# 1 *Populus* Trees

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## 1.1 Introduction

The genus *Populus*, which includes poplars, cottonwoods and aspens, is widely distributed over the northern hemisphere. Their rapid growth lends to their use as a source of fuel, fiber, lumber, windbreaks and protective stands to prevent soil erosion. Their ease of vegetative propagation means that they have been closely associated with agriculture since before the Middle Ages in the Near and Middle East. In recent years, poplars have received increasing attention as a renewable source of biomass for energy and short-fiber furnish for papermaking.

The genus also has an important role in the natural populations of their native habitat, particularly riparian ecosystems. Poplars are dioecious and wind-pollinated and produce large amounts of pollen and small, cotton-tufted seed that is dispersed by wind and water in early summer. Capable of rapidly invading disturbed sites, many species occupy habitats in the dynamic environment of riverside floodplains where they form a key component of riparian forests (Braatne et al. 1996). Others, such as the aspens, commonly colonize upland areas after intense, stand-replacing fires (Burns and Honkala 1990). All poplars also have the capacity to reproduce asexually, mostly by sprouting from the root collar of killed trees or from abscised or broken branches that become embedded in the soil.

Traditionally, *Populus* and *Salix* have been regarded as the only two members of the family Salicaceae. However, recent views include many of the genera previously assigned to Flacourtiaceae, giving more than 50, largely tropical, genera. Poplars, cottonwoods, or aspens are the common names for species of the genus *Populus*, recognized as the most abundant woody plant genus in temperate forests around the world. Some estimates put the number of species as high as 70 or more, and there are a number of naturally occurring hybrids. Many species are found in cultivation as well as numerous hybrids and cultivars (Hillier and Coombes 2002).

*Populus* spp. are fast-growing deciduous trees with alternate leaves. The flowers are extremely reduced and borne in catkins and trees are usually dioecious. For this reason *Populus* spp. have a tendency to hybridize in their natural habitat and especially in cultivation (US Environmental Protection Agency 1999; Wyckoff and Zasada 2003). The extensive interspecific hybridization and high morphological diversity in this group pose difficulties in identifying taxonomic units for comparative evolutionary studies and systematics (Hamzeh and Dayanandan 2004). *P. lasiocarpa* is one of the species that breeds true as it can be monoecious. Because of the extent of hybridization, and the fact that seeds are generally viable for only a short time, most *Populus* trees are propagated vegetatively by cuttings. Good rootability is a property of the members of two sections: Aigeiros and especially Tacahamaca.

The morphology and physiology in *Populus* species vary between geographically distinct populations and are strongly linked with the environment. It follows that any responses to stress will depend not only on the species but also on the particular genotype (Weber et al. 1985; Rogers et al. 1989; Braatne et al. 1992; Dunlap and Stettler 2001; Rowland et al. 2001). Dunlap et al. (1993), for example, looked at poplars growing in different river valleys and found that there was variation of physiological processes like photosynthesis both within and between locations.

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### 1.1.1 Taxonomic Classification

Kingdom Plantae
Subkingdom Tracheobionta (Vascular plants)
Superdivision Spermatophyta (Seed plants)
Division Magnoliophyta (Flowering plants)
Class Magnoliopsida (Dicotyledons)
Subclass Dileniidae
Order Salicales
Family Salicaceae (Willow family)
Genus *Populus* L. (cottonwood)

Bean (1976) describes five sections in the genus *Populus*, from which section Turanga, represented by *P. euphratica*, is the least cultivated as it is a species that is difficult to grow, native to North Africa through southwest and central Asia to China. The rest are mentioned briefly as follows, as they include distinctive features:

**Section Populus (Leuce)** includes the white and grey poplars characterized by a woolly leaf abaxial surface. It includes *P. alba*, *P.* x *canescens*, and *P. tomentosa*, all native to the Old World, and the Aspens, with glabrous leaves, noted for their restless movement. This subsection includes *P. grandidentata*, *P. tremula*, and *P. tremuloides*. All of these have poor rootability.

**Section Leucoides** includes a combination of American species such as *P. heterophylla* and Asiatic species such as *P. lasiocarpa* and *P. wilsonii*. The main characteristic of these species is the large and leathery leaves.

**Section Tacamahaca**, known as the balsam poplars, is a very distinctive group with fragrant buds and scented leaves for which it is named. The leaves are usually whitish and waxy on the underside. This section includes *P. angustifolia*, *P. balsamifera*, *P. koreana*, *P. laurifolia*, *P. maximowiczii*, *P. simonii*, and *P. trichocarpa*. A distinctive characteristic is that all the members of this section are easily propagated by hardwood cuttings or by suckers.

**Section Aigeiros**, known as Black poplars, includes plants with green leaves on both sides and a large petiole, nearly always in movement. This section is distributed in North America, Europe, and Western Asia. Of all the species in this group, *P. deltoides* is the only one that cannot be propagated easily by hardwood cuttings.

Although *Populus* trees share several floral features with *Salix*, molecular data show a clear separation between the two genera; the most consistent and evolutionarily significant being their mode of pollination. Wind pollination in poplars, combined with their dioecy, has led to natural hybridization, allowing interspecies gene exchange. The outbreeding habit in combination with effective seed dispersal effects the genetic variation within *Populus* species. Patterns of isozyme variation across several species in over 30 enzyme systems show a recent theme of little differentiation among populations, with over 90% of the variation being within populations. Polymorphisms within populations are also common in morphological and phenological traits.

Fossil records show evidence that ancestral poplars were widespread across North America. Members of section Abaso were the sole poplars in North America until the late Eocene, when the precursors of other sections appeared and the first Eurasian poplar fossils are recorded (Collinson 1992).

### 1.1.2 Botany

*Populus* species are single-stemmed, deciduous (or semievergreen) trees mostly spread clonally by means of rootborne sucker shoots (soboliferous). They are among the fastest growing temperate trees, a quality tied ecologically to their role as vegetational pioneers as well as functionally to their heterophyllous growth habit. Poplar shoots continue to grow after bud burst by initiating, expanding, and maturing leaves (neoformed or late leaves) throughout the growing season. Cessation of growth and bud formation is induced by photoperiod in some poplars (Pauley and Perry 1954). Preformed and neoformed leaves often differ considerably in texture, shape, and toothing, with preformed leaves often more taxonomically diagnostic than neoformed leaves. Neoformed leaves are relatively convergent among unrelated poplar species, with the exception of the unique lobed leaves of *P. alba* and its hybrids.

The separate male and female trees flower before leaf emergence in spring (except in some subtropical species) from specialized buds containing preformed inflorescences, enabling wind pollination before canopy closure. The capsules and their airborne seeds, which have a readily detached coma of cottony hairs, mature with or after the overwintered preformed leaves.

Poplars have been recognized as a group since very early times and have a unique combination of characteristics that distinguish them from all other genera of plants. The defining features are primarily in the reproductive structures. The flowers are borne in pendent racemes (catkins, aments) that vary in flower number and density among poplar species. Among temperate trees with female catkins, only poplar and willow have seeds with a coma of cottony hairs on parietal placentas in thin-walled capsules. Individual flowers of both catkin types are subtended by thin bracteoles that fall as the catkins elongate during flowering. The caduceus bracteoles distinguish the catkins of poplars from those of willows. There are no ordinary petals or sepals, but 5 to 60 stamens or solitary pistils are borne on a more or less expanded floral disk.

Overwintering vegetative and reproductive buds are covered by several bud scales. The buds are covered with exudates that are rich in a variety of hydrophobic organic chemicals (Greenaway and Whatley 1990) that are thought to be involved in winter hardiness.

The rapid, nearly continuous growth during the favorable season results in a light diffuse-porous wood structure. Poplar wood differs from that of willow in that it has homocellular rays as opposed to the heterocellular rays. The wood differs from many similar light-colored woods in that it lacks some more specialized features like the marginal parenchyma or the spiral thickening and multi-seriate rays. The bark remains thin for a longer period than in most trees.

The leaves are alternate, stipulate, and petiolate with the petiole often transversely flattened and simple with glandular teeth along the margin and often with glands at the junction of the blade and petiole. Poplars generally have a high soil moisture requirement explaining the growth of most species along floodplains of rivers or lake shores.

### 1.1.3 Poplars as Crops

Rapid growth is the hallmark of poplars. It derives from a growth system that starts with the elongation of a preformed shoot from its bud and then continues to initiate and expand shoot segments and leaves throughout the growing season. The wood is diffuse-porous, light in weight, and yet capable of building trees of 40 m height in less than 20 years.

Several of these features have made poplars attractive to humans since ancient times. Today, poplar is cultivated worldwide in plantations for pulp and paper, veneer, excelsior (packing material), engineered wood products (e.g., oriented strandboard), lumber, and energy. Grown at a commercial scale under intensive culture for 6- to 8-year rotations, production rates with hybrid poplar can be as high as 17 to 30 Mg/ha/year of dry woody biomass (Zsuffa et al. 1996), comparable to the biomass produced by row crops such as corn. Historically, poplar has been widely used in windbreaks and for erosion control. Most recently, poplars have proven to be effective in the phytoremediation of environmental toxins (Flathman and Lanza 1998) and as bioindicators for ozone pollution in the environment (Jepsen 1994).

Poplars have three main properties that make them excellent for short-rotation intensive-culture management: rapid juvenile growth, immediate response to cultural practices, and their coppicing property (Bradshaw et al. 2000). The hybrids of section Tacamahaca are considered to have particularly high water-use efficiency (Mazzoleni and Dickmann 1988). Hybrids are more drought tolerant than native cottonwoods (Hinckley et al. 1989; Wyckoff and Zasada 2003), and this characteristic makes them attractive as a crop to supplement the diminishing supply of natural hardwoods.

An important feature in poplar hybrids and species, linked to their high productivity, is their high rate of stomatal conductance ($g_{max}$ are near 600 mmol $m^{-2}$ $s^{-1}$), which suggests the transpiration of large volumes of water (US Environmental Protection Agency 1999). Therefore, considerable attention has been given to the study of water regulation in several species, hybrids and cultivars that exhibit a wide range of variation (Reich 1984; Schulte and Hinckley 1987; Schulte et al. 1987; Ceulemans et al. 1988; Mazzoleni and Dickmann 1988; Tschaplinski and Blake 1989b; Bassman and Zwier 1991; Dickmann et al. 1992; Liu and Dickmann 1992, 1996; Dunlap et al. 1993; Tschaplinski et al. 1994; Ridolfi et al. 1996). Important work has been carried out on growth and biomass rates in large-scale poplar plantations (Tschaplinski et al. 1998b; Rae et al. 2004).

The commercial planting of poplars date from half a century ago, and it occurs mainly in America, Canada, Europe, and China. In the UK, the best region in which to grow poplars is the southern half of England, where the best conditions prevail for sustaining high growth rates. It has been shown that *Populus* does

not like competition, spacing at planting range from 10 to 24 feet (ca. 3 to 7 m) but no more than 26 feet (~8 m).

Intense culture and study started in the early 1970s, shortly after the US Department of Energy (DOE) embraced the concept of SRWC (short-rotation woody crops), as a way of supplying biomass for conversion to liquid transportation fuels. At present, *Populus* is the main choice of a group of model species for SRWC that includes sycamore (*Platanus occidentalis* L.), silver maple (*Acer saccharum* Marshall), and hybrid willow (*Salix* spp.), all suitable for the Pacific Northwest, north central, and southeastern regions of the USA. Genetic improvement programs, silvicultural studies, and basic research were initiated and continue today throughout the world (Tuskan 1998), and poplar is currently accepted as a model tree in forestry (Bradshaw et al. 2000; Taylor 2002).

In addition to their high biomass yields, hybrid poplars are more drought tolerant than native cottonwoods (Tschaplinski et al. 1998a; Wyckoff and Zasada 2002). Because of this there are major selection and breeding programs, with the objectives of extending crops to marginal agricultural lands and supplementing the diminishing supply of natural hardwoods. One of the most successful hybrids created is the intersectional cross between *P. trichocarpa* and *P. deltoides*, which belong to *Populus* sections Tacamahaca and Aigeiros, respectively. The first of these contrasting species was selected from a maritime and wet climate on the Pacific coast, the second from a more continental and less humid area in the eastern United States. These two species show marked differences in both stomatal behavior and photosynthetic patterns (Hinckley et al. 1989; Dunlap et al. 1993).

Whole-plant and leaf studies have revealed that hybrid poplars close their stomata rapidly in response to atmospheric and soil water deficit, resulting in lower transpiration rates and a greater drought resistance compared to the parental species, a feature that allows hybrids to maintain higher leaf areas for longer periods (US Environmental Protection Agency 1999). Consequently the question of how poplars tolerate increasing drought stress without an impact on productivity has been raised and investigated by several authors. This issue is of particular relevance to increasing the use of marginal agricultural land and in achieving an increase in the energy conversion to liquid transportation fuel in temperate regions (Mazzoleni and Dickmann 1988; Tschaplinski and Blake 1989a, b; Tschaplinski et al. 1994, 1998b, 1999).

### 1.1.4 Economic Importance

Poplars' wood is soft, rather woolly in texture, pale in color, and inodorous. The commercial uses are as various as food containers, wagon bottoms, wheelbarrows, and colliery tubs, and they are popularly used as matches. Because of its low flammability the wood is suitable for floors of oast and for brake blocks. Uses for small logs include fiberboard, wood chipboard, and pulp. Among the most commonly planted cultivars are *P. eugenei*, *P. gelrica*, *P. robusta*, and *P. serotina*, all forms of the hybrid *P.* x *canadensis* (Bean 1976). Poplars are widely used in the pulp and paper industry, for regeneration on disturbed lands, and for biofuel (US Environmental Protection Agency 1999; Wyckoff and Zasada 2003).

### 1.1.5 Diseases

According to Bean (1976), the number of cultivars is high and they are mainly chosen for their fast growth, straight stems, and resistance to diseases. Among the most problematic diseases that attack poplars in cultivation are bacterial canker, caused by the bacterium *Aplanobacter populi* that produces cankers on the branches and main stem, generally diminishing the value of the timber; *Marssonina brunnea*, which causes the most serious foliage disease; *M. populinigrae*, which is the cause of death of branches of Lombardy poplar, defoliation, and dieback, more often occurring in the lower crown; *Melampora* species (rust), which cover the leaves with small orange pustules and can cause defoliation when abundant. Another fungus attacking poplars is *Dothichiza populnea* that can be confused with the symptoms of bacterial canker. *Cytospora chrysosperma* attacks dead wood.

### 1.1.6 Breeding

Poplars can be bred in the greenhouse on detached female branches with pollen that can be stored for several years. Each pollination can yield hundreds of seeds within 4 to 8 weeks. Seeds germinate within 24 h and give rise to 1- to 2-m-tall seedlings by the end of the same year. Few, if any, trees can match such efficiency. Poplar species within the same section, and many of the species from different sections, can be hy-

bridized. Because all members of the genus are diploid ($2n = 38$), hybrids are usually fertile and can generate $F_2$ and backcross progenies that segregate for a wide range of traits. $F_1$ hybrids often show heterosis in growth and associated characteristics that make them attractive for commercial use (Zsuffa et al. 1996).

Most poplars will not flower earlier than 4 years of age, and many will take twice that long. The long generation interval is an impediment to practical breeding and selection and the development of informative pedigrees. It limits the applicability of conventional experimental genetic techniques such as induced mutagenesis, since producing homozygotes from heterozygous mutants is impracticably slow.

Poplars are dioecious, so self-pollinations cannot be done (with a very few hermaphroditic exceptions). Classical genetic tools, such as inbred lines, cannot be produced rapidly enough to be useful.

Most poplar breeding programs have relied upon more or less random interspecific hybridization to produce large progeny arrays, followed by very intense clonal selection to identify the best clones out of perhaps 10,000 seedlings. While demonstrably effective at producing remarkable genetic gains in short order, the scarcity of well-planned, long-term, sustained breeding programs and associated breeding materials hampers genetic studies. For example, multigeneration pedigrees are the basis for genetic mapping experiments designed to identify quantitative trait loci (QTLs) affecting important tree phenotypes, yet few such pedigrees exist.

Delayed flowering represents a lost opportunity for tree geneticists to provide suitable pedigrees for physiological studies and is currently the single most limiting factor in the conventional genetic improvement of forest trees. The development of methods for inducible early flowering, either by chemical/physical treatment or genetic engineering, will make it possible to decrease the generation interval for purposes of breeding, while also preventing excessive flowering from siphoning photosynthate away from wood formation in production plantations. While some progress has been made in this area with poplars (Weigel and Nilsson 1995), much remains to be done (Rottman et al. 2000).

### 1.1.7 Genetics and Breeding Programs

Members of the genus *Populus* are becoming more important because of their suitability for genetic and environmental studies of carbon sequestration as they are some of the fastest-growing trees in the world (Stettler et al. 1996; Tuskan et al. 2002). Their ease of propagation and the intensive work in physiology, biochemistry, agronomy, and genomics around the world have led several authors to regard them as excellent models for forest trees (Bradshaw and Stettler 1995; Taylor 2002; Tuskan 2003). Genetic studies in *Populus* have advanced rapidly. *P. trichocarpa* is the first tree species with its complete genome sequenced in the United States [Tuskan et al. 1998, 2002; US Department of Energy (DOE) 2002] and has an estimated 40,000 to 50,000 coding genes and a genome only four times the size of *Arabidopsis*.

Genomics in species of *Populus* is also advanced, for example, the study of *P. tremuloides* at the University of Agricultural Sciences, in Sweden, where more than 100,000 expressed sequence tags (ESTs) have been obtained by sequencing more than 14 tissue-specific cDNA libraries from *P. tremula* × *tremuloides.* These ESTs have been used as a base to build a cDNA microarray consisting of ca 25,000 probes (Nilsson 2002), and recently the poplar root transcriptome has been profiled where 7,000 ESTs were analyzed (Kohler et al. 2003).

In order to understand the genetics of adaptation, there is a growing body of knowledge about the physiological and molecular genetics of adaptive traits, with an increasing interest in predicting the genetic response of populations to changing climates and a trend toward incorporating adaptive as well as economic traits in breeding programs (Aitken and Adams 1996; Bradshaw and Strauss 2001). Now, the physiological study of *Populus* needs to be accompanied with investigation at a molecular level. Undoubtedly, genomics and transcriptomics will help to unravel the relationships between genotypic, environmental, and adaptive traits.

It has been reported that the hybrids between *P. trichocarpa* and *P. deltoides* in Washington and Oregon in the United States had a volume growth two or three times that of the best growing parent species (Heilman and Stettler 1985; Stettler et al. 1988; Dickman et al. 1992). Exploiting the phenomenon of heterosis requires either a simple method for generating large numbers of hybrid seedlings or the ability to vegetatively (i.e., clonally) propagate desirable hybrid genotypes. Nine species of poplar – *P. angustifolia, P. balsamifera, P. deltoides, P. euphratica*, *P. fremontii*, *P. tremula*, *P. tremuloides*, *P. tomentosa* and *P. trichocarpa* – are the most used in experiments.

In order to design and implement genetic improvement strategies of interspecific hybrids in forest trees, basic information is needed on the genetics of variation in commercially important traits (Bradshaw and Grattapaglia 1994). Identifying loci contributing to genetic variance in the $F_2$ or backcross generations makes it feasible to identify loci responsible for $F_1$ heterosis, and this has been done in *Populus*. The contribution (positive or negative) and mode of action of each parental QTL allele to the phenotype of the hybrid may be determined and used to evaluate the merits of various long-term breeding plans (Bradshaw and Grattapaglia 1994). QTL mapping in *Populus* also aims to give insights into the genetic basis of hybrid vigor (Bradshaw and Grattapaglia 1994; Bradshaw and Stettler 1995).

Interspecific hybrids of poplars have proven to be particularly amenable to map-based QTL analysis. Firstly, they are fast-growing trees and the commercially important phenotypes are revealed soon after planting, while the problem of poor juvenile-mature phenotypic correlations is minimized. Secondly, they grow in a relatively homogeneous environment, resembling agriculture more than traditional extensive forestry. This reduces the nongenetic variance components of complex traits like stem volume growth and facilitates detection of the genetic basis of phenotypic variance. Thirdly, clonal propagation allows precise estimation of the broad-sense heritability and an accurate assessment of the magnitude of the effect of QTLs on the genetic variance (Bradshaw and Grattapaglia 1994).

The physical and genetic maps of *Populus* serve several important purposes: as a starting point for map-based gene cloning, as a means to determine the extent of colinearity between the *Populus* genome and that of the completely sequenced genome of *Arabidopsis* (thus making effective use of the growing knowledge of *Arabidopsis* genome structure and function), and as a scaffold for the eventual annotation of the entire *Populus* genome.

EST microarrays will allow poplar researchers to couple physiological measurements with gene expression profiles, illuminating the genes, biochemical pathways, and cellular processes that are affected by a given environmental perturbation or transgene.

Extensive collaboration between poplar geneticists, physiologists and pathologists has set a solid scientific foundation for joint efforts in the future. Shared genetic materials, genetically informative two- and three-generation pedigrees, DNA-based genetic markers, common field measurement protocols, clonal plantation trials supported by industry/government/academic partnerships, and funding from multiagency grants have established a poplar research network of proven productivity. Working groups under the aegis of the International Poplar Commission (IPC) of the Food and Agriculture Organization (FAO) of the United Nations and of the International Union of Forestry Research Organizations (IUFRO), as well as several university/industrial research cooperatives, help to coordinate the work and the exchange of information.

### 1.1.8 *Populus* Genome

The first investigation of the *Populus* genome was made in 1921, in which the haploid chromosome number was erroneously reported as four (Graf 1921). By 1924, it became clear that the base chromosome number in *Populus* was 19, based on observations in seven species (Harrison 1924). Since then, various works have revealed that all *Populus* species exist in the diploid form with $2n = 38$ (Smith 1943), with occasional cases of triploid or tetraploid genets (Einspahr et al. 1963; Bradshaw and Stettler 1993). Based on cytology studies, Van Dillewijin (1940) hypothesized that the ancestral chromosome number of *Populus* was eight. However, because *Populus* chromosomes are mostly small and lacking in distinctive morphological features (Smith 1943), there is scant information on chiasma frequencies and chromosomal dynamics to substantiate this claim.

The haploid genome size of *Populus* is 550 million base pairs (bp) (Bradshaw and Stettler 1993). The nuclear genome is relatively small (2C = 1.2 pg) (Dhillon 1987; Bradshaw and Stettler 1993), only 4 times larger than the genome of the model plant *Arabidopsis* and 40 times smaller than the genomes of conifers such as loblolly pine. The small poplar genome simplifies gene cloning, Southern blotting, and other standard molecular genetics techniques. The physical:genetic distance ratio for poplar is close to 200 kb/cM, almost identical to *Arabidopsis*, making *Populus* an attractive target for map-based (positional) cloning of genes.

### 1.1.9 *Populus* as a Model System

The woody genus *Populus* offers extremely important potential as a commercially grown tree, but it is also of tremendous value as the model tree, due to its relatively small genome size, with a highly developed number of molecular genetic maps, combined with an ability for easy genetic transformation. *Populus* may be propagated vegetatively, making mapping populations immortal and easing the ability to produce large amounts of clonal material for experiments (Taylor 2002). Hybridization occurs routinely, and in this respect *Populus* has many similarities to *Arabidopsis*. The development of large EST collections and microarray analysis, the best available globally for any tree, and the availability of mapping pedigrees for QTL detection secure *Populus* as the ideal subject for further exploitation. Of crucial importance is the availability of the poplar genome sequence released this year (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html).

Much work is being carried out in this genus to manipulate the natural variation of *Populus* to enable breeding for traits such as increased yield and improved response to biotic and abiotic stresses. The application of molecular tools has been exploited to understand the genetic resources of the genus of what is now seen as the model tree species.

## 1.2 Construction of Genetic Linkage Maps

The earliest work in molecular genetics of poplar, as was the case with most organisms, was focused on mutations of genes with large effects on the appearance. A major disadvantage with this is the limited number of markers available as a great deal of genetic variation is not identifiable at the phenotypic level. In addition to this, mutations in single genes is not easy in forest trees because of the difficulty of producing near-isogenic lines or clones differing in the mutant gene only. With the advent of molecular markers at the DNA sequence level came an increase in the number of polymorphic markers available for mapping purposes. In forest trees, where long generation time and high genetic load impede progress by traditional quantitative and classical genetic approaches, molecular genetics has much to offer.

### 1.2.1 Molecular Markers

Ideal molecular markers for linkage-map construction require that they be evenly distributed throughout the genome with selectively neutral behavior. Previously it was considered necessary to have codominant inheritance so that homozygous and heterozygous states could be discriminated, but recently mapping techniques in outbred poplars does not require this criterion.

#### Isozyme and Allozyme Markers

Allozyme analysis is based on the correlation between differences in mobility in an electric field due to differences in amino acid sequence, protein structure, and kinetic properties. (Murphy et al. 1990). This analysis is relatively easy to perform, inexpensive, and technically accessible, resulting typically in codominant markers. However, there is a limited number of loci that can be analyzed as the level of genetic polymorphism within coding sequences is relatively low and may be active only at specific physiological stages or in specific tissue.

Early molecular work in poplar relied on the use of allozymes as polymorphic markers. Such work revealed little linkage between markers (Hyun et al. 1987; Rajora 1990; Liu and Furnier 1993). This is likely to be due to the relatively small number of allozyme markers assayed across the 19 linkage groups (LGs).

#### RFLPs

Restriction fragment length polymorphism (RFLP) markers, which are based on polymorphisms observed after DNA digestion with restriction enzymes, are codominant. Although high levels of polymorphisms can be revealed, RFLP analysis is technically demanding, requires relatively large amounts of DNA, and is limited to the availability of libraries with useful probes. RFLPs have been used in *Populus* to study inter- and intraspecific variation (Keim et al. 1989) and for the production of genetic linkage maps (Bradshaw and Stettler 1993; Liu and Furnier 1993).

Sequence tagged site (STS) markers can be obtained by converting RFLP markers into PCR-based markers by use of the terminal RFLP probe sequence

as a primer resulting in codominant length polymorphisms. When the sequenced clones are cDNA, the marker is known as an expressed sequence tag (EST). Bradshaw et al. (1994) generated a genetic linkage map of hybrid poplar composed of RFLP, RAPD, and STS markers.

### SSRs

Microsatellites, or simple sequence repeats (SSRs), are tandem nucleotide repeats, polymorphic for the number of repeats. These markers are abundant and widely distributed in plant genomes. They are codominant and have been reported to have the highest polymorphism information content (PIC) of any marker system; however, the effort required to obtain the SSR flanking sequences is a drawback. Their discovery typically involves hybridization to create SSR-enriched genomic libraries followed by sequencing of selected clones and primer design based on 5' and 3' flanking sequence from microsatellite-containing fragments (Karagyozov et al. 1993). In poplar it is possible to take advantage of the growing EST and genomic databases now publicly available to use a more general computational molecular biology method, often referred to as an in silico approach, to identify repeats and their flanking regions (Tuskan et al. 2004). In *Populus* several hundred SSRs have been identified using various approaches for *P. nigra* L. (van der Schoot et al. 2000; Smulders et al. 2001), *P. tremuloides* Michx. (Dayanandan et al. 1998), and *P. trichocarpa* Torr. & A. Gray (Frewen et al. 2000). These SSRs have been derived from enriched genomic libraries and are predominantly simple di- and trinucleotide repeats.

Tuskan et al. (2004) identified and developed SSRs isolated from rapid shallow sequencing of total genomic DNA from *P. trichocarpa* and from selected bacterial artificial chromosomes (BAC) clones known to contain expressed sequences (Stirling et al. 2003). Approximately 23% of the 1,536 genomic clones and 48% of the 768 BAC subclones contained an SSR. 26.6% of the sequences contained multiple SSR motifs in complex or compound repeat structures. A survey of the genome sequence database revealed very similar proportional distribution, indicating that the limited rapid, shallow sequencing effort is representative of genome-wide patterns. The resulting SSRs developed from *P. trichocarpa* were shown to have high utility throughout the *Populus* genus and also in *Salix*, with amplification rates in excess of 70% for all *Populus* species tested. These SSRs have been used alongside other marker types to create genetic linkage maps (Yin et al 2004; Tchaplinski et al 2006).

SSR primer sequence and amplification information is publicly available for research use on the Internet: the *Populus* Molecular Genetics Cooperative (PMGC, GCPM; http://www.ornl.gov/sci/ipgc/ssr_resource.htm), Oak Ridge National Laboratory (ORPM; Tuskan et al 2004), and the Center for Plant Breeding and Reproduction Research (WPMS) (Van der Schoot et al. 2000). SSRs have also been useful for aligning genetic linkage maps with the physical sequence of poplar (Rae et al 2006; Street et al 2006).

### RAPDs

Random amplified polymorphic DNA (RAPD) is generated by PCR amplification using arbitrary primers resulting, typically, in between 5 and 20 loci per experiment. This analysis detects single nucleotide changes, deletions and insertions within the primer annealing site. PCR-based marker analysis requires less DNA for the assay and is relatively simple, quick, and inexpensive; however, the resulting markers are dominant unless they are cloned and sequenced for conversion to a sequence characterized amplified region (SCAR) that can be used as codominant markers. Also, they have been found to be difficult to reproduce due to mismatch annealing of the random primer to the DNA. RAPDs have been used in conjunction with other molecular markers to produce linkage maps in poplar (Bradshaw et al. 1994), and they have been used to establish associations between markers and fungal rust resistance (Villar et al. 1996).

### AFLPs

Amplified fragment length polymorphism (AFLP) markers were developed at Keygene (Wageningen, The Netherlands) by Vos et al. (1995). These markers assay the presence/absence of restriction enzyme sites and sequence polymorphisms adjacent to these sites.

AFLPs have the advantage over RAPDs in that a higher number of loci can be analyzed per experiment. For example, Cerverea et al. (2001) reported 24 AFLP primer combinations yielding a total of 653 segregating loci, an average of 27 loci per primer combination. There was considerable variation in the number of polymorphic AFLP markers revealed by different primer combinations, ranging from 11 to 51 markers. Pure *P. deltoides* and *P. trichocarpa* have AFLP heterozygosity levels around 20 to 30%. Mark-

ers can be codominant if the appropriate equipment and software are used to analyze the gels. It is possible to identify homo- or heterozygous loci (Cervera et al. 1996b) providing more information than dominant markers. AFLPs give highly reproducible banding patterns due to highly specific annealing of the primers to complementary adapter oligonucleotides. Associations between AFLP markers and resistance to fungal rust have been reported in *Populus* (Cervera et al. 1996a), and AFLP markers have been used alongside SSRs to produce high-density linkage maps (Cervera et al. 1996b; Yin et al. 2004)

### 1.2.2 Linkage Maps

Poplar, being dioecious and long lived, has made the development of linkage maps daunting. The first report of a linkage map for *Populus* was by Liu and Furnier (1993) using a selection of allozymes and RFLP markers in five $F_1$ crosses of trembling aspen. Work on 97 $F_1$ individuals from one of the crosses resulted in the identification of 14 LGs based on 3 allozyme and 54 RFLP markers, with a total map distance of 664 cM. This initial study showed that genetic mapping was possible in an $F_1$ generation of a highly heterozygous *Populus* species.

The first report of a linkage map identifying all 19 chromosomes of *Populus* was produced by Bradshaw et al. (1993). In recent years numerous linkage maps using a variety of mapping pedigrees have been reported.

#### Mapping Methods and Pedigrees

Much work in the early 1990s focused on the three-generation interspecific cross produced by Bradshaw et al. (1993) between *P. trichocarpa* and *P. deltoides*. Two full-sib individuals from the resulting cross were mated to produce an "inbred $F_2$" generation, family 331. Mapping in this pedigree was carried out in the same manner as for an inbred $F_2$ pedigree, assuming that there was enough marker variation between species that the heterozygous grandparents differed for alleles and could be treated as inbred lines. The $F_2$ generation was treated as though there were three possible genotypes that could occur at any locus: homozygous for parent 1; homozygous for parent 2; or heterozygous, segregating 1:2:1.

Later work on this pedigree modified the mapping procedure specifically for an outbred population structure so that a sex-averaged framework map for the $F_2$ was produced (Sewell et al. unpublished data; Tschaplinski et al. 2006). Fully informative markers (i.e., a marker that was heterozygous for different alleles in each of the $F_1$ parents) were preferentially chosen when available. A related interspecific cross using the same maternal *P. trichocarpa* but different male *P. deltoides* grandparent was produced by Frewen et al. (2000), but separate grandparental maps were constructed consisting mainly of dominant markers linked in coupling phase; however, synteny between the two repulsion phase LGs was determined by the placement of codominant AFLPs, microsatellite markers, and candidate genes.

More recently, linkage maps have been produced from $F_1$ or backcross pedigrees using the pseudotestcross strategy (Grattapaglia and Sederoff 1994). This strategy is mainly based on selection of single-copy polymorphic markers heterozygous in one parent and homozygous null in the other parent and therefore segregating into 1:1 ratio in their $F_1$ progeny as in a testcross. The term "two-way pseudotestcross" to define this mapping strategy is generally used to describe the two independent genetic linkage maps that are constructed by analyzing the cosegregation of markers in each progenitor. Examples of backcross pedigrees used to map in this way were reported by Wu et al. (2000), Yin et al. (2002, 2004), and Zhang et al. (2004). Cervera et al. (2001) used the pseudotestcross strategy for two half-sib related $F_1$ mapping pedigrees sharing the same female *P. deltoides* parent, resulting in linkage maps for the three-parental species.

#### Map Coverage

The first map reported to identify all 19 chromosomes of *Populus* was constructed by Bradshaw et al. (1994), containing 343 markers using RFLPs, STSs, and RAPDs. Marker genotypes were determined for as few as 26 and as many as 90 $F_2$ trees. MAPMAKER software resulted in the grouping of 35 LGs. The total map distance contained within the largest 19 LGs was 1,261 cM. Using the method put forward by Hubert et al. (1988), they estimated the genome length to be between 2,400 and 2,800 cM, and therefore concluded that their linkage map had ca. 50% coverage of the genome.

Using the same *P. trichocarpa* grandparent, Frewen et al. (2001) developed linkage maps for a related $F_2$ interspecific cross. Instead of producing a sex-averaged map for the pedigree, the high

percentage of dominant markers and the paucity of linkage information from dominant markers linked in repulsion led to the construction of two linkage maps representing the grandmaternal and grandpaternal maps. The *P. trichocarpa* map spanned a distance of 2,002 cM, covering 77% of the estimated genome length (Bradshaw et al. 1994) with an average marker interval of 13.6 cM, while the *P. deltoides* map was reported as 1,778 cM in length, covering 68% of the genome with an average marker spacing of 12.3 cM. There were 26 LGs found in the *P. trichocarpa* map and 24 in the *P. deltoides* map. Because the haploid chromosome number is 19 in *Populus*, some of these LGs represented different sections of the same chromosomes.

More recently a number of genetic linkage maps with improved genome coverage have been produced mainly using backcross or $F_1$ pedigrees and the pseudo-testcross strategy (Grattapaglia and Sederoff 1994).

Wu et al. (2000) constructed an integrated genetic map for a backcross population derived from two selected *P. deltoides* clones using AFLP markers, using the two-way pseudo-testcross configurations of the markers (testcross markers) heterozygous in one parent and null in the other. By using the markers segregating in both parents (intercross markers) as bridges, the two parent-specific genetic maps were aligned. A number of nonparental heteroduplex markers were detected resulting from the PCR amplification of two DNA segments that had a high degree of homology to one another but differing in their nucleotide sequences. These heteroduplex markers served as bridges to generate an integrated map that included 19 major LGs and 24 minor groups. The 19 major LGs cover a total of 2,927 cM, with an average spacing between two markers of 23.3 cM.

Yin et al. (2001) constructed RAPD-based linkage maps for an interspecific cross between two species, *P. adenopoda* and *P. alba*, based on a double pseudo-testcross strategy. In the female parent, *P. adenopoda*, 82 markers were grouped in 19 different LGs (553 cM), whereas in the male parent, *P. alba*, 197 markers established a much more complete framework map with an observed genome length of 2,300 cM covering 87% of the total *P. alba* genome.

Cervera et al. (2001) constructed linkage maps for *P. deltoides*, *P. nigra*, and *P. trichocarpa* by analyzing progeny of two controlled crosses sharing the same female *P. deltoides* parent. The two-way pseudo-testcross mapping strategy was used to construct the maps. AFLP markers that segregated as 1:1 were used to form the four parental maps. SSR and STS markers were used to align homoeologous groups between the maps and to merge LGs within the individual maps. Linkage analysis and alignment of the homoeologous groups resulted in 566 markers distributed over 19 groups for *P. deltoides* covering 86% of the genome, 339 markers distributed over 19 groups for *P. trichocarpa* covering 73%, and 369 markers distributed over 28 groups for *P. nigra* covering 61%. Several tests for randomness showed that the AFLP markers were randomly distributed over the genome.

Yin et al. (2002) reported molecular genetic linkage maps for an interspecific hybrid population produced by crosses between *P. deltoides* (mother) and *P. euramericana* (father), which is a natural hybrid of *P. deltoides* (grandmother) and *P. nigra* (grandfather). A mixed set of the testcross markers, nonparental RAPD markers, and codominant AFLP markers was used to construct two linkage maps, one based on the *P. deltoides* genome and the other based on *P. euramericana*. The two maps showed nearly complete coverage of the genome, spanning 3,801 and 3,452 cM, respectively.

Zhang et al. (2004) reported the construction of AFLP genetic linkage maps for a hybrid pedigree derived from an interspecific backcross between the female hybrid clone *Populus tomentosa* × *P. bolleana* and the male clone *P. tomentosa*. A total of 782 polymorphic fragments were obtained using 49 primer combinations. Six hundred and thirty two of these fragments segregated into an 1:1 ratio, indicating that these DNA polymorphisms are heterozygous in one parent and null in the other. In the male *P. tomentosa* framework map, 218 markers were aligned in 19 major LGs. The linked loci spanned ca. 2,683 cM (87% coverage) of the poplar genome, with an average distance of 12.3 cM between adjacent markers. For female *P. tomentosa* × *P. bolleana*, the analysis revealed 144 loci, which were mapped to 19 major LGs and covered about 1,956 cM (77% coverage), with an average distance of 13.6 cM between adjacent markers.

Jorge et al. (2005) used an $F_1$ cross between *P. deltoides* and *P. trichocarpa* to construct linkage maps using RFLP, STS, RAPD, AFLP, and SSR markers with a subset of 90 genotypes using a pseudo-testcross strategy that resulted in a *P. deltoides* map of 2,803 cM and a 2,740 cM map for *P. trichocarpa*. Eight LGs could not be linked between the female and male parental maps. The

authors reported that SSR and AFLP genotyping was extended to 253 supplementary genotypes, but they could not publish the map resulting from them.

Several other linkage maps are under construction such as a more complete map for the Bradshaw et al. (1994) *P. trichocarpa* × *P. deltoides* $F_2$ cross (Tscaplinski et al. 2006; Sewell et al. unpublished), a *P. alba* × *P. alba* $F_1$ cross (Scarascia-Mugnozza, pers. comm.), and a *P. nigra* × *P. nirga* $F_1$ cross (Scarascia-Mugnozza, pers. comm.). A summary of published linkage maps is shown in Table 1.

A number of these maps report linkage map lengths greater than that estimated as the complete length reported by Bradshaw et al. (1994). This may be a genuine effect from using different mapping pedigrees, as discrepancies may be due to differences in genome coverage, the choice of mapping function, and differences in recombination rates in the parents of the crosses (Plomion and O'Malley 1996; Echt and Nelson 1997; Remington et al. 1999). There is a tendency for angiosperm females to display higher recombination rates than males (De Vicente and Tanksley 1991; Ganal et al. 1995), and hybrids might be expected to have suppressed recombination compared to pure species because of differentiation of the homologous chromosomes of the parental species (Jackson 1985; Tenhoopen et al. 1996; Chetelat et al. 2000).

However, it is possible that these extended lengths are due to genotyping error. Errors inflate the number of apparent recombinations and expand map distances (Harald et al. 2000). This is especially severe when markers are tightly linked since a misordered marker with genotyping error is most likely to be interpreted as a double crossover in regions with high marker density. For example, it has been shown that a 3% error rate in genotyping can double the genetic map length (Brzustowicz et al. 1993). Double crossovers and possibly misscored individuals or loci can be identified by specific commands in various mapping software packages (e.g., Lincoln and Lander 1992). Therefore, a comparison of map lengths with and without error detection enabled gives some indication of the level of error in the data set. Another indication of the level of genotyping error is the number of markers that cannot be properly mapped.

Possibly the most complete and dense linkage map is that published by Yin et al. (2004) for an interspecific backcross between a hybrid *P. trichocarpa* × *P. deltoides*, backcrossed to a pure *P. deltoides*. This map includes 544 markers mapped onto 19 LGs, with all markers displaying internally consistent linkage patterns. The combined length of the 19 LGs of the maternal parent was 2,313.9 cM with the error detection function of Mapmaker enabled, and 2,564.3 cM with error detection off. The average number of recombinations observed in the progeny was 24.789, which corresponds to a genome size estimate of 2,478.9 cM. The genome length was estimated to be between 2,300 and 2,500 cM, based both on the observed number of crossovers in the maternal haplotypes and the total observed map length. Genome coverage was estimated to be greater than 99.9% at 20 cM per marker. This genetic linkage map provides the most comprehensive view of the *Populus* genome reported to date and will prove invaluable for future inquiries into the structural and functional genomics, evolutionary biology, and genetic improvement of this ecologically important model species.

## Segregation Distortion

Liu and Furnier (1993) reported finding no evidence for segregation distortion in the first reported linkage map of *Populus*; however, a number of more recent studies have reported segregation distortion (Bradshaw and Stettler 1994; Yin et al 2004). This could be due to the limited number of available markers for this earlier map.

Reasons for skewed segregation ratios of molecular markers are still not well understood but are generally believed to be related to genetic factors such as chromosome loss and structural rearrangements (Williams et al. 1995; Kuang et al. 1999), genetic isolating mechanisms (Zamir and Tadmor 1986), the presence of an allele for pollen lethality (Bradshaw and Stettler 1994), or gametic selection (Zamir et al. 1982), as well as other nonbiological factors such as sampling in finite mapping populations or scoring errors (Plomion et al. 1995).

Bradshaw and Stettler (1994) detected distortion of expected Mendelian segregation ratios in the three-generation interspecific *P. trichocarpa* and *P. deltoides* cross. An RFLP linkage map was constructed around a single locus showing severe skewing of segregation ratio against $F_2$ trees carrying the *P. trichocarpa* allele in homozygous form. Several hypotheses for the mechanism of segregation distortion at this locus were tested, including directional chromosome loss, segregation of a pollen lethal allele, conflicts between genetic factors that isolate the parental species, and inbreeding depression as a result of genetic load.

**Table 1.** Summary of linkage maps produced in *Populus* species

| Species | Pedigree | No individuals | Markers type | No markers | Total genetic distance (cM) | Reference |
|---|---|---|---|---|---|---|
| *P. trichocarpa* × *P. deltoides* | $F_2$ | 90 | RFLP, STSs, RAPDs | 343 | 1,261 | Bradshaw et al. (1994) |
| *P. trichocarpa* | $F_2$ | 55 | AFLP, SSR | 147 | 2,002 | Frewen et al. (2001) |
| *P. deltoides* | | | | 145 | 1,778 | |
| *P. deltoides* × *P.deltoides* | $BC_1$ | 93 | AFLP | 198 | 2,927 | Wu et al. (2000) |
| *P. adenopoda* | $F_1$ | | RAPD | 82 | 533 | Yin et al. (2001) |
| *P. alba* | | | | 197 | 23 | |
| *P. deltoides* | $F_1$ | 127 | AFLP, STS SSR | 566 | 2,178 | Cervera et al. (2001) |
| *P. nigra* | | | | 339 | 2,356 | |
| *P. deltoides* | | 105 | | 566 | 1,626 | |
| *P. trichocarpa* | | | | 369 | 192 | |
| *P. deltoides* | $F_1$ | 93 | RAPD, AFLP | 560 | 3,801 | Yin et al. (2002) |
| *P. euramericana* | | | | | 3,452 | |
| *P. tomentosa* | $F_1$ | 120 | AFLP | 218 | 2,683 | Zhang et al. (2004) |
| *P. bolleana* | | | | 144 | 1,956 | |
| *P. deltoides* | $F_1$ | 253 | RFLP, STS, RAPD, AFLP, SSR | 200 | 2,803 | Jorge et al. (2005) |
| *P. trichocarpa* | | | | 191 | 274 | |
| *P. trichocarpa* × *P. deltoides* | $BC_1$ | 180 | SSR, AFLP | 544 | 2,313 | Yin et al (2004) |
| *P. deltoides* | | | | | | |

A recessive lethal allele, *lth*, inherited from the *P. trichocarpa* parent, was found to be tightly linked to the RFLP marker and to cause embryo and seedling mortality.

Cervera et al. (2001) reported that markers cosegregating with a *Melampsora larici-populina* resistance gene showed a significant deviation in *P. deltoids* due to missing genotypes resulting from the death of susceptible trees.

Yin et al. (2004) detected some markers exhibiting segregation distortion that occured largely in two LGs. In fact, LG IV showed 92.1% of the chromosome was distorted with predominant alleles from *P. trichocarpa*. The distorted region of LG IV contains a locus conferring resistance against the leaf rust pathogen *Melampsora* × *columbiana* (Stirling et al. 2001; Yin et al. unpublished) and the distorted region of LG XIX contains another locus conferring resistance against *Melampsora larici-populina* (Zhang et al. 2001; Yin et al. unpublished results). The researchers hypothesized that divergent selection has occurred on chromosomal scales among the parental species used to create this pedigree and explored the evolutionary implications.

### Comparative Maps

Early maps that used anonymous and dominant markers, such as RAPDs and AFLPs, left little scope for alignment or comparative mapping between different pedigrees. Some work on later linkage maps using codominant markers, such as SSRs, has allowed the alignment of these maps. The publicly available SSRs used in many recent linkage maps has allowed alignment of these maps, and LGs have been assigned the names used for the map produced by Cervera et al. (2001). In addition to this, the SSR primer sequences can now be blasted against the genome sequence so linkage maps can be aligned to the physical sequence (Street et al 2006; Rae et al 2006).

## 1.3 Detection of Quantitative Trait Loci

Fundamental to the breeding of poplar is the understanding of the genetic control of quantitative traits, such as biomass yield and disease resistance. Such traits are often a function of many internal plant processes and their interactions with the environment. These traits are usually complex, requiring knowledge of quantitative genetics to resolve their action. Elucidation of QTLs is useful not only for analyzing important agronomic traits for breeding purposes but also for understanding fundamental aspects of genetic control and about the nature of the QTLs, such as genome position, what they do, and how they act and interact, and can provide a good starting point for future studies on individual genes and genomic regions or for focusing on the inheritance and evolution of specific traits of interest.

QTL analysis involves looking for associations between the trait and molecular marker alleles segregating in a population. This requires accurate data and effective statistical software. Most QTL analyses in plants involve populations derived from pure lines, and several different approaches have been developed to associate QTLs with molecular markers (Kearsey and Farquhar 1998). The difficulties of QTL discovery in *Populus* can never be underestimated due to their outbreeding and dioecious nature. The quantitative genetics of outbreeding species is complicated by their heterozygosity so that up to four alleles may be segregating at each locus.

Early work to identify QTLs in *Populus* used similar methods to those used for pure breeding inbred lines, making the assumption that, in the case of an interspecific $F_2$ pedigree, the grandparents showed enough variation between species to be treated as purebred parents. An example of this is the work by Wu et al. (1997) on leaf traits in an $F_2$ pedigree between *P. trichocarpa* and *P. deltoides*. The species differed enough both in genetic polymorphisms used to construct a linkage map and in the leaf traits being analyzed to assume that heterozygous alleles within the grandparental species differed between species. This method assumes that most of the genetic variance for the traits of interest is partitioned between the grandparental species rather than among individuals within the species, but there are disadvantages of treating the parental clones as inbred lines as it fails to take into account that *Populus* clones are highly heterozygous. The advantage of treating the two grandparental species as inbred lines is that any marker that is polymorphic between the two species is informative for QTL mapping under the "simplified" model, and such "fixed" polymorphisms are more abundant than the multiallelic markers necessary for a more thorough analysis of QTL inheritance (Bradshaw and Grattapaglia 1994).

In the same way that the methods for construction of genetic linkage maps have been developed

for outbred pedigrees, so have the methods for QTL mapping, particularly with the use of $F_1$ or backcross pedigrees using the pseudo-testcross approach put forward by Grattapaglia and Sederoff (1994).

### 1.3.1 Disease

Infection by rust fungus has a devastating impact on poplar plantations worldwide. Leaf rust caused by the fungus *Melampsora larici-populina* causes premature defoliation and can reduce growth by more than 20%. Trees defoliated early in the growing season become more susceptible to secondary pathogen infection and environmental stress. Therefore, durable resistance to rust pathogens is one of the main objectives of poplar breeding in Europe. Both qualitative and quantitative forms of *M. larici-populina* resistance have been described often with qualitative resistance inherited from the North American species *P. deltoides*.

Early works studied the qualitative resistance of poplar. Molecular markers were linked to simple traits for the disease resistance provided by a single gene with the discrete phenotypes for resistant and susceptible. For example, Villar et al. (1996) identified RAPD markers involved in qualitative resistance to rust fungus, *Melumpsora larici-populina*. Cervera et al. (1996a) identified three AFLP markers tightly linked to the locus for rust resistance for three races of *M. larici-populina* in the hybrid progeny derived from an interspecific cross between a resistant *P. deltoides* female and a susceptible *P. nigra* male, using both high-density marker analysis and bulked segregant analysis (BSA).

Newcombe (1998) used the three-generation *P. trichocarpa* × *P. deltoides* hybrid poplar pedigree to investigate the genetic control of resistance to an isolate of *M. medusae* sp. *deltoidae*. Necrotic flecking and rust severity were evaluated over 2 years in field and growth-room experiments and a laboratory assay of leaf-discs. Necrotic flecking in the field and growth-room experiments was found to be governed by a single, dominant gene inherited from the *P. trichocarpa* grandparent, using both nonparametric and QTL analyses of rust severity in the field and growth-room experiments. In contrast, the leaf-disc assay did not support a simple genetic interpretation.

Later works focused on the quantitative trait of rust tolerance rather than qualitative trait rust resistance. Jorge et al. (2005) worked with an $F_1$ progeny from an interspecific *P. deltoides* × *P. trichocarpa* cross consisting of 343 individuals. The *P. deltoides* parent was attributed to have quantitative and qualitative resistance, while the *P. trichocarpa* had quantitative resistance only (Dowkiw and Bastien 2004). Using a pseudo-testcross strategy, composite interval mapping (CIM) (Zeng 1993; Jansen and Stam 1994) was employed for QTL analysis so that the presence of the qualitative resistance could be fixed as a major genetic cofactor, so as to minimize the negative effect in the detection of other minor QTLs. Two QTLs with broad-spectrum effects were identified, one inherited from *P. deltoides* and one from *P. trichocarpa*. An additional seven QTLs with limited and specific effects were also detected.

Works have also been carried out to understand the genetic control of resistance to leaf spot caused by *Septoria populicola*. Hybrid $F_1$ clones of *P. trichocarpa* × *P. deltoides* are usually intermediate in disease phenotype between their susceptible *P. trichocarpa* and resistant *P. deltoides* parents. Newcombe and Bradshaw (1996) used the three-generation *P. trichocarpa* × *P. deltoides* pedigree to evaluate the percentage of leaves affected by leaf spot. QTL analysis revealed that a two-QTL model explained 68.3% and 61.2%, and 71.9% and 70.3%, of phenotypic and genetic variance, respectively, in the $F_2$ generation over the 2 years.

### 1.3.2 Stem and Growth Traits

Much early work focused on the identification of QTLs for easily measured canopy, stem, and biomass traits. Bradshaw and Stettler (1995) reported QTLs for stem growth and form in an interspecific $F_2$ pedigree using the inbreeding mapping software MAPMAKER/QTL 1.1 (Lincoln et al. 1992). The study reported several QTLs of large effect, responsible for a large portion of the genetic variance. For example, 44% of the genetic variance in stem volume was controlled by just two QTLs. The authors suggested that, instead of the expected polygenic model (the combined action of environmental effects and multiple genes of small and cumulative effects), the traits studied were under oligogenic control (few QTLs with relatively large effect). This is backed up by QTL analysis on canopy structure (Wu and Stettler 1998). However, later studies using the same pedigree reported a larger number of detected QTLs with smaller effects for stem growth (Rae et al. personal communication). The earlier study was

carried out with a considerably smaller sample size (90 $F_2$ individuals compared to 210) and so was less likely to detect QTLs of small effect. Also later studies have made use of outbred mapping methods (Grattapaglia et al. 1996; Knott et al. 1997) and software such as QTLexpress (Seaton et al. 2002).

Wullschleger et al. (2005) reported 31 QTLs for distribution of biomass between stems, branches, leaves, and coarse and fine roots in the interspecific backcross of a hybrid *P. trichocarpa* × *P. deltoides*, backcrossed to a pure *P. deltoides*. The high-density linkage map produced by Yin et al. (2004) was analyzed using QTL cartographer. The percentage phenotypic variation explained by single QTLs ranged from 7.5 to 18.3%.

Allometric analysis in this study highlighted that as trees increased in age, biomass was preferentially distributed to stems and branches, while distribution to the roots decreased. This brings about the question as to whether QTLs identified early in the development of long-lived tree species can be taken as a good indication of genetic control later in development.

Wu et al. (2003) presented a statistical model for mapping QTLs underlying age-specific growth rates. A maximum likelihood approach based on the mechanistic relationship between growth rates and ages established by a variety of mathematical functions was developed to provide the estimates of QTL position, growth parameters characterized by QTL effects, and residual variances and covariances. The effects of the identified QTLs for growth were described as a function of age, so that age-specific changes in QTL effects can be readily projected throughout the entire growth process. The QTLs displayed increased effects on growth as trees aged, yet the timing of QTL activation was found to be earlier for stem height than diameter, which is consistent with the ecological viewpoint of canopy competition.

### 1.3.3 Leaf Growth

A number of QTL analyses have been carried out on leaf growth and development traits due to the great variation in leaf morphology between species and the availability of interspecific hybrids showing high levels of leaf area heterosis. Wu et al. (1997) reported the mapping of several leaf QTLs from the interspecific $F_2$ pedigree between *P. trichocarpa* and *P. deltoides*. The grandmaternal species is known for its long lanceolate, as opposed to deltoid leaves with short round petioles. The leaves differ in that the *P. trichocarpa* has few large cells with a low stomatal density while *P. deltoides* has many small cells with high stomatal density. The $F_1$ combine the two leaf cell types to result in heterosis in leaf area, and the $F_2$ generation shows much variation and transgressive segregation. The variation within this mapping pedigree has been utilized in other QTL mapping studies to understand leaf response to drought (Street et al. 2006) and $CO_2$ level (Ferris et al. 2002; Rae et al. 2006). Figure 1 shows the alignment of the genetic linkage map and physical sequence used by Rae et al. (2006) to compare the QTL positions to differentially expressed genes under elevated $CO_2$ conditions.

### 1.3.4 QTLs and the Environment

QTL analysis has been carried out across different environments and treatments to study the genetic control of tree response to the environment. Ferris et al. (2002) reported QTLs for epidermal cell size and number, and stomatal density, in the hybrid pedigree of *P. trichocarpa* × *P. deltoides* in both ambient and elevated concentrations of $CO_2$.

Genotype by environment interactions have been studied in a number of QTL mapping experiments in poplar using specially designed software such as multiQTL (www.multiQTL.com; Korol et al. 1998). For example, Rae et al. (personal communication) mapped QTLs for stem growth traits across three contrasting sites and tested for differences in additive genetic effects of QTLs mapped at the three sites. Another approach is the mapping of QTLs for "plasticity parameters" or "response QTLs." An example of this is the use of an additive main effects and multiplicative interaction (AMMI) model (Gauch 1988) to determine genotype by environment (GxE) interactions that can be used as measures of trait plasticity and used to identify QTLs that occur in response to altered atmospheric $CO_2$ (Rae et al. personal communication).

Similar work by Rae et al. (2006) reported QTLs for a number of leaf-growth traits and senescence when the interspecific $F_2$ *P. trichocarpa* × *P. deltoides* pedigree was grown in ambient and elevated $CO_2$ conditions. *P. trichocarpa* showed a greater response to elevated $CO_2$. In the $F_2$ generation, leaf development and quality traits, including leaf area, leaf shape, epidermal cell area, stomatal number, specific leaf area,

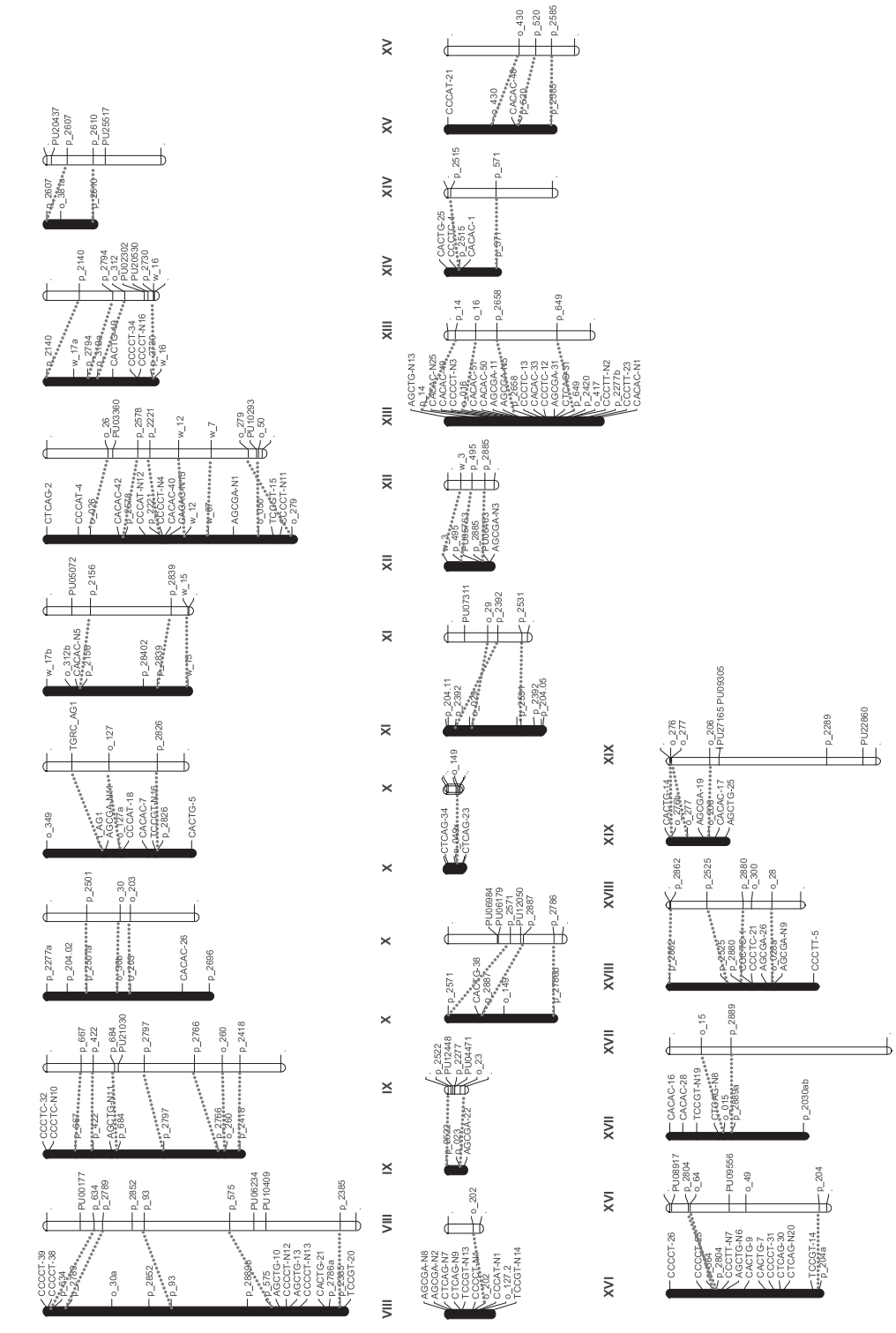

**Fig. 1.** Linkage map and physical map of *Populus trichocarpa* aligned using microsatellite primer sequences. The linkage map is shown in black showing microsatellites and AFLP markers and the physical sequence is in white showing microsatellites and candidate genes differentially regulated in elevated $CO_2$

and the canopy senescence index, were sensitive to $CO_2$ level. Many QTLs for leaf and senescence traits mapped to similar positions under ambient and elevated $CO_2$ conditions, but some genomic regions showed differential control backed up by the mapping of "response QTLs," which is the percentage difference between trees grown under elevated and control $CO_2$ conditions calculated for each individual and the results used as a trait for which response QTLs could be mapped. Two candidate genes identified as being differentially expressed under ambient and elevated $CO_2$ conditions in the grandparents (Taylor et al. 2005) were found to collocate to the regions in which response QTLs were mapped.

The response of *Populus* to drought stress has been studied in several experiments (Monclus et al 2006; Tschaplinski et al. 2006), and QTLs were mapped for such traits as specific leaf area, osmotic potential, and relative growth rate.

Street et al. (2006) utilized different responses of the grandparental species of the $F_2$ pedigree produced by Bradshaw and Stettler (1994). *P. trichocarpa* comes from the wetland region of the western USA, whereas *P. deltoides* comes from the dryer eastern regions of the USA. The $F_2$ pedigree has been shown to display transgressive segregation for drought response. This work mapped QTLs for leaf abscission, leaf expansion, photosynthesis, and transpiration when trees were grown under control and drought conditions. In addition to the comparison of QTL location for drought and control trees, response QTLs were also mapped. For each trait, the percentage effect between control and drought was calculated and used as a trait to map QTLs. A number of response QTLs mapped to regions of the genome to which drought QTLs mapped, suggesting that these regions were involved in the control of drought stress response. A transcriptome experiment was run alongside this work to identify genes differentially expressed between $F_2$ individuals showing the extreme responses to drought stress. SSR markers were used to align the genetic linkage map with the physical sequence of poplar. The degree of differentially expressed genes that collocated to genomic regions identified by QTL analysis was examined. The authors hypothesized that the occurrence of collocation could be due to differences in *cis*-acting elements (promoter sequences) in differentially expressed genes, i.e., the genes may be involved in the control of drought response, but the expression may be regulated in *trans*-acting by changes in a transcription factor regulating the drought response. Work is now under way to map the expression of the genes identified in this study to identify the genes of greatest interest and potential expression QTL (eQTL) mapping as carried out in eucalyptus (Kirst et al. 2004).

### 1.3.5 Phenology QTLs

Much of the work done to identify candidate genes for mapped QTLs has concentrated on phenological traits, such as bud burst and bud set. The first report of aligning QTLs with putative genes was detailed by Frewen et al. (2000). An interspecific *P. trichocarpa* × *P. deltoides* $F_2$ was used to map QTLs. Five candidate genes involved in photoperiod perception and transduction were mapped to the linkage map. Two candidate genes were found to collocate with QTLs effecting bud set and bud flush.

Dormancy-related QTLs were also reported to collocate with candidate genes for abscisic acid response signals by Chen et al. (2002)

### 1.3.6 Metabolite QTLs

Metabolite profiling has gained much attention as a powerful functional genomics tool to unravel gene function. Metabolite levels are controlled by both genetic and environmental factors, enabling Morreel et al. (2006) to consider metabolite concentrations as quantitative traits in a search for metabolite quantitative trait loci (mQTLs) that control their abundance and suggest that QTL analyses of the concentrations of all intermediates in a given biochemical pathway can reveal flux-regulating control points. In contrast to other complex traits, the molecular structure of metabolites and the knowledge of the pathway architecture may already suggest the function of the gene underlying the mQTL. Using the two half-sib $F_1$ pedigrees and linkage maps described by Ceverea et al. (2001), Morreel et al. (2006) detected four robust mQTLs that control flavonoid levels. A multivariate QTL analysis was used due to its better performance than single-trait analyses in cases of functionally related traits, such as the concentration of pathway intermediates. AFLP and SSR markers from the linkage maps were aligned with the genome sequence.

The chemical structure of the flavonoids, coupled with current knowledge of the pathway architecture

and in silico mapping of candidate genes, allowed the tentative assignment of a function to three of these mQTLs. The data indicate that the combination of metabolite profiling with QTL analysis is a valuable tool to identify control points in a complex metabolic pathway of closely related compounds.

### 1.3.7 Candidate Genes

Candidate genes for QTLs have been suggested using a number of methods such as the collocation of QTLs and genes mapped to the linkage map (Frewen et al. 2000) and alignment of genetic maps with the physical genome sequence using maker sequence information. Candidate genes have then been identified from the literature (Taylor et al. 2006) or transcriptome studies (Rae et al 2006; Street et al 2006).

The release of the full genome sequence for poplar has allowed an estimation of total gene number to be calculated. Based on initial gene model data, it is estimated that the poplar genome consists of around 30,000 genes, but the actual number may be lower due to duplicated regions of the genome containing nonfunctional genes (S. Jansson personal communication). Taking into account the average length of the linkage maps used to map QTLs, it can be estimated that 1 cM distance may contain ca. 15 to 20 genes.

## 1.4 Advanced Studies

Traditional breeding for *Populus* has mainly focused on the selection of trees for fast growth traits. Work based on the analysis of phenotypes has provided a wealth of information on genetic material through multigeneration pedigrees. Molecular technologies and genetic linkage maps have led to new information for breeding strategies in *Populus*. The release of the complete poplar genome sequence and functional genomic information can be exploited to directly target candidate genes putatively involved in the control of the traits of interest, thus increasing the power of marker-assisted selection (MAS) (Strauss et al. 1992). SSR markers have been shown to provide an ideal bridge for map comparison and direct links to the genomic sequence. Surprisingly, there are no reports yet of actual application of MAS in tree species.

### 1.4.1 Genomic Resources for Poplar

During the past decade research and resource development in poplar has forged ahead beyond all other forest tree species (Bradshaw et al. 2000; Taylor 2002; Wulshleger et al. 2002; Brunner et al. 2004). As a result, numerous genomic resources are now publicly available that facilitate more rapid candidate-gene selection and collocation with mapped QTLs, for example. Most important among these are the poplar genome sequence, EST collections, and expression microarrays.

### 1.4.2 The Poplar Genome Sequence

The sequencing of the poplar genome, specifically the female *P. trichocarpa* Nisqually 1, was recently reported by Tuskan et al. (2006) and is hosted by the Joint Genomes Initiative (JGI) at http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html. Genome assembly was achieved through shotgun sequencing combined with genetic mapping to allow chromosome reconstruction with the assembled genome containing 450 Mb and an estimated 45,555 protein-coding loci. Roughly 89% of predicted gene models contained homology to protein sequences held in NCBI, although some 12% (5,248) contained no evidence of homology to known genes in *Arabidopsis thaliana*, suggesting that these may represent tree-specific genes. The reader is referred to Tuskan et al. (2006), and especially the supplementary information available, for further details. A finding of great interest from the genome sequencing project, particularly in relation to the genetic architecture of trait control, was that of the shared Salicaceae genome-duplication event in addition to an older duplication event shared with *A. thaliana* (termed the salicoid and eurosid duplication events by Tuskan et al. 2006). A substantial number of paralogous genes resulting from the more recent salicoid event were identified, and the transcriptional activity and specificity (from a tissue/development/response perspective) of such paralogs must now be examined and considered when interpreting the architecture of trait control. Re-examination of some of our own QTL results suggests that duplicated regions of the genome may be exerting control for some traits (unpublished data). These paralogs also need to be considered carefully when

**Library distribution:** POPLAR.226

This cluster contains 46 Clones (48 sequences)

| Library | Clones | expected | Clones/LibSize |
|---|---|---|---|
| Cambial zone (A + B): | 1 | 2.73 | 0.000167 |
| Young leaves (C) | 1 | 2.22 | 0.000206 |
| Flower buds (F) | 11 | 2.97 | 0.001687 |
| Senescing leaves (I) | 4 | 2.52 | 0.000723 |
| Apical shoot (K) : | 2 | 2.46 | 0.000371 |
| Cold stressed leaves (L): | 2 | 1.86 | 0.000489 |
| Roots (R) : | 1 | 2.64 | 0.000173 |
| Shoot meristem (T): | 11 | 3.82 | 0.001313 |
| Male catkins (V): | 2 | 2.23 | 0.000409 |
| Dormant buds (Q): | 2 | 2.66 | 0.000342 |
| Female catkins (M) : | 1 | 2.79 | 0.000163 |
| Petioles (P): | 4 | 2.95 | 0.000618 |
| Imbibed seeds (S): | 3 | 2.84 | 0.000482 |
| Virus/fugus-infected leaves (Y): | 1 | 0.64 | 0.000716 |

**Library distribution:** POPLAR.398

This cluster contains 18 Clones (23 sequences)

| Library | Clones | expected | Clones/LibSize |
|---|---|---|---|
| Senescing leaves (I) | 14 | 0.99 | 0.002530 |
| Bark (N): | 1 | 0.87 | 0.000204 |
| Male catkins (V): | 1 | 0.87 | 0.000205 |
| Female catkins (M) : | 1 | 1.09 | 0.000163 |
| Petioles (P): | 1 | 1.15 | 0.000155 |

**Fig. 2.** Library distributions of two EST sequences annotated as ACC oxidase in PoplarDB (http://www.populus.db.umu.se). Such digital northerns provide evidence that ESTs such as these have become functionally differentiated within the *Populus* genome

designing single nucleotide polymorphism (SNP) or other sequence-based gene markers to ensure specificity to only one gene copy. Roughly a third of salicoid duplicated genes represented in *Populus* EST libraries show evidence of differentiated expression, a finding that was supported by examination of the expression patterns of duplicated genes using DNA microarrays (Tuskan et al. 2006).

The availability of a genome sequence makes some gene-finding strategies at least partially superfluous and redundant. For example, chromosome walking and map-based cloning could be considered as offering little purpose as the order and location of virtually all genes are already known and large-scale differences in gene order and content are not expected between members of a genus or even family. Evidence for

the near-perfect colinearity and synteny between both fellow members of the *Populus* genus and between *Salix* and *Populus* has been provided by a number of QTL studies using families produced from interspecific crosses, including that used for the chromosome reconstruction of the genome sequence.

Marker development is also greatly facilitated by the genome sequence with sequence repeats being visually displayed within the JGI genome viewer and the obvious ability to develop primers for SNP genotyping. SNP marker development offers a rapid means of adding candidate genes to genetic maps for subsequent QTL mapping, and the availability of intron-exon boundary information means that markers spanning both can be designed to maximize the possibility of SNP detection. Of course, it is also true that exon-specific markers can also be designed for RT-PCR analysis of transcript levels for expression QTL mapping (see below) and for transcript-trait correlation analysis.

### 1.4.3 EST Resources

A number of extensive EST collections have been developed from a range of poplar species (Table 2). These have facilitated much research as well as playing an important role in the sequence assembly and annotation of poplar (Tuskan et al. 2006). The most advanced EST resource is that available in Populusdb from the Umeå Plant Science Centre (UPSC), which offers extensive search options (annotation, homologs, library, BLAST) as well as the ability to view the library distribution of an EST of interest – something that offers potential insight into gene function and that can be used to produce a digital northern (for example, Street et al. 2006 used digital northerns to determine that genes induced by drought stress show greater similarity to genes associated with winter dormancy than with cold stress). Such library distribution patterns are extremely useful when identifying candidate genes for genomic and genetic studies such as QTL-candidate gene collocation (discussed below). For example, examination of ESTs annotated as ACC oxidase in Populusdb reveals that many appear to have tissue-specific expression patterns, and such knowledge drastically increases the ability to select a functionally relevant candidate (compare clusters POPLAR.3983 and POPLAR.2266) (Fig. 2).

These EST libraries have been constructed from a wide range of library types representing many poplar species, as well as a wide variety of tissue types. In general, there is a bias toward woody tissues, especially differentiating xylem, but across all the collections most tissue types are represented, and a range of treatment conditions have also been used to enrich libraries for genes associated with both abiotic (particularly the *P. euphratica* libraries detailed in Brosche et al. 2005) and biotic (Ralph et al. 2006) stress responses.

### 1.4.4 Expression Microarrays

The use of microarrays to examine the transcriptional response of organisms caused something of a paradigm shift in biology, and this trend has held true in poplar. There are now a number of microarray resources available for poplar representing nearly the full cross-section of array technologies. The first developed microarray resource was derived from the UPSC EST collection described in Sterky et al. (2004). The Populusdb EST database was used to identify a unigene set of representative EST clones for spotting as cDNA onto glass microarrays. The first generation of developed arrays (referred to as POP1) contained 13,490 clones selected from 7 of the 19 libraries contained in Populusdb. A second-generation array (POP2) was then constructed containing 24,912 spotted clones selected from all 19 libraries and representing ca. 40% of all predicted gene models in the poplar genome (Street et al. 2006). These arrays have been used to examine a range of processes including wood formation (Schrader et al. 2004; Moreau et al. 2005), response to abiotic infection (Smith et al. 2004), gene-expression changes associated with autumnal senenscence (Andersson et al. 2004), and response to biotic factors including the effects of elevated atmospheric [$CO_2$] (Taylor et al. 2005; Druart et al. 2006) and drought stress (Street et al. 2006). Another cDNA array resource has been developed at INRA that was initially spotted as a nylon membrane macroarray and used to examine the poplar root transcriptome (Kohler et al. 2003) and elevated [$CO_2$] and [$O_3$] responses (Gupta et al. 2005). This macroarray was produced from the EST clones represented in PoplarDB (Kohler et al. 2003). These clones have now been donated to the PICME project (www.picme.at) and are available spotted alongside the EST collection

**Table 2.** Sources of EST sequence data for Populus

| EST Resource | # ESTs | Website address | Key reference |
|---|---|---|---|
| Populusdb | 121,495 | www.populus.db.umu.se/ | Sterky et al. (2004) |
| PoplarDB | 20,005 | http://mycor.nancy.inra.fr/PoplarDB/ | Kohler et al. (2003)<br>Brosche et al. (2005) |
| AspenDB | 5,410 | http://aspendb.mtu.edu/ | Ranjan et al. (2004) |
| DFCI Poplar Gene Index | 371,518 | http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=poplar | Amalgamation of multiple EST resources |
| Treenomix | ~90,000 | http://www.treenomix.ca/cDNA-sequencing/<br>No public database, ESTs submitted to GenBank | Ralph et al. (2006) |
| Arborea | 11,591 | http://www.arborea.ulaval.ca/research_results/est_sequencing_in_poplar/ | |
| GenBank | 376,600 | As of 26/10/2006 (search term 'populus') | http://www.ncbi.nlm.nih.gov/ |
| Sputnik | 137,728 | http://sputnik.btk.fi/ | |

described in Brosche et al. (2005) as a cDNA glass-based microarray representing an estimated 9,500 genes (F. Martin personal communication). There are additionally two short oligomer microarrays available from Affymetrix (www.affymetrix.com) and Nimblegen (www.nimblegen.com). These are both whole-genome arrays with oligo probe sequences having been designed based on predicted genemodel sequences from V1.1 of the genome (Tuskan et al. 2006) as well as EST sequences (in the case of Nimblegen, these are detailed in Tuskan et al. 2006 and for Affymetrix EST sequences stored by TIGR [see DFCI entry in Table 2]). A notable gap in the availability of microarrays is the lack of an oligo expression array, although a 70-mer genomewide array is currently in development (N. Street personal comm.).

As well as the physical availability of microarrays, access to existing microarray data is a key genomics resource, as any user of Genevestigator (https://www.genevestigator.ethz.ch) or other such services will attest. Beyond actual access to the intensity values for arrays, a means of analyzing and interpreting these is also crucial if microarrays are to be exploited to their full potential. In the case of poplar, a microarray resource for the community has been established in the form of UPSC-BASE (www.upscbase.db.umu.se). This is a combined Web-based GUI resource integrating the open-source BASE (http://base.thep.lu.se) project for the storage of microarray data alongside the implementation, normalization, analysis, interrogation, and visualization methods, and the annotation and functional classification information stored in Populusdb. Public access is offered for all published microarray data, and it is intended that this resource be developed to serve the poplar community.

### 1.4.5 Applying Genomic Resources for Candidate-Gene Discovery

Microarrays are being used for an ever expanding range of applications including the mapping of expression QTL (eQTL), such as was demonstrated in Kirst et al. (2005) in a backcross *Eucalyptus* family. Street et al. (2006) proposed an alternative use for microarrays within an eQTL context whereby they compared the transcriptome in response to drought stress of a highly sensitive and tolerant set of genotypes from an $F_2$ interspecific population to identify what can be termed gene expression markers (GEMs) (West et al. 2006). They proposed the subsequent mapping of GEMs as eQTL using RT-PCR as this reduces the use of microarrays significantly, making the approach cheaper and statistically less demanding, although such an approach does limit the potential insight offered by mapping eQTL for noncandidate genes. The use of eQTL mapping represents a potentially productive method of elucidating the genetic architecture of complex trait control and can answer questions as to whether the control of a trait response is the result of a *cis*-acting polymorphism within a structural gene or due to a *trans*-acting

factor. In the case of a *trans*- effect, mapping eQTLs for multiple genes with collocating QTLs may identify the locating of the *trans*- acting factor. There are additional uses to which microarrays can be put if they are used for hybridizing genomic DNA rather than cDNA (Hazen and Kay 2003). The genomic DNA of the parents of a mapping population can be hybridized to expression arrays in order to identify single feature polymorphisms (SFPs) (Hazen and Kay 2003). If SFPs are identified, the approach can be extended to use for bulked segregant analysis (BSA) by forming two pools of DNA from groups of genotypes lying at contrasting ends of a phenotypic trait distribution, hybridizing these pools, and subsequently identifying polymorphism between them. This has the potential to produce a set of polymorphisms that densely cover a genome and can subsequently be used to map the location of the causal polymorphism with reasonable resolution. Such an approach has recently been demonstrated in *Arabidopsis thaliana* (Borevitz et al. 2003; Singer et al. 2006) and yeast (Brauer et al. 2006; Gresham et al. 2006) but has yet to be extended to forest trees. The approach is most applicable to use on short oligomer arrays such as those made by Affymetrix and Nimblegen.

The above methods of eQTL mapping and array-assisted BSA have particular appeal for use in poplar as they help to overcome or reduce the inherent limitations that often plague bridging the QTL-gene barrier in forest trees. Traditional gene-mapping approaches are hard to apply in forest trees due to the problems of producing and maintaining mapping families of adequate size to represent enough recombination events to ensure adequate mapping resolution. Forest tree species are not only problematic due to their long generation times and the cultural demands of planting and maintaining populations but also because their typically outbreeding nature, with resulting inbreeding depression, limits the type of populations that can be constructed – NILs and RILs are the jealous desire of many a tree geneticist. Genomic resources enable new approaches to be taken to attempt to identify genes containing the causal polymorphisms identified by QTL location. EST databases and microarray analysis both represent powerful means of identifying candidate genes for which the potential collocation to QTL can be examined. In the case of EST libraries, candidate-gene selection will be based on a priori knowledge of gene function, whereas microarrays allow hypothesis-independent identification of genes associated with a response trait or developmental process. In the case of poplar, the availability of the physical genome sequence allows for the immediate physical location of the candidate gene to be known. Genetic maps can then be aligned against the physical map, and the collocation of candidate genes on the physical map and QTL on the genetic map can then be examined (Street et al. 2006). Alternatively, the candidates can be sequenced in the parents of the mapping family, and sequence polymorphisms (SNPs, indels, etc.) can be identified and utilized to develop sequence-based markers. The mapping population can then be genotyped for these markers and added to the genetic map used for QTL analysis, and collocation can be examined in this way. This has advantages over the physical-genetic comparison method as it takes into account local recombination frequencies. In such a case, the candidate gene should represent the closest flanking marker to the peak QTL location score in order for it to represent a convincing candidate. This approach is limited due to the large average size of QTL intervals (10 to 30 cM) that often can contain several hundred to a few thousand genes depending on local recombination frequency. For example, Street et al. (2006) acknowledge that the QTL regions within which there are collocating candidate genes contain an average of 433 genes, making the likelihood of collocation by chance relatively high. Another option made available by the genome sequence is that of identifying all genes lying between the flanking markers of a QTL region and the annotation-based selection of candidates from these using a priori knowledge of their function. Ultimately all of these methods fail to provide causal proof, which is ideally provided through functional means such as over- or under-expression analysis in transgenic plants. Poplar has been shown to be highly transformable, with an increasing number of species and hybrids being successfully transformed using a range of technologies (Han et al. 1997; Rottmann et al. 2000; Groover et al. 2004; Jing et al. 2004; Meyer et al. 2004; Nowak et al. 2004; Strauss et al. 2004; Busov et al. 2005; Filichkin et al. 2006).

These approaches represent powerful means of identifying candidate genes for the control of traits of commercial importance, such as wood quality, biomass yield, and biotic pest resistance, as well as identifying genes responsible for the vast diversity that exists within the *Populus* genus. The identification of ecologically important genes is likely to become increasingly important as a result of the need to understand plant-level responses to climate change

as well as requiring the development of new elite varieties that can achieve commercially viable yields in the context of a changing climate.

Comparative genomics approaches comparing species exposed to selection pressures in contrasting natural ranges with, for example, differing precipitation regimes represent a method of identifying existing genetic adaptations that could be exploited to provide commercial benefit, either through means of transgenic manipulation or directed breeding. It can be expected that the *Salix* genome will be largely colinear with that of *Populus* (Tuskan et al. 2006), which should allow for comparative mapping between the two genera. The recent divergence of *Salix* and *Populus* also means that genomic resources developed in one genus, particularly long-oligo and cDNA microarrays, are suitable for use in both (Sterky et al. 2004), making possible a number of interesting comparative studies across the whole of the Salicaceae. Street et al. (2006) recently showed that this approach has the potential through the use of cDNA microarrays to examine the transcriptional drought response of two contrasting *Populus* species whose natural ranges differ in mean annual precipitation and temperature. They showed that microarrays could identify genes with contrasting responses when the two species are exposed to drought, suggesting that gene-expression control is important in the process of speciation. They additionally showed that large-scale differences in transcriptional response could be identified for drought response within an $F_2$ population formed from a cross between the two species. Many of the transcriptional differences they identified can be expected to be the result of control exerted by few *trans*-acting elements, and subsequent work to identify these represents an excellent method to identify candidate genes for manipulation to produce trees with a desired response to drought.

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