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ELECTROPHORETIC CONFIRMATION OF INTERSPECIFIC HYBRIDIZATION IN *AESCULUS* (HIPPOCASTANACEAE) AND THE GENETIC STRUCTURE OF A BROAD HYBRID ZONE

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Abstract.—Within a broad (>200 km wide) hybrid zone involving three parapatric species of *Aesculus*, we observed coincident clines in allele frequency for 6 of 14 electrophoretic loci. The cooccurrence of alleles characteristic of *A. pavia, A. sylvatica,* and *A. flava* was used to estimate genetic admixtures in 48 populations involving various hybrids between these taxa in the southeastern United States. High levels of allelic polymorphism (up to 40% greater than the parental taxa) were observed in hybrid populations and also in some populations bordering the hybrid zone. A detailed analysis of a portion of the hybrid zone involving *A. pavia* and *A. sylvatica* revealed a highly asymmetrical pattern of gene flow, predominantly from Coastal Plain populations of *A. pavia* into Piedmont populations of *A. sylvatica.* Computer simulations were used to generate expected genotypic arrays for parental, F_1, and backcross individuals, which were compared with natural populations using a character index scoring system. In these comparisons, hybrid individuals could be distinguished from either parent, but F_1 and backcross progeny could not be distinguished from each other. Most hybrid populations were found to include hybrids and one of the parental taxa, but never both parents. Three populations appeared to be predominantly hybrids with no identifiable parental individuals. Hybrids occurred commonly at least 150 km beyond the range of *A. pavia,* but usually not more than 25 km beyond the range of *A. sylvatica.* Introgression, suggested by genetically hybrid individuals and significant gene admixtures of two or more species in populations lacking morphological evidence of hybridization, may extend the hybrid zone further in both directions. The absence of one or both parental species from hybrid populations implies a selective disadvantage to parentals in the hybrid zone and/or that hybridization has occurred through long-distance gene flow via pollen, primarily from *A. pavia* into *A. sylvatica.* Long-distance pollen movement in plants may generate hybrid zones of qualitatively different structure than those observed in animals, where gene flow involves dispersal of individuals.

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Interspecific hybridization has long been considered an important process in the evolution of flowering plants for at least three reasons (Stebbins, 1959, 1969). First, novel genotypes are created from the crossing and subsequent interactions of similar, but distinct, genomes. The resulting progeny may have phenotypic characteristics unlike either of the parents, display heterosis or enhanced physiological stability (Barber, 1970; Schwartz and Laughner, 1969), possess selective advantages in novel habitats, or exploit resources unused by either parent (Straw, 1955). Second, hybridization followed by repeated backcrossing to parental and later generation hybrids (introgression; Anderson, 1949, 1953) can result in a transfer of genetic information across the usual species boundaries (Stebbins, 1959; Heiser, 1973; Levin, 1975). This may contribute to the maintenance of larger quantities of genetic variation, allowing a more rapid response to selection, and possibly promote major range expansions (Lewontin and Birch, 1966; Hardin, 1975). Third, stabilization of hybrid populations, with or without polyploidy, can lead directly to the formation of new biological species as hypothesized for many crop and noncrop plants (Stebbins, 1959, 1969; Levin, 1979).

Despite a long-standing interest in examples of hybridization and introgression and their potential significance in plants (Anderson and Stebbins, 1954; Stebbins, 1959, 1969; Barber, 1970; Knobloch, 1972; Heiser, 1973; Levin, 1979; Giannasi and Crawford, 1986), many of the properties of hybrid population systems are still unstudied. Little is known, for example, of the characteristics of hybrid zones in plants: their size, shape, and whether few or many events of hybridization led to their origin. The effects of hybridization on genetic variability, particularly in studies involving introgression in diploid taxa, are largely un-

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quantified. With a few notable exceptions (Levin, 1975; Bloom, 1976; Millar, 1983; Werth et al., 1985; Heywood, 1986; Wheeler and Guries, 1987), detailed knowledge of the genetic structure of hybrid plant populations is almost completely lacking. This lack of knowledge is most surprising in view of the abundance of theoretical interest in hybrid zones and the excellent population genetic studies of hybridization in many animal taxa, recently reviewed by Barton and Hewitt (1985, 1989) and Hewitt (1988).

A classic and often cited example of hybridization in the woody plant genus, *Aesculus* ($n = 20$), was first described in detail by Hardin (1957*c*, 1957*d*). This genus is represented by five species in the southeastern United States: *A. flava* Solander (syn. *A. octandra* Marshall), *A. glabra* Willdenow, *A. parviflora* Walter, *A. pavia* L., and *A. sylvatica* Bartram. Throughout most of their ranges, each is easily recognized by a number of diagnostic morphological traits, including flower color, size, and shape (Hardin, 1957*a*, 1957*b*, 1957*d*; dePamphilis and Wyatt, 1989). However, in areas where their distributions approach each other and overlap to a very limited extent, hybridization can occur, as evidenced primarily by the occurrence of plants combining the diagnostic traits of two or more species (Hardin, 1957*c*, 1957*d*). A degree of hybrid unfitness (Barton and Hewitt, 1985), suggested by a greater frequency of meiotic irregularities and inviable pollen in plants from the hybrid zone (Hardin, 1957*d*; dePamphilis and Wyatt, 1989), also supports the notion that this is indeed a zone of secondary contact. Hardin's (1957*d*) studies of morphological variation indicated that hybridization in this region mainly involves the red-flowered *A. pavia*, and the yellow-flowered *A. sylvatica* and *A. flava*, which appear to form complex 2- and 3-species hybrid populations throughout a large area of west-central and northern Georgia (Fig. 1). Morphological variation also suggests that the distribution of hybrids is asymmetrical with respect to the distributions of the species: apparent hybrids with the *A. pavia* often occur far beyond the range of this species, 100 km or more into the Piedmont, while relatively few hybrids are found in the Coastal Plain, where *A. pavia* occurs naturally (Fig. 1). At many locations, only a small amount of intergradation is seen, which was interpreted by Hardin (1957*d*) as evidence of introgression of genes for morphological traits of one species into populations of another. *Aesculus glabra*, which is rare south and east of Tennessee, can hybridize with these species, but only appears to do so west and north of the region shown in Figure 1 (Hardin, 1957*d*). *Aesculus parviflora* is distantly related to the above species and is not known to hybridize with them despite occurring sympatrically with *A. pavia* throughout much of central and southeastern Alabama (Hardin, 1957*b*, 1957*d*).

Here we present the results of an examination of allozyme variation in 51 populations of *Aesculus* that provide a comprehensive sample of the complex hybrid zone and the putative parental species. The major questions addressed by the present study are: (1) Do the patterns of allozyme variation support the existence of a complex zone of hybridization involving three species of *Aesculus* and can this zone be described quantitatively? (2) How does the shape and size of the hybrid zone, defined allozymically, compare with that defined on morphological grounds? (3) What is the influence of hybridization on genetic variability? (4) Can the allozyme data be used to distinguish cases of introgressive hybridization as opposed by hybridization without introgression?

MATERIALS AND METHODS

Populations Sampled

A previous study of allozyme variation in 24 populations of *Aesculus* outside the hybrid zone provided baseline information for each of the possible parental species (dePamphilis, 1988; Appendix 1). For this study, 27 additional populations were sampled in and around the complex hybrid zone involving *A. pavia*, *A. sylvatica*, and *A. flava*. The sample included 11 populations from the hybrid zone between *A. pavia* and *A. sylvatica* (Fig. 1). Also included were five populations identified morphologically as *A. pavia*, but which were located just outside of the hybrid zone (henceforth referred to as "border" *A. pavia*), and four populations of *A. sylvatica* and one of *A. flava* near the

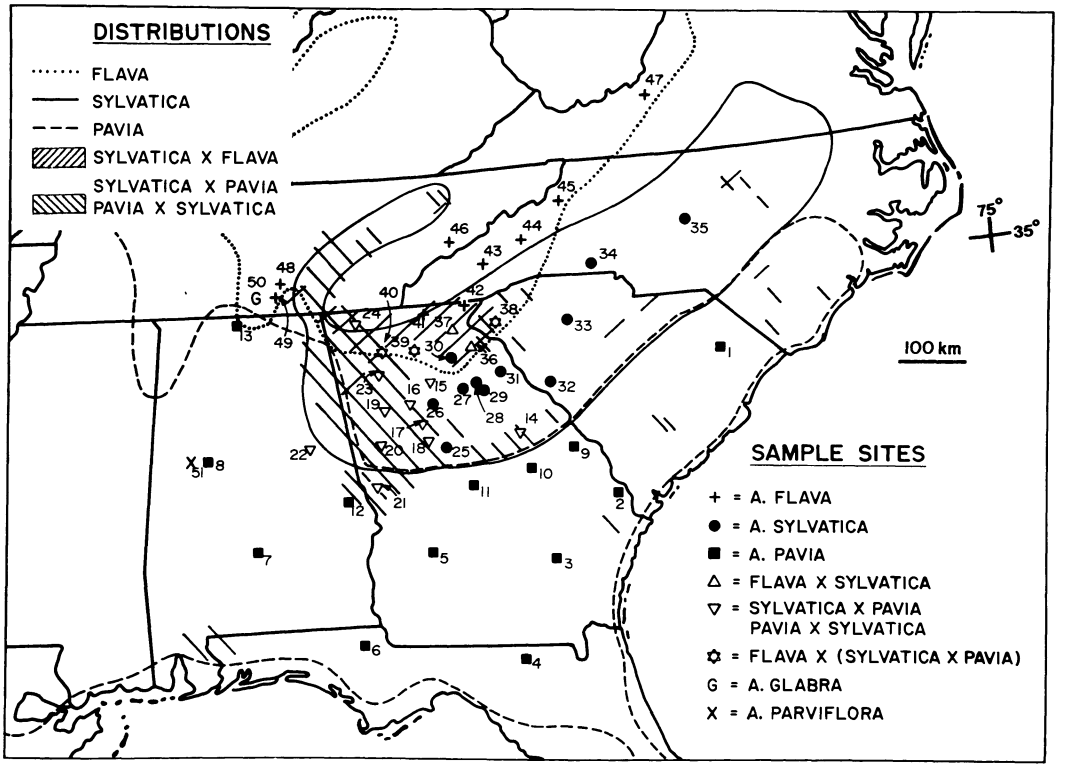

FIG. 1. Distribution of *Aesculus* species and hybrids (indicated by cross-hatching) in the southeastern United States (based on Hardin, 1957*b*) and location of sample sites used in the electrophoretic survey. Populations 1–8, 28, 29, 31–35, 41, 43–48, 50, 51 were characterized electrophoretically by dePamphilis (1988). *Aesculus parviflora* occurs in Alabama and, rarely, in Georgia and South Carolina (Hardin, 1957*b*; Wyatt, 1985), while the range of *A. glabra* includes interior states of the eastern United States (Hardin, 1957*b*).

hybrid zone ("border" *A. sylvatica* and "border" *A. flava,* respectively). Two sample populations were identified as *A. sylvatica* × *A. flava* and three others were probable triple hybrid swarms that combined the diagnostic morphological traits of *A. pavia, A. sylvatica,* and *A. flava.* An apparent hybrid population between *A. flava* and *A. glabra* (#49) was also sampled in Franklin County, TN. Six of the hybrid populations (16, 18, 20, 24, 36, 40) and three of the parental populations (2, 3, 28) were included in Hardin's (1957*d*) earlier study.

Electrophoresis

Within each population, 30–50 plants (usually 30) were selected at random (Schoen and Fruchter, 1983) and two or more late-winter terminal buds were collected from each sample plant. The buds were frozen immediately on dry ice and transferred to the laboratory. Subsequent sample storage, sample preparation, and procedures for starch gel electrophoresis of proteins obtained from the buds are described elsewhere (dePamphilis, 1988).

Nine enzyme systems encoded by 20 putative loci were assayed for each individual tree. Fourteen of the loci could be reliably stained and scored and all analyses were performed with this set. Inheritance of allozyme variants was previously verified using single-parent progeny arrays (dePamphilis, 1988). The enzyme systems and locus designations are as follows: Amino acid transferase (*Aat2, Aat3*), acid phosphatase (*Acp1, Acp2*), alcohol dehydrogenase (*Adh1*), leucine aminopeptidase (*Lap1*), malic enzyme (*Me1, Me2*), peroxidase (*Px2*), phosphoglucoisomerase (*Pgi1, Pgi2*), phosphoglucomutase (*Pgm2, Pgm3*), and shikimate dehydrogenase (*Shk2*). The staining pro-

cedures, variation patterns, and scoring rules are described by dePamphilis (1988).

Analyses

Patterns of allele frequency variation were examined carefully for evidence of hybridization in the putative hybrid populations. A previous analysis of allozyme variation in the parental species had revealed 34 alleles to vary significantly in frequency among *A. pavia, A. sylvatica,* and *A. flava* (dePamphilis, 1988). A number of these alleles were common in one taxon but absent or nearly so in the other taxa. These alleles were used as genomic markers. We considered the cooccurrence of marker alleles of each of the putative parents as the minimum evidence required to support the hypothesis of hybridization. Detailed analyses of individual multilocus genotypes were also performed for the populations of *A. pavia, A. sylvatica,* and the portion of the hybrid zone involving these taxa (described below).

Additional evidence for testing the hypothesis of hybridization came from patterns of unbiased genetic distance (D) among populations (Nei, 1978; Swofford and Selander, 1981). Hybrid populations were expected to have genetic distances intermediate between the putative parental species and not closer to any other potential parent.

The relative genetic contribution of each parental species to each study population was estimated using a least squares procedure developed to describe hybridization and gene flow among human populations (Roberts and Hiorns, 1965; Elston, 1971). The procedure uses a matrix $\mathbf{X}$ of allele frequencies for two or more reference taxa (here, three) and a row vector $\mathbf{y}$ of allele frequency differences between a study population and the reference taxa. The least-squares estimator of gene admixture, $\mathbf{m}$, is a row vector defined as

$$\mathbf{m} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{y}$$

provided $\mathbf{X}'\mathbf{X}$ is nonsingular. $\mathbf{m}$ has as its elements the least squares estimates of the proportion of genes in the study population derived from each of the reference taxa. Two restrictions, imposed for increased biological realism, are that all (elements of) $\mathbf{m}$ sum to 1.0, and all $\mathbf{m} \geq 0$.

The restricted least-squares procedure of the Statistical Analysis System (SAS Institute, 1985) was used to estimate $\mathbf{m}$ and its standard error for each population in the study under the first restriction. The mean allele frequencies for populations of *A. pavia, A. sylvatica,* and *A. flava* outside the hybrid zone were used for the reference taxa. *Aesculus glabra* and *A. parviflora* were eliminated from this analysis on morphological and distributional grounds, but patterns of allele variation (dePamphilis, 1988) also eliminated these species from consideration. To avoid a downward bias to the standard errors of the estimates, we used only those alleles that were informative for distinguishing the reference taxa by one-way analysis of variance of the arcsine square root-transformed allele frequencies (dePamphilis, 1988). To implement the second restriction (all $\mathbf{m} \geq 0$), we followed the suggestion of Elston (1971) to first calculate $\mathbf{m}$ without this restriction and inspect the result. If negative estimates were obtained for any element of $\mathbf{m}$, the most negative element is set to zero and $\mathbf{m}$ is recalculated. The procedure is repeated until an $\mathbf{m}$ is obtained that meets both restrictions. While slightly improved estimates of $\mathbf{m}$ could be obtained by applying both restrictions in a simultaneous solution, the iterative procedure we chose is computationally much simpler and less expensive. When negative estimates of $\mathbf{m}$ were observed, the values were very small (usually <0.05), so the precise method of estimation appears to be unimportant.

Standard errors of $\mathbf{m}$, also obtained from SAS (SAS Institute, 1985) were used in two-tailed t tests of the null hypothesis H_0: $\mathbf{m} = 0$ for each of the reference populations. Interpretation of standard errors must consider that the statistical estimator assumes equal variances of allele frequencies among loci and zero variance in allele frequencies among populations of each reference taxon. Neither condition was rigidly met in this study, so the statistical tests leading from the standard errors were viewed conservatively.

Measures of within-population genetic variation, including the mean proportion of polymorphic loci (P) and the mean number of alleles per locus (A), were calculated for each population. Expected heterozygosity (H_e) and mean heterozygosity on a direct count basis (H_{dc}) were estimated from the

allele frequencies and individual genotypes, respectively (Swofford and Selander, 1981).

Individual variations within populations of *A. pavia, A. sylvatica,* and the hybrid zone between these taxa were examined using a composite character index similar to that of Sage and Selander (1979). Individuals were given a score based on the presence of alleles characteristic of each taxon. At a particular locus, one negative point was given for the presence of partially diagnostic *A. pavia* alleles (up to two points per locus) and positive points were given for *A. sylvatica* alleles. The character index was the sum of scores across all loci.

To provide a framework for the quantitative evaluation of patterns of character index scores within populations, we generated expected multilocus genotypes of parental, F_1, and backcross individuals using Monte Carlo computer simulations. Random mating was simulated by selecting gametes at random from the appropriate gene pools, here defined by the mean frequency of alleles in populations of *A. pavia* and *A. sylvatica* (dePamphilis, 1988; Appendix 1), and combining them to form diploid individuals representative of the parental allele frequencies. F_1s were generated by combining randomly chosen gametes from the two parental gene pools, and backcrosses were made by combining gametes from randomly selected F_1s and one of the parents. One thousand of each category were simulated and, for each individual, a character index score was calculated as above. An assumption of unlinked loci adopted for these simulations appears to be realistic based on the infrequency of segregation distortions in progeny of doubly heterozygous maternal parents (dePamphilis, 1988).

Results

Allele Distributions

Earlier studies of *A. pavia, A. sylvatica,* and *A. flava* outside the hybrid zone (dePamphilis, 1988) revealed substantial differences between these taxa in allele frequencies at a number of structural gene loci. Several of the largest of these differences form steep clines in allele frequency (Fig. 2) that are coincident through the parental species and the hybrid zone. Each of the three species differs considerably in at least one of the alleles illustrated, but *A. pavia* appears to be the most highly differentiated of the three. The hybrid zone, which includes primarily those populations in central and west Georgia (Fig. 1), occupies a position of steep changes in frequency for each of the alleles examined.

Of 14 loci examined, all but three appeared to be sufficiently differentiated to provide useful information for studies of interspecific hybridization. The amount and nature of the genetic differences varied substantially, however, among loci (Appendix 1). While no locus was fixed for alternative alleles in the taxa examined, two loci, *Shk2* and *Me2,* were nearly diagnostic. Frequencies of $Shk2^g$ averaged 0.87 in *A. pavia,* whereas frequencies of $Shk2^e$ averaged 0.90 or higher in *A. sylvatica* and *A. flava.* Because of additional alleles at this locus, actual overlap in allele frequencies was less than that suggested by the frequency of these two alleles alone. Several populations from the hybrid zone (14, 15, 16, 21, 22, 23) possessed substantial proportions of both alleles in frequencies not observed in the parental species. $Me2^c$, the common allele in *A. pavia* (mean frequency 0.88) was much less common in *A. sylvatica* and *A. flava,* where $Me2^d$ was the major allele. Populations 14, 15, 23, 27, and 36 from the hybrid zone all possessed relatively high frequencies of both alleles.

At *Aat3,* allele *h* is the major allele in most *A. pavia* populations (mean frequency = 0.52) with alleles *a, b,* and *e* typically present at a lower frequency (0.07, 0.27, and 0.13, respectively). In populations of *A. sylvatica* and *A. flava,* allele *e* predominates and allele *h* is uncommon, while alleles *a* and *b* are rare or unrecorded. An additional allele, $Aat3^c$, was recorded only in *A. sylvatica* at an average frequency of 0.07. Populations 16, 17, and 20 from the hybrid zone had combinations of these alleles expected from a mixing of these genomes.

At six other loci, certain alleles occurred in substantial frequency in populations of one or two taxa but were absent or nearly so in populations of the other taxa. This type of allele distribution, which we will call "partially diagnostic," was observed for *A. pavia* ($Px2^{b,c,e}$, $Pgi2^d$, $Pgm2^e$), *A. sylvatica* ($Acp1^b$, $Lap1^{d,e}$, $Me1^b$, $Px2^{c,e,g}$, $Pgm2^{e,b,c}$) and *A. flava* ($Lap1^{d,e}$, $Px2^{g,i}$, $Pgm2^{a,b}$). When considered jointly, these loci also suggested

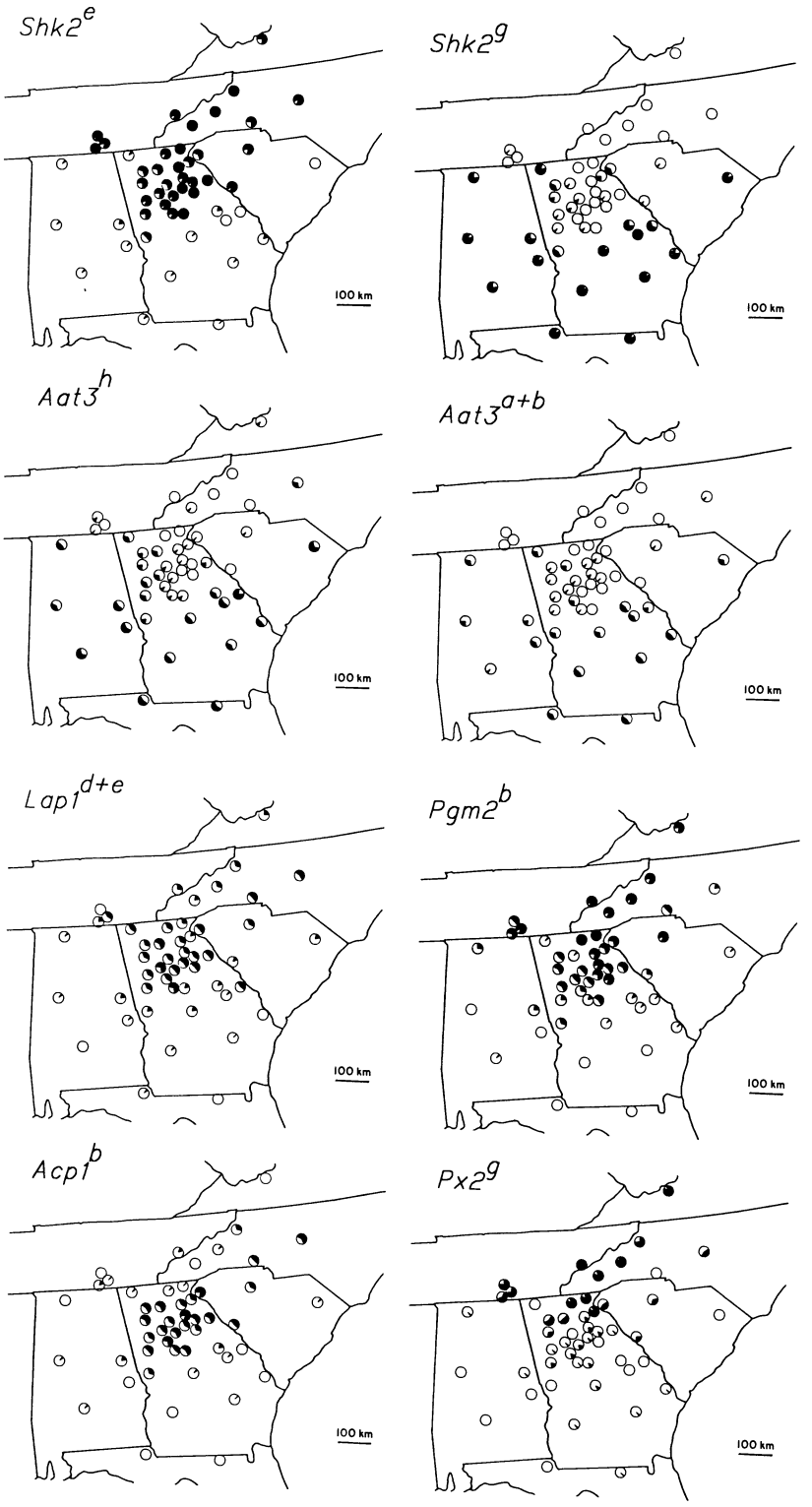

FIG. 2. Pie diagrams showing, in black, the frequency of eight partially diagnostic alleles in 50 populations of *Aesculus*. Populations are as given in Figure 1, except that values for *A. parviflora* (dePamphilis, 1988) are not included.

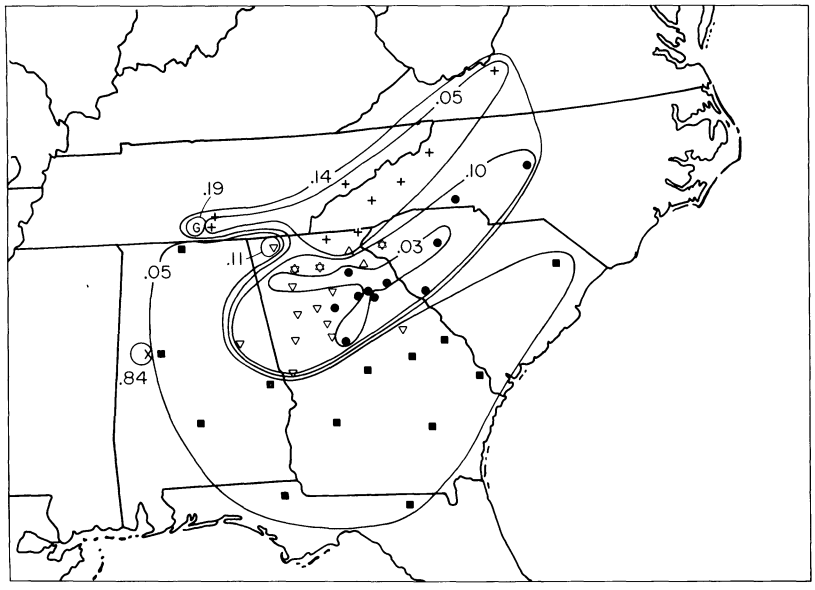

FIG. 3. Geographical distributions of major groupings from a UPGMA cluster analysis using Nei's (1978) unbiased coefficients of genetic distance (*D*). Clusters are enclosed by *D* values corresponding to the base of the cluster except for the single population of *A. glabra* and one hybrid population, for which *D* values are indicated. Population numbers are given in Figure 1.

a mixed background for many of the populations from the hybrid zone. For example, $Acp1^b$ is present in populations 14–23 at fairly high frequencies, suggesting some contribution of *A. sylvatica,* but populations 14, 17, 21, 22, and 24 also have an allele common only in *A. pavia,* $Pgi2^d$, at frequencies far above the mean of 0.01 observed in populations of *A. sylvatica.* Similar patterns were observed for allele combinations in populations 36–40, all of which appeared to be of mixed ancestry. Population 49 had all of the alleles that were common only in population 50 of *A. glabra* (Adh^h, $Me1^f$, $Pgi1^d$), and also a number of alleles that occurred in *A. flava* but were absent from population 50 ($Acp1^b$, $Lap1^{d,e}$, $Px2^i$, $Pgi2^a$). Most of these alleles found in *A. flava,* however, are also common in *A. sylvatica* and *A. pavia.*

A UPGMA cluster analysis (Swofford and Selander, 1981), based on Nei's (1978) unbiased genetic distance, *D,* was used to create a map showing the major clusters of genetic relatedness (Fig. 3). All of the populations of *A. pavia* plus two of those from the hybrid zone (14 and 24) form a cluster with a *D* of 0.05 or less. Similarly, all of the *A. flava* populations, one of the *A. flava* × *A. sylvatica* hybrids (37) and the single *A. flava* × *A. glabra* hybrid population (49) cluster with *A. flava.* Most of the populations in the hybrid zone, including most of the *A. sylvatica* × *A. pavia* hybrids and the putative triple hybrid swarms, clustered with the populations of *A. sylvatica.* These three major clusters coincide closely with the natural ranges of the three species: *A. pavia, A. sylvatica,* and *A. flava* (Fig. 1). In addition, *A. pavia* is seen to be relatively distinct from *A. sylvatica, A. flava,* and *A. glabra.* None of the suspected hybrid populations clustered with *A. glabra* or the genetically distinct *A. parviflora* (dePamphilis, 1988).

Genetic Admixtures

Estimates of gene admixture proportions were largely in agreement with expectations based on morphology, with a few notable exceptions (Table 1). Most of the populations outside the hybrid zone were estimated to be pure representatives of the species in question. Estimates for populations 33 and 45, however, included significant contributions from both *A. sylvatica* and *A. flava.* Reexamination of flowering

TABLE 1. Genetic admixture proportions (Roberts and Hiorns, 1965; Elston, 1971) for 48 populations of *A. pavia, A. sylvatica, A. flava,* and hybrids. Admixtures are based on mean allele frequencies of *A. pavia, A. sylvatica,* and *A. flava* as presented in Appendix 1. *P* values indicate the level of significance of the admixture proportion (see text).

Taxon and population[1]	Admixture proportion		
	A. pavia	*A. sylvatica*	*A. flava*
A. pavia			
1 P-FLO-SC	0.901****	0.098	0.000
2 P-EFF-GA	0.955****	0.045	0.000
3 P-APP-GA	1.000****	0.000	0.000
4 P-HAM-FL	1.000****	0.000	0.000
5 P-LEE-GA	1.000****	0.000	0.000
6 P-JAC-FL	1.000****	0.000	0.000
7 P-LOW-AL	0.977****	0.000	0.023
8 P-TUS-AL	0.988****	0.000	0.012
Border *A. pavia*			
9 P-BUR-GA	0.924****	0.060	0.015
10 P-JEF-GA	0.905****	0.094	0.000
11 P-TWI-GA	0.806***	0.027	0.167
12 P-LEE-AL	0.980****	0.000	0.020
13 P-LIM-AL	0.866***	0.000	0.134**
Hybrid *A. sylvatica* × *A. pavia*			
14 SP-GLA-GA	0.699****	0.301****	0.000
15 SP-GWI-GA	0.188****	0.761****	0.051
16 SP-DEK-GA	0.311****	0.676****	0.012
17 SP-HEN-GA	0.039	0.961****	0.000
18 SP-BUT-GA	0.166**	0.834****	0.000
19 SP-DOU-GA	0.077	0.923****	0.000
20 SP-COW-GA	0.457****	0.482****	0.060
21 SP-HAR-GA	0.559****	0.321****	0.120
22 SP-RAN-AL	0.789****	0.056	0.154*
23 SP-BAR-GA	0.159**	0.729****	0.112
24 SP-CAT-GA	0.646****	0.354***	0.000
Border *A. sylvatica*			
25 S-JAS-GA	0.000	1.000****	0.000
26 S-MON-GA	0.197*	0.546***	0.257*
27 S-OCO-GA	0.000	0.772****	0.228
30 S-JAC-GA	0.000	1.000****	0.000
A. sylvatica			
28 S-CLA1-GA	0.011	0.989****	0.000
29 S-CLA2-GA	0.000	0.785****	0.215
31 S-ELB-GA	0.000	1.000****	0.000
32 S-MCC-SC	0.013	0.987****	0.000
33 S-UNI-SC	0.004	0.686****	0.310****
34 S-RAN-NC	0.021	0.979****	0.000
35 S-GAS-NC	0.040	0.960****	0.000
A. sylvatica × *A. flava*			
36 SF-BAN-GA	0.164*	0.546****	0.290**
37 SF-HAB-GA	0.114	0.243*	0.643****
A. flava × (*A. sylvatica* × *A. pavia*)			
38 FSP-OCO-SC	0.119	0.713****	0.168
39 FSP-DAW-GA	0.191*	0.809****	0.000
40 FSP-GOR-GA	0.135	0.620****	0.246*
Border *A. flava*			
42 F-RAB-GA	0.000	0.000	1.000****
A. flava			
41 F-UNI-GA	0.000	0.000	1.000****
43 F-JAC-NC	0.045	0.000	0.954****

TABLE 1. Continued.

Taxon and population[1]	Admixture proportion		
	A. pavia	*A. sylvatica*	*A. flava*
44 F-BUN-NC	0.037	0.000	0.963***
45 F-WAT-NC	0.000	0.274****	0.726****
46 F-SEV-TN	0.000	0.067	0.933****
47 F-GIL-VA	0.000	0.000	1.000****
48 F-FRA-TN	0.021	0.000	0.979****

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.
[1] Identifications follow Hardin (1957*d*). Population codes are given as first letters of species–county–state.

specimens from these populations failed to detect morphological evidence of hybridization in either population. Several other populations at a considerable distance from *A. flava* (11, 21, 22, 23, 26, 27, 29) were also estimated to contain a fairly large proportion (>10%) of *A. flava* genes, but not all of these admixture coefficients were statistically significant. Three species populations bordering the hybrid zone (26, 36, 37) also had significant admixture estimates from unexpected sources. Again, these populations showed no morphological evidence of hybridization.

Most of the populations from the *A. sylvatica* × *A. pavia* hybrid zone combined significant proportions of genes from both species. Significant proportions of *A. pavia* genes ranged from 16 to 79%, while significant admixtures of *A. sylvatica,* seen in all but one population (22), varied from 30 to 96%, the latter representing basically pure *A. sylvatica.* Interestingly, not all of the populations judged from morphology to be triple hybrid swarms (38, 39, 40) were judged so on genetic grounds. While *A. sylvatica* was the primary genetic entity in all three populations, admixtures of *A. pavia* and *A. flava* >10% were observed in 38 and 40 only. However, inspection of allele frequencies for population 39 showed that the relatively unambiguous markers of *A. flava, Px2*^{g} and *Px2*^{h}, were also present in this population.

A separate analysis of gene admixture proportions was performed for population 49, using local populations of *A. flava* (48) and *A. glabra* (50), and the mean allele frequencies of *A. pavia* and *A. sylvatica* as the base populations. Estimates of gene admixture for population 49 were: population 48, 0.289 ($P < 0.0001$); population 50, 0.576 ($P < 0.0001$); *A. sylvatica,* 0.135 ($0.05 < P < 0.10$); and *A. pavia,* 0.000.

Variation within Populations

Mean numbers of alleles per locus (A) and the percentage of polymorphic loci (P) throughout the hybrid zone were high, but similar to populations outside the hybrid zone (Table 2). Measures of heterozygosity (H_e and H_{dc}), already quite high in the parental taxa, were even greater in the hybrid zone and in border populations of *A. pavia.* Most of the hybrid populations had $H_e > 0.30$ and H_{dc} was typically >0.25. Similar fixation indices (F) in hybrid populations (or lower in populations involving *A. flava*) were not statistically different from the corresponding parental species. Population 49, the *A. flava* × *A. glabra* hybrid population, had markedly higher H_e relative to nearby populations of *A. flava* and *A. glabra,* but H_{dc} was not as elevated, resulting in a much larger F in this population.

A. sylvatica × A. pavia *Hybrid Zone*

The character index scoring system used to examine individual variation in *A. pavia, A. sylvatica,* and the hybrid zone is summarized in Table 3. The maximum possible scores could range from −8 (for a plant with two diagnostic alleles of *A. pavia* at each of four loci) to +14 (for a plant with two diagnostic alleles of *A. sylvatica* at each of seven loci). A hybrid plant, which would have some alleles from both taxa, would receive an intermediate score.

A range of character index scores was observed for each of the simulated genetic categories, including parental *A. pavia* and *A. sylvatica,* F_1 hybrids, and backcross individuals (Table 4). These variable scores reflected in part the large amount of allelic

TABLE 2. Mean number of alleles per locus (A), percentage of loci polymorphic (P), heterozygosity measures (H), and fixation indices (F) for populations of *Aesculus*. Values for species means are from dePamphilis (1988). Population (POP) numbers and taxon abbreviations are given in Table 1.

Taxon	POP	A[1]	P	H_{dc}[2]	H_e[3]	F[4]
p	**Mean**	**2.5**	**80.4**	**0.187**	**0.228**	**0.180**
Border *p*	9	2.4	78.6	0.223	0.289	0.228
	10	2.6	100.0	0.159	0.232	0.315
	11	3.1	85.7	0.289	0.337	0.142
	12	2.1	71.4	0.195	0.217	0.101
	13	2.6	71.4	0.226	0.297	0.239
	Mean	**2.6**	**81.4**	**0.218**	**0.274**	**0.205**
s × *p*	14	2.5	78.6	0.294	0.355	0.172
	15	2.8	92.9	0.283	0.381	0.257
	16	3.1	92.9	0.301	0.389	0.226
	17	2.9	85.7	0.242	0.306	0.209
	18	3.1	100.0	0.198	0.273	0.275
	19	2.3	78.6	0.255	0.296	0.139
	20	2.6	85.7	0.279	0.318	0.123
	21	3.0	78.6	0.278	0.376	0.261
	22	2.6	78.6	0.304	0.349	0.129
	23	2.7	85.7	0.318	0.364	0.126
	24	2.5	64.3	0.219	0.255	0.141
	Mean	**2.7**	**83.8**	**0.270**	**0.333**	**0.187**
Border *s*	25	2.3	78.6	0.210	0.252	0.167
	26	2.7	78.6	0.278	0.342	0.187
	27	2.4	71.4	0.265	0.328	0.192
	30	2.4	85.7	0.248	0.288	0.139
	Mean	**2.4**	**78.6**	**0.250**	**0.302**	**0.171**
s	**Mean**	**2.6**	**83.7**	**0.263**	**0.307**	**0.144**
s × *f*	36	2.6	92.9	0.277	0.338	0.180
	37	2.4	78.6	0.264	0.318	0.170
	Mean	**2.5**	**85.8**	**0.270**	**0.328**	**0.175**
f × (*s* × *p*)	38	2.6	85.7	0.237	0.319	0.257
	39	2.8	78.6	0.288	0.306	0.059
	40	2.6	85.7	0.294	0.318	0.075
	Mean	**2.7**	**83.3**	**0.273**	**0.314**	**0.130**
Border *f*	42	2.2	85.7	0.152	0.190	0.200
f	**Mean**	**2.1**	**71.4**	**0.184**	**0.229**	**0.206**
f × *g*	49	2.6	85.7	0.222	0.319	0.304
g	50	2.1	71.4	0.229	0.258	0.112

[1] A locus is considered polymorphic if more than one allele was observed.
[2] Direct count.
[3] Hardy–Weinberg, unbiased estimate (Nei, 1978).
[4] $F = (H_e - H_{dc})/H_e$.

variability observed in these taxa, but also reflected the fact that few of the genetic markers of one taxon were totally absent from the other taxon (Appendix 1). Nevertheless, the distribution of scores for *A. pavia* (mean score = −4.90) and *A. sylvatica* (mean score = +8.37) were completely separated and the overlap between these classes and the simulated F_1s (mean score = +1.68) was restricted mainly to the tails of the score distributions. Scores for backcrosses overlapped considerably with the F_1s and their respective parents, but there were some classes of scores (−3 to −1 and +4 to +6) where backcrosses predominated. While there was too much overlap to distinguish each of the five categories, certain ranges of scores were still informative. Plants receiving a score from 0 to +3 were very likely to be hybrids (98.6%), even though it was not possible to distinguish F_1s from backcrosses. Scores of −8, −7, and −6, and +9 to +13 had a 90% or better chance of being *A. pavia* and *A. sylvatica,* respectively. Our

TABLE 3. Summary of scoring procedure for the calculation of individual character-index scores.

Locus	*A. pavia*		*A. sylvatica*	
	Allele	Maximum possible score	Allele	Maximum possible score
Aat3	*a, b, f, h* (−1)	−2	*c, e* (+1)	+2
Acp1	—	0	*b* (+1)	+2
Lap1	—	0	*d, e* (+1)	+2
Me1	—	0	*b* (+1)	+2
Me2	*c* (−1)	−2	*d* (+1)	+2
Pgi2	*d, e, g* (−1)	−2	—	0
Pgm2	—	0	*a, b, c* (+1)	+2
Shk2	*c, g, i* (−1)	−2	*a, e, j* (+1)	+2
Total		−8		+14

analysis of individual variation in natural populations concentrated on these three major categories.

The distributions of character index scores for populations of *A. pavia* and *A. sylvatica* outside the hybrid zone (Table 5) were very close to those expected for random progeny from these taxa (Table 4). A single *A. sylvatica* individual with a score of +2 probably was not a hybrid, because the simulation study suggested that about 1% or 1.4 *A. sylvatica* individuals in a sample of this size will score +2 in the absence of hybridization. This individual also lacked any of the diagnostic alleles of *A. pavia,* further suggesting that this plant was an *A. sylvatica* with an unusual genotype, but not a hybrid. In contrast, a low level of gene flow from *A. sylvatica* into one or more of the *A. pavia* populations is indicated by the character index scores. Four individuals with scores of 0 to +2 were observed in the first eight populations. Approximately 1.8 individuals (0.8% of 226) are expected in the 0 to +1 category, but a score of +2, received by one plant each in populations 7 and 8, was not expected in the absence of hybridization. Inspection of the genotypic arrays of these individuals showed that each did indeed possess several of the *A. sylvatica* marker alleles and, thus, they were probably hybrids.

Index scores for populations of *A. sylvatica* and *A. pavia* bordering the zone of hybridization resembled those of the respective taxon outside the zone, except for a larger number of individuals classified in the hybrid categories. Fifteen of the *A. pavia* border plants (11.7% of total) and four of the *A. sylvatica* border plants (4% of total) were classified as hybrids, and many of these individuals possessed alleles diagnostic for both species. However, we note that the border *A. pavia* all occur in close proximity to *A. sylvatica* (Fig. 1), while the "border" *A. sylvatica* populations are well up into the Piedmont because of the great width of the hybrid zone within the range of *A. sylvatica.* A large range of character index scores was observed for populations from the hybrid zone, as expected from the admixture estimates. Mean scores ranged from −2.50 (population 22) to +7.53 (population 17), a value close to that expected of pure *A. sylvatica.* A total of 66 of 253 plants in this region (26%) were unambiguous hybrids, but because about 80% of the plants in the flanking categories (−3 to −1 and +4 to +6) are probably F_1s or backcrosses (Table 4), the true proportion of hybrids is likely to be much higher.

In every population where hybrids were detected, one or both of the parents was missing. Near the edge of the hybrid zone, three populations (14, 21, and 22) consisted of *A. pavia* individuals and hybrids, but no *A. sylvatica* individuals were observed. Five other populations (15, 17, 18, 19, 23) included *A. sylvatica* and hybrids but no individuals of *A. pavia.* A similar situation was observed in populations outside the hybrid zone, where probable hybrids were always detected in the absence of the other putative parental taxon. Within the hybrid zone, three populations (16, 20, 24) had no individuals classified as either parent. These populations consisted entirely of plants with intermediate scores, and most plants had

TABLE 4. Character index scores for simulated *A. pavia* (*p*), *A. sylvatica* (*s*), F_1, and backcross individuals. The simulation was based on the allele frequencies for *A. pavia* and *A. sylvatica* given in Appendix 1. Sample size was 1,000 for each simulation category.

Simulation category	Character index score																					Mean score
	−8	−7	−6	−5	−4	−3	−2	−1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	
p	21	114	306	170	218	76	67	20	6	2													−4.90
Back-*p*	1	8	36	68	105	145	165	178	126	70	61	20	12	4	1								−1.66
F_1					3	11	37	57	151	202	225	161	92	45	12	4							+1.65
Back-*s*							3	10	16	39	77	101	147	162	159	120	87	51	17	6	4	1	+5.12
s											3	7	24	47	80	130	220	193	169	100	19	8	+8.37

the marker alleles of both *A. pavia* and *A. sylvatica.*

The genetic effect of hybridization, as indicated by changes in population mean character index scores, is primarily seen in an area from about 25 km below to about 150 km above the Fall Line (Fig. 4). The Fall Line is the physical boundary between the Coastal Plain and Piedmont physiographic provinces, where the elevation changes rapidly from about 75 m to about 250 m above sea level. It forms the approximate border between morphologically pure *A. pavia* and *A. sylvatica.* As the Fall Line is approached from the Coastal Plain and within the range of *A. pavia,* little change in the mean character index is seen until just before the Fall Line, at about −25 km. Above this, a highly variable situation is observed, reflecting the fact that populations a given distance from the Fall Line may be relatively pure *A. sylvatica* or may include obvious hybrids (Table 5). Although a few hybrid plants are observed in certain populations of *A. pavia,* almost all of the hybrids are found in the Piedmont with *A. sylvatica.*

DISCUSSION

Shape and Structure of the Hybrid Zone

The complex hybrid zone in *Aesculus* is characterized by coincident clines in allele frequency in an area of major morphological transition and increased meiotic abnormalities (Hardin, 1957*d*). In this paper, we have shown that populations considered on morphological grounds to be of hybrid origin do indeed possess combinations of alleles characteristic of two or more parents and that these combinations can be used to estimate quantitatively the genetic contribution of the possible parental taxa. Although the distributions of the parental species are nearly parapatric, a very broad (>200 km) hybrid zone involving *A. pavia, A. sylvatica,* and *A. flava* extends far beyond the range of *A. pavia* and *A. flava* to include most of the range of *A. sylvatica* in northern Georgia. In addition, a detailed analysis of genotypic variation within *A. pavia, A. sylvatica,* and the broad hybrid zone between them has allowed us to demonstrate that unambiguous hybrids are common in the

TABLE 5. Character index profiles for 35 populations of *A. pavia* (*p*), *A. sylvatica* (*s*), and hybrids. Probable identifications of individuals with certain character index scores are based on results of the computer simulations summarized in Table 5. The approximate least significant difference between two means ($\alpha = 0.05$) is 1.72 index units.

Taxon and population			Character index scores and probable identifications[1]										
			A. pavia			Hybrids				*A. sylvatica*			Mean character index
		N	(−)8–6	5–4	3–1	0–1	2–3	4–6	7–8	9–10	11–12	13–14(+)	
p	1	26	7	8	11								−4.00
	2	20	11	6	2	1							−4.95
	3	35	25	10									−5.86
	4	39	27	4	8								−5.10
	5	29	18	8	3								−5.41
	6	26	14	10	2								−5.42
	7	18	5	8	4		1						−4.33
	8	33	13	12	6	1	1						−4.91
Border *p*	9	24	5	8	10		1						−3.54
	10	23	2	4	13	4							−2.44
	11	32	3	9	11	7	2						−2.22
	12	26	18	7	1								−5.69
	13	23	6	10	6	1							−4.13
s × *p*	14	27	3	4	9	6	3	2					−1.41
	15	35			2	3	4	12	9	4	1		+5.26
	16	12		1	2	1	3	3	2				+2.08
	17	17						6	6	3	2		+7.53
	18	28					2	10	9	6	1		+7.07
	19	15				1		7	5	2			+5.93
	20	26			3	6	11	6					+2.04
	21	17	1	3	2	2	2	6	1				+1.35
	22	24	3	6	11	3	1						−2.50
	23	25				3	1	11	5	4	1		+5.92
	24	27			12	9	5	1					−0.07
Border *s*	25	27						3	7	15	2		+8.70
	26	17				2	1	10	1	3			+5.12
	27	18						5	6	6	1		+7.78
	30	27					1	5	7	7	7		+8.37
s	28	29						4	12	11	2		+8.21
	29	7							5	2			+7.86
	31	20						2	8	6	4		+8.75
	32	15						2	2	10	1		+8.73
	33	11					1	2	4	3		1	+7.64
	34	32						4	11	14	3		+8.53
	35	25						8	7	7	2	1	+7.76

[1] Probability of misidentification is discussed in the text.

morphological hybrid zone and to an extent, beyond it. From this analysis, we were able to gain novel insights into the detailed genetic structure of the hybrid populations.

Recent efforts to develop population genetic models of hybrid zones have generally considered the width of a zone to reflect a dynamic equilibrium between selection against hybrids and dispersal by parentals into the zone (Barton and Hewitt, 1985). Nevertheless, as these authors point out, very broad hybrid zones present a challenge to these models. Very long-distance dispersal is necessary to generate broad hybrid zones even when selection is quite weak. An alternative mechanism, proposed to explain a broad hybrid zone in gophers (*Thomomys*) is that selection favors hybrids and thus promotes expansion of the zone (Barton and Hewitt, 1985). It is likely that such selection will favor relatively few genotypes, resulting in unequal clines through the zone. In contrast, broad clines maintained primarily by dispersal should be coincident across loci (Barton and Hewitt, 1985). In *Aesculus,* the shapes and widths of the individual allele clines are similar to the overall cline seen in Figure 4 (data not shown).

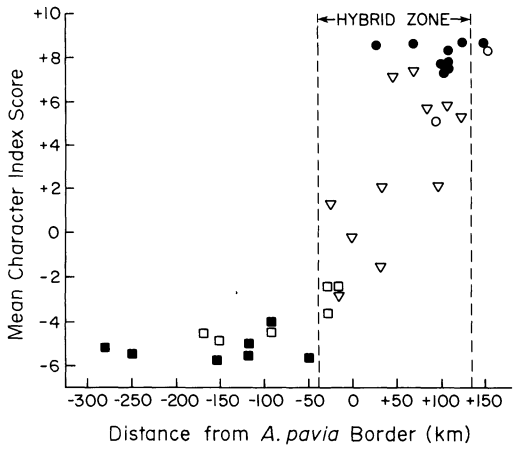

FIG. 4. Variation in mean character index scores for populations of *Aesculus*, showing asymmetry of the hybrid zone between *A. pavia* and *A. sylvatica*. The border between the distribution of *A. pavia* and *A. sylvatica* (Fig. 1) corresponds generally to the Fall Line, which marks the boundary between the Piedmont and Coastal Plain provinces and is indicated here by 0 km. (■) *A. pavia*; (□) *A. pavia* with hybrids; (△) hybrid zone populations; (○) *A. sylvatica* with hybrids; (●) *A. sylvatica*. Approximate width of the zone is indicated by broken vertical lines.

This suggests that dispersal, presumably across distances of many kilometers, maintains the broad hybrid zone observed in *Aesculus*.

The portion of the hybrid zone involving *A. pavia* and *A. sylvatica* is also strongly asymmetrical: most of the hybrids are found in populations within the natural range of *A. sylvatica*, but outside the range of *A. pavia*. Such asymmetries in hybrid zones are common (Hunt and Selander, 1973; Millar, 1983; Barton and Hewitt, 1985). Two factors that can generate asymmetrical zones are (1) differing selection regimes that alter the chances of success for hybrids in parental populations or (2) differences in the ability of two species to disperse their genes over long distances (Nagylaki, 1976). We have no evidence bearing on the first possibility, which could be addressed by reciprocal transplant experiments involving seedlings from hybrid and both parental populations. The second possibility, differential gene dispersal, appears very likely to contribute to the observed asymmetry. *Aesculus pavia, A. sylvatica,* and their hybrids are pollinated in part by ruby-throated hummingbirds (*Archilochus colubris*). This bird migrates from the Coastal Plain through the Piedmont in the early spring (Bertin, 1982), when both *A. pavia* and *A. sylvatica* are flowering, and may carry viable pollen from *A. pavia* north to *A. sylvatica* at that time. Pollen movement by migratory hummingbirds, which may explain the asymmetry of the hybrid zone, could also account for the large dispersal distances expected to maintain such a broad zone. Evidence in support of this hypothesis is detailed elsewhere (dePamphilis and Wyatt, 1989).

We suggest that plant species that can disperse viable pollen over great distances, albeit rarely, may form hybrid zones that are qualitatively different from those observed in animals and plants that disperse pollen only over short distances. Hybridization in the latter necessarily requires sympatry of both parental taxa at some time; hybrid swarms would typically consist of both parents and various hybrid offspring. The absence of one parent would imply its loss from the population. In a plant population where the initial event of hybridization results from pollen transfer from a distant population, the genetic structure of the population should consist of one parent, F_1s, and whatever later generation hybrids and backcrosses may result. The absence of the second parent from the hybrid population does not imply its loss because, strictly speaking, it was never present. Precisely this type of mechanism may help to explain the unique structure of the hybrid zone between *A. sylvatica* and *A. pavia,* in which one or both parental species was absent from virtually every population in which hybrids were detected. The absence of one of the parentals from hybrid populations is most uncommon in animals (Barton and Hewitt, 1985), but has been suggested for several plants based on morphological (Stebbins, 1950; Grant, 1981), biochemical (Flake et al., 1978), and genetic evidence (Wheeler and Guries, 1987). There are too few detailed analyses of hybrid zones in plants to determine if this is a characteristic difference between plant and animal hybrid zones.

The selective loss of parental genotypes from at least some populations in the hybrid zone is suggested by the presence of three hybrid populations that appeared to lack

individuals of either parental species. The existence of "stabilized" hybrid populations persisting in the absence of parental species has been suggested for a number of plant taxa (Stebbins, 1950; Heiser, 1973), including *Aesculus* (Hardin, 1957*d*). Given the limited sample sizes within each population, it is not possible to say that parental individuals are absent, but it is clear that the populations are predominantly hybrids.

Genetic Admixtures and Introgressive Gene Flow

Admixture proportions for each of the study populations provided a way of estimating the relative number of genes contributed by each of the possible parental taxa and the approximate statistical significance of each contribution. As such, they can be an appropriate means to examine suspected hybridization involving two, three, or more parental taxa, when each of these is genetically divergent. A strength of this approach is that information from many loci, even ones for which the parental taxa differ only in allele frequency, may be used in the analysis. Genetic admixtures in *Aesculus* confirmed initial expectations that the hybrid zone is complex and that it involves the mixture of *A. pavia, A. sylvatica,* and *A. flava.* Curiously, several populations were estimated to include significant proportions of *A. flava* genes, even when there was no morphological evidence to suggest involvement of that species. This observation could mean that gene flow from *A. flava,* perhaps in the form of introgression, is actually greater than morphological evidence would suggest. However, we note that *A. flava* and *A. sylvatica* are very similar allozymically: the two taxa share a high genetic identity (dePamphilis, 1988), and almost all of the major alleles of *A. flava* are also present in *A. sylvatica* (Appendix 1). The relatively few unambiguous markers of *A. flava* are not always present in populations with unexpected admixtures with this species (e.g., populations 13, 33). Therefore, it is also possible that some of the significant estimates of *A. flava* in the absence of morphological evidence are due to the difficulty inherent in distinguishing between these two similar genomes. Stronger evidence of introgressive gene flow from *A. sylvatica* into *A. flava* is seen in population 45, where in addition to a significant admixture of *A. sylvatica* genes in the absence of morphological hybrids, this population does have some of the allelic markers of *A. sylvatica* and also the greatest heterozygosity ($H_{dc} = 0.239$) among the *A. flava* populations sampled (dePamphilis, 1988).

A population with the morphological characteristics of *A. flava* × *A. glabra* (Hardin, 1957*d*; dePamphilis and Wyatt, 1989), population 49, also had a few plants with light red flowers, suggesting the possibility of introgression from *A. pavia* (Hardin, 1957*d*). This population had a number of the alleles found in local samples of *A. glabra* (population 50) and *A. flava* (population 48), but the possible markers of *A. flava* were ambiguous because they represented alleles also shared by *A. pavia* and/or *A. sylvatica.* Gene admixture analysis suggested that *A. flava, A. glabra,* and possibly *A. sylvatica* were represented, but no contribution of *A. pavia* was found. This is consistent with the qualitative observation that all of the unique markers of *A. pavia* were missing from this population (Appendix 1), and suggests that the reddish flower color sometimes seen in populations of *A. sylvatica, A. flava,* and *A. glabra,* is not sufficient evidence of introgression from *A. pavia.*

A critical assumption of our admixture studies, and indeed of all attempts to calculate admixture proportions, is that the allele frequencies of the parental taxa (base populations) are known without error. Lack of absolute knowledge (as is always the case in natural hybridization) will generate errors in the estimates insofar as the true parental taxa and the hybrid populations have diverged since the time of hybridization. Similar arguments can be made concerning attempts to simulate the genetic products of hybridization, as we have also done in this paper. In our case, the goodness of fit of the least squares models was generally quite high ($r^2 > 0.9$). In addition, an examination of the total allelic distribution in hybrids relative to the parents (Appendix 1) revealed few alleles unique to the hybrid zone. While these observations do not provide any strict validation of the use of extant population structures for these analyses, they suggest

that this source of error did not strongly influence the results.

Another potential source of error in attempting to infer the parents of a hybrid population is heterogeneity in allele frequencies among subpopulations of the parental species. If such heterogeneity has a distinctive geographical component and the hybrids more closely resemble a subset of the putative parents, it can identify more precisely the correct parental population system (e.g., Werth et al., 1985). In the case of *Aesculus,* allele frequencies do vary significantly among populations of *A. pavia, A. sylvatica,* and *A. flava* from outside the hybrid zone, but the variation appears to be mostly random (dePamphilis and Wyatt, 1989). Such heterogeneity increases the error variance of an admixture estimate above that from the fit of the model alone (Krieger et al., 1965). We attempted to examine the influence of such heterogeneity by eliminating highly heterogeneous enzyme systems (*Me1, Pgm2*) from the analysis one at a time and recalculating the admixtures. We found small differences in the final solutions, but no systematic improvements in terms of goodness of fit of the models.

Genetic Variation

Significantly greater values of H_e were observed both in the hybrid zone and in border populations lacking morphological evidence of hybridization. These relatively modest increases in heterozygosity were similar to those observed in hybrid populations of *Pinus* (Wheeler and Guries, 1987), but were much smaller than the 7- to 25-fold increases seen in hybrid populations of *Phlox* (Levin, 1975). In *Aesculus,* the parental species are highly polymorphic, far more so than *Pinus* and *Phlox,* with average H_e values of 0.11 and 0.05, respectively. Hybridization in *Aesculus* appears to increase heterozygosity at already polymorphic loci, rather than to create new polymorphisms.

Slightly greater heterozygote deficiencies in some hybrid populations could have resulted from incomplete mixing of partially incompatible genomes. However, there is no evidence from our results that this is the case, because populations with the largest *Fs* (15, 18, 21) do not appear to have different patterns of character index scores from those with small *Fs* (19, 20, 22, 23, 24). Intensive sampling within populations would allow studies of linkage disequilibrium, to examine the nature of associations among loci (Barton and Hewitt, 1985). This might provide insight into the large *Fs* observed in some hybrid populations and could also help to interpret the role of natural selection in maintaining the very wide hybrid zone observed in this group (Barton and Hewitt, 1985).

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LITERATURE CITED

ANDERSON, E. 1949. Introgressive Hybridization. Wiley, N.Y.

———. 1953. Introgressive hybridization. Biol. Rev. Cambridge Philos. Soc. 28:280–307.

ANDERSON, E., AND G. L. STEBBINS. 1954. Hybridization as an evolutionary stimulus. Evolution 8:378–388.

BARBER, H. N. 1970. Hybridization and the evolution of plants. Taxon 19:154–160.

BARTON, N. H., AND G. M. HEWITT. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113–148.

———. 1989. Adaptation, speciation, and hybrid zones. Nature (London) 341:497–503.

BERTIN, R. I. 1982. The Ruby-throated Hummingbird and its major food plants: Ranges, flowering phenology, and migration. Can. J. Zool. 60:210–219.

BLOOM, W. L. 1976. Multivariate analysis of the introgressive replacement of *Clarkia nitens* by *Clarkia speciosa polyantha* (Onagraceae). Evolution 30: 412–424.

DEPAMPHILIS, C. W. 1988. Hybridization in woody plants: Population genetics and reproductive biology in Southeastern buckeyes (*Aesculus*: Hippocastanaceae). Ph.D. Diss. Univ. Georgia, Athens.

DEPAMPHILIS, C. W., AND R. WYATT. 1989. Hybridization and introgression in buckeyes (*Aesculus*: Hippocastanaceae): A review of the evidence and

a hypothesis to explain long-distance gene flow. Syst. Bot. 14:593–611.

ELSTON, R. C. 1971. The estimation of admixture in racial hybrids. Ann. Human Genet. (London) 35: 9–17.

FLAKE, R. H., L. URBATSCH, AND B. L. TURNER. 1978. Chemical documentation of allopatric introgression in *Juniperus*. Syst. Bot. 3:129–144.

GIANNASI, D. E., AND D. J. CRAWFORD. 1986. Biochemical systematics II. A reprise. Evol. Biol. 20: 25–248.

GRANT, V. 1981. Plant Speciation, 2nd ed. Columbia Univ. Press, N.Y.

HARDIN, J. W. 1957*a*. A revision of the American Hippocastanaceae. Brittonia 9:145–171.

———. 1957*b*. A revision of the American Hippocastanaceae. II. Systematic treatment. Brittonia 9: 173–195.

———. 1957*c*. Studies in the Hippocastanaceae. III. A hybrid swarm in the buckeyes. Rhodora 59:45–51.

———. 1957*d*. Studies in the Hippocastanaceae. IV. Hybridization in *Aesculus*. Rhodora 59:185–203.

———. 1975. Hybridization and introgression in *Quercus alba*. J. Arnold Arbor. 56:336–363.

HEISER, C. B. 1973. Introgression re-examined. Bot. Rev. (Lancaster) 39:347–366.

HEWITT, G. M. 1988. Hybrid zones—Natural laboratories for evolutionary studies. Trends Ecol. Evol. 3:158–167.

HEYWOOD, J. S. 1986. Clinical variation associated with edaphic ecotones in hybrid populations of *Gaillardia pulchella*. Evolution 40:1132–1140.

HUNT, W. G., AND R. K. SELANDER. 1973. Biochemical genetics of hybridisation in European house mice. Heredity 31:11–33.

KNOBLOCH, I. W. 1972. Intergeneric hybridization in flowering plants. Taxon 21:97–103.

KRIEGER, H., N. E. MORTON, M. P. MI, E. AZEVÊDO, A. FREIRE-MAIA, AND N. YASUDA. 1965. Racial admixture in north-eastern Brazil. Ann. Human Genet. 29:113–125.

LEVIN, D. A. 1975. Interspecific hybridization, heterozygosity and gene exchange in *Phlox*. Evolution 29:37–51.

———. 1979. Editor's comments. *In* Hybridization, an Evolutionary Perspective. Dowden, Hutchinson & Ross, Stroudsburg, PA.

LEWONTIN, R. C., AND L. C. BIRCH. 1966. Hybridization as a source of variation for adaptation to new environments. Evolution 20:315–336.

MILLAR, C. V. 1983. A steep cline in *Pinus muriata*. Evolution 37:311–319.

NAGYLAKI, T. 1976. Clines with variable migration. Genetics 83:867–886.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

ROBERTS, D. F., AND R. W. HIORNS. 1965. Methods of analysis of the genetic composition of a hybrid population. Human Biol. 37:38–43.

SAGE, R. D., AND R. K. SELANDER. 1979. Hybridization between species of the *Rana pipiens* complex in central Texas. Evolution 33:1069–1088.

SAS INSTITUTE INC. 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc., Cary, NC.

SCHOEN, D. J., AND D. FRUCHTER. 1983. A calculator-assisted method of random sampling. Ecology 64: 205–206.

SCHWARTZ, D., AND W. J. LAUGHNER. 1969. A molecular basis for heterosis. Science 166:626–627.

STEBBINS, G. L. 1950. Variation and Evolution in Plants. Columbia Univ. Press, N.Y.

———. 1959. The role of hybridization in evolution. Proc. Am. Philos. Soc. 103:231–251.

———. 1969. The significance of hybridization for plant taxonomy and evolution. Taxon 18:26–35.

STRAW, R. M. 1955. Hybridization, homogamy, and sympatric species. Evolution 9:441–444.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. Biosys-1: A FORTRAN computer program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72: 281–283.

WERTH, C. R., S. I. GUTTMAN, AND W. H. ESHBAUGH. 1985. Electrophoretic evidence of reticulate evolution in the Appalachian *Asplenium* complex. Syst. Bot. 10:184–192.

WHEELER, N. C., AND R. P. GURIES. 1987. A quantitative measure of introgression between lodgepole and jack pines. Can. J. Bot. 65:1876–1885.

WYATT, R. M. 1985. *Aesculus parviflora* in South Carolina: Phytogeographical implications. Bull. Torrey Bot. Club 112:194–195.

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APPENDIX 1

Allele frequencies and sample sizes (*N*) for 14 loci in 27 populations of *Aesculus*. Abbreviations are first letter of specific epithet.

Locus	Allele	Species marker	PAVIA[1]	Border *A. pavia*					*A. sylvatica* × *A. pavia* hybrid zone								
				9	10	11	12	13	14	15	16	17	18	19	20	21	22
Aat2	(*N*)			*28*	*32*	*36*	*26*	*20*	*30*	*40*	*28*	*25*	*38*	*21*	*27*	*48*	*26*
	−*b*		.02	.09	.03	.08				.01	.02	.02	.01		.02		
	−*c*		.98	.91	.97	.89	1.00	1.00	1.00	.99	.98	.98	.97	1.00	.98	1.00	1.00
	−*d*					.03							.01				
Aat3	(*N*)			*28*	*32*	*37*	*30*	*32*	*30*	*43*	*29*	*28*	*39*	*28*	*27*	*47*	*30*
	−*a*	P	.07	.02	.09	.11	.20	.17	.25			.21			.02	.09	.13
	−*b*	(P)	.27	.18	.25	.15	.13	.13	.18	.06	.05	.04	.04	.05	.06	.20	.07
	−*c*	S								.01	.16	.04	.10		.02		
	−*d*																
	−*e*	S, F	.13	.04	.13	.31	.07	.27	.18	.81	.50	.61	.72	.59	.59	.50	.28
	−*f*	(P)	.01								.02						
	−*g*											.02				.02	
	−*h*	P	.52	.77	.53	.43	.60	.44	.38	.12	.28	.09	.12	.36	.32	.19	.52
	−*j*												.03				
Acp1	(*N*)			*28*	*32*	*37*	*29*	*31*	*30*	*43*	*41*	*28*	*35*	*23*	*27*	*43*	*29*
	−*a*																
	−*b*	S	.02		.03	.11			.17	.41	.45	.70	.46	.39	.50	.30	.19
	−*c*		.03	.20			.12	.05	.03	.01							
	−*d*		.95	.75	.94	.89	.88	.94	.75	.58	.55	.30	.54	.61	.50	.69	.78
	−*f*		.01	.05	.03			.02	.05							.01	.03
Acp2	(*N*)			*28*	*32*	*37*	*30*	*24*	*30*	*43*	*28*	*28*	*38*	*22*	*27*	*48*	*29*
	−*a*	(S)															
	−*b*		.01		.06			.02					.01				
	−*d*		.98	1.00	.94	1.00	1.00	.98	1.00	.94	.96	.98	.99	1.00	1.00	1.00	1.00
	−*e*		.01								.04	.02					
	−*f*	(S)															
	−*g*									.06							
Adh1	(*N*)			*26*	*32*	*37*	*30*	*29*	*29*	*43*	*44*	*27*	*35*	*24*	*27*	*47*	*29*
	−*b*		.01														
	−*c*																
	−*d*		.16	.10	.08	.42	.10	.24	.50	.31	.41	.50	.10	.31	.17	.14	.16
	−*e*				.02	.01											.02
	−*f*		.83	.90	.91	.57	.90	.76	.50	.69	.59	.50	.90	.69	.83	.86	.83
	−*g*																
	−*h*																
	−*i*																

APPENDIX 1.

Extended.

		A. sylvatica × *A. pavia* hybrid zone		Border *A. sylvatica*					Border								
									s × *f*		*f* × (*s* × *p*)			*f*		*f* × *g*	*g*[3]
Locus	Allele	23	24	25	26	27	30	SYLVA[2]	36	37	38	39	40	42	FLAVA[2]	49	50
Aat2	(*N*)	*27*	*34*	*28*	*35*	*21*	*30*		*34*	*9*	*20*	*28*	*25*	*27*		*10*	*19*
	−*b*	.02		.05					.03						.02		.26
	−*c*	.98	1.00	.95	1.00	.98	.93	.99	.97	1.00	1.00	1.00	.96	1.00	.98	1.00	.74
	−*d*					.02	.07	.01					.04				
Aat3	(*N*)	*27*	*34*	*28*	*35*	*21*	*30*		*33*	*11*	*32*	*28*	*28*	*34*		*27*	*28*
	−*a*		.21							.05							
	−*b*	.04	.09				.10	.02	.06	.05		.23	.05				
	−*c*	.19		.02				.07		.05	.03	.02					
	−*d*						.02										
	−*e*	.56	.38	.88	.91	1.00	.85	.84	.80	.82	.91	.52	.75	.99	.98	.96	.89
	−*f*																
	−*g*				.01				.08		.02			.02			
	−*h*	.22	.32	.11	.07		.03	.07	.06	.05	.05	.23	.20		.02	.04	.11
	−*j*																
Acp1	(*N*)	*27*	*34*	*28*	*35*	*21*	*28*		*34*	*9*	*37*	*28*	*26*	*33*		*25*	*28*
	−*a*		.02														
	−*b*	.63	.04	.57	.60	.41	.84	.49	.40	.33	.78	.63	.58	.02	.08	.14	
	−*c*				.01			.01		.11					.04		.04
	−*d*	.37	.94	.43	.39	.60	.16	.50	.60	.56	.22	.38	.42	.99	.87	.86	.96
	−*f*							.01									
Acp2	(*N*)	*27*	*33*	*28*	*35*	*21*	*28*		*18*	*8*	*31*	*28*	*27*	*33*		*27*	*27*
	−*a*							.01				.02					
	−*b*		.03											.06		.54	
	−*d*	1.00	.97	.91	1.00	1.00	1.00	.96	.97	1.00	1.00	.98	1.00	.94	1.00	.43	1.00
	−*e*			.09				.03	.03							.04	
	−*f*							.01									
	−*g*																
Adh1	(*N*)	*27*	*29*	*28*	*34*	*21*	*27*		*32*	*8*	*35*	*25*	*27*	*34*		*26*	*28*
	−*b*														.01		
	−*c*							.01				.14					
	−*d*	.32	.28	.20	.29	.48	.39	.34	.36	.38	.14	.14	.22	.24	.25	.27	.25
	−*e*		.05			.05										.02	
	−*f*	.69	.67	.80	.71	.48	.61	.64	.64	.63	.86	.72	.78	.77	.74	.65	.66
	−*g*															.02	
	−*h*							.01								.04	.09
	−*i*							.01									

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Allele frequencies and sample sizes (*N*) for 14 loci in 27 populations of *Aesculus*. Abbreviations are first letter of specific epithet.

Locus	Allele	Species marker	PAVIA[1]	Border *A. pavia*					*A. sylvatica* × *A. pavia* hybrid zone								
				9	10	11	12	13	14	15	16	17	18	19	20	21	22
Lap1	(*N*)			*28*	*32*	*37*	*28*	*28*	*29*	*43*	*42*	*28*	*38*	*23*	*27*	*47*	*28*
	−*a*					.01											
	−*b*		.38	.16	.50	.27	.18	.50	.24	.08	.06	.14	.07	.07	.11	.39	.55
	−*c*		.58	.39	.44	.54	.79	.43	.62	.52	.24	.43	.22	.57	.48	.40	.27
	−*d*	S, F	.04	.34	.03	.18	.04	.04	.14	.20	.42	.14	.34	.11	.22	.06	.05
	−*e*	S, F	.01	.11	.03			.04		.20	.29	.27	.36	.26	.19	.14	.13
	−*g*											.02	.01				
Me1	(*N*)			*28*	*32*	*37*	*30*	*32*	*30*	*43*	*29*	*28*	*39*	*26*	*27*	*46*	*30*
	−*b*	S	.01			.04	.02	.03	.20	.15	.03	.04	.13	.27	.02	.11	
	−*d*		.95	1.00	.98	.92	.98	.97	.80	.85	.97	.96	.86	.73	.98	.89	.97
	−*f*		.04		.02	.04							.01				.03
	−*h*																
Me2	(*N*)			*26*	*32*	*35*	*30*	*32*	*29*	*42*	*14*	*23*	*36*	*28*	*27*	*43*	*30*
	−*c*	P	.88	.81	.42	.51	1.00	1.00	.53	.42	.57		.11		.98	.80	.80
	−*d*	S, F	.12	.15	.58	.49			.47	.58	.18	1.00	.89	1.00	.02	.20	.20
	−*e*			.04							.25						
Px2	(*N*)			*28*	*32*	*37*	*28*	*27*	*30*	*39*	*42*	*23*	*37*	*20*	*26*	*33*	*28*
	−*a*					.11											
	−*b*		.05			.16		.13		.09	.12	.02		.10		.08	.07
	−*c*	P, S	.21	.48	.11	.42	.63	.19	.18	.26	.26	.15	.22	.45	.19	.35	.30
	−*d*																
	−*e*	P, S	.72	.52	.89	.19	.38	.59	.72	.65	.57	.67	.76	.40	.62	.55	.61
	−*g*	F, S	.01			.10		.04			.05	.15	.03	.05	.19		.02
	−*i*	F	.01			.03		.06	.10							.03	
	−*j*	(F)															
Pgi1	(*N*)			*28*	*32*	*37*	*28*	*28*	*30*	*43*	*43*	*28*	*38*	*25*	*27*	*48*	*28*
	−*a*		.99	1.00	.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.97	.98	1.00	1.00	1.00
	−*b*		.01		.02												
	−*c*													.02			
	−*d*												.03				
Pgi2	(*N*)			*28*	*32*	*37*	*28*	*28*	*30*	*42*	*43*	*28*	*38*	*25*	*27*	*47*	*25*
	−*a*		.14	.20	.25	.32	.09	.38	.27	.24	.16	.13	.08	.08	.13	.17	.32
	−*b*																
	−*c*		.64	.71	.59	.50	.70	.41	.62	.76	.79	.75	.91	.90	.78	.66	.54
	−*d*	P	.21	.09	.16	.16	.21	.20	.12		.04	.13	.01	.02	.09	.17	.14
	−*e*	(P)	.01					.02			.01						
	−*g*	(P)	.01			.01											
	−*h*	(F)															

APPENDIX 1.

Extended.

		A. sylvatica × *A. pavia* hybrid zone		Border *A. sylvatica*					Border								
									s × *f*		*f* × (*s* × *p*)			*f*		*f* × *g*	*g*[3]
Locus	Allele	23	24	25	26	27	30	SYLVA[2]	36	37	38	39	40	42	FLAVA[2]	49	50
Lap1	(*N*)	*27*	*34*	*28*	*31*	*21*	*30*		*30*	*9*	*35*	*28*	*26*	*19*		*26*	*28*
	−*a*						.02										
	−*b*	.11	.19		.13	.21	.08	.10	.27		.20	.05	.33	.26	.14	.67	1.00
	−*c*	.52	.40	.75	.19	.24	.55	.47	.38	.83	.29	.34	.33	.47	.55	.14	
	−*d*	.13	.25	.02	.26	.38	.05	.24	.18		.17	.14	.02	.21	.18	.06	
	−*e*	.24	.16	.14	.26	.17	.30	.18	.17	.17	.34	.46	.29	.05	.13	.14	
	−*g*			.09	.16								.04				
Me1	(*N*)	*27*	*34*	*28*	*35*	*20*	*30*		*33*	*11*	*32*	*28*	*26*	*32*		*26*	*28*
	−*b*	.02		.13	.06	.18	.18	.25	.06	.09	.11	.09	.08	.11	.07	.64	.50
	−*d*	.98	1.00	.88	.94	.83	.80	.73	.86	.91	.86	.89	.92	.89	.92	.35	.27
	−*f*							.01	.08		.03	.02			.01	.02	.23
	−*h*						.02										
Me2	(*N*)	*26*	*34*	*28*	*23*	*20*	*30*		*27*	*11*	*31*	*28*	*25*	*27*		*27*	*28*
	−*c*	.40			.80	.53	.32	.16	.69	.18	.37			.11	.27		
	−*d*	.60	1.00	1.00	.20	.48	.68	.84	.32	.82	.63	1.00	1.00	.89	.73	.98	1.00
	−*e*														.01	.02	
Px2	(*N*)	*27*	*32*	*28*	*28*	*21*	*30*		*14*	*9*	*36*	*26*	*27*	*32*		*21*	*28*
	−*a*																
	−*b*	.02	.11		.46	.26	.02	.04	.14					.09			
	−*c*	.28	.11	.16	.07	.07	.02	.14	.11		.06	.15	.04	.02	.02		.07
	−*d*																
	−*e*	.43	.78	.70	.29	.60	.73	.64	.57	.06	.49	.33	.22	.02	.01	.02	.14
	−*g*	.28		.13	.18	.07	.23	.17	.14	.89	.44	.50	.59	.86	.87	.62	.77
	−*i*			.02					.04	.06	.01	.02	.15	.02	.08	.36	
	−*j*														.01		.02
Pgi1	(*N*)	*27*	*34*	*28*	*35*	*21*	*30*		*34*	*11*	*36*	*28*	*28*	*35*		*26*	*28*
	−*a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.96	1.00	.98	1.00	1.00	.92	.77
	−*b*																
	−*c*																
	−*d*										.04		.02			.08	.23
Pgi2	(*N*)	*27*	*34*	*28*	*35*	*21*	*30*		*34*	*11*	*36*	*28*	*25*	*33*		*27*	*28*
	−*a*	.07	.18	.05	.37	.07	.23	.18	.38	.32	.28	.23	.22	.32	.46	.20	
	−*b*				.01												
	−*c*	.91	.29	.95	.61	.81	.77	.81	.59	.46	.64	.70	.78	.68	.51	.69	1.00
	−*d*	.02	.49			.12		.01	.03	.05	.01	.07			.02	.11	
	−*e*		.04														
	−*g*									.18	.07						
	−*h*														.02		

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Allele frequencies and sample sizes (*N*) for 14 loci in 27 populations of *Aesculus*. Abbreviations are first letter of specific epithet.

Locus	Allele	Species marker	PAVIA[1]	Border *A. pavia*					*A. sylvatica* × *A. pavia* hybrid zone								
				9	10	11	12	13	14	15	16	17	18	19	20	21	22
Pgm2	(*N*)			*26*	*23*	*34*	*29*	*30*	*30*	*42*	*44*	*28*	*36*	*26*	*27*	*45*	*28*
	−*a*	F, (S)															
	−*b*	F, S	.01	.04	.04	.04		.27	.13	.50	.47	.29	.17	.58	.20	.34	.23
	−*c*	S		.02		.02		.20		.04	.01		.01		.06		.16
	−*d*	(P, S)	.02				.02		.05	.02	.17	.04	.01		.02		.02
	−*e*	P, S	.96	.94	.96	.94	.98	.53	.82	.44	.35	.68	.81	.42	.72	.63	.59
	−*f*	(P)	.01													.02	
Pgm3	(*N*)			*26*	*23*	*35*	*29*	*31*	*30*	*41*	*43*	*28*	*36*	*24*	*27*	*45*	*27*
	−*a*										.13					.01	
	−*b*		.89	.79	.94	.87	.64	1.00	.90	.50	.80	.77	.86	.96	.57	.57	.72
	−*c*		.01							.04						.07	
	−*d*		.09	.21	.04	.13	.36		.10	.44	.06	.23	.11	.04	.41	.34	.28
	−*e*	(P)	.01								.01						
	−*g*		.01		.02					.02			.03		.02	.01	
Shk2	(*N*)			*26*	*32*	*37*	*30*	*27*	*29*	*41*	*44*	*28*	*36*	*20*	*27*	*39*	*29*
	−*a*	(S)															
	−*b*		.01			.01				.18	.03		.04	.08	.19	.03	
	−*c*	F, (P)	.04		.02	.03	.05	.19		.05	.02	.02					.12
	−*d*			.37													
	−*e*	S, F	.05			.01	.07	.06	.21	.65	.76	.93	.86	.88	.74	.51	.16
	−*f*					.01			.05			.02				.03	
	−*g*	P	.87	.64	.98	.93	.88	.76	.72	.12	.13		.08	.05	.07	.40	.72
	−*h*								.02							.01	
	−*i*	(P)	.02								.06	.02	.01			.03	
	−*j*	(S)										.02					

[1] Mean of eight populations examined by dePamphilis (1988).
[2] Mean of seven populations examined by dePamphilis (1988).
[3] Single population examined by dePamphilis (1988).

APPENDIX 1.

Extended.

		A. sylvatica × *A. pavia* hybrid zone		Border *A. sylvatica*					Border								
									s × *f*		*f* × (*s* × *p*)			*f*		*f* × *g*	*g*[3]
Locus	Allele	23	24	25	26	27	30	SYLVA[2]	36	37	38	39	40	42	FLAVA[2]	49	50
Pgm2	(*N*)	*26*	*32*	*28*	*35*	*21*	*27*		*18*	*8*	*27*	*28*	*26*	*32*		*23*	*27*
	−*a*		.02	.04				.01			.02		.02		.06	.04	.02
	−*b*	.60	.05	.57	.44	.71	.87	.55	.78	.63	.70	.05	.44	.98	.90	.70	.52
	−*c*	.12	.02	.07		.21		.05				.04	.02			.02	
	−*d*	.02			.09			.02				.02					
	−*e*	.27	.92	.32	.47	.07	.13	.38	.22	.38	.28	.89	.52	.02	.04	.24	.46
	−*f*																
Pgm3	(*N*)	*26*	*432*	*27*	*35*	*19*	*27*		*18*	*6*	*20*	*28*	*26*	*26*		*20*	*25*
	−*a*	.04				.13							.02				
	−*b*	.64	1.00	.72	.60	.24	.85	.68	.78	.83	.93	.98	.85	.17	.51	.65	.40
	−*c*				.01	.03		.02									
	−*d*	.33		.28	.34	.61	.15	.28	.22	.17	.08	.02	.10	.83	.48	.35	.60
	−*e*																
	−*g*				.04			.02					.04		.01		
Shk2	(*N*)	*27*	*32*	*28*	*34*	*21*	*27*		*34*	*9*	*36*	*27*	*26*	*34*		*28*	*28*
	−*a*				.04			.03		.06	.01						
	−*b*	.06			.04			.04				.09		.02	.04		
	−*c*	.02	.08		.02				.02	.06		.07			.05		
	−*d*							.01									
	−*e*	.74	.09	1.00	.90	1.00	.87	.90	.99	.67	.63	.70	.65	.99	.91	1.00	.98
	−*f*						.13					.07					
	−*g*	.19	.83					.02		.22	.36	.06	.35				.02
	−*h*																
	−*i*																
	−*j*							.01									