

Hybridization and genome size evolution: timing and magnitude of nuclear DNA content increases in *Helianthus* homoploid hybrid species

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Summary

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- Hybridization and polyploidy can induce rapid genomic changes, including the gain or loss of DNA, but the magnitude and timing of such changes are not well understood. The homoploid hybrid system in *Helianthus* (three hybrid-derived species and their two parents) provides an opportunity to examine the link between hybridization and genome size changes in a replicated fashion.
- Flow cytometry was used to estimate the nuclear DNA content in multiple populations of three homoploid hybrid *Helianthus* species (*Helianthus anomalus*, *Helianthus deserticola*, and *Helianthus paradoxus*), the parental species (*Helianthus annuus* and *Helianthus petiolaris*), synthetic hybrids, and natural hybrid-zone populations.
- Results confirm that hybrid-derived species have 50% more nuclear DNA than the parental species. Despite multiple origins, hybrid species were largely consistent in their DNA content across populations, although *H. deserticola* showed significant interpopulation differences. First- and sixth-generation synthetic hybrids and hybrid-zone plants did not show an increase from parental DNA content. First-generation hybrids differed in DNA content according to the maternal parent.
- In summary, hybridization by itself does not lead to increased nuclear DNA content in *Helianthus*, and the evolutionary forces responsible for the repeated increases in DNA content seen in the hybrid-derived species remain mysterious.

Key words: *C*-value, flow cytometry, genome size, *Helianthus*, hybridization, nuclear–cytoplasmic interaction, nuclear DNA content, speciation.

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Introduction

Nuclear DNA content in angiosperms (2*C*-value) ranges from 0.431 pg for *Arabidopsis* (Schmuths *et al.*, 2004) to 254.8 pg for *Fritillaria assyriaca* (Bennett & Leitch 2003). This range in *C*-value is not entirely dependent on changes in ploidy, nor is it correlated with organismal complexity. For example, within diploid species in Poaceae, genome sizes range from 1.11 pg in *Oryza sativum* to 20.90 pg in *Secale cereale* (Bennett & Leitch 2003), with no apparent differences in complexity between these taxa. This variation in nuclear DNA content and its lack of correlation with gene number has been termed

the ‘*C*-value paradox’ (Thomas, 1971) or ‘*C*-value enigma’ (Gregory, 2001).

Adaptive explanations have been sought for differences in genome size. Differences in genome size are correlated with slope aspect in *Ceratonia siliqua* (Bures *et al.*, 2004), with frost resistance in the British flora (MacGillivray & Grime, 1995), with calyx size in *Silene* (MeaGher & Costich, 1996) and with elevation in Patagonian species of *Berberis* (Bottini *et al.*, 2000) and European species of *Dactylis* (Reeves *et al.*, 1998). Studies on invasive pines have found that smaller genomes are correlated with smaller seed size and higher invasiveness (Rejmanek & Richardson, 1996; Grotkopp *et al.*, 2004).

Alternatively, nonadaptive explanations have posited that increases in genome size are the result of deleterious mutations which fix via drift in small populations (Lynch & Conery, 2003). The observation that rare and endangered species, which usually have reduced population sizes, have larger genomes than more common species within their families (Vinogradov, 2003) is in accord with this proposal.

In the past decade, research has shifted to understanding the mechanisms behind genome size changes in plants. Polyploidy has been seen as a major source of increasing genome size. However, studies of natural polyploids within Asteraceae have shown that, while total DNA content increases on average with increasing ploidy level, the DNA content of each genome (i.e. the G_1 nuclear DNA content divided by the ploidy level) in the polyploid nucleus *decreases* (Leitch & Bennett, 2004). Differences in nuclear DNA content within diploid plants have been linked to differences in intron size (Petrov, 2001) and transposon copy number (Bennetzen, 2002). Decreases in genome size correlate with a mutational bias towards deletions over insertions (Petrov, 2001), and illegitimate recombination has been shown to eliminate retrotransposon sequences (Bennetzen, 2002; Devos *et al.*, 2002; Ma *et al.*, 2004).

Newly synthesized plant polyploids may undergo extensive genomic changes. Hybridization followed by chromosome doubling leads to loss of DNA sequences in *Brassica* and *Aegilops* (Song *et al.*, 1995; Liu *et al.*, 1998a,b; Ozkan *et al.*, 2001). In addition, some polyploid lineages exhibit increased retrotransposon activity (Ozkan *et al.*, 2001). However, polyploidization does not automatically entail genomic restructuring: studies of resynthesized cotton polyploids (*Gossypium*; Liu *et al.*, 2001) and recently derived natural polyploids (*Tragopogon mirus* and *Spartina anglica*) failed to find genomic changes (Baumel *et al.*, 2002; Soltis *et al.*, 2004).

Several studies have explored the timing of genomic change in polyploid lineages, and it now appears that some genomic changes are initiated in first-generation diploid hybrids, whereas others are exclusive to polyploidization (e.g. Song *et al.*, 1995; Ozkan *et al.*, 2001; Osborn *et al.*, 2003). In *Aegilops*, for example, elimination of sequences unique to one of the parental genomes but found on multiple chromosomes begins in F_1 plants and is completed in just two or three generations after polyploidization (Ozkan *et al.*, 2001; Shaked *et al.*, 2001). In contrast, sequences unique to a single chromosome from one parental genome are maintained in diploid hybrids, but are rapidly lost following polyploidization (Ozkan *et al.*, 2001). Similar patterns have been reported from studies of diploid hybrids. Some plant and animal hybrids show genomic changes upon hybridization, including fruit flies, wallabies, and beans (Rogers & Bendich, 1987; Petrov *et al.*, 1995; O'Neill *et al.*, 1998; Labrador *et al.*, 1999), whereas others are entirely stable (Guerreiro, 1996).

Despite substantial research on the genomic consequences of hybridization and polyploid speciation, genome size changes

in diploid or homoploid hybrid species remain to be explored. Sims & Price (1985) reported nuclear DNA contents for 19 diploid sunflower (*Helianthus*) species, and it was later shown that three of these (*Helianthus anomalus*, *Helianthus deserticola*, and *Helianthus paradoxus*) are diploid hybrid derivatives of the same parents, *Helianthus annuus* and *Helianthus petiolaris* (Rieseberg, 1991). Intriguingly, the three homoploid hybrid species were reported to have substantially more DNA than their parents. However, only three individuals were analyzed in each species. Also, discovery of the influence of plant secondary compounds on the estimation of DNA content by both Fuelgen densitometry and flow cytometry (Greilhuber, 1988; Price *et al.*, 2000) has led to changes in practice and created uncertainty regarding many earlier reported C -values.

Hybrid sunflowers offer several advantages for the study of genome-size evolution in homoploid hybrid species and its potential adaptive consequences. First, two of the three species (*H. anomalus* and *H. deserticola*) appear to have arisen multiple times in nature (Schwarzbach & Rieseberg, 2002; Gross *et al.*, 2003), so we can ascertain whether the same genomic changes have occurred independently in the wild. Secondly, numerous hybrid zones between the parental species exist naturally. These zones can be exploited to ask whether the genome size variation found in the ancient hybrid species occurs in natural hybrid zones. Finally, genetic mapping studies indicate that the genomes of the hybrid species were extensively restructured during the speciation process (Rieseberg *et al.*, 1995, 1996, 2003). Glasshouse experiments have shown that the chromosomal changes that separate the hybrid species from the parents can be largely duplicated after just four generations of fertility selection (Rieseberg *et al.*, 1996; Rieseberg, 2000). Thus, we can use this unique germplasm to ask whether genome size variation can be replicated in the glasshouse and whether the 'genomic shock' caused by hybridization can generate DNA content variation.

In this study, we examined multiple individuals from multiple populations of the three homoploid hybrid sunflower species and the two parents to confirm previous reports of a DNA-content shift and to assess variation among independently derived populations. We also examined individuals from natural hybrid zones, synthetic F_1 individuals, and synthetic F_6 individuals in order to understand the timing of changes in genome size in the speciation process.

Materials and Methods

Study system

Helianthus paradoxus Heiser, *H. anomalus* Blake, and *H. deserticola* Heiser are among a handful of well-documented homoploid hybrids (Rieseberg, 1997). All three species result from hybridization between *H. annuus* L. and *H. petiolaris* Nutt. ssp. *fallax* Heiser (Rieseberg, 1991). Estimates of the age of origin based on microsatellite diversity of the three species

Table 1 *Helianthus* taxa used, source information, and nuclear DNA content and standard deviation (SD) as measured by flow cytometry

Taxon	Identifier and source	Location	2C (pg) (SD)	N	Notes
<i>H. anomalus</i>	USDA AMES 26095/LD ANO2	Little Sahara, UT	11.52 (0.19)	8	
	LD-ANO1	20 km N of Hanksville, UT	11.32 (0.15)	4	
	DR-ANO3	15 km S of Hanksville, UT	11.38 (0.23)	5	
<i>H. annuus</i>	EJB-HSD	Hermosa, SD	7.15 (0.17)	5	
	USDA PI 413024	Limon, CO	7.13 (0.27)	5	
	USDA Ames 14400	Tucson, AZ	7.34 (0.21)	4	
	USDA PI 468477	Bushland, TX	7.19 (0.06)	4	
	USDA PI 468607	Leeds, UT	7.40 (0.15)	3	
<i>H. deserticola</i>	USDA Ames 26094	Anderson, UT	11.14 (0.17)	4	
	BLG-DES1	Page, UT	9.93 (0.09)	3	
	BLG-DES2	Bigwater, UT	10.03 (0.15)	4	
	BLG-DES4	Toquerville, UT	11.01 (0.10)	4	
	BLG-DES1261	Little Sahara, UT	11.13 (0.12)	4	
	BLG-DESC	Fallon, NV	11.28 (0.27)	4	
<i>H. paradoxus</i>	LHR 1300	Grants, NM	10.78 (0.12)	3	
	LHR 1302	Santa Rosa, NM	10.78 (0.34)	5	
	LHR 1303	Bitterlake NWR, NM	10.95 (0.29)	4	
	LHR 1304	Ft. Stockton, TX	10.60 (0.47)	4	
<i>H. petiolaris</i> spp. <i>fallax</i>	USDA PI 468815	Kanab, UT	6.81 (0.18)	4	
	EJB-TXM	Monohans, TX	6.28 (0.09)	4	
	EJB-NMP	Puerto de la Luna, NM	6.70 (0.14)	4	
<i>H. petiolaris</i> ssp. <i>petiolaris</i>	USDA PI 586912	Terry, MT	6.47 (0.37)	6	
	USDA PI 586920	Carr, CO	6.81 (0.14)	4	
	USDA PI 435809	Channing, TX	6.86 (0.25)	5	
	USDA PI 435807	Bushland, TX	6.88 (0.06)	4	
Hybrid zone	NK1	Dunes National Monument, CO	6.87 (0.12)	20	Three collections
	KR1	Kent Road, KS	6.85 (0.15)	12	Five collections
Synthetic					
<i>H. annuus</i> × <i>H. petiolaris</i> F ₁	LDH1-5	Laboratory of Lisa Donovan	7.26 (0.18)	12	Three crosses
Synthetic		Laboratory of Lisa			
<i>H. annuus</i> × <i>H. petiolaris</i> F ₁	LDH6-8	Donovan/Loren Rieseberg	6.91 (0.16)	17	Five crosses
Synthetic					
<i>H. annuus</i> × <i>H. petiolaris</i> F ₆	LHR1-3	Laboratory of Loren Rieseberg	7.26 (0.22)	11	Three crosses

range from 60 000 to 200 000 years ago (Schwarzbach & Rieseberg, 2002; Welch & Rieseberg, 2002; Gross *et al.*, 2003). The three species occur in the south-western USA, in Arizona, New Mexico, Utah, and Nevada. *H. paradoxus* has a single origin, based upon all populations sharing a single chloroplast haplotype with *H. annuus* and high interpopulation crossability (Welch & Rieseberg, 2002). *H. anomalus* probably had multiple origins, as different populations have different chloroplast haplotypes that are shared with either *H. annuus* or *H. petiolaris*, and *H. anomalus* populations hypothesized to have separate origins have decreased fertility when crossed (Schwarzbach & Rieseberg, 2002). *H. deserticola* is ambiguous in its origins: patterns of microsatellite and chloroplast DNA variation can be accounted for by multiple origins or by a single origin followed by introgression with the parental species. However, partial crossing barriers imply that this taxon also arose multiple times (Gross *et al.*, 2003).

The two parental species, *H. annuus* and *H. petiolaris*, frequently hybridize in the wild, although hybrid fertility is very low (< 1%) (Rieseberg, 2000). However, F₁ and back-cross plants can be found in many locations (Rieseberg *et al.*,

1998). Despite equal chromosome counts ($2N = 34$ for all five species), the three hybrid-derived species have strong crossing barriers with their parental species: F₁ fertility ranges from < 1% for crosses between *H. anomalus* and *H. annuus* to c. 25% for crosses between *H. deserticola* and *H. annuus* (Rieseberg, 2000; L. H. Rieseberg, unpublished). These barriers are probably caused by several chromosomal rearrangements in each hybrid species. Fourth-generation synthetic hybrid lineages, selected for fertility, had high fertility when crossed with *H. anomalus* (Rieseberg, 2000), but strong barriers when crossed to the parental species, reflecting the chromosomal evolution that took place during the four generations of selection.

Study populations

We selected three to five populations from each of the hybrid-derived species. Populations selected spanned the range of the species. In addition, we chose four to five populations of the parental species from the general region of interest (Table 1). Two hybrid zones were sampled, one from Colorado and one from Kansas. In addition, F₁ seeds from eight *H. annuus* ×

H. petiolaris crosses were analyzed, as well as individuals from three sixth-generation synthetic hybrid lineages; these are the same lineages analyzed for genomic composition and crossability by Rieseberg *et al.* (1996) and Rieseberg (2000), but extended by two generations of selfing.

Flow cytometry

We used flow cytometry to estimate nuclear DNA content, following recommendations for the use of internal standards chopped with each sample (Bennett *et al.*, 2000; Price *et al.*, 2000). To establish appropriate conditions for flow cytometry, juvenile leaves from glasshouse plants and/or germinating seeds were used. Tests with all species revealed that DNA content estimates were consistent between the two tissue types. *Hordeum vulgare* cv. 'Sultan' (barley) was used as an internal standard ($2C = 11.12$ pg) (Johnston *et al.*, 1999). Samples and the internal standard were chopped together in a buffer containing 50 mM Tris, 1 mM $MgCl_2$, 0.1% Triton X-100, 0.1% polyvinylpyrrolidone (PVP; average molecular weight 40 000), and 0.5 mM spermine at pH 7.2, then filtered through two layers of Miracloth (CalBiochem, Pasadena, CA, USA). The filtrate was then stained with 50 ppm propidium iodide and 1 mg ml^{-1} RNase. Samples were kept on ice and run on a Becton-Dickinson FACSCalibur (Becton Dickinson, Franklin Lakes, NJ, USA). At least 5000 nuclei were counted for each sample.

Initial trials revealed that the G_1 nuclear DNA content of the hybrid species was very close to the barley standard, making DNA quantification difficult. For these species, French green lentils (*Lens culinaris* ssp. *culinaris*) ($2C = 9.0$ pg; calibrated using *H. vulgare* cv. 'Sultan') were used as an alternate internal standard, with samples of the parental species run with both standards to verify consistency.

Sampling design and analysis

We quantified nuclear DNA content from at least four individuals per population. We tested for differences between

populations within each species using analysis of variance (ANOVA) (SAS PROC GLM; SAS Institute, Cary, NC, USA). Differences between species were tested using nested ANOVA, with population within each species treated as a random effect (SAS PROC MIXED; SAS Institute). Pre-planned contrasts tested for differences in means for synthetic F_1 lineages with *H. annuus* or *H. petiolaris* as the maternal parent, and for the synthetic hybrids compared to the three homoploid hybrid species. All other posthoc pairwise comparisons of means were corrected for multiple tests.

Results

Parents and hybrid species

Homoploid hybrid species showed a consistent pattern of increased nuclear DNA content (Fig. 1). After adjustment for multiple tests, the three hybrid species were each significantly different from their parents, *H. annuus* (7.23 pg; $P < 0.0001$) and *H. petiolaris* (6.68 pg; $P < 0.0001$). The three homoploid hybrid species all showed increases compared with the parental species, but differed from each other: *H. anomalus* had a higher $2C$ -value (11.46 pg) than either *H. deserticola* (10.79 pg; $t = 4.36$, $P = 0.002$) or *H. paradoxus* (10.78 pg; $t = 3.90$, $P = 0.009$), after adjustment for multiple contrasts.

Synthetic hybrids and hybrid-zone plants

The DNA contents of natural and synthetic hybrids did not show increases similar to those found in the hybrid species (Fig. 2). Correspondingly, the three hybrid species were each significantly different from all synthetic and natural hybrid populations ($P < 0.0001$ in all cases). F_1 plants derived from *H. annuus* maternal plants had a mean $2C$ -value of 7.26 pg, while F_1 seeds from the reciprocal cross had a mean $2C$ -value of 6.91. In an analysis treating each cross as a random factor nested within the maternal species, this difference was significant ($P = 0.008$). The synthetic F_6 lineages, derived

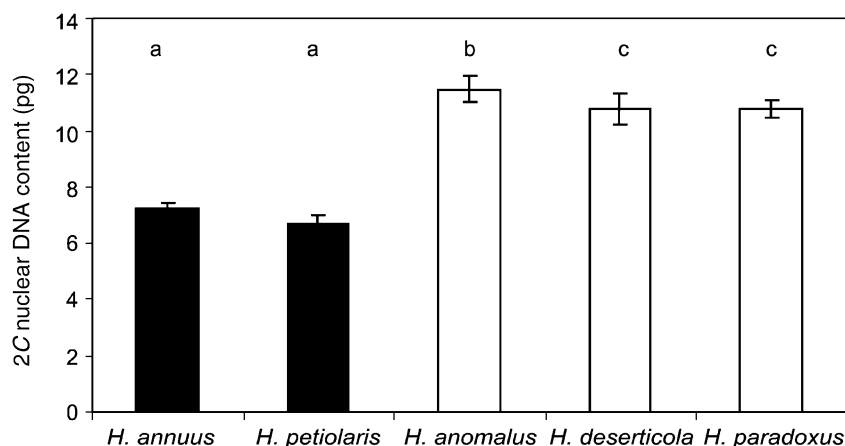


Fig. 1 Nuclear DNA content of sunflower (*Helianthus*) parental species and hybrid descendants. Mean $2C$ -value and standard deviation are shown. Differences in $2C$ -value significant at $P < 0.0005$ in a mixed model ANOVA, treating population as a random nested factor within species, are indicated by different letters. Solid bars, parental species; open bars, hybrid-derived species.

Fig. 2 Nuclear DNA content of sunflower (*Helianthus*) synthetic hybrid lineages, hybrid-zone plants, and (for comparison) one of the ancient homoploid hybrid species, *Helianthus paradoxus*. Mean $2C$ -value and standard deviation are shown. Synthetic lineages differed in nuclear DNA content depending on their maternal parent ($P = 0.008$) in a mixed model ANOVA treating each cross as a random nested factor within the maternal parent, as indicated by the different letters. Solid bars, natural hybrid-zone plants; gray-shaded bars, synthetic hybrids; open bars, hybrid-derived species.

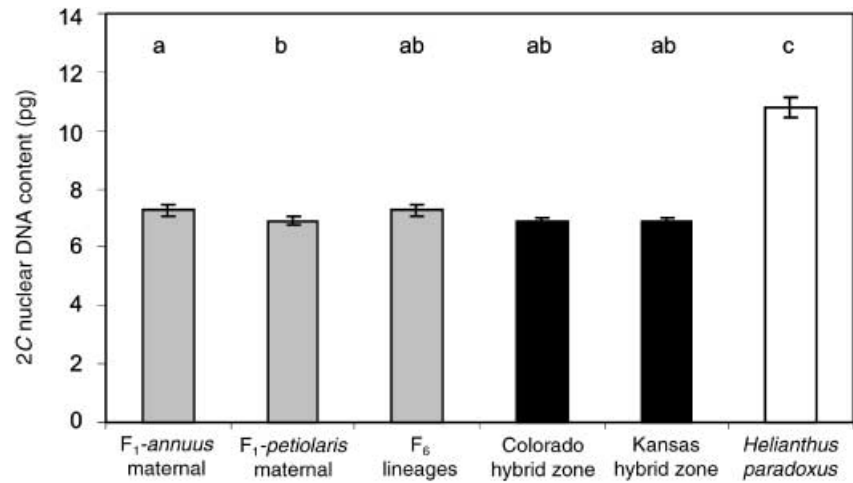
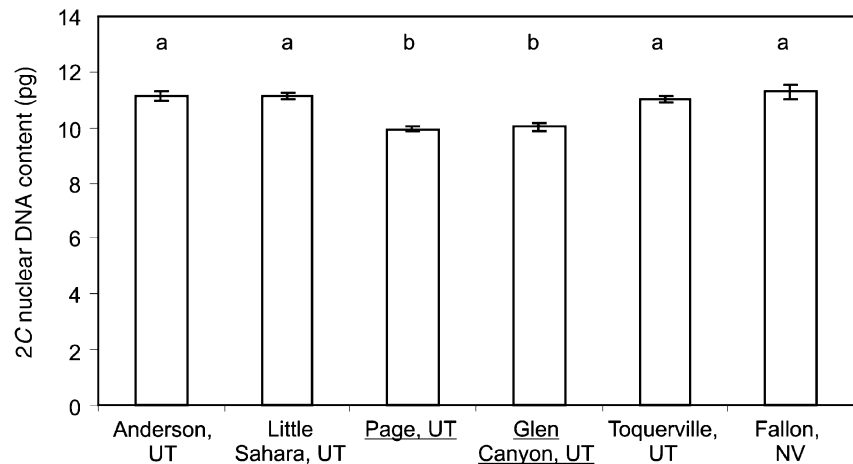


Fig. 3 Nuclear DNA content of populations of *Helianthus deserticola*. Mean $2C$ -value and standard deviation are shown. Populations differing in mean DNA content in a one-way ANOVA ($P = 0.0001$) are indicated by different letters. The two populations occurring near Glen Canyon are underlined.



from *H. annuus* maternal plants in their initial cross but back-crossed to both *H. annuus* and *H. petiolaris*, had the same mean nuclear DNA content as the *H. annuus* maternal F₁ plants (7.26 pg), indicating no genomic size changes in the first six generations following hybridization. Plants from natural hybrid zones had values near that of *H. petiolaris* (6.86 pg).

Intraspecific variation

Within each species, populations did not differ significantly in C -value for *H. annuus*, *H. paradoxus*, and *H. anomalus* (Table 1). However, interpopulation differences were found for the two remaining species. For *H. petiolaris* ($F_{6,24} = 4.51$, $P = 0.003$; Table 1), the $2C$ -value of the Monahans, Texas population of ssp. *fallax* was lower than that of any other population, and significantly lower than the values of four of the other six populations. The two *H. petiolaris* subspecies did not differ consistently: the Montana *H. petiolaris* ssp. *petiolaris* population had the second-lowest C -value (Table 1). Populations also differed significantly in *H. deserticola* ($F_{5,17} = 49.84$,

$P = 0.0001$); two populations with significantly lower C -values were located near Glen Canyon in southern Utah (Fig. 3).

Discussion

Our results reveal three phenomena. First, one of the hybrid taxa (*H. deserticola*) had populations that significantly differed in DNA content. Secondly, the nuclear DNA content of F₁ hybrids between *H. annuus* and *H. petiolaris* differed depending on the direction of the cross, suggesting nuclear–cytoplasmic interactions. Thirdly, the three hybrid-derived species showed a marked increase in DNA content compared with their parents, while synthetic hybrids showed no increase. We discuss each of these patterns in turn.

Intraspecific variation in genome size

Before 2000, intraspecific genome size variation was reported for many taxa (Price *et al.*, 1983; Price & Johnston, 1996). However, the observed variation declined in many species with

the use of internal standards (Baranyi & Greilhuber, 1999; Schmutz *et al.*, 2004). Using internal standards, we found a 10% difference in nuclear DNA content within *H. deserticola*. The two populations with decreased genome size occur at the southern-most part of the range in Utah, and appear to be basal in the phylogeny of the species (Gross *et al.*, 2003). Other data suggest that these populations may have had an independent origin from the rest of the species (Gross *et al.*, 2003).

Nuclear–cytoplasmic interactions in hybrid DNA content

We found an effect of maternal species on the *C*-values of hybrid plants. To our knowledge, this is the first case in which DNA content has been found to depend on the identity of the maternal parent in hybrid plants, although related phenomena have been reported in other contexts. Variation from the mid-parent genome size that depended upon the identity of the parental plants was seen in *Dasyphyrum villosum* (Caceres *et al.*, 1998). Work on synthesized wheat polyploids has shown that one genome may be stable while the other undergoes rearrangement (Levy & Feldman, 2004), but this is not dependent on the direction of the cross. Synthesized *Brassica* polyploids show a maternal effect on genome change (Song *et al.*, 1995). The ease of crossing *H. annuus* and *H. petiolaris*, the repeatability of the apparent nuclear–cytoplasmic interaction in determining nuclear DNA content, and the availability of natural hybrid zones make this an excellent study system for unraveling the mechanism behind this pattern.

Nuclear DNA content of hybrid species

We found that *Helianthus* species derived from interspecific hybridization had significantly higher nuclear DNA contents than their parent species. The three hybrid taxa are derived from the same parental species, but evidence points to multiple origins within two of the three hybrid taxa. Therefore, the increase in nuclear DNA content occurred independently and repeatedly.

Unfortunately, we cannot assess how common this pattern is for homoploid hybrid species. Of the handful of well-documented cases (Rieseberg, 1997), only those in *Helianthus* have published *C*-values for both the parental and descendant hybrid taxa. We are currently seeking seed sources to establish whether the increases seen in the *Helianthus* hybrid species are exceptional. Evidence from some animal hybridization studies indicates that large changes in genome size can occur in a single generation through transposon replication (O'Neill *et al.*, 1998; Labrador *et al.*, 1999), although other animal and most plant studies have failed to find similar patterns (Guerreiro, 1996; Rayburn *et al.*, 1993; Williams *et al.*, 2002). Given the repeated independent emergence of larger genomes in hybrid sunflower taxa, we expected to see frequent increases in genome size in F_1 hybrids. We did not: F_6 hybrids did not differ from

the F_1 hybrids, and naturally occurring hybrid-zone plants showed a nonsignificant decrease in genome size compared with the parental plants. These results suggest several hypotheses: (1) DNA increases occur much later than the F_6 generation; (2) DNA increases occur rapidly following hybridization, perhaps as early as the F_6 generation, but occur infrequently or depend on particular genotypes not included in this study; and (3) DNA increases occur in the field in response to environmental conditions which were lacking in the glasshouse environment.

Potential evolutionary forces underlying genome size increases in *Helianthus*

The repeated large increases in genome size may be attributable to a ratchet effect. Hybrid speciation probably proceeds through a tight bottleneck. If a rare, large-genome plant existed during the bottleneck, larger genome size may persist if it is selectively neutral, or even if it is disadvantageous (Lynch & Conery, 2003). While several mechanisms, including unequal and illegitimate recombination, can eliminate genes, it is unclear whether selection would favor genomes that were 0.0001% smaller as a result (Bennetzen, 2002). Repeated evolution of large genome size across multiple origins argues against this mechanism.

Alternatively, large genomes may have been directly favored in the transition of the hybrid sunflowers to their new habitats. The three hybrid species inhabit extreme environments relative to their parents. *H. deserticola* inhabits the desert floor, *H. anomalus* is found in sand dunes, and *H. paradoxus* occurs in salt marshes. Perhaps these distinct harsh environments all favor larger genomes, and so selected for increased genome size. Supporting the plausibility of this hypothesis, larger genomes are found in more variable environments in *C. siliqua* (Bures *et al.*, 2004). However, the opposite pattern is more frequent, with plants in more stressful environments exhibiting lower rather than higher DNA contents (e.g. Price, 1988; Castrojimenez *et al.*, 1989).

As a third alternative, we propose that increased genome size in the *Helianthus* ancient hybrids might have been indirectly favored by selection. This might have occurred in two ways. *H. annuus* and *H. petiolaris* differ by numerous chromosomal rearrangements (Burke *et al.*, 2004). As a consequence, recombination in F_1 hybrids generates mostly inviable gametes because of the presence of large chromosomal deletions (Chandler *et al.*, 1986). Genomic redundancy provides a simple way of reducing the initial fitness costs of these rearrangements. Thus, there might have been strong selection for early-generation hybrids that carried large-scale chromosomal duplications.

Increased genome size might also have been indirectly favored if it increased recombination between homeologous chromosomes. Selection for increased fertility in the glasshouse leads to rapid genomic restructuring and recovery of fertility

(Rieseberg *et al.*, 1996). We also know that the successful generation of the novel phenotypes that characterizes each of the hybrid species requires recombination of parental quantitative trait loci (Rieseberg *et al.*, 2003). If an increase in genome size (e.g. the amplification of repetitive elements) increased recombination and chromosome pairing, then selection might favor larger genomes, not because of the favorable effects of larger genomes *per se*, but because of the favorable recombinations or increased fertility that resulted from increased chromosome pairing. The ecological differentiation and establishment of homoploid hybrids may have required the fortuitous involvement of individual lineages that were predisposed to repetitive element increases, allowing increased recombination and thus the formation of new, recombinant genotypes. This proposal is supported by work in other systems linking the quantity of repeated elements to recombination rates. Disease resistance clusters have been shown to have repetitive elements that may favor recombination (Richter & Ronald, 2000). In allopolyploid wheat, deletions of repeated elements may decrease homoeologous pairing (Ozkan *et al.*, 2001).

Future prospects

Here, we have shown that genomic organization in three *Helianthus* hybrid species differs from that of their parents and early-generation hybrid lineages (both natural and synthetic). Genome size increases are therefore not automatic in the first six generations following hybridization. Future work could aim to distinguish between the alternative possibilities that genome size increase occurs early but infrequently (requiring large-scale sampling of early generation hybrids), occurs later in the process (requiring maintenance of hybrid lineages for many generations), or only occurs under particular ecological conditions, for example in stressful environments.

Whether or not the timing of genome size changes is ultimately resolvable, we show here that hybrid sunflowers also offer the opportunity *experimentally* to examine the mechanisms and potential adaptive roles of genome size change. Synthetic hybrid lineages have high fertility when crossed with the natural hybrid species (Rieseberg, 2000), yet differ in genome size by 50%. By crossing these synthetic lineages with the hybrid species, it should be possible to generate a range of genome sizes, and then to examine the effect of genome size on both recombination rates and ecological performance.

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