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Note

Allele-Specific, Bidirectional Silencing of an Alcohol Dehydrogenase Gene in Different Organs of Interspecific Diploid Cotton Hybrids

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ABSTRACT

Interspecific hybridization is a common and important process that generates phenotypic novelty and can lead to hybrid speciation as well as to changes in gene expression. Using two different interspecific cotton (Gossypium) diploid hybrids, we show organ-specific, bidirectional allelic silencing at a heterozygous *Adh* locus, with alternate alleles being silenced in leaves and many floral organs, respectively. These results show that developmental regulation of gene expression is changed immediately upon hybridization between diploid species, possibly due to epigenetic factors or regulatory mismatch.

HYBRIDIZATION between different species is prevalent in plants and is thought to play an important role in adaptive evolution (Arnold 1997, 2004; Cronn and Wendel 2004; Hegarty and Hiscock 2005; Rieseberg 1997). Interspecific hybridization may lead to chromosomal rearrangements (Rieseberg *et al.* 1996; Shaked *et al.* 2001), transposable element mobilization (Liu and Wendel 2000; Shan *et al.* 2005), DNA methylation changes (Salmon *et al.* 2005), and a broad array of new and sometimes transgressive phenotypes (Rieseberg *et al.* 1999, 2003) and ecological innovations (Rieseberg *et al.* 2003). Hybridization between two species can result in changes in gene expression. Up- and downregulation of expression has been shown in triploid Senecio hybrids, in diploid and triploid maize hybrids, and in diploid wheat hybrids compared with their parents (Wu *et al.* 2003; Auger *et al.* 2005; Hegarty *et al.* 2005). Hybridization also can affect allelic expression. Allele-specific differences in expression levels of nonimprinted genes have been documented in F_1 hybrids of mouse (Cowles *et al.* 2002), Drosophila (Wittkopp *et al.* 2004), and *Saccharomyces cerevisiae* (Ronald *et al.* 2005). In plants, expression levels of alleles (of nonimprinted genes) have been explored in intraspecific hybrid lines but not in interspecific diploid hybrids. In maize diploid hybrids, several genes displayed unequal expression in heterozygotes or silencing of one allele (Guo *et al.* 2003, 2004), whereas two other genes exhibited allelic expression level differences that varied by organ type (Guo *et al.* 2004).

In allopolyploid hybrids expression levels of homeologous gene pairs (*i.e.*, genes duplicated by polyploidy) relative to each other have been found to be variable by organ type (Adams *et al.* 2003, 2004). The most extreme form of organ-specific partitioning of expression between homeologs is exemplified by the alcohol dehydrogenase gene *AdhA* in cotton, where there is silencing of one homeolog in some organs and of the other homeolog in other organs, suggesting subfunctionalization (Adams *et al.* 2003). The organ-specific partitioning of gene expression could be caused by hybridization, chromosome doubling, or both; diploid hybrids provide a means to separate these factors.

To determine if organ-specific alteration in the expression of individual alleles can occur in response to interspecific hybridization between two diploid species, without chromosome doubling, we assayed expression of *AdhA* in several organs of two different diploid F_1 hybrids in the cotton genus (Gossypium). Two F_1 hybrids (Figure 1A) were synthesized by pollinating emasculated flowers of the maternal species with pollen from the paternal species and growing the resulting seed to generate F_1 hybrid individuals. The success of the crosses was verified by morphology and allozymes. To assay gene expression, we used a combination of RT-PCR and SSCP-cDNA approaches, as described (Adams *et al.* 2003; Cronn and Adams 2003).

Bidirectional, organ-specific allelic silencing: As shown in Figure 1, interspecific diploid hybrids display allele-specific expression changes at the first generation. In the

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Gossypium trilobum × *G. gossypioides* F_1 hybrid, only the *G. gossypioides* (paternal) *AdhA* allele was expressed in petals, stamens, and stigmas and styles (Figure 1B), although the genomic DNA control demonstrates that both alleles are present. *AdhA* is expressed in the equivalent organs of the *G. trilobum* parent (Figure 1D). These results indicate that silencing of one allele can occur in the first generation after hybridization. In the *G. raimondii* × *G. gossypioides* hybrid, expression of the two *AdhA* alleles ranged from approximately equal in the petals to expression of one allele *or* the other in different organs (Figure 1C, Table 1). The *G. raimondii* allele was silenced in the bracts (epicalyx), stigmas and styles, and ovules, whereas the *G. gossypioides* allele was silenced in leaves. *AdhA* is expressed in the equivalent organs of the *G. raimondii* and *G. gossypioides* parents (Figure 1D). This organ-specific, reciprocal silencing of alternative alleles at a single locus in F_1 hybrids is analogous to the previously reported silencing of homeologous *AdhA* genes in natural and synthetic allopolyploids (Adams *et al.* 2003). A key distinction between the present phenomenon and that reported earlier is that in the present case the reciprocal silencing affects alternative alleles at a heterozygous locus, as opposed to duplicated genes residing in different, coresident genomes of a polyploid nucleus.

Functional and evolutionary significance: The functional consequences of organ-specific allelic silencing are unknown, as is the scope of the phenomenon. In the present example, there are no differences in the inferred *AdhA* amino acid sequences among the parents and hybrids surveyed, so one might suggest that the phenomenon has little relevance from an evolutionary perspective. We can, however, envision evolutionarily relevant dimensions to the observations reported here. One key question would appear to be whether the expression pattern segregates in the F_2 and subsequent generations. These populations were not available for this study, but in principle an F_2 progeny could be generated for the reportedly fertile (Brown and Menzel 1952; Menzel and Brown 1954) *G. raimondii* × *G. gossypioides* hybrid (the other hybrid is sterile). To the

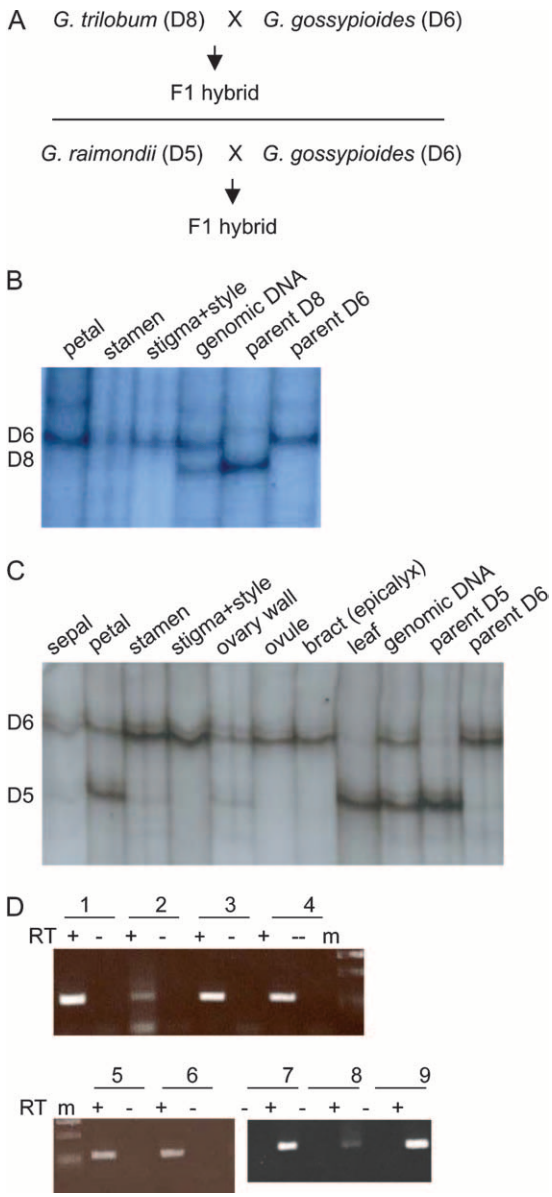

Figure 1.—(A) Diagram showing synthesis of two different interspecific hybrids. (B and C) SSCP-cDNA gels showing transcript levels of alcohol dehydrogenase A (*AdhA*) alleles in the F_1 hybrids of *G. trilobum* × *G. gossypioides* (B) and *G. raimondii* × *G. gossypioides* (C). D6, D8, and D5 correspond to the species used in the crosses (see A) and hence reveal gel migration positions of the alleles derived from each parent. (D) Agarose gels showing *AdhA* RT-PCR products from parental species. Reactions were performed with (+) or without (−) reverse transcriptase. Lanes are numbered in pairs as follows: 1, D5 bract; 2, D5 stamen; 3, D5 stigma and style; 4, D5 ovule; 5, D6 leaf; 6, D8 stamen (repeat); 7, D8 petal; 8, D8 stamen; and 9, D8 stigma and style. "m" indicates size marker. Plant materials: *G. gossypioides* (accession HC8065), *G. raimondii* (Wendel laboratory stock), and *G. trilobum* (accession D8-1) were crossed as described in the text and as shown in A. The same perennial *G. raimondii* and *G. gossypioides* individuals used to make the hybrids and a plant from the accession of *G. trilobum* were used for RNA extractions. Two progeny plants derived from the same cross were used as biological replicates for each of the diploid hybrids. All plants were grown under the same conditions in the greenhouse at Iowa State University. Methods: RNA was extracted from the plants (flowers were collected between 10:00 am and noon on the day of flower opening), the quality was checked by electrophoresis on agarose gels, and the concentration was estimated with a spectrophotometer as previously described (Adams *et al.* 2003). The RNA was treated with DNase to remove residual DNA and then it was reverse transcribed also as previously described (Adams *et al.* 2003). PCRs were performed with cDNA templates, using primers that amplify both alleles, as described in Adams *et al.* (2003). Primer sequences were 5′-GGTGAGG GTGTGAATGATTTG-3′ and 5′-GTACATATCCCACAGCTAA TGG-3′. RT-PCR products were separated by SSCP-cDNA (Cronn and Adams 2003); gels included 2% urea and were run at 4°. Bands were quantified from dried gels by phosphorimaging using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). RT-PCR products showing one allele on the SSCP gels were directly sequenced to verify the presence of only one allele.

TABLE 1

Expression levels of *AdhA* alleles in cotton F_1 diploid hybrids

Hybrid	Allele	Sepal	Petal	Stamen	Style and stigma	Ovary wall	Ovule	Epicalyx	Leaf	Genomic DNA
D8 × D6, rep 1	D6		100	100	100					51
	D8		0	0	0					49
D8 × D6, rep 2	D6		100	100	100					52
	D8		0	0	0					48
D5 × D6, rep 1	D6	85	42	91	100	74	100	100	0	41
	D5	15	58	9	0	26	0	0	100	59
D5 × D6, rep 2	D6	83	44	95	100	77	100	100	0	43
	D5	17	56	5	0	23	0	0	100	57

Numbers represent transcript proportions derived from each parental allele, as determined by phosphorimaging. Each pair of rows represents a biological replicate (rep). Allelic expression was not assayed in some organs of the D8 × D6 hybrid.

extent that organ-specific silencing of alleles is controlled by *cis*-regulatory elements (Wittkopp *et al.* 2004), heterozygotes in subsequent generations are expected to display the bidirectional silencing reported here, while parental patterns should be restored in both classes of parental homozygotes. When extended across the genome to include hundreds to perhaps thousands of independently assorting genes, each exhibiting the type of regulatory effects shown here, one can imagine that populations derived from the initial hybridization event will exhibit a remarkable diversity of combined expression patterns. Thus, one potential basis of the morphological, physiological, and ecological novelty that characterizes hybrid populations and species may be the cumulative effect of developmentally regulated, reciprocal allelic expression biases.

Organ-specific partitioning of aggregate ancestral expression patterns, as observed for *AdhA* in cotton polyploids (Adams *et al.* 2003), may be considered a form of subfunctionalization. In an analogous fashion, the reciprocal silencing of *AdhA* alleles reported here in the cotton F_1 hybrids raises the possibility of instantaneous subfunctionalization that could lead to preservation of both parental alleles in subsequent generations. Thus, an additional evolutionarily relevant consequence of this process, to the extent that it operates, would be the preservation of higher levels of intrapopulation allelic diversity than would otherwise occur.

Possible causal factors: From a mechanistic perspective, what might be the cause of allelic silencing in F_1 hybrids in an organ-specific manner? At present no data specifically address this question, but clearly epigenetic factors, such as changes in DNA cytosine methylation, histone modifications (including deacetylation and methylation), or small RNAs may play a role. Another possibility is that sequence variation in the *cis*-regulatory elements of the alleles might cause differential binding of regulatory factors, causing different stoichiometric biases and regulatory mismatches that vary by organ and tissue (Birchler *et al.* 2005; Veitia 2005).

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