Inheritance of Juvenile Morphological Traits in Crosses of Chinese and American Chestnut

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ABSTRACT. Progeny from crosses of American and Chinese chestnut were examined for the occurrence of the following traits: green or red stem color; stipule size; stipule dehiscence; bud shape; density of simple hairs on twigs (twig hairs) and lower leaf midribs and secondary veins (vein hairs); and, occurrence of simple hairs on interveinal areas of lower leaf surfaces (interveinal hairs). All traits appear to be controlled by two genes, with two exceptions. These were that occurrence of interveinal hairs and high density of vein hairs on Chinese chestnut may be controlled by one and three dominant genes, respectively. High density of twig hairs on Chinese chestnut is probably controlled by two dominant genes. Red stem color on American chestnut may be controlled by two epistatically interacting, partially dominant genes. Small stipule size on American chestnut may be controlled by two incompletely dominant genes. Large stipules on Chinese chestnut take much longer to senesce and dehisce than small stipules on American chestnut. This appears to be strictly related to stipule size and controlled by the same gene(s). Squat bud shape in Chinese chestnut may be controlled by two dominant genes. The three hair traits, bud shape and stem color were linked to each other. Stem color also was linked to stipule size. The stem color determinations were the most interesting from a pathological perspective. Data also are presented on bud break at one date prior to the first frost-free date in spring.

The American Chestnut Foundation is attempting to transfer the blight resistance of the Chinese chestnut tree, *Castanea mollissima* Bl., into the American chestnut tree, *Castanea dentata* (Marsh.) Borkh., but otherwise recover the traits of the American parent. This recovery is accomplished by repeated cycles of backcrossing to the American parent, after an initial hybridization step. Each cycle of backcrossing increases the proportion of American chestnut traits by a factor of 1/2, on average. However, the individuals in each backcross generation will vary in their proportion of American chestnut characteristics. By selecting for American chestnut traits other than blight susceptibility in each segregating generation, we will be able to accelerate the recovery of American-type chestnut trees, bypassing several generations of backcrossing.

Selection for American traits can be accomplished using either traditional genetic markers or markers developed with recombinant deoxyribonucleic acid (DNA) techniques, or both. Markers also would be useful for

detecting pollen contamination in controlled crosses and detecting errors in identification of parents. Since data for many morphological markers are easier and cheaper to obtain than data for recombinant DNA markers, it is preferable to use morphological markers where possible. However, it is unlikely that a sufficient number of morphological markers could be found to span the chestnut genome. If both types of markers could be mapped on the chestnut genome, one could substitute the less-expensive morphological markers for closely linked DNA markers.

This study was undertaken to develop methods for rating traits that are polymorphic in crosses of Chinese and American chestnut, and to begin a genetic map of chestnut. A preliminary report has been made on the inheritance of one trait, interveinal leaf hairs (3). This paper presents further preliminary data and analyses.

MATERIALS AND METHODS

Controlled pollination were made in 1989 and 1990 using methods described by Rutter (7), with the following exceptions. Pollination bags were fastened to stems with jumbo paper clips, without any cotton sealant. All leaves in the bag were removed, and bags were left on until harvest. Every tenth bag was left unpollinated as a control to indicate pollen contamination. Pollination were made with fresh catkins, rubbed directly on female flowers. Catkins were kept fresh for up to 5 days by placing cuttings in water. Cuttings were covered with paper bags to minimize pollen movement from one group of cuttings to another.

Pollen was obtained from the highly blight resistant Chinese chestnut cultivars 'Meiling' and 'Nanking' located at the old Horticulture Farm of Virginia Polytechnic Institute and State University, Blacksburg, Va. Additionally, pollen was obtained from existing F1 hybrids of Chinese and American chestnut, and two first backcrosses (B1) to American chestnut, located in plantings of the Connecticut Agricultural Experiment Station (CAES). The F1 hybrids included tree OTR1T7, located at the White Memorial Foundation in Litchfield, Conn., and trees R4T23, R4T31, R4T49, and R4T52 located in the Spring lot of Sleeping Giant Chestnut Plantation in Hamden, Conn. The B1 trees were two grafts of the 'Clapper' tree, which has been described previously (6), and the undescribed 'Graves' tree. The 'Graves' tree came from a cross made by Hans Nienstaedt at CAES in 1953. Both the `Graves' and 'Clapper' trees have levels of blight resistance similar to that of F1 hybrids. Pedigrees and maps of trees are available from the author.

Mother trees included some of the aforementioned pollen sources, American chestnut trees located at the CAES Lockwood Farm in Connecticut, at the Jefferson National Forest in southwestern Virginia, and in Iowa.

The F2 progeny for which results are given were intercrosses of trees R4T31, R4T49 and R4T52. These three F_1 hybrids are descended from the 'Mahogany' Chinese chestnut at CAES (PI # 70315), which has not been described, and from the Roxbury East and Roxbury West American chestnut trees. The Roxbury trees are located very near each another and are probably related quite closely. The 'Mahogany' tree also is the source of blight resistance for the 'Graves' tree. It was planted in 1929 and is highly blight resistant.

In 1990 and 1991, nuts were planted directly at orchard spacing and tended using methods described by Hebard and Rutter (4). Three orchards were planted in completely randomized designs. Each orchard contained Chinese and American chestnut trees and their first hybrids. Ratings of morphological traits were pooled if assessments were homogeneous for a cross type common to the three orchards.

Morphological traits for which seedlings were rated include: stem color; bud shape; stipule size and dehiscence; density of simple hairs on twigs (twig hairs) and lower leaf midribs and secondary veins (vein hairs); and occurrence of simple hairs on interveinal areas of lower leaf surfaces (interveinal hairs). On one date, April 29, 1992, seedlings also were assessed for bud break. Ratings of bud morphology were made in April, 1992. In most cases, the other traits were rated in early August, 1991. Twig and vein hairs were reassessed in September, 1992.

Trees were rated as having interveinal hairs when clusters of simple hairs were visible with a 10x hand lens on the lower leaf surface between secondary veins. A leaf would be rated as lacking interveinal hairs only if a single hair were observed. On 0-yr-old seedlings, hairs were most evident near the midrib, toward the tip of young leaves. Most 0- and 1-yr-old seedlings lack stellate hairs. These ratings were made on leaves growing in full sun. Even pubescent species, like *C. crenata* Sieb. and Zucc., lack hairs on leaves growing in the shade.

Seedlings were rated as having a high density of twig hairs when simple hairs, visible without a hand lens, were abundant below the third or fourth node from the stem apex; a medium density when long (ca 1 mm) simple hairs that were not oppressed to the stem occurred below the first or second node; a low density when short (< 0.5 mm) hairs oppressed to the stem were visible anywhere on the stem or long, non-oppressed hairs occurred above the first or second node. Some trees lacked twig hairs. A 10 x hand lens was used.

Trees were rated as having a high density of vein hairs when simple hairs on the midrib were strongly overlapping on full-sized leaves, a medium density when most overlapped on 7-cm-long leaves, and a low density when most did not overlap on 7-cm-long leaves. A 10x hand lens was used. It was often difficult to distinguish leaves with medium and low vein hair density, which was the critical break for inheritance studies. In one orchard, two assessments were made; when these did not agree, up to three further assessments were made before a plant was classified. The other orchards have not been reassessed yet.

Stipules were rated as large if the base (proximal end) was more than 8-mm wide, moderately large if the base was between 4 and 8 mm wide, moderately small if the base was more than 1-mm wide and wider than the top (distal

third), and small if they were about 1-mm wide from top to bottom. Small diameter stems had smaller stipules than large diameter stems; the above figures are for stems 3-5 mm in diameter. The first three or four stipules on newly emerged stems were wider than others and were not rated.

Buds were rated as elongated in shape if they were longer than wide when viewed laterally, as squat if not. Bud tips were classified as flat if the upper portion sloped more than 45° from vertical and pointed when it did not. When rating tips, buds should be viewed facing the lower surface. I did not learn this prior to bud break in the spring, so data are reported only for bud shape. Buds were rated as having emerged (broken) if an internode was visible.

Stems were rated as red, greenish-red, reddish-green and green. The basic colors of red and green were distinguished by examining stipules and the tops of petioles in midsummer. The subsidiary classifications were made by examining the colors of stems. Red stems were rated greenish-red if they were not a dark red. Green stems with a hint of red were classified as reddish-green. Cold weather induces a brownish color in leaves and stems of Chinese chestnut, which can confound color determinations if they are not performed in midsummer. Some Chinese chestnut cultivars, such as 'Kuling,' may have reddish-green stems, but the tops of petioles are always green.

Recombination percentages were calculated by the product (1) and scores (5) methods, as appropriate. When percentages were combined, they had first been found to be homogeneous by a chi-square test. One trait (vein hairs) appeared to be controlled by two linked genes in progeny of the 'Graves' and 'Clapper' trees. The linkage of this trait to others was determined using the following frequencies of zygote types for the backcross case: AB = (1—r + pr)/2; Ab = (r—pr)/2; aB = (1— pr)/2; and ab = pr/2, where p is the recombination fraction in repulsion between the gene for trait A and the nearest gene for trait B, r is the recombination fraction in repulsion within the two genes for trait B, AB is the zygote with dominant alleles for both traits; Ab is the zygote with a dominant allele for trait A, and no dominant alleles for trait B, etc. The gene for trait A was assumed not to be flanked by the genes for trait B. The zygotic series for the case where the gene for trait A is flanked was also developed, but did not fit the data. Zygotic series for more complicated situations than the backcross were derived from the above basic series using standard methods.

RESULTS AND DISCUSSION

Interveinal hairs. The lower, interveinal leaf surface on sun leaves of American chestnut is glabrous whereas Chinese chestnut leaves are pubescent. Lower surface interveinal hairs were present on first hybrids (F_1) between the two species (Table 1). The 'Clapper' tree was reported to lack interveinal hairs (6), but sun leaves of the tree possess them. The 'Graves' tree also has interveinal hairs. Segregation of interveinal hair occurrence fit a 1:1 ratio in backcrosses (B2) of the 'Graves' and 'Clapper' trees to American chestnut; it fit a 3:1 ratio in intercrosses (B1— F2) of the two trees. These data indicate that inheritance of interveinal hairs in the 'Graves' and 'Clapper' trees is controlled by a single gene from Chinese chestnut. Interveinal hairs segregated 1:1 in first backcrosses, but segregation was between 3:1 and 15:1 in the F2 populations checked. This suggests that the occurrence of interveinal hairs in Chinese chestnut is controlled by a single gene, with additional modifier *genes.* This conjecture is supported by the observation of a decrease in interveinal hair density with an increase in the fraction of American chestnut parentage, in crosses progressing from F1 to B2. Unusual segregation patterns are not uncommon in interspecific crosses.

Table 1. Occurrence of simple hairs on interveinal lower leaf surfaces of Chinese and American chestnut trees and hybrids.

Tree	Hairs		Hypothesis	χ^2†
	Present	Absent		
American	0	35		
Chinese	30	0		
F_1	18	0		
F_2	176	23	3:1	19.2**
B_1 (3/4 American)	68	50	1:1	2.7
B_2 (3/4 American)	137	149	1:1	2.5
B_1–F_2 (3/4 American)	312	112	3:1	0.5

†One asterisk indicates rejection of the hypothesis at the 5% level. Two asterisks indicate rejection at the 1% level.

Leaves of seedling Chinese chestnut and its hybrids with American chestnut lack stellate hairs, which are abundant on leaves of mature Chinese chestnut. It is possible that development of simple hairs on interveinal surfaces will also reflect this trend, such that a larger fraction of trees will have leaves with interveinal hairs as the trees mature.

Interveinal hair data can be used to assess the degree of pollen contamination in controlled crosses. Three F1 hybrids were not hairy; this characteristic and others indicated that these three trees were pure American chestnut rather than hybrids. Data on them were not included in the tables. Additionally, progeny of a backcross of the `Clapper' tree to American chestnut, made in 1989, had significantly more hairless than hairy progeny. This, in combination with the occurrence of nuts in unpollinated controls, suggests contamination with American chestnut pollen. Results for the cross are not included in the tables, except Table 5.

Twig hairs. Twigs of Chinese chestnut are conspicuously hairy while hairs are not visible with the naked eye on twigs of American chestnut. First hybrids between the two species show twig hair density in between the two parents (Table 2). In the F2, twigs with high or medium hair density segregated 15:1 to twigs with slight or no hairs. In first and second backcrosses to the American parent, twig hair density segregated 3:1, while it segre-

Table 2. Occurrence of simple hairs on twigs of Chinese and American chestnut trees and hybrids.

Tree	Twig Hair Density				Hypothesis high+med: low+none	χ^2†
	High	Medium	Low	None		
American	0	0	13	3		
Chinese	34	1	0	0		
F_1	7	7	0	0		
F_2	68	121	11	0	15:1	0.2
B_1 (3/4 American)	3	93	20	2	3:1	2.5
B_2 (7/8 American)	4	203	64	1	3:1	0.2
B_1–F_2 (3/4 American)	72	327	22	0	15:1	0.8

†One asterisk indicates rejection of the hypothesis at the 5% level. Two asterisks indicate rejection at the 1% level.

Table 3. Occurrence of simple hairs on veins of lower leaf surfaces of Chinese and American chestnut trees and hybrids.

Tree	Vein Hair Density			Hypothesis high+med: low	χ^2†
	High	Medium	Low		
American	0	3	13		
Chinese	36	0	0		
F_1	12	2	0		
F_2	137	56	6	35:1	0.0
B_1 (3/4 American)	63	37	19	5:1	0.0
B_2 (7/8 American)	79	106	82	2:1	0.8
B_1–F_2 (3/4 American)	182	190	52	8:1	0.6

†One asterisk indicates rejection of the hypothesis at the 5% level. Two asterisks indicate rejection at the 1% level.

gated 15:1 in crosses of the 'Graves' and 'Clapper' first backcrosses. These data indicate that twig hair density is controlled by two genes in Chinese chestnut.

Vein hairs. The midrib and secondary veins of Chinese chestnut sun leaves are covered with dense simple hairs whereas vein hairs are sparse on American chestnut. Vein hairs were dense on F1 hybrids (Table 3). Simple one, two or three-gene models could not explain the data. A three-gene hypothesis, with two of the genes linked, could, if it was additionally posited that the 'Clapper' and 'Graves' trees had only the two linked genes. These trees were the parents of the B2s and B1—F2s in Table 3. A recombination percentage of 33.33% led to the hypotheses in Table 3. This percentage was very close to the percentage that gave the best fit to the data (33.1332 ± 1.48%).

Most backcross progeny described in Table 3 (as well as the other tables) were located in one orchard. This was the orchard where up to five assessments of vein hair density were performed. All the pure American chestnut trees in this orchard were rated as having slight vein hair density.

Stipules. Stipules on Chinese chestnut are 5 to 10 mm wide at the base, tapering to a point over a length of 10 to 20 mm, whereas stipules on American chestnut are about 1 mm wide over most of their length of 5 to 10 mm, tapering to a point. The stipules on Chinese chestnut are widest just above the point of attachment to the stem. In first hybrids, stipule size was intermediate between the two parents (Table 4). A fairly large proportion of F2 progeny had medium-small and small stipules. The segregation of large versus medium-large plus medium-small plus small stipules in F2 fit a model of two incompletely dominant genes controlling small stipule size in American chestnut. This model can only be confirmed by examining stipule size in backcrosses to Chinese chestnut and in F3 progeny. We have attempted the backcrosses to Chinese chestnut, but they have been plagued with pollen contamination thus far.

Stipules on American chestnut dehisce within 1 mo of leaf expansion whereas stipules on Chinese chestnut often remain on the stem through winter. Early dehiscence was associated almost perfectly with moderately small and small stipule size (data not shown), and appears to be caused by the small area of attachment of the stipules.

Stem color. American chestnut stems have a reddish color while Chinese chestnut stems are green or tan colored. There were no green-stemmed F1 hybrids between the two species, although about one-half of the individuals did not have a deeply red stem (Table 5). The number of red plus greenish red versus green plus reddish green stems in F2 fit a 9:7 ratio, suggesting that red stem color is controlled by two epistatically interacting genes. Like the F1, some first backcrosses of Fi hybrids to American chestnut neither had a deep red stem color, nor did some second backcrosses of the 'Graves' B1 to American

Table 4. Stipule size on Chinese and American chestnut trees and hybrids.

Tree	Size				Hypothesis large:med-large+med-small+small	χ^2†
	Large	Medium Large	Medium Small	Small		
American	0	0	1	34		
Chinese	32	13	0	0		
F_1	2	13	1	1		
F_2	13	96	57	28	1:15	0.0
B_1 (3/4 American)	1	16	40	51		
B_2 (American × 'Graves')	1	6	43	113		
B_2 (American × 'Clapper')	0	3	12	90		
B_1–F_2 (3/4 American)	4	115	167	138		

†One asterisk indicates rejection of the hypothesis at the 5% level. Two asterisks indicate rejection at the 1% level.

Table 5. Stem color on Chinese and American chestnut trees and hybrids.

Tree	Color				Hypothesis Green+Reddish Green:Greenish Red+Red	χ^2†
	Green	Reddish Green	Greenish Red	Red		
American	0	0	0	37		
Chinese	46	1	0	0		
F_1	0	5	4	8		
F_2	17	68	15	101	7:9	0.2
B_1 (3/4 American)	1	13	18	87		
B_2 (American ×'Graves')	0	9	7	161		
B_2 (American × 'Clapper')	0	0	2	295		
B_1–F_2 (3/4 American)	6	29	20	375		

†One asterisk indicates rejection of the hypothesis at the 5% level. Two asterisks indicate rejection at the 1% level.

chestnut. However, there were no green or reddish green stems in second backcrosses of the 'Clapper' B1 to American chestnut and only two greenish red stems. This suggests that the 'Graves' first backcross is heterozygous for red stem color while the 'Clapper' first backcross is homozygous for the genes controlling red stem color. This hypothesis is supported by the fact that the frequency of non-red stems in intercrosses of the 'Graves' and 'Clapper' trees was not significantly independent ($p = 0.19$) of the frequency of non-red stems in backcrosses of the `Graves' tree to American chestnut. There are several examples in the literature of intermediate flower colors being observed in heterozygotes, where two epistatically interacting genes controlled color (8). In the present study, I conclude that I was unable to classify the heterozygotes precisely.

The red stem color of American chestnut is probably due to the presence of flavanoids. Condensed tannins, which are polymerized flavanoids, that occur in American chestnut but not Chinese chestnut (2). Since the 'Clapper' tree appears to be homozygous for red stem color and is partially resistant to blight like the 'Graves' tree, this suggests that the difference in blight resistance between Chinese and American chestnut is not directly related to the difference in their content of condensed tannins.

Bud shape. Buds on American chestnut are longer than they are wide (elongated), round in cross section, and have pointed tips whereas buds on Chinese chestnut are not longer than they are wide (squat), are elliptical in cross section, and have flat tips. Buds on American chestnut are free of the stem over much of their length and are skewed off the axis of the stem, whereas buds on Chinese chestnut are oppressed to the stem and their main axis is parallel to the stem axis when viewed face on. Buds on Chinese chestnut tend to be as wide as the stem whereas buds on American chestnut tend to be narrower than the stem. The elongated vs. squat trait was the only one rated successfully prior to bud break this spring. The oppressed and non-skewed traits were lacking in some Chinese chestnut seedlings. In F1 crosses, buds on all trees were squat (Table 6). There was segregation of the trait in F2 and backcross generations, but reliable screening methods were not developed in time to rate all our crosses prior to bud break this spring. Data for the B is suggest that squat versus elongated bud shape is controlled by two genes.

Bud break. As of April 29, 1992, leaves had emerged on 100% of 23 Chinese chestnut trees but only 19% of 26 American chestnut trees. Emergence was 64% on 11 first hybrids between the two species. The differences in emergence between the species and their first hybrid were

highly significant ($p < 0.01$). Similar results (with an exception discussed below) were obtained for numerous other American and Chinese chestnut trees growing at the Meadowview farm and environs.

The 29th of April is one week prior to the first frost-free date for the Meadowview area, so there is a strong selective advantage for the trees whose leaves had not emerged. On the night of April 28, a moderate frost killed the emerged shoots of trees at the farm located toward the bottom of hills.

Table 6. Bud shape on Chinese and American chestnut trees and hybrids.

Tree	Buds		Hypothesis	χ^2†
	Squat	Elongated		
American	5	17		
Chinese	39	5		
F_1	17	0		
B_1 (3/4 American)	84	31	3:1	0.2

†One asterisk indicates rejection of the hypothesis at the 5% level. Two asterisks indicate rejection at the 1% level.

Emergence was 67% on 97 first backcrosses of Chinese-American chestnut hybrids to American chestnut, and 66% on 404 second backcrosses. These rates were similar to the rate for the first hybrid. One might expect the backcrosses to have shown rates closer to the recurrent parent, but many of their American chestnut parents were not from the Meadowview area. Leaf emergence on American chestnut trees originating from Kentucky, which were growing at the Meadowview farm, was 100% in contrast to the 19% rate presented above, which was for American chestnut trees originating near Meadowview. It also is possible that leaf emergence has a low heritability.

Recombination. The traits identified in this study are summarized in Table 7, which also assigns symbols to the genes controlling their expression. The three hair traits (I, T and V), bud shape (B) and stem color (C) were linked with each other; stem color also was linked with stipule size (S) (Tables 8A and 8B). Figure 1 depicts the deduced order of the linked genes and map distances, where these could be calculated. Genes *V3, T2, S2* and *B2* assort independently and are not mapped. Gene *T1* appeared to be identical to gene *V1,* although the standard error of the map distance was large. However, gene *T1* was 13.5 ± 3.01

Table 7. Number of genes, dominant parent, and symbol for traits observed in crosses of Chinese and American chestnut.

Trait	Number of Genes	Dominant Parent	Symbol
Leaf Interveinal Hair Occurrence	1	Chinese	*I*
Leaf Vein Hair Density	3	Chinese	*V1, V2, V3*
Twig Hair Density	2	Chinese	*T1, T2*
Stipule Size	2	American	*S1, S2*
Stem Color	2	American	*C1, C2*
Bud Shape	2	Chinese	*B1, B2*

Table 8A. Numbers of individuals in the four phenotypic classes for character pairs from crosses of Chinese and American chestnut, contingency chi-squares, recombination percentages, and their standard errors.

	Type of Cross									
	F_2							B_1		
	Character Pairs									
	I×S	I×C	V×S	V×C	T×S	T×C	S×C	I×B	V×B	T×B
Phenotype†										
AB	156	91	172	108	168	104	106	54	74	69
Ab	13	84	13	84	13	84	73	13	22	24
aB	22	23	6	6	11	11	2	30	9	15
ab	0	0	0	0	0	0	11	18	9	7
χ^2††	1.8	19.2	0.5	4.6	0.8	8.5	9.5	4.7	5.6	0.3
%Recomb.‡							10.8	33.4	6.6	41.5
Recomb. s.e.							7.64	7.71	20.01	13.35

†"A" denotes occurrence of the dominant phenotype for the first trait in a pair, "a" the recessive phenotype. "B" and "b" correspondingly denote the phenotypes of the second trait in a pair.

††Chi-square values exceeding 3.8 and 6.6 are significant at the 5% and 1% levels, respectively.

‡Recombination percentages are not given for the other character pairs for the following reasons. The percentage was not estimable by the product or scores methods for the T×C character pair. The T×B and T×S pairs showed no signs of linkage. There were so many genes in the V×S and V×C pairs that the standard error would have been more than 50%. The I trait did not show Mendelian segregation for the I×S and I×C pairs.

Table 8B. Numbers of individuals in the four phenotypic classes for additional character pairs from crosses of Chinese and American chestnut, contingency chi-squares, recombination percentages, and their standard errors.

	Character Pairs											
	I×*V*				*I*×*T*				*V*×*T*			
	Type of Cross											
	B_1	B_2	B_1–F_2	F_2	B_1	B_2	B_1–F_2	F_2	B_1	B_2	B_1–F_2	F_2
Phenotype†												
AB	69	121	306	176	63	121	308	170	83	162	359	184
Ab	1	6	5	0	5	6	1	6	14	23	9	9
aB	31	63	66	17	32	84	91	18	11	39	40	4
ab	18	75	47	6	17	59	20	5	8	42	12	2
χ^2††	26.8	76.7	123.2	47.3	13.9	49.1	53.8	13.1	7.9	47.4	40.8	9.2
%Recomb.‡	3.5	9.1	5.2			13.5			0.0	0.0	0.0	
Recomb. s.e.	4.06	2.79	2.22			3.02			19.81	7.23	9.90	

†"A" denotes occurrence of the dominant phenotype for the first trait in a pair, "a" the recessive phenotype. "B" and "b" correspondingly denote the phenotypes of the second trait in a pair.

††Chi-square values exceeding 3.8 and 6.6 are significant at the 5% and 1% levels, respectively.

‡For the *I*×*T* character pair, the scores method was used to estimate one value from the three crosses; the "Ab" phenotype was assumed missing in the B_1–F_2 cross for the *I*×*T* character pair. Recombination percentages were not calculated for the F_2 cross because the *I* trait did not segregate in a simple Mendelian fashion and because there were too many genes in the *V*×*T* character pair.

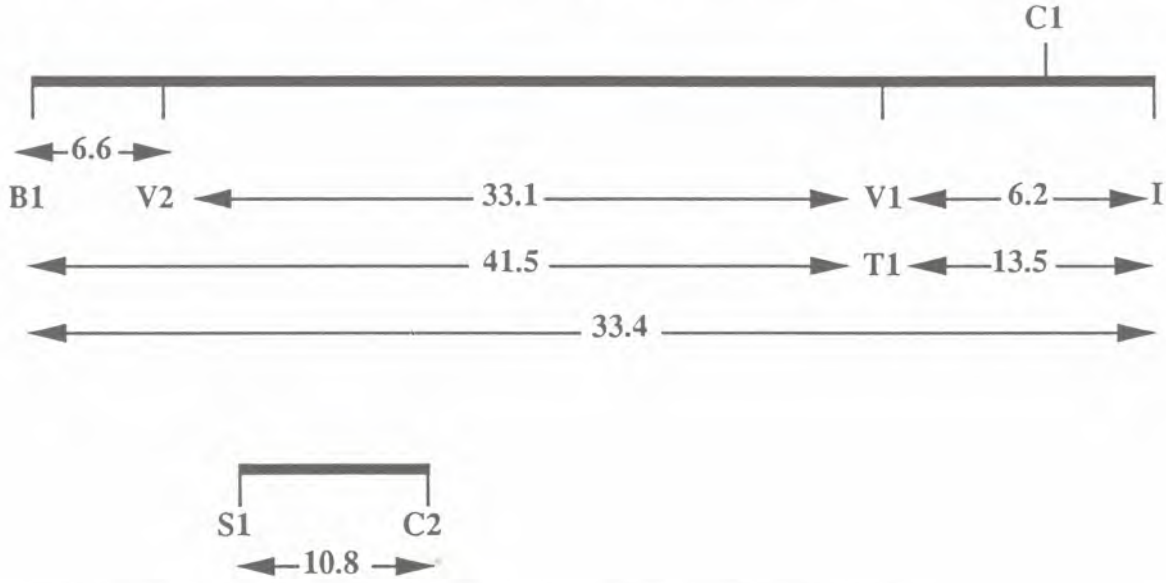

Figure 1. Preliminary mapping of morphological traits segregating in crosses of Chinese and American chestnut.

map units from gene *I* while gene *VI* was 6.2 ± 1.60 units from gene *I*. This difference was significant ($t = 5.2$, $p < .05$). I also considered a set of models (zygotic series) for the Inh x Vnh character pair where there were no double crossover events, one between the *I* and *V1* genes and one between the *VI* and *V2* genes, due to interference. These increased the recombination percentage a maximum of 3% (in other words by a factor of 1.03). As mentioned in the materials and methods, the zygotic series for the case where the *T1* genes of the *B1* gene was flanked by the *V1* and *V2* genes did not fit the data.

The multigenic inheritance of most of these traits does not make them very suitable for precise mapping. Additionally, the contingency chi-squares from which some map orders were deduced may be affected by the mode of inheritance. On the other hand, one chromosome is fairly extensively mapped, and, potentially, markers are located on five others.

The fact that genes for all traits but stipule size were linked to each other helps explain why the complex of hairy leaves and twigs, squat buds and slightly greenish stems is so prevalent in trees with Chinese chestnut parentage. It is possible that this character complex, and large stipule size, is related to the lack of red color in Chinese chestnut. Anthocyanins in many plants are thought to protect developing photosynthetic systems from direct sunlight. The large stipules of Chinese chestnut shield the apical meristem, which is not so shielded in American chestnut. Likewise, the dense mats of hairs on stems and leaves of Chinese chestnut may have a similar function.

The buds on Chinese chestnut also are less exposed to sunlight than buds on American chestnut.

Taking all traits together, 3.4% of the B1s and 8.8 % of B2s had all American morphological traits, namely, no interveinal hairs, a low density of vein and twig hairs, small stipules, red stems and cylindrical buds. Of the B1—F2s, 1.4% had all American traits. No Fes had all American traits, but 3.2% had all Chinese traits; this may reflect the fact that all of the traits chosen for this study are dominant in the Chinese parent, or incompletely dominant in the American parent.

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