crosses between informative allozyme genotypes suggests tetrasomic inheritance at one locus and disomic inheritance at another locus implying that the genome has become partly diploidized (Galloway et al. 2003, unpubl. data). Partial diploidization of inheritance combined with the lack of diploid populations suggests the ploidy event has not been recent. Common garden studies have demonstrated genetic divergence among populations with respect to morphological and phenological characters across its geographic distribution (Kalisz and Wardle 1994; Galloway and Etterson 2005). Plants are visited primarily by Hymenopteran pollinators (Galloway et al. 2002), and the mating system is highly outcrossing (Galloway et al. 2003).

AMONG-POPULATION CROSSES

Seeds were collected from five populations at the following locations: (1) Wintergreen Resort, Augusta County, VA (VA-W), (2) Salt Pond Mountain, Rt 700, Giles County, VA (VA-700), (3) Bean Field Mountain, Rt 613, Giles County, VA (VA-613), Blue Ridge Parkway, Allegany County, NC (NC), and (5) Bloomington, Monroe County, IN (IN). All possible pairwise crosses were done for two sets of three populations: Trio 1 (VA-W, NC, and IN) and Trio 2 (VA-613, VA-700, and IN). In Trio 1, VA-W and NC are closest in proximity (280 km) and both are distant from the IN population (922 km and 811 km, respectively). In Trio 2 VA-613 and VA-700 are separated by only 1.5 km and are each 555 km from IN. Reciprocal F1, F2, and backcross generations (F1 × parental) were produced for Trio 1, and reciprocal F1 and F2 hybrids were produced for Trio 2. So that all seeds were a product of the same number of generations of experimental crosses and the same environments, F1 and parental lines were reproduced with each generation of crosses. Parental lines were initiated by crossing 10 founder genotypes per population. Five genotypes from each population were used to create the among-population crosses. Reciprocal F1 and F2 hybrids were created using the same individuals as pollen donors in one crossing direction and pollen recipients in the other. To produce backcross lines, reciprocal F1 plants served as pollen donors for crosses onto the two parental lines (i.e., four backcross cross-types produced by $F1_{VA-W \times IN}$ and $F1_{IN \times VA-W}$ crossed onto P_{VA-W} and P_{IN}). For Trio 1, there were 27 cross-types in total (three parental, six F1, six F2, and 12 backcross), yielding 10 cross-types between each pair of populations. Reciprocal F1s differed in only one case when contributing to the backcross generation (seed number, $P_I \times F1_{I,VA-W} \neq P_I \times F1_{VA-W,I}$, $P = 0.003$) and therefore were pooled in the analyses. For Trio 2, there were 14 cross-types in total (three parental, six F1, and five F2), yielding six cross-types between each pair of populations. One exception was the cross between IN × VA-700, which was missing the $F2_{IN \times VA-700}$ cross-type because few F1 hybrids carrying the IN cytoplasm flowered, and those that did flower did not produce seed. All crosses were done in a greenhouse at the University of Virginia (UVA); Trio 1 offspring were raised at the University of Minnesota Duluth whereas Trio 2 offspring were raised at UVA.

The planting design for Trio 1 consisted of 50 seeds per parental, backcross, and F1 cross-type and 100 seeds per F2 cross-type (50 × 3 parental populations + 50 × 6 reciprocal F1 + 50 × 12 backcrosses + 100 × 6 F2 = 1650 seeds). The design for Trio 2 included 50 seeds per parental and F1 cross-type and 75 seeds per F2 cross-type (50 × 3 parental population + 50 × 6 reciprocal F1 + 75 × 5 F2 = 825 seeds). A greater number of F2 seeds were included because of the increased variance expected in F2 hybrids following recombination and independent assortment. Seeds were drawn from families within cross-type as evenly as possible.

Seeds were individually weighed to the nearest 0.001 mg, planted in a randomized block design into plug trays filled with Promix HP (Premier Horticulture, Dorval, QC, Canada), and germinated in growth chambers (21–22°C during day/11–12°C during nights, 12-h days). The trays were misted daily and checked for germination. A seed was scored as germinated when the hypocotyl was above the soil surface and the cotyledons were separated.

Germinated seedlings were monitored for the presence of chlorophyll deficiencies and other deformities including three cotyledons or malformed cotyledons. After 50 days, the seedlings were vernalized for six weeks at 5°C before being transplanted into 15.25-cm containers filled with 50:50 Promix HP and Turface and placed in a greenhouse (16 h days). Length of the longest leaf was measured on rosettes after vernalization and mortality noted. Number of days to first flower was recorded and hand-pollinations were conducted within each cross-type. Seed number per fruit (average of three fruits for Trio 1) and aboveground biomass (dry) at the time of seed maturation were also scored.

STATISTICAL ANALYSIS

Comparison of generation means

For continuous data, analyses of variance were used to determine whether trait expression varied among cross-types (JMP 5.1, SAS Institute 2004). For categorical data, log-linear analyses were conducted assuming a binomial distribution and a logit link (PROC GENMOD, SAS Institute 2005). Blocking factors were included (eight and four blocks in Trio 1 and 2 respectively). Seed weight and days to germination were used as covariates in the analyses of later traits. This was done because early traits often displayed complex genetic architecture (e.g., Fig. 1), and we wanted to evaluate genetic effects on later traits independent of traits expressed earlier. Family was not included as a factor in the analyses because the majority of the genetic variance for hybrid performance was assumed to exist between populations and not between families within populations.

If there was significant variation among cross-types, a series of a priori hypotheses were tested using contrasts. First, we tested whether trait expression varied among the parental populations. Second, we tested whether reciprocal hybrids differed within generations. Reciprocal hybrids share the same nuclear genetic content on average, but differ in their cytoplasmic composition. Differences between reciprocal crosses of same-generation hybrids therefore reflect differentiation in the cytoplasmic genes or in interactions between the cytoplasmic and hybrid nuclear genomes. Third, we tested whether the hybrid generations differed from expected values assuming additive gene action. Expected means for F1 and F2 hybrids were calculated as 1/2 ($P_i + P_j$) and for backcrosses, for example onto P_i, were calculated as 3/4 P_i + 1/4 P_j. Data were transformed as necessary to conform to the ANOVA assumptions of normality of the residuals and constant variance (square-root: seed weight, days to germination [Trio 2 only], and seed number per fruit [Trio 2 only]; natural-log: days to germination [Trio 1 only], leaf length, and biomass).

Cumulative fitness

To estimate the overall effect of interpopulation hybridization, we calculated an index of cumulative fitness for each individual as

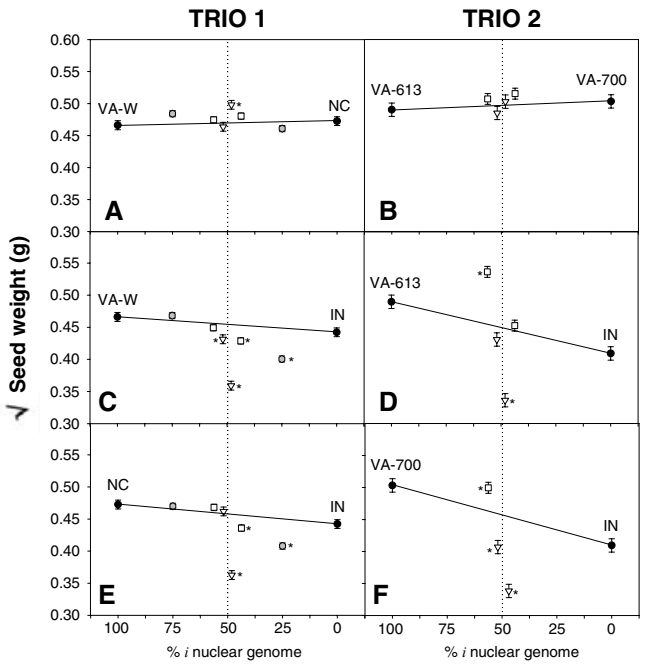

Figure 1. Least squares means of seed weight (± SE) for two trios of among-population crosses in *Campanulastrum americanum*. Parental populations are represented with filled circles and the solid line connecting them indicates the expectation for hybrids given an additive genetic model. Hybrids with open symbols contain, on average, half of each parental genome and are expected to express the phenotypic mean of the parental lines, which is indicated by the intersection of the solid and dotted line (F1 = triangles, F2 = squares). The hybrid means are shown offset from this expectation to the right or left according to which cytoplasmic genome the reciprocal hybrid bears. Backcrosses are shown with gray hexagons. Stars indicate that the hybrid mean differs significantly from the expected mean according to an a priori contrast following a significant main effect of cross-type (see Materials and Methods for details).

w = germination (0/1) × survival (0/1) × biomass × seeds per fruit. Because fruit number was not assessed in this greenhouse study, biomass was used to scale the estimate of seed production. Biomass indicates plant size that is significantly correlated with fruit number in nature ($r = 0.75$, $P < 0.0001$, $N = 76$). An index of the effect of interpopulation hybridization was calculated as $\delta_{\text{hybridization}} = 1 - (\bar{x}_{\text{hybrid}}/x_{\text{expectation}})$ where values significantly different than zero indicate outbreeding depression ($\delta > 0$) or hybrid vigor ($\delta < 0$). The expected hybrid fitnesses were calculated as in the contrasts described above. This index is analogous to estimates of reproductive isolation (e.g. Moyle et al. 2004). Because the observed distribution of cumulative fitness did not meet normality assumptions due to an excess of zeros, significance levels are based on 10,000 bootstrap samples for each cross-type tested against the null hypothesis $\delta = 0$ (MATLAB 7.0, The Mathworks 2006).

Line-cross analysis

For continuously distributed traits, we used line-cross analysis to estimate the relative contribution of maternal, additive, dominance, and epistatic effects to population divergence. Specifically, we used least-squares regression to model genetic effects starting with maternal effects due to crossing direction (M) and a simple additive mode of gene action (A). Maternal effects were included to accommodate differences among reciprocal F1 and F2 crosses. Given that seeds were produced in a common environment, maternal effects are likely due to cytoplasmic genetic effects rather than maternal environmental effects. If this initial model was not adequate to fit the data, model building then proceeded in a stepwise fashion to evaluate more complex models incorporating dominance (D) and three digenic interactions: epistasis between alleles at different loci (AA); epistasis between a specific allele at one locus with the genotype at another locus (AD); and epistasis between genotypes at different loci (DD) (Mather and Jinks 1982). Dominance and epistatic effects were added sequentially to the model in the order listed above. However, in cases in which the inclusion of AA did not significantly improve model fit and there were sufficient degrees of freedom, AD was also tested. The adequacy of successively more complex models in explaining the observed data were evaluated at each step using joint-scaling tests (Lynch and Walsh 1998) with chi-square test statistics in which the degrees of freedom were equal to the number of different line means minus the number of estimated parameters. For example, in Trio 1 the starting null model was tested with five degrees of freedom [eight line means: $\{P_i, P_j, F1_{ij}, F1_{ji}, F2_{ij}, F2_{ji}, B_i, B_j\}$ − three estimated parameters (intercept, M, and A)]. If the null model was rejected, the next genetic effect was added (i.e., D). The process was continued until the genetic model was not rejected or the degrees of freedom were exhausted. When evaluating model fit, we adopted a conservative approach that accepted the current model when $P \geq 0.1$ for the Chi-square goodness-of-fit test. If $P \leq 0.1$, then we proceeded with model building, but only when the next parameter added significantly enhanced model fit. Analyses were done on MathCad 11.2 (Mathsoft Engineering & Education, Inc. 2003).

This diploid model does not account for all possible gene interactions in an autotetraploid species. For example, a genotype with four alleles per locus, A_{ijlk}, has six potential diallelic interactions analogous to dominance deviations of a diploid (*ij*, *ik*, *il*, *jk*, *jl*, *kl*), four three-allele interactions (*ijk*, *ijl*, *ikl*, and *jkl*) and one four-allele interaction (*ijkl*) as compared to a diploid species that has a single dominance interaction per locus (*ij*) (Kempthorne 1969). Polyploids also have greater opportunity to express epistatic variance than diploids. Considering only two loci, three terms are required to describe epistatic interactions in diploids (i.e., AA, AD, and DD) whereas 10 terms are required to fully describe all possible epistatic interactions in an autotetraploid: AA, AD, DD, AT (trigenic), AQ (quadragenic), DD, DT, DQ, TT, TQ, QQ (Kempthorne 1955, 1969). Estimation of the full set of parameters outlined above would require an extensive breeding design involving 21 cross-types (Killick 1971), in contrast to the 10 cross-types for Trio1 and six for Trio 2 in the current study. To our knowledge, this breeding design has not previously been employed, and in allopolyploid crops the diploid model is often used (e.g., cotton, Devey and Roose 1987; Dani and Kohel 1989; Percy et al. 1996). The diploid model may be most appropriate for older polyploid lineages that have become diploidized in their inheritance patterns (Qu et al. 1998; Wang et al. 2005) or in cases in which duplicated genes have diverged in function over time or have been silenced. For many polyploids the suitable genetic model is not clear because inheritance is neither fully polysomic or disomic. Here we employ a diploid model and discuss consequences of this model choice for our interpretations.

Results

COMPARISON OF GENERATION MEANS

Significant variation among cross-types was found for every continuously distributed trait for all population combinations in this study except for % germination in Trio 1 and seed number per fruit in Trio 2, both of which were marginally significant at $P < 0.1$ (see online Supplementary Table S1). Combinations of parental populations differed for some traits but not for others and the pattern did not correspond to geographic distance between the populations (Table 1). For example in Trio 1, the VA-W population differed for similar numbers of traits when compared to populations in close proximity (VA-W × NC: leaf size and biomass), and those separated by longer distances (VA-W × IN: seed weight and seeds per fruit). NC and IN were the most distinct, differing for seed weight, leaf size, date of first flower, and biomass. Likewise, the closest populations in Trio 2 differed for the same number of traits as one of the long-distance population pairs (VA-613 and VA-700: days to first flower, biomass, and seed number; VA-700 and IN: seed weight, timing of germination, and seed number). IN and VA-613 were the most distinct in this trio differing significantly for seed weight, leaf size, biomass, and timing of germination and flowering.

Experiment-wide, hybridization among these populations more often had negative (78%) than positive effects (Table 1). All hybrid means that differed from expectation assuming additive gene action had a lower percent germination (Fig. 2), slower germination, more deformities, smaller rosette leaves, and reduced survival (Fig. 3). However, hybridization had mixed effects on seed weight (Fig. 1), biomass, days to first flower, and seed number per fruit (Fig. 4), with some hybrid generations exhibiting enhanced performance and others exhibiting reduced performance. Interpopulation crossing more often had positive effects on

Table 1. Summary of a priori contrasts conducted to test for deviation from an additive genetic model for two trios of among-population crosses in *Campanulastrum americanum*. Significant variation among parental populations (P) are indicated with stars. Subscripts *i* and *j* indicate which population served as a female in reciprocal crosses to produce hybrid generations. If crossing direction did not matter, reciprocal F1 or F2 means were averaged ($\bar{x}_{ij}$). The presence of an arrow in any cell indicates that the generation differed from expectation assuming additive gene action. The direction of the arrow indicates whether the hybrid generation exhibited significantly better (↑) or worse (↓) performance than expected (df = 1, >155; $P < 0.05$). Dashes show absence of one cross-type due to reproductive failure. Of the 153 contrasts conducted, one would expect to erroneously assign significance in 7.7 cases by chance alone. +: $P < 0.06$; ∗: $P < 0.05$; ∗∗: $P < 0.01$; ∗∗∗: $P < 0.001$.

Cross *i*, *j*	Trio 1										Cross *i*, *j*	Trio 2								
	P	F1			F2			B				P	F1			F2				
		i	$\bar{x}_{ij}$	*j*	*i*	$\bar{x}_{ij}$	*j*	*i*	$\bar{x}_{ij}$	*j*			*i*	$\bar{x}_{ij}$	*j*	*i*	$\bar{x}_{ij}$	*j*		
VA-W × NC											VA-613 × VA-700									
Seed weight				↑							Seed weight									
Germination (yes/no)											Germination (yes/no)			↓			↓			
Days to germination											Days to germination									
Deformed (yes/no)	+										Deformed (yes/no)									
Leaf size	∗∗										Leaf size									
Days to first flower										↑	Days to first flower	∗∗								
Biomass	∗										Biomass	∗∗								
Seed number per fruit					↓					↑	Seed number per fruit	∗								
Survival (yes/no)											Survival (yes/no)									
VA-W × IN											VA-613 × IN									
Seed weight	∗	↓		↓			↓			↓	Seed weight	∗∗∗			↓	↑				
Germination (yes/no)			↓								Germination (yes/no)			↓		↓				
Days to germination			↓							↓	Days to germination	∗∗	↓		↓					
Deformed (yes/no)			↓				↓				Deformed (yes/no)			↓			↓			
Leaf size			↓			↓				↓	Leaf size	∗				↓				
Days to first flower					↑						Days to first flower	∗								↑
Biomass				↓							Biomass	∗		↓			↓			
Seed number per fruit	∗				↑						Seed number per fruit									
Survival (yes/no)							↓				Survival (yes/no)			↓			↓			
NC × IN											VA-700 × IN									
Seed weight	∗∗			↓	↑		↓			↓	Seed weight	∗∗∗	↓		↓	↑				–
Germination (yes/no)				↓							Germination (yes/no)		↓		↓					–
Days to germination		↓		↓	↓		↓	↓		↓	Days to germination	∗∗∗		↓						–
Deformed (yes/no)											Deformed (yes/no)			↓						–
Leaf size	∗∗∗		↓		↓					↓	Leaf size					↓				–
Days to first flower	∗				↑			↑			Days to first flower				↓					–
Biomass	∗∗										Biomass			↑						
Seed number per fruit					↓		↑			↑	Seed number per fruit	∗∗		↑						–
Survival (yes/no)											Survival (yes/no)					↓				–

timing of flowering and seed number; 80% of hybrids that differed significantly from expectation flowered earlier and 71% had more seeds per fruit.

Overall 33% of F1, 30% of F2, and 20% of backcross means differed significantly from the expected means in Trio 1 and 48% of F1 and 28% of F2 differed in Trio 2 (Table 1). The most common pattern in these data was poor performance in the F1 but better than expected or not different from expected performance in the F2 and/or backcrosses. This pattern is particularly apparent for the early life-history traits of the long-distance crosses and occurs repeatedly for the variables: seed weight (Fig. 1C–F), percent germination (Fig. 2C–F), and days to germination. F2 recovery appears less consistently in later life-history traits but is evident in biomass (VA-W × IN). In fewer instances, both the F1 and F2 performed poorly (e.g., Figs. 2D, 3D; also see Table 1 VA-W × IN: leaf size; NC × IN: days to germination; VA-613 × IN:% deformed and biomass), however in half of these cases, the F1 exhibited significantly poorer performance than the F2.

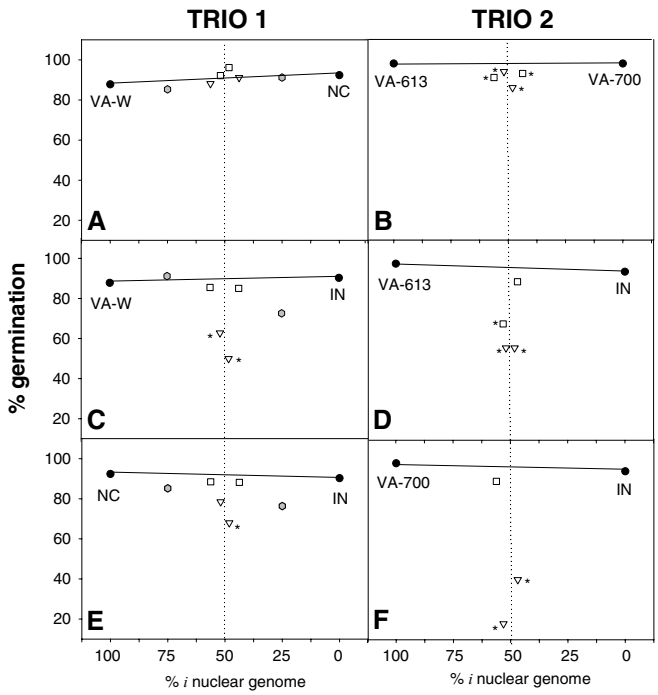

Figure 2. Percentage germination for two trios of among-population crosses in *Campanulastrum americanum*. See Figure 1 for details.

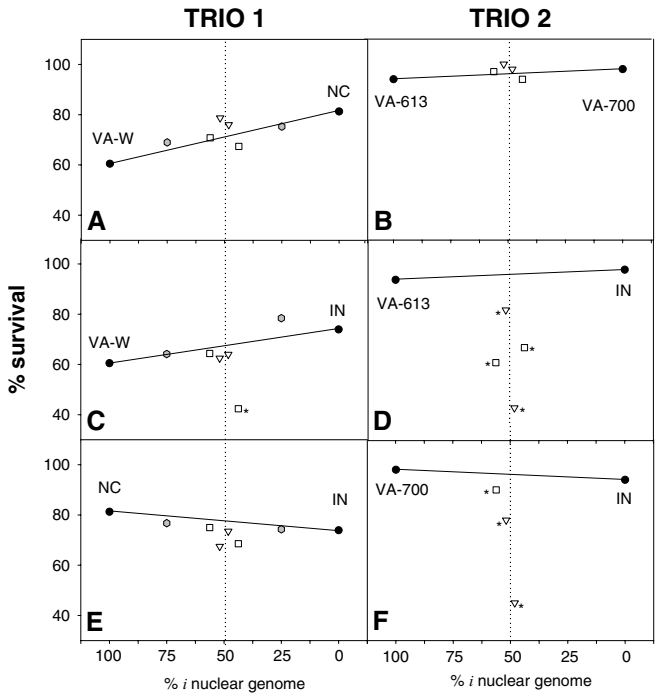

Figure 3. Percentage survival for two trios of among-population crosses in *Campanulastrum americanum*. See Figure 1 for details.

Less frequently, the F1 mean conformed to expectations but the F2 mean was lower than expected (leaf size: VA-613 × IN and VA-700 × IN; seed number per fruit: VA-W × NC and NC × IN; survival: VA-W × IN and VA-700 × IN).

Crossing direction influenced almost all traits in this study. However, the magnitude of differences between reciprocal hybrids was often determined by the distance between populations. For the populations in closest proximity, VA-613 × VA-700, there was no significant effect of crossing direction for any trait (Table 1). The crossing direction also rarely mattered for hybrids between populations at intermediate distance, VA-W × NC, and when it did, the effects were more often positive than negative (e.g., Table 1, Fig. 1A). However, for many longer distance crosses, performance differed between the reciprocal hybrids. A striking pattern is the poor performance of interpopulation hybrids bearing IN cytoplasm on a hybrid nuclear background. These hybrids accounted for 71% of the cases of lower than expected line means.

The overall effect of hybridization on cumulative fitness, $\delta_{\text{hybridization}}$, shows a pattern of strong outbreeding depression in the F1 generation, substantial outbreeding depression in the F2, and recovery of fitness in the F1-backcrosses (Table 2). This pattern is related to the distance between parental populations. In both trios, hybridization among populations in close proximity did not result in significant outbreeding depression except for the F2 generation between VA-W and NC. In contrast, hybrids produced by long-distance crosses showed significant outbreeding depression on cumulative fitness for all but one F1 cross-type and in half of the F2 cross-types. The greatest cumulative outbreeding depression occurred in crosses between Indiana and both VA-613 and VA-700 where F1 hybrids had up to a 90% reduction in fitness relative to their parental populations. Strikingly, there is no significant outbreeding depression in the F1-backcrosses.

LINE-CROSS ANALYSIS

Simple additive models with maternal effects provided a good fit for the populations in closest proximity for days to first flower, biomass, and seed number per fruit (VA-613 × VA-700; Table 3 and Fig. 4B). Models with additive and dominance gene action explained the pattern of line means in seven other instances, mostly involving later life traits and crosses with the IN population (Table 3).

Genetic models that included various forms of epistasis provided good fit to the pattern of line means for 13 population/trait combinations (Table 3). Models with epistasis between alleles at different loci (AA) fit the data best for nine population/trait combinations. Inclusion of effects of epistatic gene action between a specific allele at one locus and the genotype at another locus (AD), provided good fit to the line means for one other population/trait combination (VA-W × IN: days to germination). In another case, the best-fit model included both AD and epistasis between genotypes at different loci (DD) but not AA (VA-W × NC: days to first flower; biomass). The preponderance of models in which the best fit included only AA epistasis still holds in a comparison of crosses

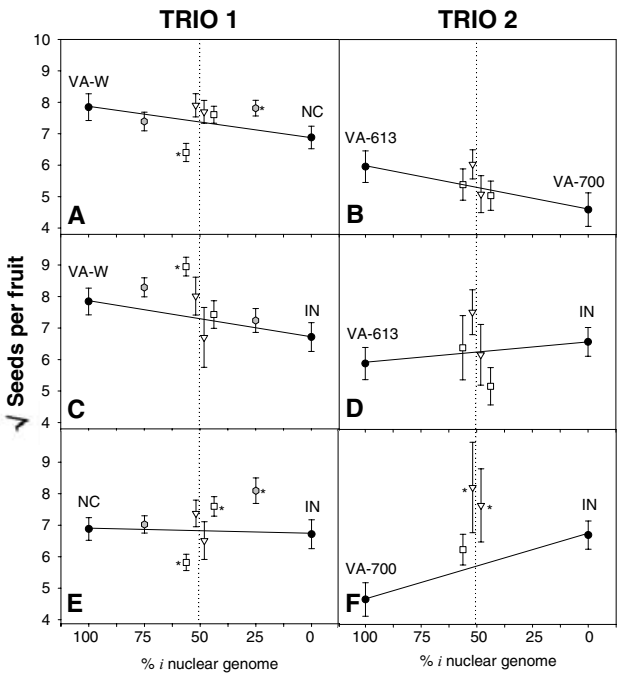

Figure 4. Least squares means of seed number per fruit (± SE) for two trios of among-population crosses in *Campanulastrum americanum*. See Figure 1 for details.

in which all three types of epistasis were tested (e.g., Trio 1). Overall, the probability of detecting epistasis was lower in Trio 2 because it had a more limited genetic design (no backcrosses) and was missing one of the long-distance F2 lines.

We did not find an adequate genetic model for 11 models across five of the traits of these interpopulation crosses (Table 3). In eight of these models, all possible forms of epistasis that could be tested given the genetic design were included (seed weight: VA-W × NC, VA-W × IN, NC × IN; days to germination: NC × IN, VA-613 × IN; leaf size: VA-613 × IN; biomass: VA-W × IN; seeds per fruit: VA-w × NC, NC × IN). For three models for Trio 2, there were insufficient degrees of freedom to test for epistatic gene action (Table 3). However, it is often assumed that a lack of fit for an additive/dominance model indicates the presence of epistasis.

Discussion

Between-population hybrids of *C. americanum* often performed poorer than the expected average of the parental populations, especially for long-distance crosses. Outbreeding depression was particularly strong among F1 hybrids for early life-history traits leading to as much as a 90% reduction in cumulative fitness relative to the parental populations, and often differed between the reciprocals of a cross. However, hybrid performance was fully recovered in the F2 generation in more than half of these cases. This pattern was not completely consistent; there were some examples of poor performance in both F1 and F2 generations as well as hybrid breakdown only expressed in the F2 generation. Nevertheless, our index of the effect of hybridization on cumulative fitness ($\delta_{hybridization}$) strongly reinforces our general conclusions. Overall, these results support expectations for polyploids to show decreased heterosis and enhanced outbreeding depression relative to diploids.

Divergence among populations for gene interactions is supported by the line-cross analyses that frequently found that quantitative gene action could not be accounted for with simple genetic models that included additivity. With few exceptions (e.g., VA-613 and VA-700), more complex models that included dominance and various combinations of digenic epistasis were necessary to adequately model the patterns of line means. Even with the inclusion of epistasis, however, models that provided good fit to the data were only found for 66% of the population/trait combinations (24 out of 36; Table 3). The complex patterns of gene action we observed are consistent with the notion that polyploids should experience an increased effect of gene interactions relative to diploids. Because *C. americanum* is an autotetraploid, it has the potential to express more than three times as many intralocus and two-way interlocus interactions than were tested here (Killick 1971). Testing such large numbers of interactions would require many more

Table 2. The effect of interpopulation hybridization, $\delta_{hybridization} = 1 - (w_{hybrid}/w_{expected})$, of *Campanulastrum americanum* grown in the greenhouse. Bold numbers show significant outbreeding depression ($\delta > 0$) and hybrid vigor ($\delta < 0$) according to two-tailed *t*-tests.

Trio 1					Trio 2			
Pop $_{i,j}$	♀Pop	F1	F2	Backcross	Pop $_{i,j}$	♀Pop	F1	F2
VA-W × NC	*i*	−0.23	−0.01	0.05	VA-613 ×VA-700	*i*	−0.45	0.21
	j	−0.38	**0.23*****	−0.31		*j*	0.29 +	0.08
VA-W × IN	*i*	**0.47****	**0.54*****	−0.12	VA-613 ×IN	*i*	**0.65*****	**0.75*****
	j	**0.88*****	−0.24	0.23 +		*j*	**0.90*****	**0.89*****
NC × IN	*i*	0.06	−0.04	0.00	VA-700 × IN	*i*	**0.89*****	
	j	**0.58*****	**0.30****	0.06		*j*	**0.81*****	0.22

+: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Table 3. Summary of line-cross analyses for two trios of among-population crosses in *Campanulastrum americanum* showing the modes of gene action included in the best-fit models. The genetic effects are: M, maternal (cytoplasmic) effects; A, additivity; D, dominance; AA, additive by additive epistasis; AD, additive by dominance epistasis; DD, dominance by dominance epistasis. *P*-values from goodness of fit tests indicate whether estimated line means differed significantly from actual line means in the final genetic model (models which show good fit to the data are shown in bold). Dashed lines in any cell indicate that there were not sufficient degrees of freedom to test the effect.

	Trio 1 genetic effects								Trio 2 genetic effects				
Cross	M	A	D	AA	AD	DD	P_{model}	Cross	M	A	D	AA	P_{model}
VA-W × NC								VA-613 × VA-700					
Seed weight	X	X	X	X	X	X	0.002	Seed weight	**X**	**X**	**X**	**X**	**0.26**
Days to germination	**X**	**X**	**X**	**X**			**0.06**	Days to germination	**X**	**X**	**X**	**X**	**0.33**
Leaf size	**X**	**X**	**X**				**0.16**	Leaf size	**X**	**X**	**X**	**X**	**0.07**
Days to first flower	**X**	**X**	**X**		**X**	**X**	**0.44**	Days to first flower	**X**	**X**			**0.27**
Biomass	**X**	**X**	**X**		**X**	**X**	**0.48**	Biomass	**X**	**X**			**0.24**
Seed number per fruit	X	X	X	X	X	X	0.02	Seed number per fruit	**X**	**X**			**0.26**
VA-W × IN								VA-613 × IN					
Seed weight	X	X	X	X	X	X	<0.0001	Seed weight	**X**	**X**	**X**	**X**	**0.24**
Days to germination	**X**	**X**	**X**	**X**	**X**		**0.17**	Days to germination	X	X	X	X	<0.0001
Leaf size	**X**	**X**	**X**	**X**			**0.34**	Leaf size	X	X	X	X	0.05
Days to first flower	**X**	**X**	**X**				**0.10**	Days to first flower	**X**	**X**	**X**		**0.39**
Biomass	X	X	X	X		X	<0.0001	Biomass	**X**	**X**	**X**		**0.09**
Seed number per fruit	**X**	**X**	**X**	**X**			**0.19**	Seed number per fruit	**X**	**X**	**X**	**X**	**0.46**
NC × IN								VA-700 × IN					
Seed weight	X	X	X	X	X	X	<0.0001	Seed weight	X	X	X	- - -	<0.0001
Days to germination	X	X	X	X	X	X	<0.0001	Days to germination	X	X	X	- - -	<0.0001
Leaf size	**X**	**X**	**X**	**X**			**0.10**	Leaf size	X	X	X	- - -	0.008
Days to first flower	**X**	**X**	**X**	**X**			**0.07**	Days to first flower	**X**	**X**	**X**		**0.17**
Biomass	**X**	**X**	**X**	**X**	**X**	**X**	**0.34**	Biomass	**X**	**X**	**X**		**0.28**
Seed number per fruit	X	X	X	X	X	X	<0.0001	Seed number per fruit	**X**	**X**	**X**		**0.15**

lines and thus necessitate large and complex breeding designs, making these added interactions very difficult to estimate. Similarly, diploid genetic models applied to allopolyploid agricultural species typically fail to find good models of gene action (Devey and Roose 1987; Dani and Kohel 1989; Percy et al. 1996). The complex nonadditive gene action observed in intraspecific hybrids of *C. americanum* suggests that differences in genetic architecture may cause polyploid populations to diverge in a fundamentally different way than diploids.

The finding of F1-hybrid breakdown corroborates previous analyses conducted after one generation of hybridization among these populations (Galloway and Etterson 2005). However, this finding contradicts the classic observation of fewer postzygotic barriers to hybridization among polyploid lineages relative to diploid lineages (Stebbins 1950). The low fitness of F1 hybrids is not attributable to the dilution of local adaptation (i.e., gene × environment interaction; Templeton 1986) because, as in the previous study, progeny were raised in a controlled environment. Instead, poor performance of F1 hybrids is likely due to intrinsic genetic factors. In diploids, F1-hybrid breakdown is unexpected, as many studies find that intraspecific F1 hybrids display heterosis and outperform parental populations (Hufford and Mazer 2003). This is because combining genomes shelters recessive or partially recessive deleterious alleles in heterozygous genotypes, causing the performance of F1 hybrids to exceed parental values (Lynch 1991). The same may hold true for polyploids if the duplication event is old and deleterious alleles are partially recessive (Otto and Whitton 2000). However, if the duplication event is relatively recent or deleterious alleles are mostly recessive, then polyploids may already mask their load within populations by virtue of the large number of allele copies per locus. Increased sheltering within populations would cause hybrids to derive little to no benefit from heterosis. In *C. americanum,* the mating system is almost exclusively outcrossing ($t_m = 0.94$; Galloway et al. 2003) and controlled crosses within populations reveal a large genetic load ($\delta = 0.92$–0.95; Galloway and Etterson 2007). Thus, even though substantial load exists within *C. americanum* populations, it consists of highly recessive mutations masked by one or more dominant alleles at a locus. This sheltering of load within populations causes heterosis to be a weak force in hybrids. A lack of heterosis may magnify hybrid breakdown by not balancing the negative impacts of other genetic effects, such as the creation of novel negative interactions

within loci (underdominance) and between loci (epistatic incompatibilities), or the loss of positive additive by additive epistasis (Lynch 1991).

Underdominance can arise from chromosomal rearrangements that are fixed in different populations causing structural heterozygotes in interpopulation F1 hybrids with reduced fitness (reviewed in Fishman and Willis 2001). Independent assortment is expected to lead to partial fitness recovery in the F2, mirroring patterns found here (e.g., Figs. 1, 2). Within diploid populations it may be difficult for such chromosomal inversions to initially become established because interbreeding with other population members would produce offspring with low fitness (Coyne and Orr 1998). However, increased genome rearrangement following chromosome doubling (e.g., Song et al. 1995), together with the genetic redundancy associated with polyploidy, may increase tolerance for chromosomal rearrangement (Levy and Feldman 2002) and allow these mutants to become established more readily than in a diploid population. Recent theoretical models also show that inversions can spread rapidly within populations if they encompass locally adapted alleles (Kirkpatrick and Barton 2006). Currently there is not sufficient cytological data to evaluate the frequency of chromosomal rearrangements for *C. americanum*. Although differences in genome size among these populations have previously been reported, they are not associated with interpopulation cross compatibility (Galloway and Etterson 2005).

The diversity in *C. americanum*'s hybrid performance clearly suggests that multiple mechanisms are in operation (Table 1). For example, in some instances hybrid fitness was only reduced in the F2 generation. This pattern is consistent with complexes of coadapted genes that differ among parental populations and have been broken up by recombination (i.e., loss of positive epistasis, Lynch 1991). Polyploidy may facilitate the formation of coadapted gene complexes, as gene duplication relaxes selection on any one copy and allows for functional divergence of paralogues (Wendel 2000). Divergence in expression at one duplicated locus may exert selection for parallel divergence at another locus, resulting in the concerted divergence of genes involved in interaction networks (Blanc and Wolfe 2004). Hybridization and recombination would disrupt such interaction networks and result in the mis-pairing of genes that lack a recent history of concerted divergence.

Alternatively, reduced F2 performance may result from the formation of negative epistatic interactions between alternate alleles that have been fixed in different populations by selection or drift. If the effect of these alleles depends upon the genetic background in which they arose, hybridization and expression on a composite genetic background can result in outbreeding depression in the form of Dobzhansky–Muller incompatibilities (Coyne and Orr 1998; Turelli and Orr 2000). Gene duplication provides an ideal substrate for generating Dobzhansky–Muller incompatibilities, as degenerative mutations are predicted to cause loss of function or subfunctionalization (partial function of paralogues that require expression of all copies for full performance) of different alleles among populations (Lynch and Force 2000; Burke and Arnold 2001). Such alternate gene silencing creates no fitness disadvantages until silenced alleles are combined in hybrid offspring. Further, if degenerative mutations occur with higher probability than mutations with beneficial effects, epistatic incompatibilities may arise frequently among duplicated genes relative to the beneficial mutations necessary to generate coadapted gene complexes (Lynch and Force 2000). Thus, alternate silencing of duplicated genes in polyploids make them likely candidates for evolving reproductive isolation among populations via Dobzhansky–Muller incompatibilities (Werth and Windham 1991). Both loss of positive epistasis and the creation of negative epistatic interactions are more likely to result in outbreeding depression in recombinant hybrid generations, although disruption of coadapted complexes among homologous chromosomes and dominant Dobzhansky–Muller incompatibilities may also be expressed in F1 hybrids (Lynch and Walsh 1998).

In contrast to hybrid breakdown, fitness seemed to recover in the F2 of some traits, at times even exceeding the average of the parents (e.g., seed weight, days to first flower, seed number per fruit; Table 1). One possible mechanism is that highly favorable dominance × dominance epistatic relationships were established during recombination to produce the F2 (Lynch 1991). Recovery in the F2 could also reflect loss of underdominance caused by structural rearrangements, as discussed previously. Alternatively, heterozygosity in autopolyploids continues to accrue into the F2 and more advanced hybrid generations at a rate that depends upon inheritance patterns and the degree of inbreeding in the parental populations (Bingham 1980). In contrast to diploids in which maximum heterosis is reached in a single generation, heterosis in polyploids is progressive and is not fully attained until later hybrid generations (Bingham et al. 1994). It is also possible that higher-order epistatic interactions that are unique to polyploids influenced these patterns and resulted in the production novel genotypes with high fitness. If this were true, polyploids may possess greater potential to explore rough adaptive topographies, such as those posited in Wright's shifting balance theory (Wright 1931; Whitlock et al. 1995).

Finally, it cannot be ruled out that recovery in the F2 is due to viability or fertility selection that caused attrition of F1 genotypes with particularly low fitness. When this experiment was advanced to the F2, F1 individuals of some cross-types grew poorly, did not survive to flower, or produced no seeds following hand pollination. In one extreme case, poor F1 performance precluded the production of an F2 cross-type (VA-700 × IN). Furthermore, in the final greenhouse assay, F1 genotypes had consistently low rates of germination and high rates of deformities and mortality. Nonrandom selection with respect to interacting loci affecting trait

expression may purge genotypes with low fitness and contribute to the rebound in performance observed in the F2 generation. Similar recovery of fitness after hybrid breakdown in the F2 has been observed up to the F6 generation in interpopulation crosses in *Chamaecrista fasciculata* (Erickson and Fenster 2006). These authors point out that the effects of selective purging and the creation of new positive epistatic interactions are not mutually exclusive and postulate their combined effects may play an important creative role in adaptive evolution.

Cytoplasmic and cytonuclear genetic effects strongly influenced trait expression in this study and, like other epistatic effects, appeared most notably in crosses involving the geographically distant Indiana (IN) population. None of the reciprocal F1 or F2 hybrids differed among the populations in closest geographic proximity and few differed for the populations at intermediate distances. However F1, F2, or both reciprocal hybrids frequently differed for crosses between eastern populations and the IN population located >550 km away. In particular, those bearing IN cytoplasm had exceptionally low performance. Strong asymmetry in the phenotype of reciprocal hybrids suggests that populations are differentiated for interactions between cytoplasmic and nuclear genes, especially the IN population versus the others. Again, it is not clear if cytonuclear epsitasis manifested during hybridization results from the formation of incompatibilities between genomes (e.g., Burke and Arnold 2001) or the disruption of intergenomic coadaptation (Rawson and Burton 2002).

Ample evolutionary opportunity exists for genomic coadaptation in plants because many enzymes that perform essential functions in the choloroplast and mitochondria are constructed from polypeptides encoded in both the organelle and nuclear genomes (Borst et al. 1983; Herrmann et al. 2003). Given that the processes of photosynthesis and respiration underlie many quantitative traits (e.g. CO_2 assimilation and biomass), disruption of these beneficial intracellular genomic interactions can have profound effects that are expressed throughout the life span. In particular, the presence of variegated and albino seedlings, 98% of which occurred among hybrid offspring, implicates disruption of chloroplast function (Wong-Staal and Wildman 1973). Both the finding of asymmetrical phenotypes in reciprocal hybrids for many quantitative traits and the predominance of chlorophyll mutants among hybrids suggest that the cytoplasmic genomes contribute significant genetic variance for fitness traits, either singly or epistatically with the nuclear genome. Cytonuclear epistasis is a common cause of male sterility in plants (Frank 1989) and its impact on other phenotypic characteristics is well known for agricultural species (Kihara 1957; Ashri 1964), but less well documented for intraspecific variation in native species (but see Galloway and Fenster 1999). Although cytonuclear interactions are thought to be important to the performance of plant hybrids (Burke and Arnold 2001; Tiffin et al. 2001), and perhaps polyploids specifically (Wendel 2000), few studies have addressed this issue. Therefore, there is little basis for comparison as to whether the frequent contribution of cytonuclear interactions to trait expression in *C. americanum* is influenced by the species ploidy level or whether further investigation may reveal similar interactions in diploid taxa. Nevertheless, our results suggest that cytoplasmic genomes contribute to population divergence, often in concert with the nuclear genome, and thus are integral components of the genetic architecture of plant performance and fitness.

In conclusion, intraspecific hybrids in this polyploid species support a different pattern of genetic divergence than is typically reported for diploid taxa. In particular, F1 hybrids often expressed poor performance, in contrast to the expected heterosis, and in many cases, there was recovery in the recombinant hybrid generations. The genetic architecture also suggests that strong nonadditive gene action differentiates these populations. An enhanced role of epistasis in population differentiation may be the norm in polyploids that have more gene copies. Duplicate gene copies may allow divergence to be less constrained by a function, leading to positive epistasis, as well as differential silencing among populations creating negative epistatic interactions in population hybrids. Further work exploring the genetic architecture of polyploid taxa is needed to determine the generality of these findings.

ACKNOWLEDGMENTS

We appreciate the help of S. Foltz, E. Hurst, G. High, and M. Peshman in creating the hybrid seed and NSF DEB-9974126 and DEB-0316298 to LFG and the Undergraduate Research Opportunities Program at the University of Minnesota Duluth for financial support. We thank M. E. Etterson for providing MATLAB code for bootstrap analyses.

LITERATURE CITED

Adams, K. L., and J. F. Wendel. 2005. Novel patterns of gene expression in polyploid plants. Trends Genet. 21:539–543.

Ashri, A. 1964. Intergenic and genic-cytoplasmic interactions affecting growth habit in peanuts. Genetics 50:363–372.

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. Vol. 8.

Bingham, E. T. 1980. Maximizing heterozygosity in autopolyploids. Pp. 471–491. *in* W. H. Lewis, ed. Polyploidy: biological relevance. Plenum Press, New York.

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

Blanc, G., and K. H. Wolfe. 2004. Functional divergence of duplicated genes formed by polyploidy during Arabidopsis evolution. Plant Cell 16:1679–1691.

Borst, P., J. F. Taback, and L. A. Grivell. 1983. Organelle DNA. Pp. 71–84. *in* S. P. Gregory, and R. A. Flavell, eds. Eukaryotic genes: their structure activity and regulation. Butterworth, London.

Butruille, D. V., and L. S. Boiteux. 2000. Selection-mutation balance in polysomic tetraploids: impact of double reduction and gametophytic selection on the frequency and subchromosomal localization of deleterious mutations. Proc. Natl. Acad. Sci. USA 97:6608–6613.

Burke, J., and M. L. Arnold. 2001. Genetics and the fitness of hybrids. Ann. Rev. Genet. 35:31–52.

Comai, L., A. P. Tyagi, K. Winter, R. Holmes-Davis, S. H. Reynolds, Y. Stevens, and B. Byers. 2000. Phenotypic instability and rapid gene silencing in newly formed *Arabidopsis* allotetraploids. Plant Cell 12:1551–1567.

Coyne, J. A., and H. A. Orr. 1998. The evolutionary genetics of speciation. Philos. Trans. R. Soc. Lond. Ser. B 353:287–305.

Dani, R. G., and R. J. Kohel. 1989. Maternal effects and generation mean analysis of seed-oil content in cotton (*Gossypium hirsutum* L.). Theor. Appl. Genet. 77:569–575.

Devey, M. E., and M. L. Roose. 1987. Genetic analysis of *Verticillium* wilt tolerance in cotton using pedigree data from three crosses. Theor. Appl. Genet. 74:162–167.

Erickson, D. L., and C. B. Fenster. 2006. Intraspecific hybridization and the recovery of fitness in the native legume *Chamaecrista fasciculata*. Evolution 60:225–233.

Fishman, L., and J. H. Willis. 2001. Evidence for Dobzhanzky-Muller incompatibilites contributing to the sterility of hybrids between *Mimulus guttatus* and *M. nasutus*. Evolution 55: 1932–1942.

Frank, S. A.,1989. The evolutionary dynamics of cytoplasmic male-sterility. Am. Nat. 133:345–376.

Galloway, L. F., T. Cirigliano and K. Gremski. 2002. The contribution of display size and dichogamy to potential geitonogamy in *Campanula americana*. Int. J. Plant Sci. 163: 133–139.

Galloway, L. F., and J. R. Etterson. 2005. Population differentiation and hybrid success in *Campanula americana*: geography and genome size. J. Evol. Biol. 18:81–89.

———. 2007. Inbreeding depression in an autotetraploid herb: a three cohort 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.

Galloway, L. F., and C. B. Fenster. 1999. The effect of nuclear and cytoplasmic genes on fitness and local adaptation in an annual legume, *Chamaecrista fasciculata*. Evolution 53:1734–1743.

Herrmann, R. G., R. M. Maier, and C. Schmitz-Linneweber. 2003. Eukaryotic genome evolution: rearrangement and coevolution of compartmentalized genetic information. Philos. Trans. R. Soc. Lond. B 358:87–97.

Hufford, K. M., and S. J. Mazer. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends Ecol. Evol. 18:147–155.

Kalisz, S., and G. M. Wardle. 1994. Life history variation in *Campanula americana* (Campanulaceae): population differentiation. Am. J. Bot. 81:521–527.

Kempthorne, O. 1955. The correlation between relatives in a random mating population. Proc. R. Soc. Lond. B. 143:103–113.

———. 1969. An introduction to genetic statistics. Iowa State Univ. Press, Ames, IA.

Kihara, H. 1957. Completion of genome-analysis of three 6x species of *Aegilops*. Zeiken Ziho 15:1–12.

Killick, R. J. 1971. The biochemical genetics of autotetraploids I. Generations derived from a cross between two pure lines. Heredity 27:331–346.

Kirkpatrick, M., and N. Barton. 2006. Chromosome inversions, local adaptation and speciation. Genetics 173:419–434.

Levy, A. A., and M. Feldman. 2002. The impact of polyploidy on grass genome evolution. Plant Phys. 130:1587–1593.

Lynch, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45:622–629.

Lynch, M., and A. G. Force. 2000. The origin of interspecific genomic incompatibility via gene duplication. Am. Nat. 156:590–605.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Associates Inc., Sunderland, MA.

Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science 264:421–424.

Mather, K., and J. L. Jinks. 1982. Biometrical genetics. Chapman and Hall, London.

Mathworks 2006. MATLAB R2006 a. Natick, MA.

Moody, M. E., L. D. Mueller, and D.E. Soltis. 1993. Genetic variation and random drift in autotetraploid populations. Genetics 134:649–657.

Moyle, L. C., M. S. Olson, and P. Tiffin. 2004. Patterns of reproductive isolation in three angiosperm genera. Evolution 58: 1195–1208.

Osborn, T. C., J. C. Pires, J. A. Birchler, D. L. Auger, Z. J. Chen, H-S. Lee, L. Comai, A. Madlung, R. W. Doerge, V. Colot, and R. A. Martienssen. 2003. Understanding mechanisms of novel gene expression in polyploids. Trends Genet. 19:141–147.

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

Percy, R. G., Z. M. Lu, J. W. Radin, E. L. Turcotte, and E. Zeiger. 1996. Inheritance of stomatal conductance in cotton (*Gossypium barbadense*). Phys. Plant. 96:389–394.

Qu, L., J. F. Hancock, and A. Sharma. 1998. Evolution in an autopolyploid group displaying predominantly bivalent pairing at meiosis: genomic similarity of diploid *Vaccinium darrowi* and autotetraploid *V. corymbosum* (Ericaceae). Am. J. Bot. 85:698–703.

Rawson, P. D., and R. S. Burton. 2002. Functional coadaptation between cytochrome *c* and cytochrome *c* oxidase within allopatric populations of a marine copepod. Proc. Natl. Acad. Sci. USA 99:12955–12958.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363–372.

SAS Institute. 2004. JMP IN Version 5.1.2. SAS Institute, Cary, NC.

———. 2005. SAS/STAT Version 9.1. SAS Institute, Cary, NC.

Seagraves, K. A., J. B. Thompson, P. S. Soltis, and D. E. Soltis. 1999. Multiple origins of polyploidy and the geographic structure of *Heuchera grossulariifolia*. Mol. Ecol. 8:253–262.

Soltis, D. E., and P. S. Soltis. 2003. Advances in the study of polyploidy since *Plant Speciation*. New Phytol. 161:173–191.

Soltis, D. E., P. S. Soltis, J. C. Pires, A. Kovarik, J. A. Tate, and E. Mavrodiev. 2004. Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. Biol. J. Linn. Soc. 82:485–501.

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. USA 92:7719–7723.

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

———. 1969. The significance of hybridization for plant taxonomy and evolution. Taxon 18:26–35.

Straub, S. C. K., B. E. Pfeil, and J. J. Doyle. 2006. Testing the polyploid past of soybean using low-copy nuclear gene—is *Glycine* (Fabaceae: Papilionoideae) an auto- or allopolyploid? Mol. Phylogenet. Evol. 39:580–584.

Templeton, A.R. 1986. Coadaptation and outbreeding depression. Pp. 105–116. *in* M. Soulé, ed. Conservation biology: science of scarcity and diversity. Sinauer, Sunderland, MA.

Tiffin, P., M. S. Olson, and L. C. Moyle. 2001. Asymmetrical crossing barriers in angiosperms. Proc. R. Soc. Lond. B. 268:861–867.

Turelli, M., and H. A. Orr. 2000. Dominance, epistasis and the genetics of postzygotic isolation. Genetics 154:1663–1679.

Wang, X., S. Xiaoli, H. Bailin, G. Song, and L. Jingchu. 2005. Duplication and DNA segmental loss in the rice genome: implications for diploidization. New Phyt. 165:937–946.

Wendel, J. F. 2000. Genome evolution in polyploids. Plant Mol. Biol. 42:225–249.

Werth, C. R., and M. D. Windham. 1991. A model for divergent allopatric speciation of polyploid pteridophytes resulting from silencing of duplicate gene expression. Am. Nat. 137: 515–526.

Whitlock, M. C., P. C. Phillips, F. B. G. Moore, and S. J. Tonsor. 1995. Multiple fitness peaks and epistasis. Ann. Rev. Ecol. Syst. 26:601–629.

Wong-Staal, F., and S. Wildman. 1973. Identification of a mutation in chloroplast DNA correlated with formation of defective chloroplasts in variegated mutant of *Nicotiana tabacum*. Planta 113:313–326.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97–159.

———. 1938. The distribution of gene frequencies in populations of polyploids. Proc. Natl. Acad. Sci. USA 24:372–377.

Zhu, T. J. M. Schupp, A. Oliphant, and P. Keim. 1994. Hypomethylated sequences: characterization of the duplicate soybean genome. Mol. Gen. Genet. 244:638–645.

Associate Editor: J. Kohn

Supplementary Material

The following supplementary material is available for this article:

Table S1. Supplementary Linear model test statistics for continuous traits (analysis of variance) and discrete traits (log-linear analysis) measured on parental and hybrid generations produced by among-population crosses of *Campanulastrum americanum*. Analysis of data for crosses among populations in trio 1 and 2 were conducted separately.

Appendix Literature Cited.

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/ 10.1111/j.1558-5646.2007.00234.x
(This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.