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ORIGINS, DISTRIBUTION, AND LOCAL CO-OCCURRENCE OF POLYPLOID CYTOTYPES IN *SOLIDAGO ALTISSIMA* (ASTERACEAE)[1]

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There is growing realization that intraspecific polyploidy in plants has important implications for the evolution of plant diversity and for plant and plant–herbivore community dynamics. Nonetheless, the rate at which polyploid cytotypes arise and their fate in local mixed-cytotype populations are not well understood. Although within- and especially among-population cytotype variation has been documented in many plants, particularly at the largest (continental) spatial scales, local and regional distributions of cytotypes have been well characterized only for a handful of species. Furthermore, few if any studies have addressed both local and regional patterns in the same plant species with sufficient power to detect cytotype variation on both scales. We assessed the co-occurrence and frequency distributions of diploid, tetraploid, and hexaploid cytotypes of *Solidago altissima* (Asteraceae) at local and regional spatial scales, sampling across a zone of presumed ploidy-form overlap in the midwestern United States. Determining ploidy by flow cytometry, we found many local populations containing two or three cytotypes, with the relative frequencies of cytotypes varying dramatically even among neighboring populations. AFLP marker data suggest that polyploid cytotypes of *S. altissima* likely have multiple origins from different diploid lineages. Our results are consistent with recent perspectives on polyploidization as an evolutionarily dynamic phenomenon with frequent multiple origins of higher-ploidy cytotypes.

Key words: AFLP; Asteraceae; coexistence; flow cytometry; goldenrod; polyploidy; *Solidago altissima*.

Polyploidization has been a major source of genetic and phylogenetic diversity along many branches of the tree of life, and has been especially important in the evolution of angiosperm plants. A substantial fraction, and likely a majority, of angiosperm species are of polyploid origin (estimates range from 30–70% and even higher; Stebbins, 1950; Grant, 1981; Masterson, 1994; Bennett, 2004). Cytotypic variation is also rampant within many angiosperm species or species complexes, and where it occurs it can shape both plant evolutionary dynamics (Otto and Whitton, 2000; Dobeš et al., 2004; Yeung et al., 2005) and the community ecology of plants and their insect herbivores (e.g., Nuismer and Thompson, 2001; Thompson et al., 2004; Halverson et al., 2007). Despite the evolutionary and ecological importance of intraspecific polyploidization, little is known about the rates at which new cytotypes arise, about cytotype variation at local or regional spatial scales, or about the fate of new cytotypes in local mixed-cytotype populations. In particular, neither the local distribution nor the evolutionary origins of intraspecific polyploid cytotypes have been well documented for more than a handful of species.

The codistribution of intraspecific cytotypes is extremely important in setting the stage for their ecological interaction, for gene flow (Herrera et al., 2004; Martonfiova, 2006) or gamete wastage due to cross-pollination (Levin, 1975; Baack, 2005a, b), and for potential community-level effects where herbivores, pathogens, or other interacting species prefer, or perform better on, one cytotype relative to another (Nuismer and Thompson, 2001; Thompson et al., 2004; Halverson et al., 2007). In many cases, cytotypic variation is exclusively geographic, with local populations consisting of a single cytotype (e.g., Yeung et al., 2005; Yang et al., 2006). In other cases, mixed-cytotype populations are known, although it is often not clear whether co-occurrence of cytotypes is typical for the species (e.g., Keeler, 2004), widespread but infrequent (e.g., Rothera and Davy, 1986; Lumaret et al., 1987; Schranz et al., 2005), or restricted to a few populations in ecotonal habitats or at contact zones (e.g., Borrill and Lindner, 1971; Husband and Schemske, 1998; Hardy et al., 2000; Stuessy et al., 2004). Studies capable of distinguishing these alternatives are fairly uncommon, because doing so requires both intensive (many plants per site) and extensive (many sites) sampling and studies attempting to infer intrapopulation cytotype distribution based on just a handful of collections (e.g., Rothera and Davy, 1986; Murray et al., 1989; Hopkins et al., 1996; Stuessy et al., 2004) may underestimate the tendency for codistribution of cytotypes. As well-sampled studies have accumulated (largely since the introduction of flow cytometric methods for cytotype determination), some suggest an apparent paradox: surprisingly widespread co-occurrence of cytotypes in some species (e.g., Keeler, 2004) despite theoretical arguments that without niche separation or prezygotic isolation between cytotypes, local co-occurrence should be unstable and minority cytotypes should be lost to extinction (Fowler and Levin, 1984; Rodriguez, 1996; Husband, 2000; Kennedy et al., 2006). It is not yet clear whether persistent mixed-cytotype populations are rare exceptions or are common enough to challenge theoretical understanding of cytotype dynamics.

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Another open question of fundamental importance is whether polyploid cytotypes typically have single or multiple evolutionary origins, with the latter being especially plausible where polyploids are widely codistributed with presumptive ancestral diploids. Polyploidization might be a rare event, with its role primarily macroevolutionary and most polyploids having single, relatively ancient origins (e.g., Widmer and Baltisberger, 1999; Hardy et al., 2000). Alternatively, polyploidization might be frequent on evolutionary time scales, with polyploid cytotypes originating repeatedly within a single parent species (for autopolyploids) or between the same pair of parent species (for allopolyploids). Recent reviews (Soltis and Soltis, 1993; Ramsey and Schemske, 1998) have suggested, on both empirical and theoretical grounds, that rates of polyploidization are often high enough for multiple origins of cytotypes to be common; and well-documented cases of multiple origins are available for both allopolyploids (e.g., Brochmann et al., 1992; Soltis et al., 2004) and autopolyploids (e.g., Segraves et al., 1999; Dobeš et al., 2004).

In this paper, we describe within- and between-population patterns in distribution of cytotypes of *Solidago altissima* L. (Asteraceae), a widespread and abundant goldenrod. Diploid, tetraploid, and hexaploid cytotypes are known to occur at a continental scale in *S. altissima* (Semple and Cook, 2006) and to co-occur in some regions, but little is known about the codistribution of these cytotypes at local scales. *Solidago altissima* and its close relative *S. gigantea* have been widely used as a model system for the study of plant–insect interactions (e.g., Abrahamson and Weis, 1997; Stireman et al., 2005, 2006; Heard et al., 2006), and cytotypic variation may represent an important form of genetic structure in *S. altissima* populations (with direct consequences for the distribution of insect herbivores; Halverson et al., 2007). Here, we document patterns of co-occurrence of the three cytotypes within and among populations across a 570-km transect through a zone of cytotypic overlap in the Midwest of the United States. In addition, we use amplified fragment-length polymorphism (AFLP) marker profiles to test the hypothesis of single, ancient origins for each polyploid form.

MATERIALS AND METHODS

Study species—*Solidago* (Asteraceae: Astereae) is a primarily North American genus of ca. 100 species, in which cytotypic variation is rampant within species (Semple and Cook, 2006). (Proper taxonomic treatment of "intraspecific" cytotypic variation in plants is contentious and can depend on the resolution of conflicting biological, morphological, and phylogenetic species concepts; however, this debate does not concern us here, and we use an intraspecific terminology for simplicity.) *Solidago altissima* (late goldenrod) belongs to the *S. canadensis* complex of subsect. Triplinervae and is a rhizomatous, herbaceous perennial with a broad geographic range (Nova Scotia to Florida, west to Texas and Alberta; Fig. 1). It is self-incompatible and hence obligately outcrossing, with most floral visitors being generalist pollinators such as bees (Gross and Werner, 1983). It is a common species of prairies, woodland edges, and open areas, and in association with human development, old fields, roadsides, and similar disturbed areas. *Solidago altissima* has long been known to include diploid ($2n = 18$), tetraploid ($2n = 36$), and hexaploid ($2n = 54$) cytotypes (Semple and Cook, 2006), with the higher ploidies most likely autopolyploids (J. C. Semple, University of Waterloo, ON, Canada, personal communication). While the taxonomic treatment of the species has been complex, recent treatments (Semple and Cook, 2006) have recognized two subspecific taxa. *Solidago altissima* subsp. *altissima* is distributed across the woodlands of eastern North America and west into the prairies roughly to the eastern Dakotas and Texas, while *S. altissima* subsp. *gilvocanescens* is primarily western but extends east to overlap with subsp. *altissima* primarily in the tallgrass prairie region (Minnesota south to Missouri and east into Indiana; Fig. 1). These subspecific taxa appear to be associated with cytotypic variation, with subsp. *gilvocanescens* reported as diploid and tetraploid across its range and subsp. *altissima* primarily hexaploid (a few tetraploids have been reported at the western edge of the distribution and across the southeastern US; Semple and Cook, 2006). The result is an expected continental-scale pattern of hexaploids in the east, with diploids and tetraploids in the west, and a zone of overlap broadest across Iowa and Illinois where all three cytotypes may co-occur (Fig. 1). This pattern of cytotypic variation, however, is tentative: it is based on rather few published chromosome counts (~100 across the entire range), and data on local-scale co-occurence of cytotypes are entirely lacking. It is, furthermore, unknown whether diploid, tetraploid, and hexaploid individuals are interfertile, whether polyploid cytotypes represent ancient or new lineages, or whether each cytotype arose only once or repeatedly.

Sampling of goldenrod populations—We surveyed *S. altissima* populations for cytotypic variation across a 570-km transect through the midwestern USA, in the zone where subsp. *altissima* and subsp. *gilvocanescens* were presumed to overlap (and hence all three cytotypes were expected). Our focus was on local and regional co-occurrence rather than on the continental biogeography of cytotypes, so we did not attempt to map the extent of the overlap zone—an enterprise that would have required vastly expanded sampling. We sampled *S. altissima* populations in the summers of 2003–2004 (except Grinnell, IA collections in 2001), collecting *S. altissima* material from 16 sites distributed along an east–west transect running through Illinois, Iowa, and Nebraska (Fig. 1). At each site, we haphazardly sampled $N = 18$–34 individuals over an area of approximately 1 ha, with a minimum of 10 m between samples to minimize the probability of sampling multiple ramets from individual genets. From each ramet, we took several leaves, which were refrigerated or flash-frozen in liquid nitrogen and stored at −80°C until the cytotype could be determined via flow cytometry (procedure detailed in the next section).

These initial sample sizes gave us good power to detect even uncommon cytotypes: for $N = 20$ and $N = 30$, we had 80% power to detect a cytotype present at 7.7% and 5.2%, respectively. However, to better quantify the relative frequencies of different cytotypes at local scales, we returned to six of the eight sites for which the initial analyses indicated the presence of more than one cytotype. We sampled additional ramets at each of these sites, this time collecting at 10-m intervals on a 40 × 120-m grid (JST, BVL, NNA, SWA), 5-m intervals on a 40 × 120-m grid (RAL), or 5-m intervals on a 40 × 80-m grid (MFP; we used different grid shapes and spacings to accommodate differences among sites in the size and shape of goldenrod patches). With these additional specimens and allowing for samples that proved unsuitable for flow cytometry, our final sample sizes at the eight mixed-cytotype sites ranged from 21 to 83 ramets (Table 1). In total, we determined chromosome number for 500 *S. altissima* ramets—five times as many as all previously published counts for the species.

Detailed location data for all collection sites are provided in Appendix 1.

Flow cytometric determination of ploidy—We determined the cytotype of each *S. altissima* ramet using flow cytometry. Flow cytometric methods measure DNA content of nuclei via incorporation of propidium iodide (a fluorescent nuclear stain). DNA content can then usually be interpreted in terms of chromosome number, especially when known-cytotype standards are available (de Laat et al., 1987; Akinerdem, 1991; Arumuganathan and Earle, 1991). For each *S. altissima* ramet sampled, we placed chilled leaf tissue in a petri plate with 25 μL of Galbraith buffer (Galbraith et al., 1983). We finely chopped the leaves with a new razor blade, added 2 mL of Galbraith buffer, and filtered all through 50-μm and 20-μm microfilters into polystyrene tubes. Tubes were centrifuged at 800 × *g* for 8 min at 4°C, the supernatant removed, and the pellet resuspended in 0.60–1.00 mL of 10% w/v propidium iodide. The stained samples were vortexed and analyzed on a Beckman-Coulter (Fullerton, California, USA) Epics XL-MCL flow cytometer in the Iowa State University Flow Cytometry Facility. The cytotype was determined by measuring the propidium iodide fluorescence (488 nm excitation) of ~3000 nuclei. We converted fluorescence to chromosome number using *S. altissima* standards (collections with cytotype determined by conventional examination of mitotic root-tip squashes) provided by J. C. Semple (University of Waterloo). In all cases, standards yielded the expected patterns of relative fluorescence intensity, and sampled individuals were easily categorized as diploid, tetraploid, or hexaploid.

Statistical analysis of cytotype frequencies and spatial pattern—We used a *G* test of association to compare frequencies of cytotypes among the mixed-cytotype sites (the null hypothesis being that, at all sites, we were sampling from a common cytotype distribution). We omitted single-cytotype sites from

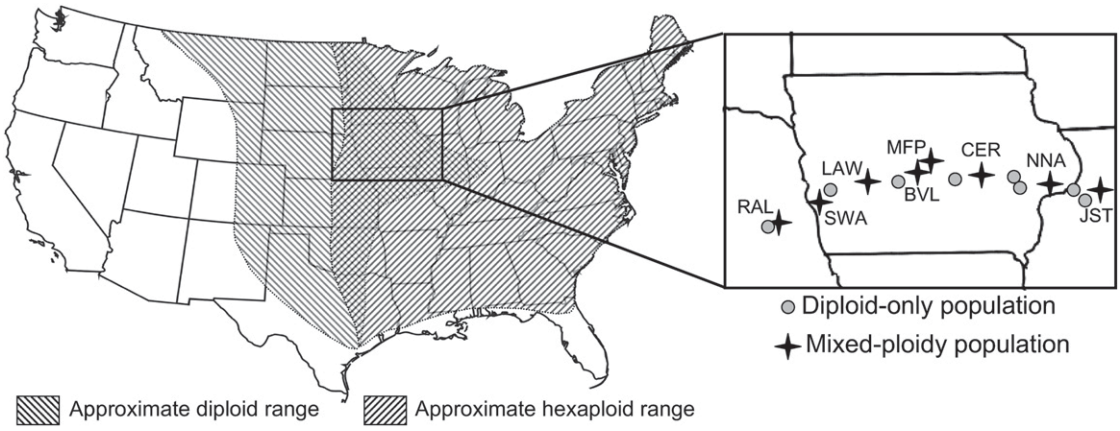

Fig. 1. Approximate ranges of *Solidago altissima* diploids (subsp. *gilvocanescens*) and hexaploids (subsp. *altissima*) in the USA, and locations of 16 sampled populations (right; see Appendix 1 for site names and descriptions). Ranges of both diploids and hexaploids extend into Canada, although the northern limits in the boreal forest region are uncertain. Tetraploids (not shown) are infrequent in subsp. *altissima* across the southern part of its range and common in subsp. *gilvocanescens* in at least the eastern part of its range. Range data from Semple et al. (1984) and J. C. Semple (University of Waterloo, ON, Canada, personal communication).

this analysis. Following this overall *G* test, we conducted separate *G* tests (with Williams' correction) at each mixed-cytotype site to test the hypothesis of locally equal frequencies for the three cytotypes.

For each of the six mixed-cytotype sites at which we sampled goldenrod ramets on a grid, we tested for spatial aggregation of plants by cytotype. Such aggregation (in which plants of a given cytotype tend to occur in one or more clumps, and therefore tend to be close neighbors of one another) would be expected if seed dispersal were limited or if the cytotypes differ in their microhabitat associations. (Aggregation by cytotype would also be expected if we were sampling multiple ramets from a single clone of each cytotype, but AFLP banding patterns rule out this possibility; data not shown). We tested for cytotype aggregation using two different randomization analyses that address slightly different possibilities for spatial structure in our data. In the first analysis, we calculated the average distance between ramets of the same cytotype, then compared this distance to similar calculations for 1000 data sets in which cytotype labels were shuffled randomly among ramets. In the second analysis, we calculated the fraction of nearest-neighbor ramet pairs sharing a common cytotype, and then compared this fraction to similar calculations for 1000 data sets in which cytotype labels were shufled randomly among ramets. These analyses were performed using software written by one of us (S.B.H.) in QuickBASIC (Microsoft, Redmond, Washington, USA).

AFLP analysis—To test hypotheses of single vs. multiple origins for each polyploid cytotype, we determined AFLP profiles for 78 individual *S. altissima* collected from four sites at which all three cytotypes were present. Four individuals had anomalous AFLP profiles suggestive of problems with DNA digestion or amplification; we omitted these individuals, leaving a final sample size of 74 individuals including multiple individuals from each cytotype in each population (Table 2). Our AFLP sample size was much smaller than our cytotype-determination sample size because we were no longer concerned with the detection of rare cytotypes. DNA was isolated from samples via cetyl trimethylammonium bromide (CTAB) automated extraction (Iowa State University DNA Sequencing Facility) and quantified on a 2% agarose gel. These samples were subjected to an AFLP protocol optimized from standard AFLP methods (Vos et al., 1995). Approximately 200 ng of genomic DNA was digested with 10 units each of *Mse*I and *Eco*RI (New England Biolabs, Ipswich, Massachusetts, USA) at 37°C for 2 h and 65°C for 15 min, and ligated with 5 units of Biolase T4-ligase (Denville Scientific, Metuchen, New Jersey, USA) to double-stranded adapters incubated at 16°C overnight. Preselective amplification used 10 μL of restricted/ligated template (diluted up to 5× depending on the quantity of 50–800 bp restriction/ligation product as visualized by gel electrophoresis) and 5 μL of 10× PCR buffer (Biolase) in a 50-μL reaction volume containing 1.5 mM $MgCl_2$, 0.8 μM dNTP (Invitrogen, Carlsbad, California, USA), 0.8 μM each Mse+C and Eco+A primers, and 2.5 units *Taq* polymerase

TABLE 1. Sample frequencies, tests for equal cytotypic frequencies, and tests for spatial aggregation of diploid, tetraploid, and hexaploid cytotypes. Significant ($P < 0.05$) results are in boldface. The eight sites listed (from west to east) are those for which multiple cytotypes were detected; eight additional sites yielded only diploids (194 total ramets). For detailed site locations, see Appendix 1. For the spatial aggregation tests, the first *P* value is based on average distance between plants of the same cytotype; the second is based on the frequency of nearest-neighbor plants sharing a cytotype.

Site	*N*	Percentage			Test for equal frequencies[a]	Spatial aggregation by cytotype?
		Diploid	Tetraploid	Hexaploid		
Rest Area, Lincoln, NE (RAL)	33	0	33	67	***P* < 0.0001**	No (*P* = 0.69, 0.46)
Smith Wildlife Area, Council Bluffs, IA (SWA)	34	15	35	50	***P* = 0.048**	No (*P* = 0.67, 0.88)
Lake Anita Wildlife Preserve, Anita, IA (LAW)	25	76	16	8	***P* < 0.0001**	—
Beaver Lake, Dexter, IA (BVL)	32	16	34	50	***P* = 0.034**	No (*P* = 0.85, 0.06)
McFarland Park, Ames, IA (MFP)	66	85	15	0	***P* < 0.0001**	No (*P* = 0.94, 0.26)
Conard Environmental Research Area, Grinnell, IA (CER)	83	45	5	51	***P* < 0.0001**	—
Norton Nature Area, Durant, IA (NNA)	36	72	17	11	***P* < 0.0001**	No (*P* = 0.17, 0.97)
Johnson Sauk Trail State Park, Annawan, IL (JST)	21	24	19	57	*P* = 0.085	No (*P* = 0.71, 0.36)
Total	306	44	19	37		

[a]Single-classification *G* test vs. expected frequencies of 1/3 for each cytotype; $P < 0.05$ indicates significant deviation from equal frequencies.

TABLE 2. Sample sizes (number of individuals) for AFLP analysis. For detailed site locations, see Appendix 1.

Site	Diploids	Tetraploids	Hexaploids
SWA	2	10	9
BVL)	6	5	4
CER	8	5	8
JST	6	7	4

(Biolase), with a PCR cycle of 72°C for 2 min; 20 cycles of 94°C for 30 s, 56°C for 30 min, and 72°C for 1 min; and a final 30 min extension at 60°C. Two primer combinations were selected for the quality and quantity of the 108 polymorphic bands produced: TET-Eco+ACA/FAM-Eco+ACC/MSE+CAT and TET-Eco+ACA/FAM-Eco+ACC/MSE+CAA. Selective amplification was implemented using 5 μL of preselective amplicon template (diluted up to 15× depending on the quantity of preselective amplification product as visualized by gel electrophoresis) and 2.5 μL of 10× PCR buffer in a 25-μL reaction volume containing 30 μM $MgCl_2$, 1.2 μM dNTP, 1 μM Mse+C primer, 0.15 μM each Eco+ACA and Eco+ACC primers, and 0.25 units *Taq* polymerase (Biolase). The PCR cycle was 94°C for 2 min; 20 cycles of 94°C for 30 s, 65°C for 30 s (reducing by 1°C per cycle), and 72°C for 2 min; 36 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 2 min; and a final extension step at 60°C for 30 min. AFLP products were detected with an ABI 377 Perkin-Elmer Automated Sequencer (Applied Biosystems, Foster City, California, USA) on a 5% polyacrylamide gel by electrophoresis (ISU DNA Sequencing Facility). Fragments and peak sizes were visualized with GeneScan Analysis 3.1 (Perkin-Elmer, Waltham, Massachusetts, USA). Scoring was completed with Genographer software (version 1.6; http://hordeum.msu.montana.edu/genographer).

Analysis of single vs. multiple origins—We took three complementary analytical approaches to determine whether the single origin and multiple origins models best fit the data. All are based on the same data (74 AFLP profiles representing three cytotypes at each of four populations).

AMOVA analysis—We tested two contrasting models explaining genetic relationships among cytotypes and sampling locations, using the AFLP genotypes and analysis of molecular variance (AMOVA; Excoffier et al., 1992) implemented in GenAlEx 6 (Peakall and Smouse, 2006). The single origin model proposes that extant tetraploid and hexaploid cytotypes arose once each and now represent monophyletic lineages. This model also necessarily proposes that the origins of the polyploid cytotypes took place long enough ago for the resultant cytotypes to have spread across their modern range. In terms of our AFLP data, this hypothesis specifies a hierarchical AMOVA model in which genotypes are nested within sites and sites within cytotypes. Alternatively, the strictest multiple origins model proposes that polyploid cytotypes have arisen repeatedly and independently (and perhaps recently) at each geographic location. This hypothesis was tested using an AMOVA analysis in which genotypes were nested within cytotypes nested within sites. The preferred AMOVA model is that in which the higher stratum (cytotypes for the single origin model or sites for the multiple origins model) explains the greatest component of total genetic variation. We quantified this preference using the corrected Akaike information criterion (AIC_c; Burnham and Anderson, 1998), calculated based on the mean square associated with all hierarchical levels except the highest level one (cytotypes for the ancient polyploidy model, sites for the neopolyploidy model; see Appendix 2). A poor fit of the data to both models would suggest a more complex historical scenario for the evolution of polyploid forms, perhaps with limited numbers of polyploid origins followed by regional dispersal.

STRUCTURE analysis—We also analyzed our AFLP data using the computer program STRUCTURE, which clusters individuals based on their multilocus genotype using a Markov chain Monte Carlo (MCMC) algorithm (Pritchard et al., 2000; Falush et al., 2007). We used STRUCTURE version 2.2 (Falush et al. 2007), which implements algorithms accounting for genotypic uncertainty arising from the dominant behavior of AFLP markers and from copy number variation when the data include polyploid cytotypes. Under the single origin model, we would expect the *K* clusters identified by STRUCTURE to correspond to cytotypes, whereas under the multiple origins model we would expect clusters corresponding to sites. We obtained posterior probabilities of *K* for $K = 1$–8 clusters using STRUCTURE's Admixture model, which allows for potential recombination between inferred clusters. Each value of *K* was evaluated using 25 independent MCMC replicates consisting of a burn-in of 5000 iterations followed by a run of 25 000 iterations. We inferred the number of clusters following Pritchard et al. (2000) with posterior probabilities of *K* calculated assuming uniform priors on *K* and using for each *K* the maximum value of the probability of the data given *K*, ln Pr(*X*|*K*), obtained over MCMC replicates.

Phylogenetic analysis—We also visualized relationships among cytotypes and locations as a phylogenetic tree. We grouped our 74 AFLP profiles by site and cytotype into 12 "populations" (four sites × three cytotypes) and calculated population pairwise Euclidean distances, *E*, as described for binary data by Huff et al. (1993). This metric is equivalent to the binary distance used in AMOVA (Excoffier et al., 1992) and, for *n* polymorphic sites over all sample populations, may range for a pair of populations from zero (shared monomorphism at all sites) to *n* (fixed differences at all sites). From these pairwise distances, we built an unrooted neighbor-joining tree among populations. Bootstrap support was calculated from 999 replicates, resampling individuals within populations using a custom resampling program written by one of us (J.D.N.) in Python (Python Software Foundation, Hampton, New Hampshire, USA; http://www.Python.org) combined with the Neighbor and Consense modules of the program PHYLIP (Felsenstein, 2005).

RESULTS

Cytotype frequencies and spatial pattern—Of the 16 sites surveyed, initial samples of eight included only diploid plants, while eight others included two or more cytotypes (Table 1). Among the eight mixed-cytotype sites, there was very strong heterogeneity in presence and relative frequency of 2N, 4N, and

TABLE 3. AMOVA analyses associated with the (A) single origin polyploidy model and (B) strict multiple origins polyploidy model. Significant ($P < 0.05$) results in boldface type. Details of AIC_c computation are in Appendix 2.

A) Single origin polyploidy model ($AIC_c = 137.9$)						
Source of variation	df	Sum of squares	Estimated variance	% of variation	Φ	*P*
Among cytotypes	2	17.7	0.0[a]	0	0.000	0.59
Among sites w/in cytotypes	9	78.9	0.54	9	0.088	**0.001**
Individuals within sites	62	346	5.58	91		
Total	73	442				
B) Strict multiple origins polyploidy model ($AIC_c = 134.0$)						
Source of variation	df	Sum of squares	Estimated variance	% of variation	Φ	*P*
Among sites	3	40.0	0.33	5	0.053	**0.002**
Among cytotypes within sites	8	56.7	0.26	4	0.044	**0.037**
Individuals within cytotypes	62	346	5.58	91		
Total	73	442				

[a]A small but negative variance component here is set to zero.

6N cytotypes (G = 182, 14 df, $P < 0.0001$). Individual-site G tests rejected the hypothesis of equal frequencies for the cytotypes at seven of the eight mixed-cytotype sites; diploids predominated across the transect, with locally high frequencies of tetraploids and especially hexaploids (Table 1). Within mixed-cytotype sites, there was no indication of spatial aggregation of the cytotypes (Table 1), at least at the scales (5 and 10 m) on which we sampled ramets.

AFLP genotypes and polyploid origins—AFLP analysis yielded 112 scorable loci (fragments), of which 106 were variable among individuals. AMOVA results were inconsistent with the single-origin model (Table 3A): genetic variance associated with cytotype as the highest-level effect was negligible and nonsignificant. In contrast, AMOVA results for the alternative multiple-origins model (Table 3B) indicated that genetic variation associated with site as the highest-level effect was highly significant ($\Phi = 0.05$; $P = 0.002$). Consistent with this difference, AIC_c indicates that the single-origin model has considerably less support than the multiple-origins model (ΔAIC_c = 3.9; Table 3).

STRUCTURE analysis identified three clusters of individuals ($K = 3$ having a posterior probability of greater than 0.999 relative to other evaluated values of K). These clusters, however, did not correspond to the three cytotypes (Table 4). Instead, the identified clusters correspond loosely to sites: CER collections have higher membership in cluster 1 than individuals from other sites, while JST collections have the highest membership in cluster 2, and BVL and SWA collections have the highest membership in cluster 3. However, there is considerable heterogeneity in assignments among individuals within each cytotype × site combination (data not shown), suggesting in agreement with AMOVA results that genetic differentiation by collection site, though much stronger than by cytotype, is certainly not categorical.

Visualization of AFLP genetic structure as a neighbor-joining tree (Fig. 2) confirms the AMOVA and STRUCTURE analyses: *S. altissima* "populations" (defined by site and cytotype) show no sign of grouping by cytotype. Some grouping by site is apparent in the tree, although bootstrap support for nodes is generally low. The single node with high support groups tetraploids and hexaploids from the same site (JST; 81%), to the exclusion of polyploid cytotypes from other sites.

DISCUSSION

Cytotype frequencies and spatial pattern—Our results demonstrate considerable cytotypic variation both within and among *S. altissima* populations in the Midwest. Because our westernmost and easternmost populations both retain cytotype variation (and because we have not sampled along the north–south axis), our results provide a lower bound on the geographical extent of local cytotype co-occurrence in *S. altissima*.

Within mixed-cytotype sites, we found no tendency for spatial aggregation of cytotypes, at least at the spatial scales addressed by our sampling (on 5- and 10-m grids). Of course, had we sampled on fine enough scales, we would certainly have detected spatial aggregation simply because *S. altissima* is a rhizomatous clonal perennial (Maddox et al., 1989). We designed our sampling to avoid collecting multiple ramets from individual clones (with individual-ramet AFLP profiles confirming that we were successful), however, because our focus was on possible aggregation of genets by cytotype.

Our tests for spatial aggregation by cytotype serve as an indirect test for microhabitat differences among cytotypes—an important issue because theoretical studies have suggested that mixed-cytotype populations should be evolutionarily unstable except when cytotypes have highly local dispersal (Baack, 2005b), distinct microhabitat preferences (e.g., Fowler and Levin, 1984; Rodriguez, 1996), or strong prezygotic isolation,

TABLE 4. Results of STRUCTURE (version 2.2) analysis of AFLP profiles. (A) Log-likelihood estimation of cluster number for K = 1–4; $K = 3$ clusters are favored. (B) Assignments of cluster membership for each cytotype × site combination. Dashed lines highlight the sites having highest membership assignment for each cluster.

A) Log-likelihood estimation

	K (no. clusters)			
Estimate	1	2	3	4
Highest ln Pr(X|K)	−3709.4	−3509.8	−3491.5	−3692.6
△ ln-likelihood	−217.9	−18.3	0.0	−201.1
Posterior Pr(K)	2×10^{-95}	1×10^{-08}	>0.999	5×10^{-88}

B) Assignment of cluster membership

		Cluster membership			
Cytotype	Site	1	2	3	n
2	BVL	0.284	0.006	0.710	6
4	BVL	0.811	0.010	0.179	5
6	BVL	0.502	0.007	0.490	4
2	CER	0.702	0.036	0.262	8
4	CER	0.946	0.012	0.042	5
6	CER	0.871	0.005	0.124	8
2	JST	0.532	0.166	0.301	6
4	JST	0.587	0.401	0.012	7
6	JST	0.746	0.251	0.003	4
2	SWA	0.499	0.007	0.494	2
4	SWA	0.555	0.016	0.429	10
6	SWA	0.373	0.028	0.598	9

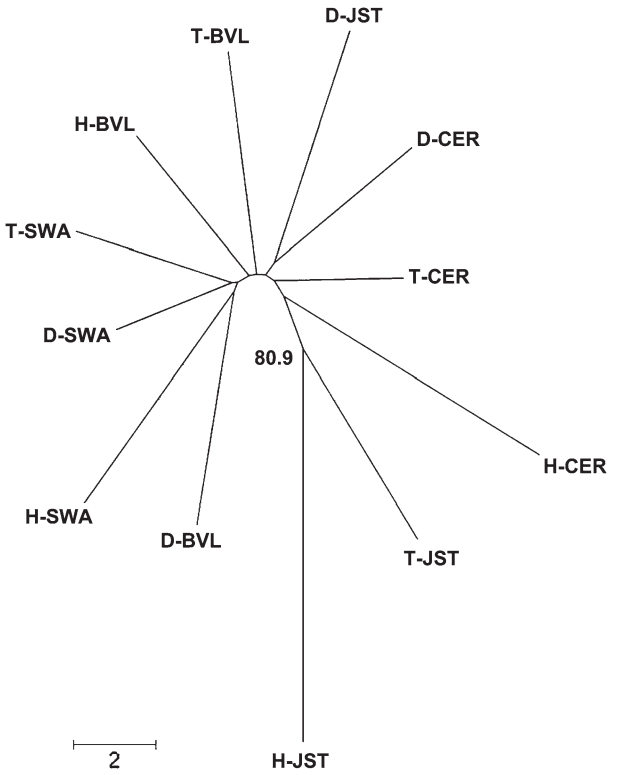

Fig. 2. Unrooted neighbor-joining tree of populations (site × cytotype combinations). Population labels show cytotype (D = 2N, T = 4N, H = 6N) and site (abbreviations as in Table 1). Number indicates bootstrap support for the node T-JST + H-JST (support values for all other nodes are <50% and are not shown). Scale bar indicates Euclidean (AMOVA) distance of Huff et al. (1993); see text for details.

for instance as enforced by pollinator movements within rather than between cytotypes (e.g., Kennedy et al., 2006). Such niche differentiation (seen in other Astereae; e.g., Chmielewski and Semple, 1983) would act to alleviate competition between cytotypes and to reduce cross-pollination and gamete wastage by pollinator movement between cytotypes. Strong niche differentiation among cytotypes is known for several other plant species (e.g., Lumaret et al., 1987; Rothera and Davy, 1986), and it can strongly reduce pollen movement between cytotypes (Kennedy et al., 2006), but our spatial data suggest that for *S. altissima* either such differentiation is weak (or absent) or it occurs on a very fine spatial scale. While we cannot rule out strong niche differentiation on spatial scales smaller than that of our sampling, we suspect that such fine-scale separation of cytotypes would be ineffective in stabilizing co-occurrence. Certainly, separation on scales of a few meters or even tens of meters would not prevent pollen movement among *Solidago* cytotypes (the generalist pollinators of *Solidago* spp. having much longer cruising ranges; S. D. Hendrix, University of Iowa, Iowa City, personal communication). Resource competition among cytotypes, if important, is also likely to occur on scales of at least a few meters, given the clonal structure of *Solidago* populations (Maddox et al., 1989).

In summary, local co-occurrence of *S. altissima* cytotypes is not a rare phenomenon, at least in the midwestern zone of regional cytotype overlap, and it appears to persist despite the lack of strong niche separation among cytotypes. This represents something of a challenge to theoretical predictions that such co-occurrence should be unstable, with minority cytotypes lost to extinction (Fowler and Levin, 1984; Rodriguez, 1996; Husband, 2000), although models differ with respect to how much niche separation is required (Rodriguez, 1996), and theoretical exploration is far from complete. There are at least four possible resolutions of this challenge. First, our current theoretical understanding may simply be incorrect. Second, gamete wastage may be reduced or even eliminated if flowering phenology differs sufficiently among cytotypes (Husband and Sabara, 2004). Such patterns have been documented for other species in the Astereae (Shackleford, 1983) and appear to exist for *S. altissima* as well (based on collection records; J. C. Semple, personal communication), with diploids flowering earliest on average, tetraploids later, and hexaploids later still. However, no data have been available to assess the overlap in flowering phenology among cytotypes at mixed-cytotype sites (and complete nonoverlap is unlikely). Third, pollinator preference for visits to a particular cytotype or gametic selection might enforce prezygotic isolation (Husband and Sabara, 2004; Kennedy et al., 2006), even though multiple cytotypes are within pollinator cruising range. Finally, the theoretical prediction (that cytotype-form coexistence is unstable) may simply be irrelevant, because plant populations have not reached the equilibrium at which all cytotypes but one are locally excluded. This possibility is of particular interest for *S. altissima*, which is a disturbance-tolerant plant of landscapes that have been, and continue to be, heavily modified since European colonization of North America. In the face of such nonequilibrial processes and frequent establishment and local extinction of *S. altissima* populations, the effects of niche differentiation or competitive exclusion promoting or limiting the co-occurrence of cytotypes, respectively, may simply be too weak to detect.

Unfortunately (because local cytotype distributions have been studied for relatively few species), we do not know whether our *Solidago* result is exceptional or whether such challenges to current theory will be commonplace. Comparative studies across many cytotypically variable species, including data on microhabitat differentiation and interfertility, may be required for a full understanding of the ecological and evolutionary consequences of cytotype codistribution in plants.

Polyploid origins—The two models of cytotype origins tested by our AMOVA analyses make dramatically different predictions about the structure of AFLP genetic variation. Under the single origin model, tetraploid and hexaploid cytotype of *S. altissima* would represent monophyletic and well-differentiated lineages, with AFLP genotypes predicted to group strongly by cytotype. (Strong differentiation is implied by the time necessary for a single polyploid clade to disperse throughout its modern range.) Under the strict multiple origins model, in contrast, polyploid cytotypes at each site are of independent and recent origin. Our AIC_c analysis suggests considerably less support for the single origin model (Table 3; $AIC_c = 3.9$), and rejection of this model is also suggested by the fact that cytotype as the top-level effect in AMOVA explains a negligible and nonsignificant fraction of total genetic variation (Table 3A). Furthermore, in STRUCTURE analysis our AFLP profiles fail to cluster by cytotype (Table 4), showing instead weak structuring by collection site. Finally, in phylogenetic analysis (Fig. 2) our populations again fail to group by cytotype, showing instead a tendency to group by site, and the one grouping with high bootstrap support is inconsistent with a single origin

of polyploids (tetraploids group with hexaploids at JST to the exclusion of polyploid populations from other sites; Fig. 2).

Although AIC_c indicates that we should prefer a multiple origins model, the fit to the strict multiple origins model was not particularly tight (Table 3B): site as the highest-level effect was significant, but explained just 5% of the total genetic variance. This is not surprising, as even under a strict multiple origins model, the top-level "site" effect could be quite weak if recent diploid spread or substantial gene flow among diploid populations has limited their divergence. However, our data are equally consistent with more complex models in which polyploid cytotypes have more limited numbers of origins, with dispersal across the modern zone of cytotype overlap—or with models in which there is ongoing gene flow between cytotypes (for a similar result, see Yeung et al., 2005).

Our data, in summary, do not provide strong support for any particular model of multiple polyploid origins (strict or more complex), but they do firmly reject the hypothesis that each polyploid cytotype has a single, ancient origin. As such, our results support an emerging consensus (e.g., Soltis and Soltis, 1993; Ramsey and Schemske, 1998) that polyploidization events need not be rare, at least on evolutionary timescales. As in *Heuchera* (Segraves et al., 1999) and *Boechera* (Dobeš et al., 2004), *S. altissima* polyploid cytotypes have likely had multiple origins, and thus each cytotype includes lineages of unknown, but not necessarily close, relatedness. This diversity of lineages within a single plant species suggests that plant polyploidy could be responsible for considerable unexplored complexity in plant ecology and in plant–herbivore interactions (Halverson et al., 2007), especially if cytotype (or genetic variation correlated with cytotypic variation) has functional consequences either for plants or for the other species that interact with them.

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APPENDIX 1. Collection site details.

Site (from west to east)	Location	Latitude/Longitude	Cytotypes detected
Spring Creek Prairie Audubon Center	NE: Lancaster Co. 5 km S. of Denton on SW 100th St.	40°40′ N, 96°51′ W	2N
Rest Area, Lincoln (RAL)	NE: Lancaster Co. N. of Lincoln, exit 405 on I-80.	40°54′ N, 96°39′ W	4N, 6N
Smith Wildlife Area (SWA)	IA: Pottawattamie Co. Just NE of Chatauqua on Hwy G8L	41°18′ N, 95°47′ W	2N, 4N, 6N
Prairie Rose State Park	IA: Shelby Co. 11 km SE of Harlan, Hwy M47 and 700th St.	41°54′ N, 95°13′ W	2N
Lake Anita State Park (ASP)	IA: Cass Co. 2 km S of Anita on Hwy 148.	41°25′ N, 94°46′ W	2N, 4N, 6N
Karl & Grace Correll Wildlife Area	IA: Guthrie Co. 2 km E of Adair on 105th St.	41°30′ N, 94°37′ W	2N
Beaver Lake Wildlife Management Area (BVL)	IA: Dallas Co. 2 km NE of Dexter.	41°32′ N, 94°13′ W	2N, 4N, 6N
McFarland Park (MFP)	IA: Story Co. 7 km NE of Ames, Hwy R63 and 180th St.	42°6′ N, 93°35′ W	2N, 4N
Conard Environmental Research Area (CER)	IA: Jasper Co. 4 km S of Kellogg, E of Hwy 224.	41°41′ N, 92°54′ W	2N, 4N, 6N
Kellogg Wildlife Area	IA: Jasper Co. 4 km SE of Kellogg, Hwy 6.	41°42′ N, 92°52′ W	2N
FW Kent Park 1	IA: Johnson Co. 6 km NW of Tiffin on Hwy 6; near NE corner of Kent Park	41°44′ N, 91°43′ W	2N
Valley View Prairie (FW Kent Park 2)	IA: Johnson Co. 6 km NW of Tiffin on Hwy 6; near NW corner of Kent Park. (3 km W of FW Kent Park 1)	41°44′ N, 91°44′ W	2N
Norton Nature Area (NNA)	IA: Cedar Co. 2 km W of Durant on Hwy 6.	41°36′ N, 90°56′ W	2N, 4N, 6N
Crows Creek Wildlife Preserve	IA: Scott Co. Just NE of Mt. Joy on Hwy F55.	41°38′ N, 90°33′ W	2N
Munson Township Cemetery	IL: Henry Co. 13 km S of Geneseo, 1300 N and 1500 E.	41°38′ N, 90°7′ W	2N
Johnson Sauk Trail State Park (JST)	IL: Henry Co. 8 km S. of Annawan on Hwy 78.	41°20′ N, 89°54′ W	2N, 4N, 6N

Note: IL, Illinois; IA: Iowa; NE: Nebraska

APPENDIX 2. AIC_c calculations for alternative AMOVA models.

Two statistical models for a set of data can be compared using Akaike's information criterion (AIC) (Burnham and Anderson, 1998). For least squares estimation with normally distributed errors,

$$\mathrm{AIC} = n \log_e(\hat{\sigma}^2) + 2K,$$

where $\hat{\sigma}^2 = \sum \varepsilon_i^2 / n = \mathrm{SSR}/n$ is the residual variance (with n data points) and K is the number of estimated parameters. For small sample sizes, an adjustment is typically made to arrive at a corrected AIC score, AIC_c:

$$\mathrm{AIC_c} = \mathrm{AIC} + \frac{2K(K+1)}{n-K-1}.$$

In our analyses, $n = 74$ and $K = 4$. The desired SSR for each AMOVA model is the total sum of squares for all effects except the top-level one; that is, for model A in Table 3, SSR = SS(among sites within cytotypes) + SS(individuals within sites) = 425, and similarly for model B, SSR = 403. Thus,

$$\mathrm{AIC_c\ (model\ A)} = 74 \cdot \ln(425/74) + 8 + 0.58 = 137.9$$

$$\mathrm{AIC_c\ (model\ B)} = 74 \cdot \ln(403/74) + 8 + 0.58 = 134.0$$

The preferred model is that with the smallest AIC_c, and other models are considered based on the difference in AIC_c between models (ΔAIC_c). As rough rules of thumb (Burnham and Anderson, 1998), when $\Delta AIC_c < 2$, both competing models are considered to have substantial support; when $4 < \Delta AIC_c < 7$, the model with the larger AIC_c is considered to have considerably less support than the preferred model, and when $\Delta AIC_c > 10$, the model with the larger AIC_c is considered to have essentially no support. For our models, model B (multiple polyploid origins) is preferred, and with $\Delta AIC_c = 3.9$, the single origin model has considerably less support.