HISTORY OF BLACK WALNUT GENETICS RESEARCH IN NORTH AMERICA

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ABSTRACT—Eastern black walnut (*Juglans nigra* L.) is an economically and ecologically important hardwood species that has been used throughout the history of settlement in North America. It was a resource that helped Native Americans in their everyday life, it helped European settlers carve a living out of the wilderness, and it has helped rural farmers and private landowners subsist and invest in the future. Described here is a brief history of black walnut breeding and molecular genetics research. Current genetic research may ultimately lead to the domestication of black walnut, an event that would be a hallmark for forest tree species.

Eastern black walnut (*Juglans nigra* L.) has been a component of North American forests since the Middle to Upper Cretaceous (Elias 1980) and has been used by humanity for centuries. This species has never held a predominant position in temperate forests, but it has been a consistent feature in the Central Hardwood Region. Almost every part of the tree has played some valuable role in helping people subsist, including consumption of the nuts and the use of nut husks for dyes, and use of the wood for fencing, railroad ties, firewood, gunstocks, and fine furniture. Black walnut also is an important tree for forest animals, providing shelter and forage especially for squirrels.

In the 20th century, black walnut researchers began to study the ecology, reproductive biology, and phenotypic variation of this valuable species. Eventually breeding programs were initiated to find black walnuts of sufficient growth rate and architecture to meet the demands of industry. Although much is now known about black walnut biology and natural history, this quest continues today, after over 60 years of effort. The purpose of this is to provide a summary of improvement programs, investigations of vegetative propagation, advances in taxonomic relationships within the Juglandaceae, parentage, gene flow, and studies of genetic diversity made possible by molecular genetics.

NUT CULTIVARS

Landowners have identified and selected walnut trees for propagation since the late 1800s (Zarger 1969). Emphasis on cultivation in the early 20th century was focused on nut tree culture and propagation (MacDaniels 1933, Talbert 1942). Gradually, an awareness grew that quality walnut trees were becoming uncommon due to the clearing of land for agriculture and overharvesting. A tree planting movement began with the goal of restoring the diminishing supply of quality black walnut (Record 1986, U.S. Forest Service 1966). Contests sponsored by the Northern Nut Growers Association and State agencies were conducted with the intent of discovering new cultivars (Crane and Reed 1937).

The Tennessee Valley Authority spent many years, from 1934-1960, identifying trees and cultivars that were well adapted to the valley (Zarger 1969). McKay (1971) published his lifelong breeding program to develop a Persian walnut and eastern black walnut hybrid with superior nut production, with little success. By the end of the 1960s, there were 18 nut cultivars that were widely used for nut production in the Central Hardwood Region (Zarger 1969), but efforts towards an improved timber variety were just beginning.

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TREE IMPROVEMENT

In the 1960s there was a shift from "mining" walnut trees from natural stands to a conscientious effort towards improving black walnut as a crop. The impetus for this was a perceived decrease in the availability of high quality walnut timber in natural stands, a condition attributed to the long-standing practice of high-grading (Clark 1965, Elias 1980). Reduced supplies of high quality trees increased the price of walnut timber leading growers and industry to recognize the need for high quality, fast-growing trees to meet increasing market demands. The increasing demand for black walnut led the USDA Forest Service to initiate their Black Walnut Genetics Project at Carbondale, IL in 1965 (Funk 1966), a research effort focused on progeny testing, seed sources, and the heritability of apical dominance. The mission was to gather and provide genetic information with which to base a breeding program for superior trees (Caraway 1976).

By the mid-1960s, several provenance studies were already underway, with plans to begin hybridization research (Clark 1965). However, while information gathered at the beginning of the century regarding pollination, vegetative propagation, and seed germination provided a sound foundation for basic research, there were virtually no data on the genetics of the species (Wright 1966). To address questions related to regional adaptability and variability, in the absence of genetic data, seed zones were proposed for the Central States based upon temperature, annual precipitation, occurrence of frost in the spring and fall, the number of frost-free days, latitude, and summer day length (Deneke and others 1981). Seed zones are regions representing a limited range of environmental variance such that the seeds collected anywhere in a zone could be planted in the same zone with some assurance of success.

By the 1970s, black walnut research had been focused on tree improvement for almost a decade, but there remained a lack of high quality product (seeds or seedlings) to meet the demands of a growing industry. In a summarization of what was known about taxonomy, evolution, sexual reproduction, and breeding and improvement programs of black walnut, Funk (1970) stated that black walnut breeding programs should focus on climatic adaptation, vigor and growth rate, form, fecundity, and pest resistance. In describing the importance of the natural phenotypic variation in walnut, both Funk (1973) and Bey (1970, 1973) found that there were more differences among seed sources than within stands or areas. For instance, Bey's research on six year-old provenance studies from six States in the Midwest indicated that the origin of seed collection affected the timing of leaf flush and leaf fall, and tree height, with trees from southern sources flushing earlier, losing their leaves later, and being generally taller and of larger diameter. Bey (1980) again used these provenances to determine that geographic origin was an important component when considering seed sources and recommended choosing trees up to 200 miles south of the planting site as the tallest 20% of the trees in his study were from sources south of the planting site. His results indicated that though sources beyond 200 miles south of the planting site were taller and bigger than trees from northern sources, they also were more prone to injury or death due to frost and cold winters. This finding was again confirmed by Geyer and Rink (1998) when they took diameter, height, and survival measurements on a 17-year-old provenance test in southern Illinois.

An outcome of Bey's (1973) research was his conclusion that future research needed to determine the importance of trade-off's in trait selection, how successful silvicultural manipulations were in addressing problems, and the effectiveness of selecting wild trees based on phenotype.

By 1982, 11 States within the walnut commercial range had established walnut improvement programs, from provenance and progeny testing, to second-generation seed orchards (Rink and Stelzer 1982). For example, nine timber varieties (thought to be superior in growth rate, form, or seed yield [Beineke 1984]) were selected and propagated at Purdue University. Moreover, in the same decade, several regional cooperatives were established: the NC-99 program, and the eight-state Fine Hardwood Tree Improvement Cooperative. NC-99 began in 1960 (then known as NC-51) and focused on determining the range and pattern of genetic diversity, breeding, selection and seed production, breeding zones, and advanced generation breeding for species of economic importance. NC-99 contributed significantly to the knowledge accumulated on breeding black walnut (Stier 1986). The eight-state Fine Hardwood Tree Improvement Cooperative began in 1986 to combine efforts towards genetic improvement of primarily black walnut and northern red oak in the north central region. The optimism that was a hallmark of the early efforts towards black walnut timber breeding was tempered in later years as it became clear that walnut was extremely site sensitive and that significant environment-genotype interactions would confound genetic effects and complicate their interpretation (Geyer and Rink 1998). Today, efforts are still underway to breed a fast-growing, straight-boled, black walnut timber variety. For more detail, see McKenna and Woeste (2004, this volume).

DISEASE MANAGEMENT

Of all the diseases and damaging agents that affect black walnut, only walnut anthracnose, caused by the fungus *Gnomonia lepostyla,* has been the subject of genetic research. Walnut anthracnose can severely damage a nut crop and hinder tree growth. Beginning in the 1970s, Black and Neely published a series of articles on walnut anthracnose and the factors influencing its spread (Neely 1979, Black and Neely 1978a). Resistance to anthracnose was considered an important goal of tree improvement programs, and Black and Neely (1978b) determined that while eastern black walnut was less susceptible than the Hinds (*Juglans hindsii*), hybrids between eastern black walnut and California black walnut varied in their resistance. For example, in a study of the disease susceptibility of walnut hybrids, black walnut crossed with the Hinds was the most susceptible (had the most lesions), while black walnuts crossed with Japanese walnut (*Juglans ailantifolia*) were the most resistant (Black and Neely 1978b). This research suggested that Persian and Japanese walnut were potential sources for resistance genes to anthracnose.

Funk and others (1980) evaluated 62 black walnut families over 4 years for anthracnose incidence by visually estimating incidence of leaflets with one or more anthracnose-caused spots, estimating premature crown defoliation due to anthracnose, and determining the electrical resistance of cambial tissue. They also evaluated individual and family correlations among disease ratings and growth rates. Their results indicated that year to year correlations for anthracnose incidence were stronger for individual trees than family means, and that selecting for faster growth resulted in a 13% decrease in anthracnose incidence, while selecting for low anthracnose incidence resulted in only a 7% growth increase.

TISSUE CULTURE

Vegetative propagation of black walnut became emphasized in the 1980s as a mechanism for circumventing problems associated with conventional propagation (Coggeshall and Beineke 1997). Walnut trees produce fewer seeds per tree than many other species, and once those seeds are harvested, only a portion is viable. Poor seed yield and losses to predation prior to nut collection often result in an undersupply of seeds to sell or plant (Beineke 1982). Vegetative propagation potentially permits the almost limitless multiplication of selected genotypes without additional recourse to seeds. Grafting had long been practiced as a method for propagating black walnut, and a high rate of grafting success provided factors as scion wood storage, rootstock condition, and grafting technique and aftercare were optimized (Beineke 1983). The multiplication of microshoots *in vitro* was pursued as a promising method for producing ramets on their own roots and in association with research into the development of a transformation and regeneration system for black walnut (Van Sambeek and others 1997). Efficient rooting of micropropagated walnuts proved difficult, but by the late 1980's somatic embryogenesis looked promising, and genetic engineering was being considered for black walnut (Rink 1989). Somatic embryogenesis experiments were in progress by 1993 (Van Sambeek and others 1990, Long and others 1995, Neuman and others 1993, Khan 1995). Since then, considerable progress has been made in tissue culture, for more detail, see Michler and Bosela (2004, this volume).

MOLECULAR GENETICS

One of the first molecular-genetic studies of black walnut was the discovery of its chromosome number (n=16, Woodworth 1930). The development of allozyme markers in the 1980s made the study of black walnut population genetics feasible. Kung and Rink (1987) used eight allozyme loci to estimate the outcrossing rate and fixation index for trees sampled within one county in Illinois. The use of molecular tools to answer population level questions and questions related to tree improvement expanded in the 1990's when researchers began to correlate isozyme allele frequencies with traits such as height and diameter (Kung and others 1991, Rink 1997). Additionally, throughout the 1990s allozyme markers were used to examine mating parameters, outcrossing rates, and fixation indices in black walnut (Rink and others 1989, 1994; Zhang 1990; Kung and others 1991; Zuo 1994; Zuo and others 1995; Busov 1996). By the late 1990s questions focused on overall levels of genetic diversity and the partitioning of genetic variation among black walnut populations (Busov 1996, Busov and others 2002).

The 1990s also marked a period in which morphological studies of walnut taxonomy were re-evaluated using molecular techniques. For example, Smith and Doyle (1995) found congruence between taxonomy of the Juglandaceae based on morphological (from literature including Manning's 1978 paper along with subsequent papers) and molecular data (chloroplast restriction site data). Their findings supported Manning's (1978) classifications within the Juglandaceae (based upon inflorescences, flowers, and fruits) with the exception of the subfamilial status of *Platycarya.* Smith and Doyle's findings suggested that either Engelhardieae should be moved to subfamilial status, or that Manning's two subfamilies, Juglandoideae and Platycaryoideae, should be reduced to the tribal level. However, it was clear that Platycarya was grouped with Juglans, Carya, and Pterocarya, and was not a separate subfamily. In 1995, Fjellstrom and Parfitt used species-level phylogenetic trees based on RFLP's (restriction fragment length polymorphisms) to detect clear distinctions between the sections of *Juglans* (e.g., old world walnuts vs. new world walnuts). Fjellstrom and Parfitt (1994b) also used RFLP's to estimate the genetic diversity of 13 *Juglans* species worldwide. Though no linkage maps exist specifically for the eastern black walnut, Fjellstrom and Parfitt (1994a) used the inheritance and linkage of 48 RFLP loci to establish a linkage map for Persian walnut. This linkage map for walnut was expanded by Woeste and others (1996) to total 107 markers in 15 linkage groups.

In the 21st century, Stanford and others (2000) described the phylogeny of *Juglans* using MATK (a chloroplast maturase-encoding gene) and ITS (Internal Transcribed Spacer) sequence data. They found all four sections of the genus branched out as commonly assumed. Rhysocaryon (i.e., old world walnuts vs. new world walnuts such as Persian walnut), to which eastern black walnut belongs, showed eastern and western species distinctions (e.g., *J. nigra* vs. *J. hindsii*), and also a distinction between temperate and tropical species (e.g., *J. nigra* vs. *J. neotropica*). Microsatellite loci were first published for black walnut by Woeste and others (2002); it is clear that microsatellites (SSRs) will replace isozymes as the molecular tool of choice to answer questions pertaining to walnut genetic diversity across regions, studies of parentage, and cultivar identification (Potter and others 2002, Ninot and Aleta 2003, Robichaud and others 2004, this volume).

THE FUTURE?

Domestication, according to Kass (1993), is associated with seven activities: silvicultural manipulation, site enhancement, pest control, natural selection, semi-natural selection, anthropogenic selection, and a correlated response to selection. If Kass is correct, black walnut is well on its way to domestication. The first four activities are undoubtedly common factors to black walnut management, whether on plantations, or in managed woodlots. Semi-natural selection refers to a response by the tree to artificial factors brought about by socio-agricultural circumstances, such as tolerance to grazing animals, local site conditions, and ease of propagation. Human selection, the 6th criterion, involves actively pursuing an ideotype that is a culmination of the desired characteristics in a particular species. In black walnut, this would be traits such as fast growth, straightness, and a high proportion of heartwood in timber varieties, or early nut bearers, consistent yield, and a high proportion of kernel in nut tree varieties. It also includes a component of predictability, which would lead to an increase in crop yield, a characteristic still under development in black walnut. Site conditions and genetic factors are still too unpredictable to strive for, and consistently maintain, a black walnut ideotype. Persian walnut, on the other hand, is much farther along in this process than black walnut and there is probably an ideotype for that species that has more or less been attained.

Kass' last criterion (a correlated response to selection by the organism) also has not been met, as tree breeding and selection programs are too recent. Furthermore, although black walnut responds somewhat to management, it is not dependent upon it for survival. Black walnut has not yet evolved any dependence upon human manipulation to survive (Reid 1997). Persian walnut, though not dependent upon anthropogenic influence for survival, has been cultivated for centuries and the best varieties only grow well in highly modified environments (Ramos 1998). For Weirsum (1997), domestication is realized as increased people-plant interactions that result in morphological and genetic changes in the organism. He makes a distinction between biological and comprehensive definitions for domestication, the former referring to a gradual change in morphological and genetic characters for specific uses and environments, and the latter referring to changes in management and exploitation of the plant that brings about changes in the morphology and genetics of the species.

So far, black walnut has been recognized as a valuable tree species and has been harvested out of natural forests, propagated on farms, in experiments, and in provenance studies and plantations. Its biology, and especially reproductive biology, has been examined, and it has been clonally propagated via tissue culture. The next step in domestication is to genetically modify black walnut (this does not necessarily imply genetic transformation) in some way to make it a better crop species, and more responsive to management activities. This has not yet been accomplished, but it has been on the horizon for some time.

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