# *Populus* Breeding: From the Classical to the Genomic Approach

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**Abstract** *Populus* breeding is distinguished by a long history in forest tree improvement and its frequent dual reliance on inter-specific hybridization and varietal selection as the prominent domestication strategy. This chapter presents a review of the genecology and the principal long-term improvement approaches considered in the manipulation of the genus' key taxa, the pertinent experimental design features of worldwide varietal evaluation programs, and the current understanding of the morphological, physiological, and pathology components of yield and the physical and chemical components of wood quality. The chapter concludes with an assessment of the molecular tools being developed for an integrated translational genomics program to improve upon present breeding and selection methodologies.

## 1 Introduction

*Populus* was the first woody perennial to gain recognition as a model for worldwide tree breeding programs because of the groundbreaking work in species hybridization, polyploid breeding, and investigations into pathogen resistance during the early part of the twentieth century (Pauley, 1949). More recently, the success that *Populus* clonal testing, selection, and deployment has achieved in boosting the trend toward worldwide varietal forestry over the last 20 years cannot be overestimated. Although tree improvement work in *Populus* may be surpassed in sophistication by today's *Pinus* and *Eucalyptus* breeding programs, the model designation remains deserved in view of the sequencing of the *Populus* genome – the first of any tree in 2006 – and the subsequent investigations into genotype-phenotype associations. This chapter presents an overview of the traditional approach to applied *Populus* breeding and the advent of translational genomics, surely the next stage in a truly fascinating story.

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Domestication of the genus began in Europe, perhaps as a consequence of the introduction of eastern cottonwood (*P. deltoides*) in the late eighteenth century and the frequency with which spontaneous – and at times valuable – hybrids with the native black poplar (*P. nigra*) (hybrid binomial – *P.* ×*canadensis*) occurred under natural conditions. That led to their cultivation for timber production to forestall widespread wood shortages, especially after the Second World War (Schreiner, 1959). During his tour of European *Populus* culture in 1952, American poplar pioneer Ernst Schreiner reported that 11 countries were heavily invested in *Populus* controlled breeding programs, saying, *"...poplar specialists and growers... generally recognize that there is an essential and continuing job to obtain better clones for future use and to replace those that may fall prey to unusual environmental conditions or to new diseases and insects."* His recognition was taken to heart in Europe and elsewhere, for over the next half century catalogues of superior cultivars complete with photographs and growth and form metrics were published for four of the five continents where *Populus* culture had spread. This acknowledgement included Europe (Koster, 1972; van Broekhuizen, 1972), North America (Roller, 1984), South America (Arreghini et al., 2000), and Asia (Chen, 2005). At the 23rd Session of the International Poplar Commission held in Beijing in 2008, it was reported that over 125 elite *Populus* cultivars were globally in use (FAO 2008).

*Populus* management is unique in that its markets include a wide range of forest products, including energy feedstock, wood chips for pulping fibers and composite panels, saw- and veneer logs, agro-forestry, and phyto-remediation as well as several other environmental applications. Presently, the worldwide *Populus* estate encompasses over 5,255,000 hectares of plantations and 3,867,000 hectares of agro-forestry and environmental plantings (FAO, 2008). The management of this estate continues to emphasize the breeding of improved cultivars. This domestication activity, in turn, relies upon the accumulated knowledge of *Populus* genecology, the physiological and morphological components of yield, the genetics of pathogen resistance, and the inheritance of quantitative and qualitative traits (Stanton, 2009). While work in each of these areas has provided insights into the genetics of adaptation and wood production, the identification of controlling genes and the characterization of selectable markers is now forging new breeding approaches that will extend *Populus*' claim as the model woody perennial (Bradshaw et al., 2000).

## 2 Genecology

*Geographic Distribution* – The genus *Populus* is made up of six sections, three of which – *Aigeiros* (cottonwoods), *Tacamahaca* (balsam poplars), and *Populus* (white poplars and aspens) – account for nearly the world's entire applied breeding work. Recent taxonomies published in the West closely agree on the total number of species, which range from 29 (Eckenwalder, 1996) to 32 (Dickmann and Kuzovkina, 2008). But in Asia a more liberal classificaion is the rule, with 47–50 species recognized in China alone (Wu and Raven, 1999; Zheng, 1985). Taxonomic rank has,

at times, been extended below the species level to geographic varieties to recognize entities with distinct morphological or physiological features. Good examples include the xeromorphic *P. nigra* var. *caudina* and *P. tremula* var. *davidiana* and *P. deltoides* var. *monilifera* that at times have been used in breeding and selection programs (Kajba et al., 2004).

Directed manipulation of the genus started with an understanding of population variation patterns in adaptive and commercial traits within each of the genus' key species that, as a rule, cover expansive geographic areas (Fig. 1). In section *Populus*, for instance, the transcontinental range of quaking aspen (*P. tremuloides*) covers approximately 110° of longitude and over 50° of latitude in North America, from Alaska's sub-arctic region and Canada's Northwest Territory to disjunct populations in central Mexico (Perala, 1990). Likewise, common aspen (*P. tremula*), its sibling species, has the most expansive range in the genus and is found throughout most of Europe and a substantial part of Asia. It spans 155° of longitude from Europe's Iberian Peninsula east to Asia's Kamchatka Peninsula, and 55° of latitude from Scandinavia to southeastern China (Boratynska and Boratynski, 1977). White poplar (*P. alba)* is also found over a large expanse of Eurasia. It is spread across a longitudinal range of approximately 115° from Spain's Atlantic Coast eastward to China's Xinjiang Uigur Autonomous Region, Afghanistan, Iran, Iraq, and Pakistan. North-to-south, *P. alba* covers approximately 30° of latitude found as far north as 54–58° N latitude in Germany, Poland, and Russia and as far south as 30° N latitude in North Africa.

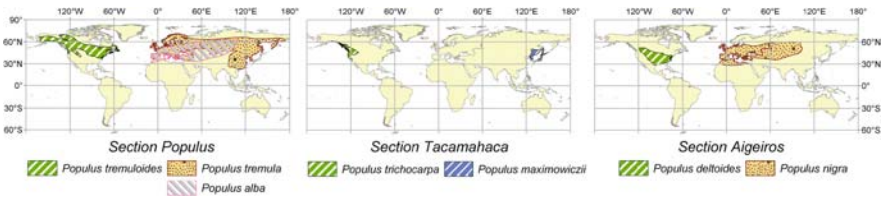


**Fig. 1** World distribution of *Populus* species most commonly used in controlled breeding programs

Extensive distributions also characterize *P. nigra* and *P. deltoides* of section *Aigeiros*. The former is found over a large portion of Europe, the Mediterranean basin, Central Asia, the Ukraine, Russia, and the northwest of China spanning more than 40° of latitude and 90° of longitude (Boratynska and Boratynski, 1977). Distribution of *P. deltoides* covers over 20° of latitude in North America between the Canadian prairie and the Gulf of Mexico and over 40° of longitude between the Atlantic seaboard and the Great Plains (Cooper, 1990). The distribution of species in section *Tacamahaca* is also substantial: Black cottonwood (*P. trichocarpa*) spans approximately 35° of latitude from Cook Inlet along the Alaskan Coast southward to the outlying populations of Mexico's Baja Peninsula, and 45° of longitude from the Rocky Mountains to the coast of southeast Alaska (DeBell, 1990). Finally, Japanese poplar (*P. maximowiczii*) ranges throughout eastern Asia, including Russia's Kamchatka Peninsula and the Kuril Islands, the Provinces of Liaoning,

Jilin, and Heilongjuang in the northeast of China, the Korean peninsula, and the Japanese Islands of Sakhalin, Hokkaido, and Honshu, covering 25° of longitude and 20° of latitude (Chiba, 1984).[1]

*Variation Patterns* – Genetic variation in adaptive traits for each of these species is commonplace and often associated with latitude as clines. Phenology is perhaps the best example, and an understanding of its variation pattern has long been a foundation of well-designed *Populus* breeding programs. Generally, southerly seed sources initiate growth later in the spring and initiate the onset of dormancy later in the fall and, as a consequence, are often less tolerant of winter temperature extremes when compared to more northerly sources in common garden experiments. A range-wide study of autumnal phenology in *P. deltoides* provenances from 30° to 45° N latitude and planted at 40° N latitude demonstrated this phenomenon. The date of leaf abscission was closely associated with seed source latitude in a north-to-south trend over which the date of leaf abscission grew progressively later (Ying and Bagley, 1976). The timing of autumnal leaf abscission was also observed to vary in a northwest-to-southeast direction within the southwestern portion of the *P. deltoides* range between 28° 51′ and 38° 25′ N latitude, in which southeastern seed sources exhibited a later date of abscission than northwestern ones (Nelson and Tauer, 1987). A similar trend was observed in the date of autumnal bud set in an investigation of *P. tremula* seed sources sampled from 56° to 66° N latitude in Sweden where a later terminal bud set date was associated with southerly sources (Luquez et al., 2008). The photoperiod of the genotype's provenance has been implicated as the controlling environmental factor. This was evident in studies of northern and southern sources of *P. trichocarpa* and *P. tremula* (∼ 34–53° N latitude for the former and ∼56–66° N latitude for the latter) where the onset of the dormancy process of the southern sources responded to a shorter day length compared with more northerly sources (Howe et al., 1995; Ingvarsson et al., 2006).

Temperature replaces photoperiod as the controlling mechanism that triggers spring phenological events. Seed sources originating at low latitudes require either a more stringent chilling requirement or higher heat sums before growth is initiated when compared with their counterparts from higher latitudes (Farmer, 1993). Farmer and Reinholt (1986) illustrated the trend in a controlled chilling study of balsam poplar (*P. balsamifera*) populations originating from 45° to 55° N latitude where the length of time to initiate shoot growth decreased with increases in seed source latitude.

The upshot of spring and autumnal adaptive patterns for controlled breeding programs is that selections moved south of their provenance – either as clones or as breeding stock – may not perform as well as local sources due to the inability to take full advantage of the growing season, while selections moved north of their

[1] Several authors in this text follow Eckenwalder's (1996) taxonomy that considers *P. maximowiczii* as a variety of Siberian poplar (*P. suaveolens*). We, however, treat *P. maximowiczii* as a distinct species following the reasoning of Dickmann and Kuzvokina (2008), because it is commonly known as such by *Populus* breeders worldwide.

provenance often exceed the performance of local selections within the constraints imposed by temperature extremes (Farmer, 1993). This holds special importance for breeding the all-important *P. deltoides* for lower latitudes of the world where inter-specific crosses with other species endemic to low latitudes, such as Himalayan poplar (*P. ciliata)* and Yunnan poplar (*P. yunnanensis*), may result in inter-specific heterosis while maintaining adaptation to local photoperiods.

Intra-specific population differences are also encountered on a more limited geographic scale as the following demonstrates: (1) across 3.80° of latitude on the Japanese island of Hokkaido (approximately 41° 36′–45° 24′ N) southern sources of *P. maximowiczii* initiate growth cessation later than northern sources (Chiba, 1984); (2) over 4.35° of latitude across a southwest to northeast gradient in the Pacific Northwest (44° 44′ N–49° 05′ N) the growth of southwesterly sources of *P. trichocarpa* remains active longer into the fall than northeastern sources (Weber et al., 1985); (3) across 3.50° of latitude in the north central region of the United States *P. balsamifera* populations from the southeast grow faster in height and set terminal buds later than those from the central and northwest sectors of the region (Riemenschneider and McMahon, 1993). Local population variation in the timing of spring growth initiation in *P. trichocarpa* is tied to changes in temperature gradients within river drainages, while autumnal events are associated with both temperature gradients and/or disease pressure dependent on the specific individual drainages (Dunlap and Stettler, 1996). These finer expressions of population variation are as important as the broader, range-wide ones in the design of *Populus* breeding programs.

Beyond phenology, genetic variation among populations within species has been reported for a variety of growth, eco-physiological, and morphological traits that impact *Populus* breeding programs. In *P. trichocarpa*, for example, a latitudinal cline in the rate of photosynthesis has been reported among coastal populations sampled between 44° and 56° N latitude where more northerly sources display a greater capacity to assimilate carbon dioxide as, perhaps, a compensatory strategy for their earlier curtailment of the growing season (Gornall and Guy, 2007). Conversely, a strong differentiation of populations was not evident in the assimilation rate of *P. balsamifera* provenances across a comparable range of latitude (43–53° N) (Schnekenburger and Farmer, 1989). The photosynthetic rate of *P. trichocarpa* also varies on a more local geographic scale with populations endemic to xeric environments of higher light intensity capable of superior rates compared with those from mesic environments of lower light intensity (Dunlap et al., 1993). An eco-physiological trait of equal importance – water use efficiency – also exhibits population variation in: (1) *P. trichocarpa*, e. g. populations from arid, continental climates possess higher efficiencies than those from moist coastal environments of mild climate (Bassman and Zwier, 1991) and (2) *P. deltoides*, e. g. clones selected from dry sites exhibit lower stomatal resistances and the ability to prolong growth under drought conditions compared to those from sites of higher moisture availability (Kelliher and Tauer, 1980). Tolerance of autumnal frosts and winter injury is a third example of an eco-physiological trait where local population variation has been studied: For example, inland sources of *P. trichocarpa*

have developed higher tolerances to both factors compared to their coastal counterparts in the Pacific Northwest (McCamant and Black, 2000). Population variation in eco-morphological traits has similarly been reported: Crown morphology of *P. trichocarpa* populations from xeric sites differs from those from mesic sites in terms of individual leaf size, crown architecture, and leaf area indices. These, too, have been exploited in selective breeding strategies (Dunlap et al., 1995).

However, eco-physiological trait differentiation may not always reflect local climatic or edaphic selection pressures, as observed in the appreciable variation in both photosynthetic and transpirational rates and tolerance of soil salinity among four populations of *P. deltoides* var. *wislizenii* from a relatively restricted part of the southwestern United States (33° 55′–36° 12′ N latitude) (Rowland, 2001; Rowland et al., 2004). Likewise, significant genotypic variation in the growth response of *P. trichocarpa* to seasonal flooding is not associated with the population of origin (Smit, 1988).

Adaptive variation in disease resistance, historically of high importance in *Populus* breeding, has been demonstrated in studies of environmental conditions conducive to pathogen selection pressure. For example, populations of *P. trichocarpa* from mesic environments are now known to be characterized by significantly higher levels of *Melampsora* leaf rust resistance compared with populations native to arid regions (Dunlap and Stettler, 1996). *P. deltoides* populations sampled from humid, wet sites in the southwestern portion of its range were shown to exhibit heightened levels of *Melampsora* rust resistance compared to populations from drier environments that evolved with less exposure to the pathogen (Nelson and Tauer, 1987).

Despite the oftentimes definitive effect of source location on such a wide range of phenological, physiological, and pathology traits, studies of the manner in which genetic resources are organized within the genus have usually shown that a sizable component of variation in each of these characteristics resides within divergent populations (Fig. 2). To illustrate, whereas variation among populations of *P. deltoides* between 30° 30′ and 34° 55′ N latitude in the lower Mississippi River Valley accounted for 5% of total phenotypic variation in growth rate, 30% of that total was attributed to variation at the level of clones-within-populations (Foster, 1986). Greater within- than among-population variation has also been noted in studies of juvenile growth in *P. tremuloides* (Thomas et al., 1997) and in those addressing winter dormancy and spring phenology in *P. balsamifera* (Farmer, 1993; Farmer and Reinholt, 1986). Molecular data also suggested a weak differentiation among North American populations of *P. tremuloides* (Cole, 2005; Yeh et al., 1995) and Italian *P. tremula* populations (Salvini et al., 2001). A study of nucleotide sequence variation at three loci in *P. balsamifera* further reinforces the finding that the majority of genetic diversity resides within populations (Breen et al., 2009). It is believed that ample gene flow partially counters the effects of natural selection that would otherwise allow populations to diverge (Weber and Stettler, 1981). However, there are exceptions. Coastal *P. trichocarpa* populations are strongly differentiated in photosynthetic rate across a latitudinal transect with little inherent residual variation (Gornall and Guy, 2007). Cathay poplar (*P. cathayana*) populations from the

**Fig. 2** A stand of *P. trichocarpa* in which individual trees exhibit varying stages of spring vegetative shoot development. Such within-population variation may be an adaptation to yearly variation in the timing of spring frosts

Qinghai-Tibetan Plateau of southeastern China show strong differentiation in microsatellite markers due to the topography of the region that creates distinct selection pressures while precluding gene flow (Peng et al., 2005). The manner in which variation is distributed among the hierarchy of genetic organization is an important consideration to building first generation breeding populations (Breen et al., 2009).

## 3 Controlled Breeding

*Reproductive Biology – Populus* species are dioecious, although reports of hermaphroditism have been filed for each of the three major sections (Fig. 3). Sex appears to be determined by a single locus or a group of tightly linked genes on chromosome XIX (Yin et al., 2008). The bisexual condition may result from a relaxation of the mechanism that suppresses recombination at this locus that otherwise keeps sex-determining multigenes intact during reduction division. Male and female reproductive structures in *P. trichocarpa* in the northern hemisphere are formed April through June of the year preceding reproduction (Boes and Strauss, 1994). Reproduction involves wind pollination of inflorescences that contain approximately 60 staminate flowers or 35 pistillate flowers (Boes and Strauss, 1994)


**Fig. 3** Sub-gynoecious *P. trichocarpa* variety 'PS-53-97'. This condition produces predominantly pistillate inflorescences with occasional staminate ones borne on the same shoot (*left* photo). Additionally, pistillate inflorescences may contain hermaphroditic flowers bearing a pistil and stamens as shown in the photo on the *right*

(Fig. 3). The process of controlled reproduction is well understood but requires varying techniques and approaches for each of the genus' major sections (Stanton and Villar, 1996, Fig. 4). Artificial crosses are made in greenhouses using pollen extracted from floral cuttings of male selections maintained in water culture (Seitz, 1958). Pollen mother cells of Simon poplar (*P. simonii*) begin meiosis within 72 h of being forced in greenhouses and complete the process at the time that one-quarter of the length of a staminate inflorescence has emerged from the bud (Wang et al., 2009). Megagametophytes of *P. tremuloides* develop to the two- to four-nucleate stage during the winter and move to the eight-nucleate stage 18 h following spring pollination under greenhouse conditions (Fechner, 1972). Seed is produced on pistillate cuttings that are set in water, grafted on to potted under-stock (Farmer and Nance, 1968), or rooted in soil (Joennoz and Vallee, 1974). In China, controlled breeding techniques for seed orchard trees using scaffolding or partially dislodged and guyed trees exceed greenhouse-based techniques in cost-savings and ease of fruit production (Zhou et al., 2008). Seed matures in one growing season, does not undergo physiological dormancy, and germinates readily under adequate temperature and moisture conditions without stratification. Breeding populations typically

**Fig. 4** An indoor *P. deltoides* female breeding orchard. In addition to the use of a rooting hormone, soil-warming pads attached to the propagation buckets speed the development of an adventitious root system necessary to sustain development of the seed crop for 8–20 weeks

achieve a level of flowering that is sufficient to initiate selective breeding within 10 years. Asexual reproduction is quite advanced within the genus; this is exploited using either adventitious field rooting of 1-year-old hardwood cuttings or 2-year-old poles (section *Aigeiros* and *Tacamahaca*), or greenhouse rooting of succulent shoots under mist propagation (section *Populus*).

Inter-specific hybridization has figured prominently in poplar breeding from its inception. Inter-sectional crosses between *Aigeiros* and *Tacamahaca* are compatible for the most part although reciprocal crossing effects can be problematic at times. For example, both the *P. deltoides* × *P. trichocarpa* and the *P. deltoides* × *P. maximowiczii* cross combinations are far more productive than crosses in which the *Tacamahaca* parent is used as the female parent (Stanton, 2005; Zsuffa et al., 1999). The same effect has been encountered in breeding the intra-sectional *P.* ×*canadensis* taxon: The *P. deltoides* × *P. nigra* cross is highly fertile but the cross is wholly ineffective when attempted in the reverse, *P. nigra* × *P. deltoides* direction, (Melchior and Seitz, 1968). Section *Populus* is, for all practical breeding purposes, reproductively isolated from *Aigeiros* and *Tacamahaca* due to incompatibility in the pollen-stigma recognition process (Gaget et al., 1984; Villar et al., 1987). However, the use of complex hybrids of section *Populus* (e.g. *P.* ×*canescens* × [*P. alba* × *P. grandidentata*]) as female parents has shown promise in effecting inter-sectional crosses with sections *Aigeiros* and *Tacamahaca* (Ronald, 1982). Species within section *Populus* are freely crossable under artificial conditions, however.

Although arguable, most of the applied breeding work is concentrated on seven species based on investment in controlled hybridization, the number of commercial cultivars in use, and the area under production plantation management. These are *P. deltoides* and *P. nigra* of section *Aigeiros*, *P. maximowiczii* and *P. trichocarpa* of section *Tacamahaca*, and *P. tremula*, *P. tremuloides*, and *P. alba* of section

*Populus*. *Populus* breeders worldwide have used them to develop the following commercial taxa: (1) *P.* ×*wettsteinii*, the intra-sectional combination of *P. tremula* and *P. tremuloides*, (2) *P.* ×*canadensis*, the intra-sectional hybrid of *P. deltoides* and *P. nigra*, (3) *P.* ×*generosa* the inter-sectional hybrid of *P. deltoides* and *P. trichocarpa*, (4) Chinese white poplar (*P.* ×*tomentosa*), the intra-sectional combination of *P. alba* and *P. tremula* var. *davidiana*[2], and (5) intra-specific hybrids of *P. deltoides* (Table 1). Two other taxa lacking assigned hybrid binomials – *P. nigra* × *P. maximowiczii* and *P. deltoides* × *P. maximowiczii* – are currently not as prevalent as the other five but are likely to soon achieve parity in terms of breeding, cultivar development, and the significance of their contribution to *Populus* cultivation.

*Breeding Strategies* – First generation ($F_1$) inter-specific hybridization combined with reciprocal recurrent selection (RRS) of the parental species is the most frequently recommended long-term improvement approach. Today RRS is being used to develop the *P.* ×*wettsteinii*, *P.* ×*canadensis*, and *P.* ×*generosa* taxa. As an alternative to $F_1$ hybridization, $F_2$ *P.* ×*canadensis* breeding is used to develop a synthetic hybrid species. This is noteworthy in view of computer simulations that suggest the advanced generation approach is a more cost-efficient route to genetic improvement than a RRS – $F_1$ program (Kerr et al., 2004). This assumes, however, that there is no breakdown of hybrid vigor in the $F_2$ generation as appears to be true of *P.* ×*canadensis*, a cross between species of the same section, *P. deltoides* and *P. nigra*. Advanced generation breeding of more distantly-related species may experience diminished hybrid performance owing to a number of causes, including the disruption of co-adapted or species-specific linkages within an otherwise integrated genome (Lester, 1973; Stettler et al., 1996).

Other breeding strategies – backcrossing, multiple-species hybridization, polyploidy, somaclonal variation – are not frequently pursued as mainline, long-term breeding approaches although they are used in short-term programs. Examples include: (1) backcrossing $F_1$ *P.* ×*generosa* hybrids to *P. deltoides* for increased resistance to *Melampsora* leaf rust (Pinon et al., 2006), (2) multiple species hybridization of the cross (*P. laurifolia* × *P. nigra*) × *P. maximowiczii* for increased site adaptability (Cagelli and Lefevre, 1995), (3) screening triploid *P.* ×*canadensis* clones for increased growth rate and fiber production (Zhang et al., 2004), and (4) induction of somaclonal variation in a *P. nigra* var. *betulifolia* × *P. trichocarpa* genotype through callus culture, followed by field evaluation and selection for *Septoria* canker resistance (Ostry and Ward, 2003). An amalgam of some of these approaches is employed in developing the *P.* ×*tomentosa* taxon in China.

The popularity of $F_1$ inter-specific hybridization is a result of the predominance of heterosis and the ease with which it can be economically exploited by vegetative propagation: Clonal selection captures the advantages of inter-specific hybrid vigor once the laborious process of controlled hybridization has been completed, while

[2]The origin of *P.* ×*tomentosa* may be in dispute; we consider it as a first-generation hybrid of *P. alba* and *P. tremula* var. *davidiana* following the analysis of Zhang et al. (1995). We assume this taxon is distinct from the *P.* ×*canescens* taxon, itself an inter-specific combination of *P. alba* and *P. tremula*.

**Table 1** Examples of commercial *Populus* cultivars of select taxa registered with FAO's International Poplar Commission

| Taxon | Cultivar | Country of origin |
|---|---|---|
| *P.* ×*canadensis* (Moench) | *P. deltoides* × *P. nigra* 'Blanc du Poitou' | France |
| | *P. deltoides* × *P. nigra* 'Koltay' | Hungary |
| | *P. deltoides* × *P. nigra* 'Luisa Avanzo' | Italy |
| | *P. deltoides* × *P. nigra* 'Manawatu Gold' | New Zealand |
| *P.* ×*generosa* (Henry) | *P. trichocarpa* × *P. deltoides* 'Beaupre' | Belgium |
| | *P. deltoides* × *P. trichocarpa* 'Donk' | The Netherlands |
| | *P. deltoides* × *P. trichocarpa* 'Generosa' | United Kingdom |
| | *P. trichocarpa* × *P. deltoides* 'Hoogvorst' | Belgium |
| *P.* ×*tomentosa* (Carriere) | *P. alba* × *P. tremula* var. *davidiana* 'Dapikongi' | China |
| | *P. alba* × *P. tremula* var. *davidiana* 'Jingxi' | China |
| | *P. alba* × *P. tremula* var. *davidiana* 'Yixiancizhu' | China |
| | *P. alba* × *P. tremula* var. *davidiana* 'Xizhi Xiaiye' | China |
| *P.* ×*wettsteinii* (Hamet-Ahti) | *P. tremula* × *P. tremuloides* 'Astria' | Germany |
| | *P. tremula* × *P. tremuloides* 'Grosshansdorf'[a] | Germany |
| | *P. tremula* × *P. tremuloides* 'Vorwerksbusch' | Germany |
| *P. deltoides* (Bartram ex Marsh.) | *P. deltoides* 'Delta Gold' | United States |
| | *P. deltoides* 'Dunav' | Serbia |
| | *P. deltoides* 'Harvard' | Italy |
| | *P. deltoides* 'Jagdish' | India |
| No hybrid binomial assigned | *P. maximowiczii* × *P. nigra* 'Geyles'[b] | New Zealand |
| | *P. nigra* × *P. maximowiczii* 'Maxfunf' | Germany |
| | *P. maximowiczii* × *P. nigra* 'Rochester' | United States |
| No hybrid binomial assigned | *P. deltoides* × *P. maximowiczii* 'Eridano' | Italy |
| | *P. maximowiczii* × *P. deltoides* 'Suwon' | The Netherlands |

[a]*P. tremula* × *P. tremuloides* 'Grosshansdorf' is a new varietal mixture of 14 individual clonal selections not yet registered with IPC.
[b]Newly-selected cultivar of The New Zealand Institute for Plant and Food Research that will be released in the spring of 2010.

allowing within-family selection with a high degree of precision. While heterosis has been widely assumed in *Populus* breeding, it has not always been substantiated with rigorous controlled studies; oftentimes conclusions are based on a limited sampling of first-generation families or comparisons involving only one parent. Nonetheless, good examples of first-generation inter-specific heterosis, presented as the mean of the $F_1$ family as a percentage of the bi-parental mean or the value of just one parent, are:

1. Increases of 75 and 177% in 2-year-old stem volume in *P.* ×*canadensis* and *P.* ×*generosa*, respectively (Dillen et al., 2009),
2. Ninety percent increase in 3-year-old stem volume of *P.* ×*wettsteinii* relative to *P. tremuloides* (Li et al., 1998; Li and Wu, 1996),

3. Fourty four percent increase in 2nd-year stem volume of *P.* ×*canadensis* (Marron and Ceulemans, 2006), and
4. Fifty percent increase in 4th-year stem volume of *P.* ×*generosa* relative to *P. trichocarpa* (Ceulemans et al., 1992).

Other reports of $F_1$ superiority are based on the performance of individual clones and have been referred to as "clonal heterosis." These, it could be argued, may be equally attributable to transgressive segregation and not hybrid vigor. Even so, Wu et al. (1992) reported clonal heterosis values of 107–123% in stem volume of *P. deltoides* × *P. simonii* clones relative to their *P. deltoides* female parent. And, Yu et al. (2001) reported a 290% increase in 5th year stem volume of four *P.* ×*wettsteinii* hybrid selections compared to local *P. tremula* selections.

Regardless of the strength of the quantitative evidence for hybrid vigor, experimentation into the morphological and physiological components of hybrid growth and development substantiates such claims. For instance, it has been accepted for some time that the inheritance of large leaf cells from *P. trichocarpa* and the greater density of leaf cells from *P. deltoides* result in their $F_1$ progeny's increased leaf area, which allows for greater light capture and superior yield in the *P.* ×*generosa* taxon (Ridge et al., 1986). In the *P.* ×*canadensis* taxon, increases in leaf increment rate and leaf area are similarly important in explaining the superiority of the $F_1$ generation (Marron and Ceulemans, 2006), although increased production of gibberellins may also be a controlling factor in the *P.* ×*canadensis* heterotic growth response (Bate et al., 1988). Differences between *P.* ×*wettsteinii* and *P. tremula* genotypes in the size of guard cells has been documented but is not clearly related to hybrid vigor in the taxon (Yu, 2001).

The financial impact of hybrid vigor is, as would be expected, of notable effect in *Populus*: Economic analyses of *Populus* genetic improvement have demonstrated internal rates of return approximating 13% for plantation operations with yield increases of 10–15%, which supports using advanced generation *Populus* breeding programs to achieve sustained advancement in heterosis (Van der Meiden, 1977). Five such long-term improvement programs illustrate the range of breeding strategies in use throughout the world today. Three are based on $F_1$ inter-specific hybridization coupled with a variety of recurrent parental species selection programs; a fourth includes a combination of advanced generation techniques and polyploid breeding; and the fifth is an intra-specific recurrent breeding program. These are: (1) the University of Minnesota's Aspen-Larch Cooperative's *P.* ×*wettsteinii* program, (2) the Italian *P.* ×*canadensis* program led by the Poplar Research Institute at Casale Monferrato, (3) GreenWood Resources' *P.* ×*generosa* breeding program, (4) Beijing Forestry University's *P.* ×*tomentosa's* breeding effort, and (5) the U.S. Forest Service-Industrial *P. deltoides* program for the southeastern United States. A critical element of all long-term breeding programs is the prediction of parental breeding values. This is especially challenging when breeding $F_1$ inter-specific hybrids where the expense of managing multiple parental species via a reciprocal recurrent breeding program may be prohibitive. To overcome this hurdle, a simple recurrent breeding program can be substituted for the more complicated and

involved reciprocal recurrent programs, especially if pure-species general combining ability estimates are a reliable gauge for general hybridizing ability (see Dungey, 2001; Nikles,1993 for a review in *Pinus*).

## *3.1 Examples of Long-Term Breeding Programs*

*P. ×wettsteinii – Modified Reciprocal Recurrent Selection* – The *P. ×wettsteinii* taxon is being developed for European plantations in Scandinavia and the Baltic States (Rytter and Stener, 2003; Tullis et al., 2007). This taxon is also viewed favorably in North America where the University of Minnesota has been hybridizing *P. ×wettsteinii* since 1952 through its Aspen and Larch Genetics Cooperative in support of the pulp and paper and the oriented strand board industries. Long-term improvement is based upon full-sib reciprocal recurrent selection of *P. tremuloides* and *P. tremula* for inter-specific heterosis in yield, in addition to improvements in wood quality and *Hypoxylon mammatum* canker resistance (Li and Wyckoff, 1991). Breeding populations are sized at 150 individuals for each parental species utilizing *P. tremula* selections from Poland, Germany, and The Netherlands, and *P. tremuloides* selections from Wisconsin, Michigan, Minnesota, and Saskatchewan. Reciprocal crossing effects are non-existent and hybrid crosses are made in both directions. The 300 genotypes of the two breeding populations are assigned to 25, 6 × 6 disconnected factorials to develop 900 full-sib $F_1$ families for the first cycle of inter-specific progeny tests. Evaluation is conducted at age five leading to the identification of 100 parents of each species that are mated intra-specifically using a circular mating design. A second evaluation of the inter-specific population conducted at age 10 is then used to choose the top 75 parents whose intra-specific progeny enter field trials. (The purpose of the two-stage selection is to accelerate the initiation of the intra-specific crosses to shorten the generation intervals; in this sense the reciprocal recurrent selection program is modified.) One hundred and fifty selections of each species are grafted into clonal breeding orchards for use as parenting stock for the second cycle of first generation inter-specific hybridization. Disconnected factorials are again used to generate 900 inter-specific families; the best 45 individuals are selected for clone deployment to commercial plantations.

*P. ×canadensis – Semi-Reciprocal Recurrent Selection* – The *P. ×canadensis* inter-specific hybrid taxon is perhaps the world's most widely planted, used in operations on all five continents where *Populus* is grown. The most advanced *P. ×canadensis* breeding program is conducted in Italy by the Poplar Research Institute at Casale Monferrato in the Po River Valley (Bisoffi and Gullberg, 1996). Traditionally bred for the veneer industry, development of the taxon now also targets renewable energy feedstock. The main selection targets are growth rate and *Marssonina* leaf spot resistance. The reciprocal crossing effect in *P. × canadensis* has defined the Italian strategy to the extent that parental inter-specific hybridizing values can only be estimated for *P. deltoides* females and *P. nigra* males (Bisoffi, 1990). Thus, the program is known as semi-reciprocal recurrent selection.

The program began with 150 selections of *P. deltoides* and an equivalent number of *P. nigra* selections that are managed as single breeding units, because interactions between planting site and parental breeding values appear unimportant. Female *P. deltoides* selections are evaluated for general hybridizing ability (GHA) in a *P. nigra* polycross mating design. But because of the inability to reproduce the *P. nigra* × *P. deltoides* cross, general combining ability (GCA) estimates are relied upon in the evaluation of *P. nigra* females using the same *P. nigra* pollen mix used in the inter-specific polycross evaluation of female *P. deltoides*. Breeding value estimation of *P. deltoides* (GCA) and *P. nigra* (GHA) males is based upon the common use of a tester mating design of six *P. deltoides* females. As an alternative to conventional polycross breeding, Wheeler et al. (2006) proposed combining this method with paternity analysis for a *P. deltoides* × *P. nigra* reciprocal recurrent selection program as a way to manage inbreeding in each of the parental species when individual components of the pollen mixes vary insubstantially in reproductive success.

One noteworthy research finding of the Italian program has been the reasonable correlation between parental genotypic values and GHA values for several traits (Bisoffi, 1990). Genotypic values are now partially relied upon as surrogates for breeding value estimates, because of the inability to reproduce the reciprocal cross. As such, clonal trials of parenting stock are important adjuncts in the management of recurrent breeding populations, although conventional inter-specific progeny tests still figure into the estimation of GHA values for female *P. deltoides* and male *P. nigra* parents. The semi-reciprocal recurrent selection program renews each generation with 300 selections of each species and a balanced sex ratio. Improvement for growth traits emphasizes selection within full-sib families while among-family selection is emphasized for *Marssonina* resistance. The Italian program also includes a simple recurrent *P.* ×*canadensis* selection program to develop a synthetic inter-specific hybrid species using the rationale that an additive model may be a more appropriate explanation for heterosis than one based on overdominance.

*P.* ×*generosa – Multiple Population Breeding* – The *P.* ×*generosa* taxon has been a staple of *Populus* culture in western Europe since the 1960s. In North America, development of clonal plantations and controlled breeding began in the Pacific Northwest in the 1980s in response to a shortage of hardwood fiber for the manufacture of high-quality communications-grade paper (Stettler et al., 1988). Drs. Reinhard Stettler of the University of Washington and Paul Heilman of Washington State University worked together on developing the region's initial hybrid varieties during the 1970–1980s. Today, GreenWood Resources manages 14,000 hectares of *Populus* operations along the lower Columbia River floodplain on the windward side of the Cascade Mountains and, on the leeward side, in the arid mid-Columbia River basin for the production of quality saw logs on 12–15 year rotations using varietal selections of the $F_1$ *P.* ×*generosa* taxon. Long-term improvement began with the assembly of breeding populations of both *P. trichocarpa* and *P. deltoides*. The *P. trichocarpa* effort began with replicated clonal testing of 1,428 genotypes assembled from 67 provenances along the windward slope of the Cascade Mountains between 48° 56′ and 42° 56′ N latitude in Washington and Oregon leading to the identification of 328 superior genotypes. Paralleling this, a *P. deltoides* breeding

population was comprised of 204 second-generation clonal selections from 104 full-sib families bred from superior first generation clonal selections originating between 35° 14′ and 30° 36′ N latitude in Tennessee, Mississippi, and Louisiana. Genotypes of each species are being re-tested to arrive at a final breeding population of 144 individuals, the upper one-third of which will be assigned to one of three multilines designed for the improvement of solidwood, bio-energy, and pulping applications. Each multiline is managed with eight female and eight male genotypes that are crossed intra-specifically using a circular mating design to develop superior selections – based on GCA estimates as well as genotypic values – for the second cycle of $F_1$ inter-specific hybridization. Parental genotypes not assigned to one of the three multilines are managed as a single unit to allow for the creation of trait combinations that would not occur otherwise.

*P. ×tomentosa – Backcross, multi-species and polyploid breeding* – *P. ×tomentosa* is a naturally-occurring hybrid of *P. alba* and *P. tremula* var. *davidiana* (Zhang et al., 1995), although some consider it a hybrid of *P. alba* and *P. adenopoda*. It is China's most valuable native *Populus* planted throughout the Yellow River drainage from Shanxi Province in the interior eastward to the coastal province of Shandong. It is managed for the plywood and pulp and paper industries and is valued for its growth rate, pest resistance, and wood quality. Few native stands remain and today's hybridization work relies upon backcross varieties developed over 50 years ago. However, a concerted effort of phenotypic selection, provenance evaluation, and family evaluation was initiated nearly 20 years ago for one of the hybrid's parental species, *P. tremula* var. *davidiana* (Li et al., 1999). Promising new crosses with *P. alba* also have been accomplished, foreshadowing the possible production of a second cycle of $F_1$ *P. ×tomentosa* hybridization.

A national tree improvement program was launched in 1983 by Beijing Forestry University. Initially 1,047 superior phenotypes were selected throughout the hybrid's natural range and established in provenance-clonal trials. This led to the release of 12 genotypes for the commercial plywood and construction industry with yield improvements of 40–50% (Zhu and Zhang, 1997). Continued breeding relies on the original germplasm collection now established in breeding arboreta and used in support of: (1) backcross hybridization of [*P. ×tomentosa* × *P. alba* 'Bolleana'] × *P. ×tomentosa* primarily for the pulp and paper industry, and (2) multi-species hybridization involving *P. ×tomentosa* × *P. alba* 'Bolleana' hybrids in crosses with *P. ×canescens*, bigtooth aspen (*P. grandidentata*), Chinese aspen (*P. adenopoda*), and *P. tremuloides* for the colder, arid region of northeastern China and Inner Mongolia. A third approach – allotriploid breeding – is conducted using diploid *P. ×tomentosa* pollen in crosses with normal haploid gametes of *P. ×tomentosa* ×*P. alba* 'Bolleana' female selections (Zhu et al., 1995). Triploids have also lately been bred using diploid *P. ×tomentosa* pollen in crosses with *P. alba* × *P. glandulosa* $F_1$ hybrid females (Wang et al., 2008). Polyploids are valued for pulp and paper applications, because of their high cellulose-to-lignin ratio, increased fiber length, and superior growth rate.

*P. deltoides – Recurrent Selection for General Combining Ability* – *P. deltoides* is the genus' most important species used as breeding and/or propagation stock in North and South America, Europe, Asia, and Australia. In North America, the

decline in natural regeneration along the Mississippi River in the 1960s gave rise to a *P. deltoides* improvement program initiated by the U. S. Forest Service's Stoneville, Mississippi Experimental Station. (Inter-specific hybrids have not performed well in this region, because of their susceptibility to *Septoria* stem canker.) The Stoneville project began with a collection of 3,700 genotypes from provenances of the Mississippi River between Memphis, Tennessee (35° 14′ N latitude) and Baton Rouge, Louisiana (30° 36′ N latitude) and from the Brazos, Trinity, and Red Rivers of east Texas and Oklahoma between 35° 57′ N and 30° 03′ N latitude. These were screened in a number of replicated clonal field trials culminating in the identification of 197 genotypes that were brought together and retested in 1980 in a series of cooperative industrial trials at Wickliffe, Kentucky (Westvaco Corporation), Fitler, Mississippi (Crown Zellerbach Corporation), and Profit Island, Louisiana (Trans Match Corporation). The objective of these so-called advanced clone trials was to determine the extent of genotype-by-environmental interactions throughout the lower Mississippi River Valley when composing breeding populations for long-term, recurrent selection and breeding (Cooper, 1980).

The goal has been a recurrent selection program for general combining ability for the production of multiple-purpose clones for pulpwood, saw timber, and veneer (Cooper, 1976). Priority selection criteria have included adventitious rooting, growth rate, resistance to *Melampsora* leaf rust and *Marssonina* leaf spot, and wood specific gravity (Robison et al., 2006). Because of an imbalanced sex ratio in the first-generation clonal collection, a male-in-female nested mating design was proposed to develop the second-generation (Foster, 1984); 50 male and 25 female selections theoretically could be mated hierarchically to generate 50 full-sib families, each represented by seven full sibs. From the resultant population of 350 second-generation genotypes, combined selection would identify 36 genotypes for assignment to four, 4 × 5 disconnected factorials to generate 80 third-generation families of seven sibs (560 in total). Yield improvements through the third generation were projected at 49% relative to unimproved base population stock.

In 2000, Mississippi State University undertook a large-scale sampling of 64 provenances throughout the southeastern United States approximately between 75° and 90° W longitude and 30° and 36° N latitude. Preliminary evaluations for leaf rust and growth rate at multiple sites has identified new selections that compete with the best commercial standards from the original U. S. Forest Service project (Jeffreys et al., 2006). Furthermore, a significant source of *Melampsora* rust resistance has been identified within populations residing in the southeast Atlantic and east Gulf regions (Land and Jeffreys, 2006).

## 4 Testing and Selection

*Experimental Techniques – Populus* genetic evaluation field trials are, in nearly all cases, clonally replicated in view of: (1) the added precision of estimating genetic parameters and increases in genetic gain during recurrent selection (Shaw and Hood,

1985), and (2) the need to expedite the selection of new varieties required by commercial ventures to substitute for the lowest-ranking varieties presently in use (Foster and Shaw, 1988). Replication of individual genotypes has the potential for creating very large test populations and, as a consequence, a multiple-stage evaluation process has been recommended to accommodate such sizable experiments (Libby, 1987). The number of genotypes is sequentially reduced between test stages with attendant increases in replication, spacing, test locations, and rotation (Cooper, 1976). Typically, testing begins with seedling populations that undergo combined family and within-family selection. Selected genotypes are then clonally replicated in first-stage field trials; these evaluate clones grown in small-sized plots where inter-genotypic competition is unchecked and are usually established at multiple sites (Riemenschneider et al., 2001). The final stage involves a limited number of highly-selected clones that are established in monoclonal plots of sufficient size to provide reliable growth and yield estimates.

Assuming that a fixed amount of resources place a limit on the number of experimental plants, the optimum combination of clones and ramets in preliminary screening trials is based upon the desired degree of genetic gain, expected level of clonal variance, and the required precision of selection. The most frequently chosen experimental design approximates dozens to several hundred clones tested in multiple, three- to five-tree row plots, and planted using a randomized complete block design of four to12 replicates (Ares, 2002; Hansen et al., 1992; Isik and Toplu, 2004; Koo et al., 2007; Riemenschneider et al., 2001; Yu and Pulkkinen, 2003; Zhang et al., 2008). Cooper (1976) reported the success of a variety of incomplete block designs, e.g. triple, balanced, and cubic lattices, in accounting for edaphic variation in *P. deltoides* clonal tests. Today, row-column designs[3] are considered preferable to the randomized complete block design for clonal evaluations based on simulation studies that show increases in precision of up to 10% (Gezan et al., 2006a). Related to the issue of optimum experimental designs is the correct number of replications and plot structure for clonal testing: Replication rates of two-to-six ramets per genotype as single-tree plots are considered to provide the greatest experimental efficiency dependent on micro-site variability and clonal repeatability (Gezan et al., 2006b; Isik et al., 2005; Russell and Libby, 1986, Fig. 5). Statistical efficiencies have also been achieved in experiments of *Populus* clonal adaptability to varying nutrition and salinity levels through the use of analyses of covariance that account for variation in the dimension of the propagation stock used to initiate field trials (Fung et al., 1998; van den Driessche, 1999). More recently, mixed model techniques have been developed to account for the covariance of within-clone residual effects to further increase the precision of *Populus* clonal comparisons (Zamudio et al., 2008).

A mid-rotation or later schedule has been the longstanding recommendation for the timing of varietal selections for growth and yield improvement in *Populus* (Mohrdiek, 1979; Zsuffa, 1975). An early report by Cooper and Ferguson (1979)

[3]Row-column designs are experimental designs that control or block site heterogeneity that occurs in two directions.


**Fig. 5** Multiple-tree plots may not lend as much experimental efficiency as single-tree plots assuming the same number of ramets per clone. In this photograph of one replicate of a five-tree row plot, little micro-environmental variation is detected among the first four ramets in the plot. This provides little additional accuracy compared to an independent randomization of the five trees as single-ramet plots over a wider area

showed that rotation-age selection for height and diameter in *P. deltoides* resulted in two to three times the magnitude of improvement when selections were made at approximately one-third of a rotation length. Yet within the context of Libby's (1987) multiple-stage test protocol, truncation of base populations using a relatively low level of selection intensity at preliminary stages to identify a subset that undergoes more concentrated testing now is in vogue in many *Populus* testing programs. Kumar and Singh (2001) suggested using a 60% truncation rate for *P. deltoides* clonal test populations at age two to maintain a reasonable certainty that the top clones are included in the final age-four evaluation. Yet when viewed from the perspective of gain-per-unit time, early selections of individual genotypes for rotation performance may stand on their own merits: An evaluation of *P.* ×*wettsteinii* demonstrated that selection of cloned genotypes for stem volume at age three was two and one-half times as efficient as selection at age nine, based on an annualized rate of improvement (Stener and Karlsson, 2004). However, these authors also point out that early selection in *P.* ×*wettsteinii* is compromised by the inability to evaluate *Hypoxylon* canker resistance that does not express itself until much later during stand development. Furthermore, inspection of *P. deltoides* clone trials in Argentina in which one-third of the clonal distribution underwent significant changes in growth ranking between the third and 9th years concluded, as did Kumar and Singh (2001), that early selections are best focused on groups of clones rather than individual genotypes (Ares, 2002). Thus the mid-rotation recommendation generally remains

valid for growth and yield traits. Matyas and Peszlen (1997) further extended this recommendation to the selection of wood quality traits where early stage selections for specific gravity, modulus of rupture, and modulus of elasticity proved unreliable.

Statistical techniques employed for *Populus* varietal evaluation have focused on three areas: (1) simultaneous selection for multiple traits, (2) classification of clonal phenotypes for extended testing, and (3) accounting for the effects of genotype-by-environment interaction. Initially, the need to select *Populus* cultivars for a multiplicity of traits was accommodated by the construction of independent culling levels. A good example is the use of selection thresholds for growth rate, resistance to *Marssonina brunnea*, and *Melampsora* spp. leaf diseases, stem form, and wood quality in the selection of clones for quality veneer log production for Portugal's match stick industry (Monteiro, 1988). Alternatively, index selection is now more frequently used in the evaluation of multiple criteria, especially in view of the developing definition of *Populus* ideotypes that has extended the range of target traits to several morphological and physiological variables. Riemenschneider et al. (1992) utilized this technique where restricted indices were used to check unfavorable correlations between growth and pathogen susceptibility that would otherwise compromise overall genetic gains in growth rate.

The need to group *Populus* genotypes for extended testing has relied upon multivariate analysis techniques. For example, Tharakan et al. (2001) used cluster analysis to select the best subset of 38 *Populus* and willow clones for yield trials based on covariances in stem volume, growing season length, and survival. Abrahamson et al. (1990) used the same multivariate technique to categorize the performance of 54 clones of diverse taxa to identify an optimum class for immediate operational use in coppice rotations, as well as a separate class for extensive site adaptability testing. Riemenschneider et al. (2001) used principal component analysis to categorize best-adapted sets of clones for regional deployment. Isik and Toplu (2004) also used principal component analysis to distinguish genotypes within a clonal collection of *P. nigra* and *P.* ×*canadensis* based on co-variation in growth, stem form, apical dominance, branching, spring and autumnal phenology.

Genotype-by-environment interactions have been reported as variously significant in *Populus* clonal testing but include notable occurrences where the interaction component of variance for growth exceeded the clonal component by 85% in one *P.* ×*wettsteinii* study (Yu and Pulkkinen, 2003), and by 51% in a study of predominately *P. deltoides* intra- and inter-specific varieties (Riemenschneider et al., 2001). Yet, it has been shown several times that individual genotypes of good commercial appeal can be found that perform well across a range of environments with striking contrasts in climate and soils (Wu and Stettler, 1997). Broadly-adapted varieties are often the selection objective. Analysis techniques to identify such genotypes have made use of standard phenotypic stability parameters – slope coefficients and mean square deviations from the linear regression of genotypic means on environmental means. This approach has been used to evaluate *P. tremula* var. *davidiana* clone trials in Korea (Koo et al., 2007) and in the analysis of *P.* ×*wettsteinii* clonal performance in Finland (Yu and Pulkkinen, 2003). Similar to genotype-by-environmental

interaction analyses, has been the treatment of *P.* ×*generosa* genotype-by-time interactions in periodic growth increments that were analyzed using a spit-plot design for repeated measurements to partition the interaction variance into its linear and quadratic components to identify selections suited to short rotation management (Stanton, 2001).

*Selection Criteria* – Reflecting the suitability of its wood for a wide diversity of markets, *Populus* breeding has focused on an equally wide array of improvement criteria. However, improvement in agronomic characteristics – yield, climatic adaptability, adventitious rooting, disease resistance, etc. – invariably has been the priority in breeding programs. Wood quality also has received attention, mostly focused on improvements in specific gravity. This focus is expanding now to include more routine evaluations of physical and chemical wood components in light of the escalating interest in *Populus* biomass energy feedstock and the advent of increasingly affordable and reliable assessment methods.

Growth rate variation in preliminary stage clone trials is usually quantified in terms of stem height and diameter as surrogates for individual tree volume, itself an indicator of stand yield. Between the two, stem diameter has proven over a lengthy period to be the more important, because it is more easily and more accurately measured and has a much larger impact on the determination of stem volume (Mohn and Randall, 1971). Clonal repeatabilities typically fall within a range of 0.40–0.70 depending whether they are calculated on an individual tree, or on a clone mean basis and whether they are reported for an entire population or a within-family basis. Predicted levels of gain are often substantial as illustrated in the following:

1. Selection of the top 10% of a *P.* ×*wettsteinii* clonal distribution evaluated across multiple sites at age nine predicted a 45% increase in stem volume based on a broad-sense heritability of 0.39 (individual tree basis) (Stener and Karlsson, 2004);
2. Selection for site-specific clone performance in *P.* ×*tomentosa* predicted a 34% improvement in 5th-year stem volume based on the use of the topmost one-third of the clonal distribution and a clone mean repeatability of 0.90 (Zhang et al., 2008);
3. Selection of the uppermost 5% of the distribution of a *P. deltoides* × *P. simonii* clonal population was associated with an estimated 51% improvement in 6th-year volume with repeatabilities of clone mean height and diameter of 0.93 and 0.95, respectively (Wu et al., 1992);
4. Selection of the top 12% of the multiple-site clone ranking in a *P.* ×*wettsteinii* experiment in which genotype-by-site interactions were highly significant led to predicted increases in 3rd-year stem diameter growth of 15% associated with a clonal repeatability of 0.52 (clone mean basis) (Yu and Pulkkinen, 2003);
5. Selection of approximately the best 8% of the clone distribution at each of three sites resulted in an estimated improvement in age six yield of 23–89% (Riemenschneider et al., 2001); and,
6. Selection of approximately the top 12% of the genotype distribution in a multi-site *P. tremula* var. *davidiana* clone trial equated to a selection differential in 12th-year stem volume of 19% (Koo et al., 2007).

Survival as a component of yield in short-rotation clonal plantations has a greater impact on productivity than individual stem volume when survival rates fall below 90% (Chambers and Borralho, 1997). Thus clonal evaluation for the ability to undergo vegetative propagation at consistently high establishment rates is quite important and, dependent upon the taxon in question, is an afterthought, challenging, or frustratingly elusive. For instance, section *Tacamahaca* and their inter-sectional hybrids propagate easily from un-rooted, 1-year-old hardwood cuttings when compared with species and intra-sectional hybrids of section *Aigeiros* (Zalesny et al., 2005). On the other hand, hardwood cutting propagation of *P. deltoides* is often quite variable, though good selection opportunities exist within southerly populations with clonal repeatabilities for number of roots (individual tree basis) varying between 0.33 and 0.53 dependent on soil type (Wilcox and Farmer, 1968). However C-effects often associated with the position from which cuttings are taken from stock plants, will potentially confound genetic effects in any clonal evaluation of adventitious rooting ability (Farmer et al., 1989; Zalesny at el. 2003). Schroeder and Walker (1991) showed, for example, that cuttings taken from the basal portion of 1-year-old nursery whips rooted with an increased frequency of 30% and produced sprouts that were 16% taller at the end of the first season when compared with cuttings of the same genotype cut from the distal portion of the whip. In section *Populus*, vegetative propagation from hardwood stem cuttings is extremely problematic, and cloning has resorted to either root cuttings or succulent stem cuttings propagated under mist as containerized, greenhouse-grown stock (Haapala et al., 2004; Stenvall et al., 2006). The genotype factor is again of critical importance. Stenvall et al. (2006) reported more than a fivefold difference in rooting percentage among *P.* ×*wettsteinii* clones grown from root cuttings. Haapala et al. (2004) reported similar findings in *P.* ×*wettsteinii* using succulent cuttings where survival varied among clones between 36 and 85% when using a hedge propagation system and between 0 and 89% when using a serial propagation method. Selection opportunities to improve the success of succulent stem cutting propagation of *P. alba* have been reported where the clonal repeatability for adventitious root initiation and establishment is 0.34 (Harfouche et al., 2007).

Another class of biomass productivity components – morphological and physiological characteristics – has also been extensively studied in *Populus*. Most notably, these are associated with leaf area, both on an individual leaf basis as well as a whole crown basis (Harrington et al., 1997; Li et al., 1997; Marron et al., 2007). The hope has been that such characteristics used as indirect selection criteria might provide for greater selection efficiency compared with clonal evaluations based solely on yield, dependent on the degree of genetic control, genotypic co-variances, and the age and cost with which they can be assessed. While an active area of research, morphological and physiological traits have not yet played a role in applied *Populus* breeding; estimates of genetic parameters will partially determine how well they fulfill their potential role. To illustrate, Monclus et al. (2005) calculated a broad-sense heritability of 0.63 for maximum seasonal leaf area in a clonal *P.* ×*canadensis* population where variation was associated with biomass production with a linear correlation coefficient of 0.74. A similar correlation coefficient of 0.84 was observed for the

linear relationship between individual leaf area and biomass production in a high-density coppice system of mostly *P.* ×*generosa* genotypes (Bunn et al., 2004). Rae et al. (2004) also demonstrated that biomass production in a *P.* ×*generosa* coppice system was associated with clonal variation in individual leaf area, the number of leaves on the dominant sprout, and leaf plastochron index that, in turn, exhibited within-family, broad-sense heritabilities varying between 0.37 and 0.62. In a limited number of *P. deltoides* and *P.* ×*canadensis* genotypes, Dowell et al. (2009) presented evidence that the more productive ones were also those that could be characterized by higher levels of cumulative leaf area indices throughout the course of the growing season. Finally, Bonhomme et al. (2008) determined linear correlations for the *P.* ×*canadensis* taxon between stem growth and several leaf traits that were non-significant in some instances, e. g. specific leaf area, $r = 0.35$, while significant in others, e.g. foliar nutrient contents, $r = 0.62$–$0.50$.

Orlovic et al. (1998) extended the list of potential indicators of biomass productivity to thickness of the palisade parenchyma leaf tissue and the number of stomata on the adaxial leaf surface in *P.* ×*canadensis* both of which exhibit moderately strong broad sense heritabilities, e.g. 0.52 – 0.62, and reasonable phenotypic correlations with yield based on genotypic means, e.g. 0.66–0.84. Regardless of whether morphological and physiological selection criteria fulfill a role as indirect selection criteria for segregating populations under evaluation they will be, nonetheless, important when choosing species for inter-specific hybridization programs based on trait complementation (Marron et al., 2007) or when defining selection objectives for specific markets or end products (Scarascia-Mugnozza et al., 1997).

Selection for pest resistance has been of great importance since the inception of *Populus* breeding due to the challenge posed primarily by a range of pathogens that infect both crown and stem. The genetics of pathogen resistance has been most extensively studied in *Melampsora* leaf rust and involves both qualitative and quantitative components (Dowkiw and Bastien, 2007; Lefevre et al., 1998; Newcombe, 1998; Newcombe and Ostry, 2001). Qualitative resistance is expressed in the host's hypersensitive response to infection conditioned by major genes whose effect is mediated by modifying genes. When the hypersensitive response is lacking, polygenic resistance comes into play as a quantitative expression of variation in latent period, sporulation rate, and the number and size of uredina. Although a number of laboratory procedures have been developed to assess the components of leaf rust resistance, genotype selection is regularly evaluated in the field using a categorical scoring method (Fig. 6). Although not measured as a true metric character, resistance has been invariably treated as such in quantitative analyses that report clonal repeatabilities of 0.50–0.80 (Jokela, 1966; Thielges and Adams, 1975). Stem-cankering pathogens are also important in *Populus* breeding, with *Septoria musiva* and *Hypoxylon mammatum* recognized as the most serious threats. Resistance evaluation involves field trials, but oftentimes incorporates artificial stem inoculations to increase the reliability of genetic selections that differentiate pathogen resistance from pathogen escape (Enebak et al., 1999; Weiland et al., 2003). Resistance to *Septoria* canker may be conditioned by the thickness of the periderm and the formation of necrophylactic periderm as a pathogen-containing response following

**Fig. 6** Selection for field resistance to *Melampsora* leaf rust can be decidedly effective. Pictured are 2-year-old test ramets of a highly susceptible clone in the foreground growing alongside a highly resistant variety in the background. The taxon is *P.* ×*generosa* and the tree spacing is 1.9 × 1.9 m

infection (Weiland and Stanosz, 2007). *Hypoxylon* canker resistance in *P. tremuloides* involves the development of a lignified response zone within proximity of the infection followed by rapid development of callus tissue (Bucciarelli et al., 1999). Unlike the *Melampsora* system where testing for resistance to a range of pathotypes is important, the same consideration seems less critical in screening for *Septoria* canker resistance where a more stable pathosystem may exist (LeBoldus et al., 2008; Ward and Ostry, 2005).

Specific gravity has been the most commonly evaluated wood quality trait included in *Populus* breeding programs, usually as a component of biomass yield (Farmer and Wilcox, 1968; Olson et al., 1985) but also as an attribute of wood quality (Mutibaric, 1971). It normally shows moderate to strong levels of clonal repeatability that can reach as high as 0.90 (Song et al., 1997; Zhang et al., 2003) but often is coupled with a limited range of variation relative to growth variables, e.g. coefficient of genotypic variation less than 10% (Pliura et al., 2007; Song et al., 1997). The relationship between specific gravity and radial growth is of keen interest and has varied from non-significant (Zhang et al., 2003) to weakly negative

(Ivkovich, 1996; Song et al., 1997), leading to the recommendation that simultaneous selection for clones of above average growth and specific gravity is possible. However, negative genotypic correlations between stem volume and wood specific gravity that are of moderate or moderately-strong effect (e. g. –0.59 to –0.74) have led to the opposing recommendation that selection should be based on an integrated measure of the two – dry fiber weight – as opposed to selection to improve both simultaneously (Olsen et al., 1985; Pliura et al., 2007). Genetic improvement opportunities for *Populus* renewable energy feedstock – specific gravity, lignin content, etc. – are now of growing interest (Davis, 2008). Frequently, these traits are being quantified for large populations under selection using near infrared spectrometry. Maranan and Laborie (2007 and 2008) reported correlations between near infrared spectral data and selected wood chemical and physical properties within a range of 0.80–0.95. Schimleck et al. (2005) also reported a similarly strong predictive relationship, i.e. $r = 0.94$, for cellulose content in *Populus* using near infrared spectroscopy.

## 5 Translational Genomics

We define translational genomics as the research and development process that bridges the basic discovery phase and the application phase in commercial breeding programs. This has often been neglected, because the motivation for application is sometimes lacking among academic researchers, and the development of genomic tools is too basic for applied programs with little or no research budgets. Translational research is an important and active area in human medical research and is increasingly seen as very important in agriculture and forestry. In traditional forest tree breeding there has been little, if any, gap between the basic and applied phases. As we saw earlier in this chapter, it was often academic researchers who developed *Populus* hybrids that were quickly used in plantations. However, with the emergence of biotechnologies and genomics sciences, it can be a long way from the discovery of a gene to the release of a new variety or improved populations. In the United States and elsewhere, research cooperatives have taken a quasi translational role, but these cooperatives have had difficulty sustaining financial support and many have disappeared. Notably in the United States, the Poplar Molecular Genetics Cooperative led by Dr. Toby Bradshaw at the University of Washington in Seattle, Washington sought to bring genomics-based breeding to application in *Populus* breeding. This program was probably too early for its time and is badly needed now that massive genomics resources are available. However there is no infrastructure currently in place to translate this resource and knowledge base into application.

In response to this widening gap between basic genomic discovery and direct application in plant breeding, the United States Department of Agriculture developed the Plant Genome Coordinated Agricultural Product (CAP) as a publicly-funded program to bring discovery to application in crop and forest tree breeding in

the United States. Each year, a research group focused on a single crop species is awarded a grant of five million dollars for four years. Awards have been made for rice, wheat, barley, Solanaceae and conifers. The conifer CAP is called the Conifer Translational Genomics Network (www.pinegenome.org/ctgn) and is a consortium of six universities, four tree breeding cooperatives, and the United States Forest Service. A similar forest tree translational genomics project also was funded in Europe. It is called NovelTree (www.noveltree.eu). A translational genomics project for *Populus* has not yet been funded but planning is underway for a *Populus* CAP in the United States.

Broadly defined, translational genomics might include all biotechnological approaches to tree improvement. These include traditional breeding, marker-based breeding, and transgenic or genetically modified trees (White et al., 2007). In this chapter, we focus entirely on marker-based breeding (see Chapter 19, White et al., 2007). Readers are referred to a number of excellent reviews on transgenic approaches (Strauss et al., 2004). Before marker-based breeding can be applied, associations between genetic markers and traits of interest must be discovered. We will first briefly review how marker-trait associations are found and summarize the state of this knowledge in *Populus*. A more detailed review of this topic can be found in Section 2.2 of this volume.

## *5.1 Discovery of Marker-Trait Associations*

There are many approaches to discovering marker-trait associations (see Chapter 18, White et al., 2007). These include: (1) two-point linkage analysis between a marker and a qualitatively inherited trait such as a disease resistance gene, (2) quantitative trait locus (QTL) mapping, and (3) association genetics. All three approaches have been used in several *Populus* species and for a variety of traits, e.g. bud phenology, water use efficiency, disease resistance, biomass production and others; however, the catalog of validated marker-trait associations is very limited (see Section 2.2). The two-point linkage approach has been used successfully a number of times to map single genes coding for resistance to *Populus* leaf rust (*Melampsora* spp.) (reviewed by Feau et al., 2007). QTL mapping has been used to identify map locations of QTLs for a number of quantitative traits but, in fact, the number of validated marker-trait associations found through QTL mapping is so limited that this knowledge base could never support a marker-based breeding program of any kind in any species. For the reasons described in Section 2.2, the QTL approach is rarely used in *Populus*.

The association genetics approach holds much more promise for identifying marker-trait associations in *Populus* and other trees (Neale and Savolainen, 2004; Neale and Ingvarsson, 2008) and is now the method of choice in *Populus* (Section 2.2). There are several steps in the implementation of an association genetics research program that we will review for *Populus*. We will also identify gaps in the *Populus* discovery stream that will need to be filled before genomic-based breeding can be fully realized. The steps in the discovery process include: (1) population

development, (2) test site establishment, (3) candidate gene identification, (4) resequencing and SNP discovery, (5) genotyping platform development, (6) phenotypic evaluation, and (7) tests for association and validation. The status of each of these steps is summarized for several species and association genetics studies around the world in Table 2.

*Population development and test site establishment* – To begin, association genetics studies have been initiated in just a few *Populus* species (*P. deltoides, P. nigra, P. tremula* and *P. trichocarpa*). Earlier in this chapter we described four inter-specific taxa that are of most interest (*P.* ×*canadensis*, *P.* ×*generosa*, *P.* ×*tomentosa,* and *P.* ×*wettsteinii*). Perhaps not so coincidentally, the parentage of these hybrids frequently involves all of the species listed above for which association genetics studies have been initiated. However, association genetic studies are lacking in three other similarly important species – *P. alba*, *P. maximowiczii,* and *P. tremuloides*. A resequencing and SNP discovery project is underway in *P. alba* as will be discussed in the next section.

Forest tree association genetics populations can be either clonal, family-based or both (Neale and Savolainen, 2004). There are relative merits to each population type (Gonzalez-Martinez et al., 2007). In *Populus*, clonal propagation is easy and is used in all of the populations listed in Table 2. This not only increases heritability but also allows for establishment of multiple test sites and estimation of genotype-by-environment interactions, something that nearly all studies have employed. Population sizes vary from 350 (*P. tremula*) to 1,100 (*P. trichocarpa*) in the ongoing United States Department of Energy's Bioenergy Science Center[4] – University of British Columbia joint study. More is always better, but these population sizes are probably of adequate power to detect associations.

*Candidate gene selection, resequencing and SNP identification* – Resequencing and SNP discovery using first generation sequencing technology dictated that a candidate gene-based approach be used, because genome-wide resequencing was not feasible. This situation has changed dramatically with the completion of the *P. trichocarpa* reference genome sequence (Tuskan et al., 2006) and the release of second generation sequencing platforms. All projects listed in Table 2 will employ the genome-wide resequencing approach as resources become available and costs go down. At this time, however, most are still using a candidate gene approach employing either first or second generation sequencing platforms. The *Populus* genome contains ∼45,000 genes, so ideally all genes will ultimately be resequenced. In practicality, a fewer number of genes have been or will be resequenced in these projects simply due to limited resources. It is expected that as second generation sequencing platforms are used and as third generation platforms become available, all projects will resequence all 45,000 genes if not entire genomes.

[4]The Bioenergy Science Center is a research affiliation of 10 organizations operating under the auspices of the United States Department of Energy and the leadership of the Oak Ridge National Laboratory.

**Table 2** Summary of worldwide *Populus* association genetics projects

| Species | Clones | Test sites | Genes | SNPs | Phenotypes | Positive associations | Reference | Lab |
|---|---|---|---|---|---|---|---|---|
| *P. trichocarpa* | 457 | 3 | 40 | 1,486 | Lignin content, S/G ratio | 37 | N/A | University California, Davis, USA, David Neale |
| *P. trichocarpa* | 1,100 | 3 | 7,000 | N/A | Lignin/cellulose, water-use-efficiency, phenology, wood properties | N/A | N/A | University of British Columbia, Canada, Carl Douglas |
| *P. trichocarpa* | 1,100 | 3 | 1,000 | N/A | Lignin/cellulose | N/A | N/A | Department of Energy, Bioenergy Science Center, USA, Gerald Tuskan |
| *P. deltoides* | 815 | 1 | N/A | N/A | N/A | N/A | N/A | University of Florida, USA, Matias Kirst |
| *P. nigra* | 612 | 2 | 40 | 1,237 | Lignin/cellulose | N/A | N/A | University of California, Davis, USA, David Neale |
| *P. nigra* | 398 | 1 | 20 | 53 | Phenology, biomass, leaf development | 3 | N/A | University Southampton, U.K., Gail Taylor |
| *P. tremula* | 350 | 2 | 40 | 100 | Phenology, senescence, herbivory | 4 | Ingvarsson et al. (2008) | Umea Plant Science Center, Sweden Stefan Jansson, Par Ingvarsson, |

Candidate gene selection is determined by a *priori* or suggestive knowledge of which genes control certain phenotypes. Three approaches are generally used for candidate gene identification: (1) known function in model systems, (2) gene expression analysis, or (3) map co-location with QTLs. For some traits, pathways and genes within pathways are quite well known, e.g. lignin and cellulose biosynthesis. This approach was used in the joint University of California, Davis – GreenWood Resource *P. trichocarpa* and *P. nigra* projects and the Umea Plant Science Center[5] *P. tremula* project because resources allowed only a few candidate genes to be sequenced using first generation sequencing platforms. The *P. trichocarpa* projects of the Bioenergy Science Center and the University of British Columbia were more recently started and both are using second generation sequencing platforms. These projects will sequence many more genes so candidate selection does not have to be so restrictive.

Once genes or genomes are resequenced in a diversity panel of some kind, it is then necessary to identify the polymorphic nucleotide position – the so-called single nucleotide polymorphisms (SNPs). Resequencing can also identify insertion/deletion (indel) polymorphism but the bioinformatics associated with indels is much more complex thus indels are often ignored. This will certainly change in the future as human geneticists are finding that indel variation can be responsible for much of the phenotypic variation in populations. An example of indel variation having a large effect on the phenotype is the *CAD* null indel that was discovered in loblolly pine that affects lignin properties (Gill et al., 2003). Presently, SNPs have been reported for just three of the active association genetics projects: the joint University of California, Davis – GreenWood Resource *P. trichocarpa* and *P. nigra* projects, and the Umea Plant Science Center *P. tremula* project. The frequency of the SNPs uncovered in these projects is roughly one in 100 base pairs. Modern population genomic methods can then be applied to estimate measures of nucleotide diversity and divergence as well as test for selection (see Section 2.2 for a comprehensive treatment of these methods and reports in *Populus*).

*SNP genotyping platforms* – Once the SNPs have been discovered in a diversity panel, each SNP can then be typed for each individual of the association population. Full genome sequences or even candidate gene sequences, for every member of an association population of 500–1,000 individuals has, to date, been prohibitively expensive. Because there are a large number of high throughput genotyping platforms on the market and these technologies change rapidly, SNP genotyping platforms will not be discussed in detail here. The only *Populus* association genetics project to date that has used one of these platforms is the University of California, Davis – GreenWood Resource *P. trichocarpa*. This project used the Illumina Golden Gate assay to type 1,536 SNPs in the full association population

[5]The Umea Plant Science Center located in Umea, Sweden is a research center in plant biology formed in 1999 from the Department of Plant Physiology, Umea University and the Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences.

of 457 clones. The other projects listed in Table 2 will use the same or similar technology. These SNP genotyping platforms can type up to 50,000 or more SNPs in parallel at a cost less than $0.01 per data point. So it is clear that *Populus* association populations will be genotyped for tens of thousands of SNPs from nearly all *Populus* genes in the next couple of years. Once this task is completed, the SNP genotype database will be somewhat complete and then all the attention focuses on the phenotype side of the process.

*Trait phenotyping* – The challenges and issues associated with trait phenotyping in association studies are not different from those in normal breeding programs, so will not be discussed in great detail here. The trait types being evaluated in association studies (Table 2) are much the same as have always been of interest (growth, biomass, wood properties, disease resistance, adaptation, etc). With the increasing interest in *Populus* as a feedstock for cellulosic ethanol, many studies are putting a high priority on lignin and cellulose quantity and quality (Davis, 2008). Other traits related to the decomposition of cellulose to simple sugars and fermentation will also be of great interest. The molecular phenotypes, e.g. transcriptome, proteome and metabolome, are also target phenotypes in association studies. By taking an integrated "omic" approach all the way through to complex, whole plant phenotypes, it becomes possible to take a network approach to association genetics and begin understanding epistatic interactions at the molecular level.

*Tests of association* – Once genotypic and phenotypic data are in hand for all members of an association population, it is possible to test for associations. The type of test performed depends on the population type. For example, the transmission disequilibrium test (TDT) has often been used with human parent-offspring data but such a test would not likely be applied in *Populus*. Rather, a variation on this test, the quantitative transmission disequilibrium test (QTDT) might be used where there is a family structure in the population (Gonzalez-Martinez et al., 2007). The populations given in Table 2 generally lack a family structure so simple regression (GLM) based methods are possible. However, the confounding effects of family and population structure must be taken in to account and corrected. The other issue that must be addressed is that of multiple testing. Generally, some type of false discovery rate probability is determined. New methods of association testing are being developed continuously and will certainly add to the power and precision of association testing. But, as always, advanced statistical approaches can never make up for poor experimental design and/or poor quality genotyping and phenotyping.

## *5.2 Marker-Assisted Selection*

Once a large number of marker-trait associations are discovered, it will be possible to develop marker-assisted selection programs in *Populus* species. White et al. (2007) distinguish between marker-assisted selection (MAS) and marker-assisted breeding (MAB). MAS is defined as the selection of superior trees based on their

molecular genotype, whereas MAB includes broader applications of markers, such as quality control and breeding designs, in tree improvement programs. We will only consider MAS in this chapter. White et al. (2007) further distinguish two general approaches to MAS: (1) indirect selection based on genetic markers linked to desirable QTLs, and (2) direct selection based on desirable alleles at genetic loci controlling target traits. Grattapaglia (2007) defines approaches (1) and (2) as *linkage equilibrium* and *linkage disequilibrium* approaches, respectively. Grattapaglia (2007) further defines *direct selection* when the functional mutation (quantitative trait nucleotide, QTN) itself can be directly selected upon. Again, we will only consider the MAS linkage disequilibrium approach in this chapter. Another approach that is being developed for application in dairy cattle and other livestock species is called *genomic selection* (Dekkers and Hospital, 2002). The general idea behind genomic selection is that a very large number of markers can be used to predict breeding values, even if marker-trait associations are not known. This approach is dependent on the extent and distribution of linkage disequilibrium in the genome.

In Section 2.2, Ingvarsson presented the concept of linkage disequilibrium and its relevance to detecting marker-trait associations in *Populus*. Furthermore, Ingvarsson describes the extremely low level of linkage disequilibrium found in *Populus* populations such that any marker (generally a SNP) associating with or controlling a phenotype would likely be within the genetic locus, or at an extremely close physical distance. If haplotypes can be established for genetic loci associating with phenotypes, full allelic discrimination is expressed at that genetic locus. Furthermore, it is possible using standard quantitative genetic methods to estimate the size and direction of effects of haplotypes (alleles). If haplotypes and haplotype effects are established for large numbers of genetic loci such that a large portion of the phenotype variance for a target trait can be accounted for, the reagents are now in place to practice direct MAS.

MAS will undoubtedly be combined with some form of phenotypic selection in either a sequential and/or combined manner. In a sequential approach, a large amount of breeding material might be screened with markers only to identify the smaller amount of material that would be field tested and phenotypically evaluated. Final selections might be made using index selection with multiple traits and markers. The exact way in which MAS will be applied in *Populus* breeding is yet to be developed, but it is quite certain that marker data will be abundant and inexpensive to obtain. The challenge remains as to how to fully capture the value of nearly complete information on a tree's genotype.

## 6 Conclusion

One significant advantage enjoyed by *Populus* breeders is the extensive knowledge of natural variation in phenological and eco-physiological traits. Complimenting this is the insight into the physiological and morphological determinants of yield and pathogen resistance that characterize segregating populations. Armed with such

extensive knowledge, *Populus* breeders can look forward to widespread application to selection programs offering greater precision and earlier schedules. However, this expectation has not yet been fully realized in most operational breeding programs, where evaluation and selection remains focused on integrated traits, e. g. climatic adaptability, biomass production and pest resistance. Although this has worked extraordinarily well to advance the number of productive and well-adapted genotypes now used commercially, the reality is that as productive genotypes continue to materialize future selection thresholds will increase. This presents a greater challenge to sustained genetic advancement. New methodologies are warranted, then, if this challenge is to be surmounted, and molecular tools are rapidly approaching their practical utility in dissecting the inheritance of complex traits that will lead to more effective manipulation and evaluation techniques (Stanton, 2009). At the same time, the field of plant phenomics is developing a rich assortment of imaging techniques to improve analyses of plant growth and performance, which should accelerate the use of molecular tools and translational genomics programs. A recent article in *Science* assessed the importance of phenomics, stating that it allows "*. . . plant physiologists to "catch up" with genomics. . .*" and plant breeders to "*. . . shift breeding into overdrive*."[6] This emphasis on phenotyping capability could propel *Populus* breeding to the next chapter of its successful story. To take full advantage of this, comparative resequencing studies of the most important species – *P. deltoides*, *P. maximowiczii*, *P. nigra*, and *P. trichocarpa* – should now become a research imperative. Such exploration will promote a superior understanding of between- and within-species allelic variation and how best to recombine the variation in both intra- and inter-specific breeding programs.

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