Genetic diversity among populations and size classes of buckeyes (Aesculus: Hippocastanaceae) examined with multilocus VNTR probes

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Received May 15, 1998
Accepted September 11, 2001

Abstract. Little is known about genetic variation in members of the genus Aesculus (Hippocastanaceae), in particular A. flava (yellow buckeye) and A. glabra (Ohio buckeye). Here, three synthetic DNA probes (composed of tandemly repeated, core sequences) that reveal alleles at multiple variable-number tandem-repeat (VNTR) loci in these two species were used to investigate: 1) levels of genetic variation in one stand of A. flava and three isolated stands of A. glabra; 2) whether the stands of A. glabra are genetically differentiated from one another; 3) whether there has been selection for more heterozygous individuals through time in one stand each of A. flava and A. glabra; and 4) whether a possible genetic bottleneck had occurred during the formation of either species of Aesculus.

First, variation of VNTR genetic markers within and among three populations of A. glabra separated by 60–180 km was examined. In each one hectare (ha) population, 22 individuals were randomly sampled. Among the three populations, the mean number of bands scored per individual was 80.35 and the average number of estimated loci surveyed was 54.17. Mean similarity and estimated heterozygosity within populations ranged from 0.634 to 0.743 and from 0.342 to 0.486, respectively. The mean similarity across populations was 0.657, while the mean estimated heterozygosity across populations was 0.484 for A. glabra. The most isolated site was the most genetically differentiated as indicated by differences in levels of similarity, heterozygosity, and Fst value comparisons.

In a separate experiment, genetic variation in 22 large (reproductively mature; dbh > 8 cm) individuals was compared with that in 22 small (not yet reproductive; dbh < 1 cm) individuals collected within one ha stands for both A. flava and A. glabra. Mean similarity values among large versus small individuals of A. flava were 0.665 versus 0.662, while for A. glabra the corresponding values were 0.686 versus 0.691, respectively. Permutation tests of these similarity data detected no evidence for size class genetic differentiation in either species (both p-values > 0.050). Further, permutation tests for the number of bands per individual (average band number should be higher in more heterozygous individuals) detected no significant differences between size classes for either species. Thus, evidence of pronounced inbreeding and/or selection altering population genetics within small relative to large individuals was not detected.

In addition, comparable similarity and heterozygosity values between these two closely related species (which still maintain an active zone of hybridization) suggests that either: 1) no extreme genetic bottleneck has accompanied the formation of these species from a common ancestor; or 2) signs of such a bottleneck have largely been
eliminated. These studies demonstrate the utility of multilocus VNTR DNA probes for investigating genetic variation within and among plant populations, between size classes within a population, and between closely related related species.

**Key words:** *Aesculus glabra*, *Aesculus flava*, Hippocastanaceae, DNA fingerprinting, genetic variation, inbreeding, minisatellite DNA, plant population genetics.

**Introduction**

Little is known about genetic variation in *Aesculus* (Hippocastanaceae), including both *A. flava* Soland. (yellow buckeye (synonym: *A. octandra* Marsh.; see Meyer and Hardin 1987)) and *A. glabra* Wild. (Ohio buckeye). These tree species were common constituents of the once continuous, pre-settlement forests of eastern North America (Fig. 1). Over the past 300 years, these forests have been greatly disturbed and reduced (e.g. >89% forest removal from Ohio), leaving a patchwork of altered remnant stands of various sizes and degrees of isolation. If this isolation disrupts gene flow among reduced populations, then the populations are expected to diverge through time due to differentiating inbreeding, selection, and/or drift (Hamrick et al. 1979). Further, as stands are reduced in size, increased inbreeding may result in inbreeding depression and selection for more fit (i.e. more heterozygous) individuals (Thornhill 1993). In such populations, levels of heterozygosity may differ between different life stages. With regard to the above issues we used modified DNA fingerprinting methods to investigate: 1) levels of genetic variation in one stand of *A. flava* and three isolated stands of *A. glabra*; 2) whether the stands of *A. glabra* are genetically

![Map of Aesculus flava and Aesculus glabra distribution](image)

**Fig. 1.** Distribution of *Aesculus flava* (after Little 1979) and *A. glabra* (after Merz 1965). Putative zone of hybridization is indicated by cross-hatching. Collection site abbreviations: A Archbold; B Medina; C Loudonville; and D Great Smoky Mountains.