Genetic Diversity among Witchhazel Cultivars Based on Randomly Amplified Polymorphic DNA Markers

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ABSTRACT. Forty selections, including 37 cultivars of *Hamamelis* spp., were evaluated for genetic similarities using randomly amplified polymorphic DNA (RAPD) markers. Cluster analysis identified seven groups, which included three groups of *H. ×intermedia* cultivars, two groups of *H. vernalis*, and one group each of *H. mollis* and *H. japonica*. Three *H. ×intermedia* cultivars, 'Arnold Promise', 'Westerstede', and 'Carmine Red', did not group closely with the other 20 cultivars of *H. ×intermedia*. Selections of the North American species *H. vernalis* were quite distinct from the Asiatic selections. However, data are presented that suggest hybridization exist between Asiatic *Hamamelis* spp. and *H. vernalis*. Genetic similarities between known half-sib families provides evidence that the cultivar pairs 'Arnold Promise'-'Winter Beauty' and 'Carmine Red'-'Hiltingbury' are, themselves, not likely half-sibs.

The Hamamelidaceae includes numerous species that are desirable as woody ornamentals. Important genera in this diverse family include *Corylopsis*, *Disanthus*, *Fothergilla*, *Hamamelis*, *Liquidambar*, *Lorapetalum*, and *Parrotia* (Lewandowski and Meyer, 1985; Wright, 1982). Within the witchhazel genus (*Hamamelis*), four major species are recognized. Plants in the genus have unusual flowers, fragrance, and bloom time that makes them desirable as landscape plants (Dirr, 1983, 1990). Flowers of *Hamamelis* consist of four strap-like petals borne either in the fall or late-winter when little else in the landscape is blooming.

Species of *Hamamelis* are native to North America and Asia. The two Asian species are *H. mollis* Oliver, which is native to central China, and *H. japonica* Sieb. & Zucc., which is scattered throughout Japan (Rehder, 1962). The two predominant North American species are common witchhazel (*H. virginiana* L.) and vernal witchhazel (*H. vernalis* Sarg.). *Hamamelis virginiana* occurs as an understory shrub or small tree in southern Canada and the eastern United States, ranging from Florida and Louisiana, west into portions of Wisconsin and Iowa, and east into Maine (Hightshoe, 1988). The range of *H. vernalis* is more limited; it is found in the Ozark Mountain region of Oklahoma, Missouri, and Arkansas (Hightshoe, 1988). Two minor North American taxa of *Hamamelis* include *H. macrophylla* Pursh. and *H. mexicana* Standley. *Hamamelis macrophylla* and are found in South Carolina to Florida, Arkansas, and Texas (Rehder, 1962), and *H. mexicana* was named from specimens located in Nuevo Leon, Mexico (Standley, 1937).

The two Asian species are freely interfertile. An interspecific hybrid between *H. japonica* and *H. mollis* from a chance seedling established at the Arnold Arboretum in 1929 was named *H. ×intermedia* by Rehder (1945). This seedling originated from seed collected from a *H. mollis*. The presumed pollen parent was an adjacent *H. japonica* 'Zuccariniana' (Bean, 1973). This hybrid was named and registered in 1963 as *H. ×intermedia* 'Arnold Promise', which today is perhaps the best known and popular spring-blooming cultivar. In addition to 'Arnold Promise', other

first generation hybrids between the two Asian species occurred independently at several botanical gardens in Denmark, Germany, and Belgium (DeBelder, 1969; Grootendorst, 1965; Hohn, 1993).

Witchhazels are popular landscape plants as shown by the >75 cultivars that have been named (Hohn, 1993). Among the cultivars, the Asian complex is best represented with 5, 13, and 47 cultivars of *H. japonica*, *H. mollis*, and *H. ×intermedia*, respectively (Hohn, 1993). The North American species have yielded only 12 *H. vernalis* cultivars, although several forms and varieties of *H. macrophylla* and *virginiana* have been designated (Hohn, 1993; Strand, unpublished).

Most *Hamamelis* cultivars were selected from open-pollinated seedlings, and pedigree information is scant. Given that the two Asian species freely hybridize and possibly are interfertile with the North American species (unpublished data), the taxonomic association of some cultivars remains in question. For example, 'Pallida' has been listed as *H. mollis* and *H. ×intermedia* (Coombes, 1996; Dirr, 1990; Hillier, 1977; Hohn, 1993; Lancaster, 1970; Strand, unpublished).

This study was designed to develop a useful set of randomly amplified polymorphic DNA (RAPD) markers to distinguish among cultivars, to help understand the similarities among cultivars, and to determine genetic affinities using cluster analysis. An additional objective was to calculate genetic similarities between known half-sib families to establish base-line information useful to compare among cultivars thought to be half-sibs.

Material and Methods

PLANT MATERIAL. Most plant material was clonally propagated as grafts budded onto understock plants of *H. virginiana*. Forty clones, including 37 cultivars, were evaluated in this study. Clones included 4, 6, 7, and 23 representatives of *H. japonica*, *H. mollis*, *H. vernalis*, and *H. ×intermedia*, respectively. In addition to the 37 cultivars, three clones were unnamed selections and included a *H. vernalis* selection designated "Boesger," a *H. mollis* designated "Renken," and a *H. japonica* obtained from the U.S. National Arboretum in Washington, D.C., as NA-25205. With this last exception, all plant material originated as budwood collected from Brotzman's Nursery, Madison, Ohio.

DNA ISOLATION AND POLYMERASE CHAIN REACTION (PCR). Young, rapidly expanding leaves were the source tissue for DNA extrac-

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tions. In general, 1.0 to 1.3 g of leaves were ground in liquid nitrogen and frozen in 50-mL centrifuge tubes at -80°C for sample storage before isolating DNA.

DNA isolations were initiated by adding 10 mL warm (60°C) 2% CTAB buffer [2% cetyltrimethylammonium bromide, 1.4 M NaCl, 0.2% β -mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0)] to the frozen and powdered leaf tissue. This mixture was incubated at 65°C for 60 min followed by an equal volume extraction with 24 chloroform : 1 isoamyl alcohol and centrifugation for 15 min at 2000 g . The aqueous phase was removed and combined with 0.55 volumes of cold isopropanol to precipitate the DNA, which was hooked (when possible) or pelleted after a 15 min centrifugation at 1400 g . DNA was washed for ≥ 30 min in 10 mL 76% ethanol with 10 mM ammonium acetate. Samples again were centrifuged for 10 min at 1800 g . The supernatant was removed, and the DNA pellet was air-dried. DNA samples were resuspended in 0.5 to 1.0 mL Tris-EDTA buffer in a 37°C water bath. This protocol closely follows the methods of Doyle and Doyle (1990).

Estimates of DNA quantity were made by loading 8 to 10 μL of each resuspended DNA sample into a 1% agarose gel with 1.25 $\text{mg}\cdot\text{mL}^{-1}$ ethidium bromide. After ≈ 2.5 h of electrophoresis at 150 constant volts, DNA samples were visually inspected above ultraviolet light, and band intensity was compared to a concentration standard (Gibco-BRL #10068-013, Gaithersburg, Md.). The estimates of sample quantity were made by comparing the standard to the DNA band with slowest mobility.

PCR amplification was determined to be near optimum with 25 ng of template DNA, 0.1 mM of each nucleotide, 2 mM MgCl_2 , 0.2 μM primer, 1 u DNA *Taq*-polymerase (*Taq*), and $1.0\times$ *Taq* buffer in a 25 μL reaction volume. Primers and *Taq* were purchased from The Univ. of British Columbia, Nucleic Acid-Protein Service Unit (Vancouver, B.C., Canada) and Gibco-BRL, respectively.

DNA amplification was performed in a MJ-Research thermal cycler (model PTC-100-60; MJ Research, Watertown, Mass.) equipped with a heated lid to minimize sample fluxing. The thermal cycler was programmed to denature DNA at 94°C for 1 min, anneal DNA to primers at 35°C for 1 min, and polymerize DNA for 2 min at 72°C . After 45 cycles, the program allowed a final extension of 5 min at 72°C before maintaining 4°C .

Amplified DNA samples were loaded into a 2% agarose gel and separated by electrophoresis at 150 constant volts for ≈ 2.5 h when bromophenol blue as a tracking dye had migrated ≈ 10 cm. Bands were visualized above a ultraviolet transilluminator and photographed for documentation. The size of prominent DNA polymorphic fragments were visually estimated using a 100-base pair ladder or high molecular mass ladder (Gibco-BRL #15628 or #15615).

Initially, 400 primers were screened with a single sample to determine useful primers (UBC primer nos. 101–500). For 131 primers that resulted in distinct banding patterns of bright intensity between 300 and 3500 base pairs, a second screening was done with nine DNA samples to identify possible polymorphisms. Next, PCR reactions were conducted using the 30 most promising primers with all 40 witchhazel samples. From these results, the best RAPD markers were chosen based on reproducibility of results within samples and between samples collected from the same clone. Repeated reactions and PCR amplification on a duplicate set of independent DNA extraction's from 10 cultivars was consistent, which assured repeatability of results. A final set of 19 primers were chosen as the most reliable and consistent.

DATA COLLECTION AND ANALYSIS. After amplification with these 19 primers, all 40 *Hamamelis* selections were scored for the presence or absence of 68 RAPD markers. Occasionally, amplifi-

cation produced equivocal results for a specific marker. Rather than assign incorrect scores, we designated "missing data" for any RAPD marker that was in question. Less than 3% of the data set was equivocal (81 of 2720 possible markers).

RAPD markers were scored as 0, 1, or 2 for absent, present, or missing, respectively, resulting in a 40×68 data matrix. Pair-wise genetic similarities were calculated for all possible pairs of *Hamamelis* clones using the Dice (1945) coefficient (NTSYS-PC software, Exeter Software, Setauket, N.Y.). The Dice coefficient is identical to that of Nei and Li (1979) [see Dudley (1994)]. The similarity coefficient, S_{ij} , equals $2N_{ij} / (N_i + N_j)$, where N_{ij} = number of positive bands shared by both individuals i and j . N_i and N_j are the numbers of fragments present in individuals i and j . Therefore, a negative match (scored 0–0) is not factored into the computation of similarity [for more information see Dudley (1994) and Rohlf (1993)].

The similarity matrix was used in two ways. First, a cluster analysis was performed in NTSYS-PC using the unweighted pair-group method using arithmetic averages (UPGMA). This software also created the phenogram presented in the results. Secondly, the similarity matrix was used to calculate an average similarity or "affinity" of each clone to *H. mollis*, *H. japonica*, *H. xintermedia*, and *H. vernalis*.

HALF-SIBS. Two half-sib families were established by collecting open-pollinated seed from plants of *H. xintermedia* 'Arnold Promise' and *H. mollis* 'Brevipetala'. Six and four open-pollinated, half-sib individuals of 'Arnold Promise' and 'Brevipetala', respectively, were grown from seed. DNA was extracted as previously described, and each was scored for the 68 RAPD markers. For each family, genetic similarities were calculated among half-sibs and to their maternal parent.

Results and Discussion

PCR RESULTS. Nineteen primers gave 68 useful markers or an average of 3.6 markers per primer. The primer numbers, estimated size of each RAPD marker, and primer sequence are provided (Table 1). In general, the primers used for these analyses gave bright amplification products (Fig. 1).

GENETIC SIMILARITIES. All possible pairwise genetic similarities were calculated between selections (Table 2), which were used in the UPGMA cluster analysis (Fig. 2). Used together, general associations between pairs and groups of cultivars can be made. Several pairs of clones were very similar ($S_{ij} > 0.95$) from pairwise analysis and include *H. vernalis* 'New Year's Gold' and 'Orange Glow' (0.980), *H. mollis* 'Boskoop' and the Renken selection from Germany (0.982), and *H. xintermedia* 'Limelight' and 'Pallida' (0.958). These high numeric similarities suggest common lineage or possibly parentage.

Seven major groups of cultivars were arbitrarily created by considering clusters that branched on the phenogram at a similarity of 0.70 (Fig. 2). These seven groups included three with *H. xintermedia* types, two groups of *H. vernalis*, one group of *H. japonica*, and one group of *H. mollis*. Of the *H. xintermedia* types, 'Carmine Red' was a group unto itself, suggesting dissimilarity to other cultivars. The *H. xintermedia* cultivars Arnold Promise and Westerstede also combined to form a group that was divergent from the major group of *H. xintermedia* types (Fig. 2).

Twenty *H. xintermedia* cultivars clustered to form the largest group (Fig. 2). Surprisingly, this large group of *H. xintermedia* types clustered first with *H. mollis* types before joining with the remaining *H. xintermedia* cultivars Arnold Promise, Westerstede, and Carmine Red (Fig. 2). As Bowers and Meredith (1996) noted,

these data sets and subsequent clustering reflect relative similarities and not necessarily evolutionary paths of origin. However, clones that have a high genetic similarity have a greater likelihood of shared parents. We suggest that the major grouping of 20 *H. xintermedia* types likely share several common lineage's that differ from those of the three *H. xintermedia* cultivars that lay outside this major cluster.

Clustering clearly separated *H. vernalis* types from the Asiatic selections (Fig. 2). *Hamamelis vernalis* types were dissimilar from the Asiatic cultivars and branched at a low numeric similarity of ≈ 0.30 . *Hamamelis japonica* types clustered tightly and joined the Asiatic types at a similarity of ≈ 0.51 (Fig. 2).

The average affinity of each clone to *H. mollis*, *H. japonica*, *H. xintermedia*, and *H. vernalis* helped establish a relative closeness between clone and taxa to provide evidence for possible interspecific hybridization (Table 3). As expected, most clones had the highest affinity to the taxa in which they belonged. For example, all *H. japonica* selections had their highest affinity to the *H. japonica* group, moderate affinity to *H. xintermedia* cultivars, and low affinity to *H. mollis* and *H. vernalis*. *Hamamelis mollis* selections had a low affinity to *H. japonica* and *H. vernalis* types with the exception of 'Brevipetala', which had a relatively high affinity to *H. vernalis* (Table 3).

Dirr (1990) speculated that 'Brevipetala' might not be a straight *H. mollis* but, rather, a hybrid. However, Dirr did not indicate the possible parental species. *Hamamelis mollis* is thought to be the least cold hardy of the major taxa of the genus (Dirr, 1990). With the exception of 'Brevipetala', *H. mollis* typically does not survive in Chicago (personal communication). Additional data that we collected, which includes more wild collected plant material, places 'Brevipetala' squarely between *H. mollis* and *H. vernalis* after principle component analysis (unpublished). Therefore, we suggest that 'Brevipetala' is a hybrid between *H. mollis* and *H. vernalis*.

Other cultivars also had an unusually high affinity to a second taxa. Within the *H. xintermedia* type, 'Carmine Red', 'Limelight', and 'Pallida' had a greater numerical affinity to *H. mollis* than to other *H. xintermedia* types (Table 3). In the literature, 'Pallida' has been placed as a *H. xintermedia* and *H. mollis* type. Most authors assign 'Pallida' to *H. mollis* (Dirr, 1990; Hillier, 1977; Hohn, 1993; Lancaster, 1970; Strand, unpublished). However, Dirr (1990) stated, 'Pallida' may be a cultivar of *H. xintermedia*, which is supported by some Ohio nurseryman. Recently, Coombes (1996) stated that 'Pallida', "although long regarded as a cultivar of *H. mollis*, is certainly a hybrid...." From the average affinities (Table 3), 'Pallida' was more similar to *H. mollis* (0.803) than to *H. xintermedia* (0.784) selections. In fact, 'Pallida' had the highest affinity to *H. mollis* among the 23 *H. xintermedia* cultivars (Table 3). Given the apparent affinity of 'Pallida' to *H. mollis*, this clone may represent a possible back-cross between *H. xintermedia* and *H. mollis* to account for the relatively high affinity to this later taxa.

However, the cluster analysis still placed 'Pallida' within the main *H. xintermedia* group (Fig. 2).

In general, *H. xintermedia* types had a higher affinity to *H. mollis* than *H. japonica* (Table 3). This is explained by a greater number of markers (28) present in *H. mollis* that were lacking in *H. japonica*. In contrast, only 13 markers were present in *H. japonica* but lacking in *H. mollis*. In addition, 30 and 15 markers were completely lacking from *H. japonica* and *H. mollis*, respectively.

PEDIGREES AND HALF-SIBS. Perhaps the best documented cultivar is *H. xintermedia* 'Arnold Promise' (see introduction). 'Winter Beauty' and 'Arnold Promise' reportedly share *H. japonica*

Table 1. Primers used to characterize cultivars of *Hamamelis*, corresponding randomly amplified polymorphic DNA fragment size, and base-pair sequence of the 10-mer primers.

Primer no.	Fragment size (base pairs)	Primer sequence
103	690, 710, 780, 1090	GTG ACG CCG C
111	1060 ^z	AGT AGA CGG G
150	540 ^z , 590, 710	GAA GGC TCT G
159	700, 750, 810, 1550, 3000	GAG CCC GTA G
164	700, 780, 850, 1190 ^z	CCA AGA TGC T
180	600, 610 ^z , 950 ^z , 1450	GGG CCA CGC T
181	490, 900, 2100	ATG ACG ACG G
189	390 ^z , 1060	TGC TAG CCT C
234	750, 3700	TCC ACG GAC G
238	690, 820, 2070	CTG TCC AGC A
278	400, 870	GGT TCC AGC T
301	400, 1000, 1600, 2000, 3000 ^z	CGG TGG CGA A
302	710, 805, 1250,	CGG CCC ACG T
327	405 ^z , 700, 900, 1000, 1400, 1600	ATA CGG CGT C
337	520, 575, 1100, 1300, 1650	TCC CGA ACC G
405	605, 625, 1020, 1270, 1500, 2300	CTC TCG TGC G
407	900	TGG TCC TGG C
455	490, 515, 600, 680, 1020, 2100	AGC AAG CCG G
493	370, 490, 650	CCG AAT CAC T

^zThe eight brightest and easiest to score markers.

Fig. 1. Typical results after polymerase chain reaction amplification and randomly amplified polymorphic DNA (RAPD) analysis of witchhazel cultivars. Lanes 1–5, DNA fragments after amplification with primer no. 405; lanes 7–12, DNA fragments after amplification with primer no. 337; lanes 14–19, DNA fragments after amplification with primer no. 180. Lanes 6 and 13 were loaded with a 100 base pair (bp) ladder. The RAPD markers that were scored included primer (no. 405) 605, 625, 1020, 1270, 1500, 2300 bp; primer (no. 337) 520, 575, 1100, 1300, 1650 bp; and primer (no. 180) 600, 610, 950, 1450. Samples chosen for amplification are a mix of cultivar types.

For many years, two European groups have had a keen interest in *Hamamelis* with a goal to develop better landscape plants. They include Hillier Nursery (England) and The Kalmthout Arboretum in Antwerp, Belgium (Cresson, 1986; Strand, unpublished). Col-

Based on the range of genetic similarities, the cultivars named at Hillier Nursery are themselves, less similar than Kalmthout's. This fact is not surprising since Hillier grew and named both *H. xintermedia* and *H. vernalis* types. From our data, most *vernalis* selections had a low affinity to Asian taxa except for 'Sandra' and 'Boesger'. These two clones had nearly twice the affinity to *H. japonica* as the other *H. vernalis* (Table 3) and the two *H. vernalis* groups bifurcate in the phenogram at a similarity of ≈ 0.67 . 'Sandra' was selected at The Hillier Nursery where Asiatic and *H. vernalis* were co-cultivated. 'Sandra' was selected, in part, for good red fall leaf color, which is generally lacking in *H. vernalis* (Dirr, 1990). Therefore, we speculate that 'Sandra' and the *H. vernalis* selection 'Boesger' represent hybrids between *H. japonica* and *H. vernalis*. Potentially, this result could be confirmed if hybrids can be made

[illegible]

Fig. 2. Phenogram of similarity among 40 clones of witchhazel after unweighted pair-group method using arithmetic averages analysis using 68 randomly amplified polymorphic DNA markers calculated with the Dice (1945) coefficient.

Table 3. Average similarity (affinity) of witchhazel clones to the four taxonomic groups studied. Averages were calculated from the genetic similarity table (Table 2) based on 68 randomly amplified polymorphic DNA (RAPD) marker determinations of 40 witchhazel cultivars using the Dice (1945) coefficient.

Species	Cultivar or selection**	Average affinity to:			
		<i>X intermedia</i>	<i>japonica</i>	<i>mollis</i>	<i>vernalis</i>
<i>H. X intermedia</i>	<i>All Gold</i>	0.765	0.685	0.650	0.353
<i>H. X intermedia</i>	<i>Aphrodite</i>	0.731	0.599	0.638	0.329
<i>H. X intermedia</i>	<i>Arnold Promise</i>	0.657	0.588	0.640	0.306
<i>H. X intermedia</i>	<i>Aurora</i>	0.782	0.555	0.711	0.287
<i>H. X intermedia</i>	<i>Barnstedt Gold</i>	0.803	0.567	0.720	0.346
<i>H. X intermedia</i>	<i>Caroline Red</i>	0.647	0.519	0.656	0.374
<i>H. X intermedia</i>	<i>Diane</i>	0.762	0.628	0.639	0.326
<i>H. X intermedia</i>	<i>Feuerzauber</i>	0.754	0.668	0.622	0.412
<i>H. X intermedia</i>	<i>Hiltingbury</i>	0.778	0.596	0.675	0.335
<i>H. X intermedia</i>	<i>Iwado</i>	0.763	0.624	0.672	0.356
<i>H. X intermedia</i>	<i>Jelena</i>	0.737	0.602	0.649	0.390
<i>H. X intermedia</i>	<i>Limelight</i>	0.770	0.482	0.776	0.267
<i>H. X intermedia</i>	<i>Luna</i>	0.761	0.545	0.668	0.299
<i>H. X intermedia</i>	<i>Moonlight</i>	0.768	0.510	0.724	0.268
<i>H. X intermedia</i>	<i>Nina</i>	0.768	0.508	0.710	0.237
<i>H. X intermedia</i>	<i>Orange Beauty</i>	0.747	0.469	0.730	0.213
<i>H. X intermedia</i>	<i>Pallida</i>	0.784	0.490	0.803	0.350
<i>H. X intermedia</i>	<i>Primavera</i>	0.795	0.623	0.687	0.354
<i>H. X intermedia</i>	<i>Ruby Glow</i>	0.773	0.588	0.712	0.370
<i>H. X intermedia</i>	<i>Sunburst</i>	0.734	0.500	0.681	0.249
<i>H. X intermedia</i>	<i>Vesna</i>	0.753	0.597	0.658	0.354
<i>H. X intermedia</i>	<i>Westerstede</i>	0.694	0.510	0.680	0.268
<i>H. X intermedia</i>	<i>Winter Beauty</i>	0.765	0.597	0.679	0.344
<i>H. japonica</i>	<i>Arborea</i>	0.592	0.816	0.379	0.397
<i>H. japonica</i>	<i>Nat'l Arb. #28203</i>	0.564	0.727	0.436	0.376
<i>H. japonica</i>	<i>Rubra</i>	0.631	0.800	0.415	0.442
<i>H. japonica</i>	<i>Sulphurea</i>	0.483	0.749	0.305	0.431
<i>H. mollis</i>	<i>Boskoop</i>	0.726	0.344	0.836	0.208
<i>H. mollis</i>	<i>Brevipetala</i>	0.642	0.456	0.714	0.616
<i>H. mollis</i>	<i>Goldcrest</i>	0.665	0.402	0.815	0.261
<i>H. mollis</i>	<i>Renken **</i>	0.728	0.351	0.833	0.246
<i>H. mollis</i>	<i>Select</i>	0.700	0.411	0.832	0.294
<i>H. mollis</i>	<i>Rochester Superba</i>	0.656	0.338	0.773	0.478
<i>H. vernalis</i>	<i>Boesger **</i>	0.474	0.610	0.388	0.635
<i>H. vernalis</i>	<i>Carnea</i>	0.251	0.306	0.329	0.782
<i>H. vernalis</i>	<i>Christmas Cheer</i>	0.236	0.303	0.297	0.788
<i>H. vernalis</i>	<i>New Years Gold</i>	0.294	0.350	0.370	0.789
<i>H. vernalis</i>	<i>Orange Glow</i>	0.289	0.343	0.364	0.784
<i>H. vernalis</i>	<i>Red Imp</i>	0.261	0.360	0.318	0.800
<i>H. vernalis</i>	<i>Sandra</i>	0.442	0.607	0.389	0.726

by controlled crosses followed by further cluster analysis.

Clearly, F_1 hybrids exist between *H. japonica* and *H. mollis*, but the taxonomic lines become blurred as primary hybrids of *H. X intermedia* backcross and progeny segregate in subsequent generations. This report has attempted to make comparisons among cultivars. *Hamamelis vernalis* types are generally dissimilar from the Asiatic complex; however, our data suggests introgression between these two groups. 'Sandra' has been regarded as *H. vernalis* but also had a moderately high similarity to *H. japonica*. 'Brevipetala' appears to be a *H. vernalis* and *H. mollis* hybrid. In addition, Marquard and Chan (unpublished) have convincing evidence that *H. vernalis* can readily hybridize with Asiatic types when grown together in a common garden.

Currently, breeding efforts are underway to develop superior cultivars of *Hamamelis* at The Holden Arboretum. Given the lack of pedigree information for *Hamamelis* cultivars, we have documented the genetic similarity among many important cultivars. In addition, popular text has been inconsistent in the taxonomic treatment of some cultivars, and this report should help to clarify some of the taxonomic associations.

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