

Salient Biological Features, Systematics, and Genetic Variation of *Populus*

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Abstract The genus *Populus* includes morphologically diverse species of deciduous, relatively short-lived, and fast-growing trees. Most species have wide ranges of distribution but tend to occur primarily in riparian or mountainous habitats. Trees from this genus are typically dioecious, flower before leaf emergence, and produce large amounts of wind-dispersed pollen or seeds. Seedlings are drought- and shade-intolerant, and their establishment depends on disturbance and high soil moisture. Asexual reproduction is common and occurs via root sprouting and/or rooting of shoots. Fossil records suggest that the genus appeared in the late Paleocene or early Eocene (i.e., 50–60 million years BP). According to one commonly used classification, the genus is comprised of 29 species divided into six sections, but a number of phylogenetic inconsistencies remain. Natural hybridization both within and among sections is extensive and is believed to have played a major role in the evolution of extant species of *Populus*. Both neutral molecular markers and adaptive traits reveal high levels of genetic variation within populations. Deviations from Hardy–Weinberg equilibrium are commonly detected in molecular marker studies. These deviations typically have small to moderate magnitudes and tend to be caused by heterozygote deficiency, indicating the possible existence of population substructure. Genetic differentiation among populations is much stronger for adaptive traits than for neutral markers, which suggests that divergent selection has played a dominant role in shaping patterns of adaptive genetic variation. Molecular and bioinformatic resources are actively being developed for multiple species of *Populus*, which makes this genus an excellent system for studying tree genetics and genomics.

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1 Dendrological Overview

1.1 Morphology

Species of the genus *Populus* (commonly known as aspens, cottonwoods, and poplars) are deciduous or, rarely, semi-evergreen trees that occur primarily in the boreal, temperate, and subtropical zones of the northern hemisphere (Eckenwalder, 1996; Dickmann, 2001; Cronk, 2005). Trees from this genus typically have tall and straight single trunks, with bark that tends to remain thin and smooth until more advanced ages than in other tree species (Eckenwalder, 1996; Dickmann, 2001). They rarely live longer than 100–200 years, but are among the fastest growing temperate trees and can reach large sizes. A notable example is black cottonwood (*Populus trichocarpa*), which can exceed 60 m in height and reach up to 3 m in diameter (DeBell, 1990).

Leaves are alternate and simple, with pinnato-palmate venation, and petioles are often transversally flattened distally (Eckenwalder, 1996). Leaf size, shape, and toothing are extremely variable among species, but also within a single tree and among trees within a species (e.g., Fig. 3 in Eckenwalder, 1996; Fig. 4 in Dickmann, 2001). Within-tree and within-species variation in leaf characteristics can largely be attributed to two sources of developmental heteromorphism (Eckenwalder, 1980). First, heteroblasty (i.e., differences in leaf characteristics between juvenile and adult trees) is common in *Populus*. Second, there is substantial seasonal heterophylly because shoots on *Populus* trees have both preformed leaves (i.e., expanded from well-formed primordia that overwinter in vegetative buds) and neoformed leaves (i.e., initiated during the current growing season). Preformed and neoformed leaves can differ substantially (e.g., Critchfield, 1960), with preformed leaf characteristics typically having higher taxonomic value (Eckenwalder, 1996).

Except for *P. lasiocarpa*, *Populus* species are mostly dioecious, although the occurrence of hermaphroditic trees has been reported in multiple species (Rottenberg, 2000; Rowland et al., 2002; Cronk, 2005; Slavov et al., 2009). Both male and female flowers are grouped in pendent catkins. Perianths are strongly reduced, with 5–60 stamens or 2–4 carpels borne on wide floral disks (Boes and Strauss, 1994; Eckenwalder, 1996). After pollination, female flowers develop into capsules that, upon dehiscence, release 2–50 light seeds (>300,000 seeds/kg; Schreiner, 1974) with cottony hairs (Boes and Strauss, 1994; Eckenwalder, 1996).

Winter twigs range from slender to moderately stout (more rarely stout), from glabrous to lightly pubescent, and can be yellow- or greenish-brown, reddish, or gray, typically with conspicuous lighter-colored lenticels (Seiler and Peterson, <http://www.cnr.vt.edu/dendro/>). Vegetative buds are long (0.5–2.5 cm), conical, and sharp-pointed (Seiler and Peterson, <http://www.cnr.vt.edu/dendro/>). They are covered by several bud scales, the most basal of which is oriented away from the stem, and are often impregnated with resinous hydrophobic exudates (Eckenwalder, 1996). Reproductive buds are often found in clusters and, in some species, are

distinguishable between the sexes and from vegetative buds based on size and shape (Stanton and Villar, 1996).

1.2 Habitat

Most species of *Populus* have wide native ranges, often spanning more than 20 degrees of latitude and a great diversity of climates and soils (Eckenwalder, 1996; Dickmann, 2001). *Populus* trees grow in a striking variety of habitats, ranging from hot and arid, desert-like sites in central Asia and northern and central Africa to alpine or boreal forests in Europe and North America (e.g., Fig. 1). They are shade- and drought-intolerant, and seed establishment typically depends on major disturbances, such as fire, floods, or ice scours (Romme et al., 1995, 1997; Rood et al., 2007).

Populus trees tend to occur in two general categories of habitats. First, many species typically grow in riparian areas and wetlands characterized by seasonal flooding and high water tables, with optimal establishment conditions occurring on fresh silt and sand, immediately following the recession of water from point bars and gravel bars (Braatne et al., 1996; Dickmann, 2001). One example is black cottonwood (*P. trichocarpa*), which is common in riparian areas of the Pacific Northwest of North America (Fig. 1a). Some riparian species, however, are extremely phreato-phytic (i.e., deep-rooting). Euphrates poplar (*P. euphratica*), for example, can grow under remarkably hot, dry, and high-salinity conditions (e.g., Fig. 1b), with water tables as deep as 10–13 m (Ma et al., 1997; Hukin et al., 2005; Ferreira et al., 2006; Thevs et al., 2008). Second, aspens and some white poplars (section *Populus*; Table 1) grow primarily in mountainous or upland habitats. The North-American quaking aspen (*P. tremuloides*), for example, occurs at elevations up to 3,500 m (e.g., Fig. 1c) and grows best on well-drained, loamy soils, with water tables between 0.6 and 2.5 m, although it can also establish and grow on ash-covered soils, shallow soils on rock outcrops, landslides, mine waste dumps, and borrow pits (Perala, 1990; Dickmann, 2001).

1.3 Life History

1.3.1 Sexual Reproduction

Under favorable conditions, *Populus* trees reach reproductive maturity within 4–8 years in intensively managed plantations and within 10–15 years in natural populations (Stanton and Villar, 1996). Reported sex ratios for natural populations of various *Populus* species range from female-biased to balanced (approximately 1:1) and male-biased (reviewed by Farmer, 1996; Braatne et al., 1996; Stanton and Villar, 1996; see also Gom and Rood, 1999; Rowland and Johnson, 2001; Hultine et al., 2007). Although no consistent pattern has emerged, several of these studies suggest

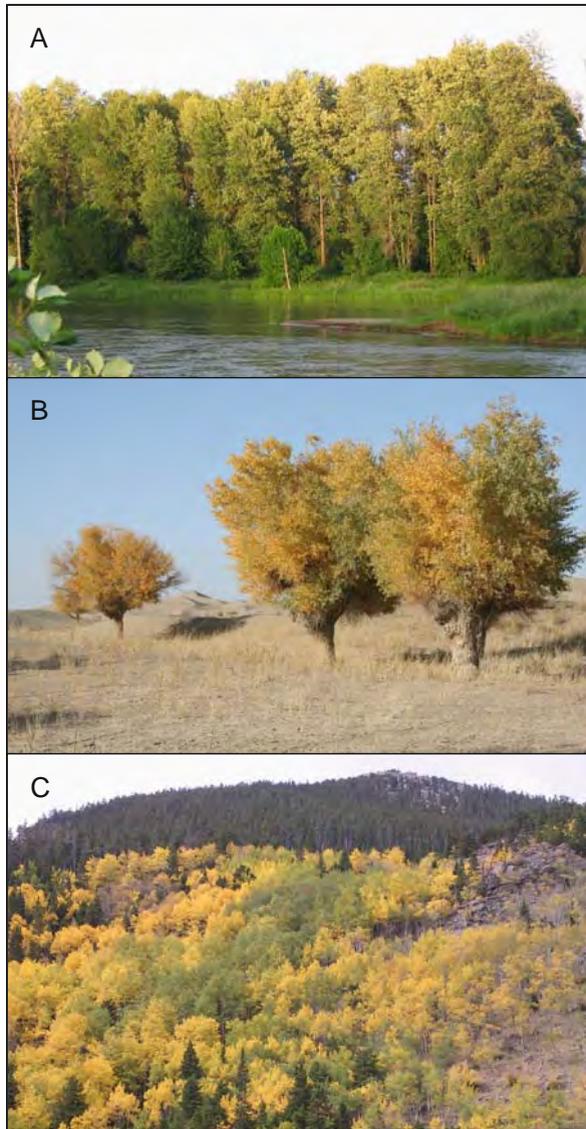


Fig. 1 An example of the diverse habitats occupied by species of *Populus*. (a): *P. trichocarpa* along the Willamette River in Oregon, USA (photograph courtesy of Steve DiFazio); (b) *P. euphratica* south of the Taklamakan Desert, Xinjiang, China (photograph courtesy of Pavel Sekerka); (c) *P. tremuloides* in the Rocky Mountain National Park, Colorado, USA (photograph courtesy of Amy Brunner)

that site-specific biases in sex ratio may be present, with female trees predominating (1) at sites with abundant moisture and nutrients and (2) at lower elevations, whereas males may be more common at high elevations, as well as in warmer, drier,

Table 1 Classification of *Populus* following Eckenwalder (1996)

Section (synonym)	Species	Distribution
<i>Abaso</i> Eckenwalder	<i>Populus mexicana</i> Wesmael	Mexico
<i>Turanga</i> Bunge	<i>P. euhratia</i> Olivier	NE Africa, Asia
	<i>P. ilicifolia</i> (Engler) Rouleau	E Africa
	<i>P. pruinosa</i> Schrenk	Asia
<i>Leucoides</i> Spach	<i>P. glauca</i> Haines <i>sl</i> ^a	China
	<i>P. heterophylla</i> L.	USA
	<i>P. lasiocarpa</i> Olivier	China
<i>Aigeiros</i> Duby	<i>P. deltoides</i> Marshall <i>sl</i> ^a	N America
	<i>P. fremontii</i> S. Watson	USA
	<i>P. nigra</i> L.	Eurasia, N Africa
<i>Tacamahaca</i> Spach	<i>P. angustifolia</i> James	N America
	<i>P. balsamifera</i> L.	N America
	<i>P. ciliata</i> Royle	Himalayas
	<i>P. laurifolia</i> Ledebour	Eurasia
	<i>P. simonii</i> Carrière	E Asia
	<i>P. suaveolens</i> Fischer <i>sl</i> ^a	NE China, Japan
	<i>P. szechuanica</i> Schneider	E Eurasia
	<i>P. trichocarpa</i> Torrey & Gray	N America
	<i>P. yunnanensis</i> Dode	Eurasia
<i>Populus</i> (<i>Leuce</i> Duby)	<i>P. adenopoda</i> Maximowicz	China
	<i>P. alba</i> L.	Europe, N Africa, Central Asia
	<i>P. gamblei</i> Haines	E Eurasia
	<i>P. grandidentata</i> Michaux	N America
	<i>P. guzmanantlensis</i> Vazques & Cuevas	Mexico
	<i>P. monticola</i> Brandege	Mexico
	<i>P. sieboldii</i> Miquel	Japan
	<i>P. simaroa</i> Rzedowski	Mexico
	<i>P. tremula</i> L.	Europe, N Africa, NE Asia
	<i>P. tremuloides</i> Michaux	N America

^a *Sensu lato*

and more extreme environments. This biologically interesting hypothesis, however, needs to be tested through replicated, large-scale studies that adequately account for (1) developmental differences between the two genders (i.e., male trees in some species may reach reproductive maturity before female trees, thus possibly skewing sex ratios; Stanton and Villar, 1996) and (2) the extensive clonality which is characteristic of many species of *Populus* (discussed below).

With the exception of some subtropical species, flowering occurs before leaf emergence in early spring (Braatne et al., 1996; Eckenwalder, 1996). Individual trees flower for 1–2 weeks (Stanton and Villar, 1996; G. T. Slavov and S. P. DiFazio, unpublished data), but the pollination period in a population can exceed one or even two months (Braatne et al., 1996). The relative timing of flowering follows a temperature-dependent progression, with populations at higher-elevations, more

northern latitudes, and more continental climates flowering later (e.g., DeBell, 1990; Perala, 1990; Zasada and Phipps, 1990; Braatne et al., 1996). Pollen is dispersed by wind, and effective long-distance pollination can be extensive (Tabbener and Cottrell, 2003; Lexer et al., 2005; Pospíšková and Šálková, 2006; Vanden Broeck et al., 2006; Slavov et al., 2009). Fertilization occurs within 24 h after a viable pollen grain has landed on a receptive stigma (Braatne et al., 1996). Capsules typically dehisce 4–6 weeks (but in some species and populations from 2–3 weeks to 3–5 months) after fertilization, which tends to coincide with snowmelt runoff, when microsites favoring seed establishment are most abundant (Braatne et al., 1996; Stella et al., 2006). Seeds are produced in great numbers (>25 million per tree per year, Braatne et al., 1996), and can potentially be dispersed over very long distances by wind and water (Braatne et al., 1996; Karrenberg et al., 2002), but direct empirical data on seed dispersal distances are limited (DiFazio, 2002). Under natural conditions, seeds retain viability for only 1–2 weeks and germination occurs within 24 h (Braatne et al., 1996; Karrenberg et al., 2002). On appropriate microsites, seedlings establish in great numbers (e.g., up to 4,000 m⁻²), but mortality in the first year is typically high (i.e., up to 77–100%), primarily as a result of desiccation, prolonged flooding, and scouring (Braatne et al., 1996; Karrenberg et al., 2002; Dixon, 2003; Dixon and Turner, 2006).

1.3.2 Asexual Reproduction

Vegetative propagation is one of the distinctive characteristics of the genus (Dickmann, 2001). The means of asexual reproduction and the extent of clonality, however, differ dramatically among species (Braatne et al., 1996; Schweitzer et al., 2002; Rood et al., 2003, 2007). The North-American quaking aspen (*P. tremuloides*), for example, propagates vegetatively through root sprouting (i.e., formation of adventitious shoots on shallow lateral roots, a process also referred to as root suckering; Perala, 1990). Because of the rare opportunities for seedling establishment, root sprouting is believed to have been the primary means of reproduction of *P. tremuloides* in the Rocky Mountains over the last century (Romme et al., 1995, 1997, 2005). The existence of extremely large, and possibly ancient, quaking aspen clones has been reported (Mitton and Grant, 1996), although the direct verification of extreme clone ages and sizes remains a technical challenge (Ally et al., 2008; Mock et al., 2008; DeWoody et al., 2008). In other species, such as the North-American cottonwoods *P. balsamifera*, *P. trichocarpa*, and *P. angustifolia*, and the European black poplar (*P. nigra*), asexual reproduction occurs commonly both through root sprouting and through rooting of shoots from broken branches or entire tree trunks that have been toppled during storms and floods and then buried in sediment (Braatne et al., 1996; Rood et al., 2003, 2007; Barsoum et al., 2004; Smulders et al., 2008). In a third group, including the North-American plains cottonwood (*P. deltoides*) and Fremont cottonwood (*P. fremontii*), asexual reproduction is relatively rare and occurs primarily via rooting of shoots (Braatne et al., 1996; Gom and Rood, 1999; Schweitzer et al., 2002; Rood et al., 2003, 2007).

2 Systematics and Evolution

2.1 Fossil Record

Fossil materials, some of which date back to the Cretaceous, have often been misclassified as belonging to *Populus* (Cronk, 2005), but the most ancient undisputed fossil records (e.g., Manchester et al., 1986, 2006) suggest that the genus appeared no later than the Eocene, and probably as early as the late Paleocene (about 60 million years BP). Fossil records dating from the Eocene and the Oligocene are relatively abundant and are available in many parts of the northern hemisphere (Manchester et al., 1986, 2006; Ramírez and Cevallos-Ferriz, 2000; Iljinskaja, 2003). Presumably, precursors of all extant sections of *Populus* (Table 1) were present by the Miocene (Eckenwalder, 1996; Cronk, 2005).

2.2 Relationships to *Salix* and Other Families

Traditionally, *Populus* and its “sister” lineage, the genus *Salix* (willows), have been considered the only two genera in the Salicaceae family, although some taxonomists have included other genera, mostly from Eastern Asia (Eckenwalder, 1996). More recently, however, the Flacourtiaceae family, the closest relative of Salicaceae, was re-classified, and a number of genera formerly included in Flacourtiaceae are now assigned to Salicaceae *sensu lato*, within the Malpighiales order of the “Eurosoid I” clade (Chase et al., 2002; Angiosperm Phylogeny Group, 2003).

The availability of molecular data and sophisticated methods of phylogenetic analysis has revolutionized plant classification (Soltis and Soltis, 2001; Angiosperm Phylogeny Group, 2003; Soltis et al., 2005). Recent molecular phylogenetic studies in Salicaceae (Leskinen and Alström-Rapaport, 1999; Hamzeh and Dayanandan, 2004; Cervera et al., 2005; Hamzeh et al., 2006) showed that *Populus* and *Salix* clearly form two separate groups. Interestingly, in one of these studies the presumably most ancient species of *Populus* (*P. mexicana*; Eckenwalder, 1996) showed higher similarity to *Salix* than to any other species of *Populus* (Cervera et al., 2005). While studies designed specifically to clarify the status of *P. mexicana* will probably resolve this issue in the near future, identifying the common ancestor of *Populus* and *Salix*, and establishing whether both genera are monophyletic natural groups remain wide-open questions.

2.3 Classification

The number of species included in the genus *Populus* varies among classifications from as few as 22 to as many as 85 (Eckenwalder, 1996). Two reasons for these drastic differences are the misclassification of natural hybrids (discussed below) as “true” species and the philosophical differences between “splitter” and “lumper” taxonomists (Eckenwalder, 1996). One classification that is commonly

used in recent years is that of Eckenwalder (1996), who recognized 29 species subdivided into six sections based on relative morphological similarity and crossability (Table 1). A consensus cladogram from the 840 most parsimonious trees built based on 76 morphological characters (Fig. 6 in Eckenwalder, 1996) provided evidence that all sections except for *Tacamahaca* are monophyletic. Section *Tacamahaca* was split into two monophyletic groups, one comprised of “typical balsam poplars” (e.g., *P. balsamifera* and *P. trichocarpa*) and the other one comprised of “narrow-leaved, thin-twiggled” species (e.g., *P. angustifolia*, *P. simonii*). Combining fossil records with information from this consensus tree, Eckenwalder (1996) speculated that (1) after the original spread of the genus from either North America or Asia in the Paleocene, the two “primitive” subtropical sections, *Abaso* and *Turanga* were split by a vicariance event, (2) temperate habitats were first invaded by species from section *Leucooides*, and (3) the remaining “advanced” sections evolved rapidly in the Miocene.

More recent molecular studies provide only partial support for this evolutionary scenario and clearly conflict with some of its aspects (Hamzeh and Dayanandan, 2004; Cervera et al., 2005; Hamzeh et al., 2006). The most parsimonious tree based on 151 Amplified Fragment Length Polymorphisms (Cervera et al., 2005), for example, suggests that section *Populus* (referred to as *Leuce* in this study) is the most “primitive” section in the genus, which is diametrically opposed to Eckenwalder’s interpretation based on morphological traits. A number of inconsistencies in the classification of *Populus* remain. Their successful resolution will likely require integration of abundant molecular genetic and genomic data with informative morphological traits and the fossil record (Soltis and Soltis, 2001; Delsuc et al., 2005).

2.4 Natural Hybridization

Hybridization is believed to have played a major role in the evolution of extant species of *Populus* (Eckenwalder, 1996; Hamzeh and Dayanandan, 2004; Cervera et al., 2005; Hamzeh et al., 2006). The existence of relict hybrids (i.e., hybrids occurring far away from the current distribution of one or both of the presumed species), and extensive contemporary hybridization both within and among sections has been documented based on morphological traits and molecular markers (e.g., Table 2; Eckenwalder, 1984a, b, c; Rood et al., 1986; Campbell et al., 1993; Martinsen et al., 2001; Floate, 2004; Lexer et al., 2005; Hamzeh et al., 2007).

Hybridization plays a key role in *Populus* domestication (Stettler et al., 1996). Naturally occurring hybrid zones, for example, provide a tremendous potential for admixture mapping, which can be a powerful complement to intraspecific genetic association studies (Lexer et al., 2004, 2007; Lexer and van Loo, 2006; Buerkle and Lexer, 2008). Finally, zones of hybridization between species of *Populus* have been among the primary study systems for the emerging field of community genetics (Whitham et al., 1999, 2003, 2006, 2008).

Table 2 Examples of naturally occurring hybrids of *Populus*

Hybrid	Scientific name
<i>P. alba</i> × <i>P. adenopoda</i>	<i>P.</i> × <i>tomentosa</i> Carrière
<i>P. alba</i> × <i>P. tremula</i>	<i>P.</i> × <i>canescens</i> (Aiton) Smith
<i>P. angustifolia</i> × <i>P. balsamifera</i>	<i>P.</i> × <i>brayshawii</i> B. Boivin
<i>P. angustifolia</i> × <i>P. deltoides</i>	<i>P.</i> × <i>acuminata</i> Rydb.
<i>P. angustifolia</i> × <i>P. fremontii</i>	<i>P.</i> × <i>hinckleyana</i> Correll
<i>P. balsamifera</i> × <i>P. deltoides</i>	<i>P.</i> × <i>jackii</i> Sargent
<i>P. deltoides</i> × <i>P. nigra</i>	<i>P.</i> × <i>canadensis</i> Moench
<i>P. grandidentata</i> × <i>P. tremuloides</i>	<i>P.</i> × <i>smithii</i> B. Boivin
<i>P. trichocarpa</i> × <i>P. deltoides</i>	<i>P.</i> × <i>generosa</i> Henry
<i>P. trichocarpa</i> × <i>P. fremontii</i>	<i>P.</i> × <i>parryi</i> Sargent

3 Genetic Variation

3.1 Molecular Markers

3.1.1 Allozymes and RFLP

As a result of their (1) obligately outcrossing mating systems, (2) relatively large population sizes, and (3) extensive long-distance pollen and seed dispersal, species of the genus *Populus* have high levels of genetic variation for neutral molecular markers. Early studies based primarily on allozyme markers and Restriction Fragment Length Polymorphisms (RFLP) depicted several basic aspects of the population genetics of *Populus* species (Table 3). First, levels of polymorphism (as measured by the average number of alleles per locus, A) and heterozygosity expected under Hardy-Weinberg equilibrium (H_e , also referred to as gene diversity; Nei, 1973) in *Populus* are close to the mean values for long-lived woody species ($A = 1.8$, $H_e = 0.15$) and are higher than those for plants in general ($A = 1.5$, $H_e = 0.11$; Hamrick et al., 1992). Second, while deviations from Hardy-Weinberg equilibrium are not uncommon and can be caused by both deficiency and excess of heterozygotes, the magnitudes of these deviations are typically small to moderate. Deviations caused by heterozygote deficiency (i.e., positive values of F_{IS}) are more common, indicating the possible existence of unaccounted population substructure (i.e., Wahlund Effect; Hedrick, 2005b). Finally, differentiation among populations as measured by F_{ST} (Wright, 1965) is typically weak, with differences among populations accounting for only 1–7% of the genetic variation. The median value of F_{ST} for the genus (0.047) is almost two times lower than the mean for long-lived woody species ($F_{ST} = 0.084$) and nearly five times lower than that for plants in general ($F_{ST} = 0.228$; Hamrick et al., 1992). The weak differentiation among populations is in good agreement with direct studies of gene flow, the findings of which suggest that long-distance pollination can be extensive in *Populus* (Tabbener and Cottrell, 2003; Pospíšková and Šálková, 2006; Vanden Broeck et al., 2006; Slavov et al., 2009).

Table 3 Allozyme and RFLP diversity and differentiation in *Populus*^a

Section	Species	N_{loci}	N_{pop}	N	A	H_o	H_e	F_{IS}	F_{ST}	References
Turanga Aigeiros	<i>P. euphratica</i>	20 ^b	3	85	1.8	0.10	0.24	0.592	–	Rottenberg et al. (2000)
	<i>P. deltoides</i>	33 ^b	9	84	1.2	0.06	–	–	–	Rajora et al. (1991)
		22 ^b	21	–	1.5	–	0.08	–	0.064	Marty (1984)
	<i>P. fremonii</i>	36 ^c	4	47	1.5	0.18	0.15	–0.175	0.074	Martinsen et al. (2001)
	<i>P. nigra</i>	8 ^b	3	146	–	–	0.16	0.113	0.063	Legionnet and Lefèvre (1996)
Tacamahaca	<i>P. angustifolia</i>	36 ^c	10	281	1.4	0.10	0.08	–0.236	0.022	Martinsen et al. (2001)
	<i>P. balsamifera</i>	17 ^b	5	248	–	–	0.04	0.061	0.014	Farmer et al. (1988)
	<i>P. trichocarpa</i>	18 ^b	10	456	1.2	–	0.09	–	0.063	Weber and Stettler (1981)
Populus	<i>P. grandidentata</i>	14 ^b	–	96	1.4	0.07	0.08	0.125	–	Liu and Furnier (1993)
		37 ^c	–	75	1.8	0.12	0.13	0.077	–	Liu and Furnier (1993)
	<i>P. tremula</i>	11 ^b	6	233	1.7	0.15	0.17	0.153	0.014	Easton (1997)
		10 ^b	5	41	1.7	0.33	0.23	–0.427	–	Lopez-de-Heredia et al. (2004)

Table 3 (continued)

Section	Species	N_{loci}	N_{pop}	N	A	H_0	H_e	F_{IS}	F_{ST}	References
	<i>P. tremuloides</i>	26 ^b	7	222	2.3	0.52	0.42	-0.238	-	Cheliak and Dancik (1982)
		15 ^b	8	200	2.7	0.13	0.24	0.462	0.068	Hyun et al. (1987)
		10 ^b	9	347	2.6	0.22	0.22	0.017	0.003	Lund et al. (1992)
		17 ^b	6	156	2.4	0.32	0.29	-0.102	0.030	Jelinski and Cheliak (1992)
		13 ^b	-	118	2.8	0.19	0.25	0.240	-	Liu and Fumier (1993)
		41 ^c	-	91	2.7	0.21	0.25	0.160	-	Liu and Fumier (1993)
	Median ^d	18	7	146	1.8	0.17	0.17	0.077	0.047	

^a N_{loci} is the number of loci used; N_{pop} is the number of populations sampled; N is the number of genets (or trees) analyzed; A is the average number of alleles per locus detected in each population; H_0 is the observed heterozygosity; H_e is the expected heterozygosity (Nei, 1973); F_{IS} is the fixation index as reported in the study or calculated as $F_{IS} = (H_e - H_0)/H_e$; F_{ST} is the among-population differentiation (Wright, 1965).

^b Allozyme markers.

^c Restriction Fragment Length Polymorphisms.

^d Because relatively few studies were included, medians were calculated in order to minimize the influence of extreme values.

Polymorphism and heterozygosity vary substantially among species of *Populus*, and even among studies in the same species (Table 3). Interestingly, however, both the number of alleles per locus and gene diversity appear to be consistently higher in *P. tremuloides* than in any other species of *Populus* (Table 3). Two life history peculiarities of *P. tremuloides* may provide an explanation for its elevated genetic variation.

First, this species is believed to reproduce almost exclusively asexually over much of its range (Mitton and Grant, 1996; Romme et al., 1995; 1997, 2005). Population genetics theory predicts that predominantly and strictly clonal organisms will have much lower genotypic diversity (i.e., fewer unique genotypes for a given number of individuals sampled) and (2) higher allelic diversity and heterozygosity (i.e., as a result of accumulation of mutations known as the “Meselson effect”) compared to organisms with similar life histories but with predominantly sexual reproduction (Balloux et al., 2003; Halkett et al., 2005; de Meeûs et al., 2007). The first prediction appears to hold only partially in *P. tremuloides*. Aspen clones spanning large areas (i.e., up to 44 ha) have been discovered in the Rocky Mountains (Mitton and Grant, 1996; Mock et al., 2008; DeWoody et al., 2008; S.P. DiFazio et al., unpublished data). However, relatively high genotypic diversities have been observed in most studies (Hyun et al., 1987; Jelinski and Cheliak, 1992; Lund et al., 1992; Liu and Furnier, 1993; Yeh et al., 1995; Namroud et al., 2005; Mock et al., 2008), suggesting that sexual reproduction may be more frequent and/or its impact on the genetic structure of aspen populations may be more persistent than previously assumed. The second prediction, which under certain conditions holds even for low rates of asexual reproduction (Yonezawa, 1997; Yonezawa et al., 2004), appears to be consistent with empirical data. The median gene diversity from six studies of *P. tremuloides* ($H_e = 0.25$) is comparable to that from two studies of its “sister” species *P. tremula* in Europe ($H_e = 0.20$), and is more than two times higher than that for other species of *Populus* ($H_e = 0.09$). This agrees with the general trend in woody plants (mean $H_e = 0.25$ for species with both asexual and sexual reproduction vs. $H_e = 0.14$ for species that only reproduce sexually; Hamrick et al., 1992).

Second, the frequency of triploid aspen trees, at least in the Rocky Mountains, may be substantially higher than previously thought (Mock et al., 2008; S.P. DiFazio et al., unpublished data). The occurrence of triploids at high frequencies is expected to result in (1) increased gene diversity and (2) heterozygote excess relative to Hardy-Weinberg predictions for a population of diploids (i.e., because phenotypes with two different alleles would occur more frequently than in a population comprised of strictly diploid individuals; Krieger and Keller, 1998; Ridout, 2000). As discussed above, gene diversity estimates for *P. tremuloides* tend to be higher than those for other species of *Populus*, but heterozygote excess (i.e., negative F_{IS}) was observed in only two studies. Thus, although both extensive clonality and triploidy appear as likely explanations for the high levels of genetic variation in *P. tremuloides*, more definitive answers about their relative or combined effects, as well as about the contribution of other factors (e.g., the possible role of past hybridization, Barnes, 1967), will come from studies designed to specifically address these questions.

Table 4 Microsatellite diversity and differentiation in *Populus*^a

Section	Species	N_{loci}	N_{pop}	N	A	H_o	H_e	F_{IS}	$F_{\text{ST}}/R_{\text{ST}}$	References
<i>Aigeiros</i>	<i>P. deltoides</i>	10	–	20	5.2	0.23	–	–	–	Rahman and Rajora (2002)
	<i>P. fremontii</i>	4	–	20	5.3	0.60	0.52	–0.146	–	G.T. Slavov (unpublished data)
	<i>P. nigra</i>	6	22	574	–	0.78	0.73	–0.077	0.047	Imbert and Lefèvre (2003)
<i>Tacamahaca</i>		12	–	60	11	0.80	0.82	0.030	–	Pospíšková and Šálková (2006)
		7	17	921	–	0.74	0.76	0.027	0.081	Smulders et al. (2008)
	<i>P. angustifolia</i>	4	–	28	4.3	0.45	0.44	–0.033	–	G.T. Slavov (unpublished data)
<i>Populus</i>	<i>P. balsamifera</i>	10	–	29	6	0.35	–	–	–	Rahman and Rajora (2002)
	<i>P. trichocarpa</i>	9	47	372	6.1	0.60	0.80	0.293	0.078/0.112	Ismail et al. (2009)
	<i>P. alba</i>	10	2	282	17.5	0.71	0.77	0.058	–	Slavov et al. (2009)
		20	2	40	–	0.38	0.39	0.021	–	Lexer et al. (2005)
		19	1	169	6.4	0.37	0.38	0.027	–	van Loo et al. (2008)

Table 4 (continued)

Section	Species	N_{loci}	N_{pop}	N	A	H_o	H_e	F_{IS}	F_{ST}/R_{ST}	References
	<i>P. tremula</i>	20	2	40	-	0.47	0.50	0.055	-	Lexer et al. (2005)
		9	3	113	-	0.35	0.41	0.120	0.117	Suvanto and Latva-Karjanmaa (2005)
	<i>P. tremulooides</i>	25	12	116	-	0.50	0.62	0.197	0.015	Hall et al. (2007)
		4	4	159	7.4	0.56	0.72	0.201	0.032/0.041	Wýman et al. (2003)
		16	11	189	4.9	0.41	0.45	0.093	0.045	Cole (2005)
		4	-	266	8.8	0.47	0.67	0.300	-	Namroud et al. (2005)
	Median ^b	10	4	116	6.1	0.47	0.62	0.055	0.047 ^c	

^a Parameter designation is the same as in Table 3. R_{ST} is an analog of F_{ST} , which is based on the Stepwise Mutation Model (Slatkin, 1995).

^b Because relatively few studies were included, medians were calculated in order to minimize the influence of extreme values.

^c Based on F_{ST} values.

3.1.2 Microsatellites

The availability of highly variable microsatellites spurred a recent wave of population genetic studies in *Populus* (Table 4). Because of the substantially higher mutation rates of microsatellite loci, results from these studies are not directly comparable to those based on allozyme and RFLP markers. The general trends discussed above, however, appear to hold in microsatellite-based studies. Observed and expected heterozygosities are generally high and fall within the broad range of values reported for other angiosperm (e.g., Dow et al., 1995; Brondani et al., 1998; Streiff et al., 1998) and gymnosperm (e.g., Elsie et al., 2000, Table 3 in Slavov et al., 2004) trees. Heterozygote deficiency is the more common cause for departures from Hardy-Weinberg equilibrium and is slightly more prevalent than for allozyme and RFLP markers, presumably because of the much higher rates of null alleles and allele “drop-out” at microsatellite loci (Ewen et al., 2000). Differentiation is typically weak and comparable to levels observed for allozyme and RFLP markers, despite the constraint on F_{ST} imposed by the higher heterozygosities of microsatellite markers (Hedrick, 1999; Hedrick, 2005a). Unlike for allozyme and RFLP markers (Table 3), *P. tremuloides* does not appear to have higher microsatellite polymorphism and gene diversity than other species of *Populus* (Table 4). It is very likely, however, that this difference exists but remains undetected. Two of the three microsatellite studies in which polymorphism and diversity were reported for *P. tremuloides* were based on the same four loci, two of which are tri-nucleotide repeats. Tri-nucleotide microsatellites tend to be less variable than di-nucleotide microsatellites (Chakraborty et al., 1997; Schug et al., 1998), which were used in most other studies. The third study used 16 loci, all of which were developed for other species of *Populus*. Transferring microsatellites across species of *Populus* can be very successful (Tuskan et al., 2004) but markers tend to be much less variable in the recipient species than in the species in which they were developed (e.g., González-Martínez et al., 2004), presumably because of ascertainment bias (Ellegren et al., 1995). Because of the inherently high heterogeneity of microsatellite markers, empirical data need to be expanded considerably before meaningful comparisons among studies and species can be made.

3.1.3 Nucleotide Diversity

The whole-genome sequence of *Populus trichocarpa* (Tuskan et al., 2006), which has been integrated with a detailed genetic map (Kelleher et al., 2007), provides an excellent resource for understanding the population genetics of the genus. Information on nucleotide diversity and linkage disequilibrium in *Populus* is still less abundant than in other model organisms, but the growing interest in genetic association studies (Howe et al., 2003; DiFazio, 2005; González-Martínez et al., 2006; Neale and Ingvarsson, 2008; Ingvarsson et al., 2008; see also Chapter “Nucleotide polymorphism, linkage disequilibrium and complex trait dissection in *Populus*” by Ingvarsson in this volume) will probably create an avalanche of Single

Nucleotide Polymorphism (SNP) data. Levels of nucleotide diversity appear to vary substantially among species and genes (Ingvarsson, 2005a, b, 2008; Ingvarsson et al., 2006; Gilchrist et al., 2006; Hall et al., 2007; Chapter “Nucleotide polymorphism, linkage disequilibrium and complex trait dissection in *Populus*” by Ingvarsson in this volume), but are generally comparable to those in other tree species (González-Martínez et al., 2006; Savolainen and Pyhäjärvi, 2007; Chapter “Nucleotide polymorphism, linkage disequilibrium and complex trait dissection in *Populus*” by Ingvarsson in this volume). Interestingly, nucleotide diversity in trees does not seem to be substantially higher than in other plants, including *Arabidopsis thaliana*, an annual characterized by high levels of self-fertilization. Presumably, this is because (1) longer generation cycles in trees translate into lower neutral substitution rates per year than in plants with shorter life cycles and (2) the genomes of many tree species, including those in the genus *Populus*, may still be affected by past demographic oscillations (Savolainen and Pyhäjärvi, 2007; Ingvarsson, 2008).

3.2 Adaptive Traits

Extensive genecological studies have revealed that forest trees typically have high levels of adaptive genetic variation both within and among populations, and *Populus* is no exception (Farmer, 1996; Morgenstern, 1996; Howe et al., 2003; Savolainen et al., 2007; Aitken et al., 2008). These studies also provided compelling indirect evidence for the existence of local adaptation (i.e., genotypes originating from a given habitat tend to have higher fitness in that habitat than genotypes originating from other habitats; Kawecki and Ebert, 2004). First, genotype-by-environment (GxE) interactions, a necessary condition for local adaptation, are commonly detected (Morgenstern, 1996; White et al., 2007). Second, differentiation among populations is generally much higher for adaptive traits than for neutral genetic markers (Fig. 2; Merilä and Crnokrak, 2001; McKay and Latta, 2002; Howe et al., 2003; Savolainen et al., 2007), which suggests that divergent selection has played a dominant role in shaping adaptive genetic variation. Finally, and most importantly, genecological studies have revealed strong and repeatable correspondence between clinal genetic variation for adaptive traits and climatic and geographic factors believed to be important agents of natural selection (Morgenstern, 1996; St.Clair et al., 2005; Aitken et al., 2008).

Because gene flow is believed to be extensive in most forest trees, the prevalence of local adaptation is a paradox. This apparent contradiction can be explained by (1) reproductive isolation by distance and phenological asynchrony between populations growing under different climatic conditions, (2) very strong divergent selection, or most likely (3) a complex interaction between these two factors. Unraveling the relative roles of gene flow and natural selection, as well as the molecular underpinnings of adaptive genetic variation will be critical for our basic understanding of the evolution of *Populus* and other forest trees, and thus for designing adequate conservation and domestication strategies.

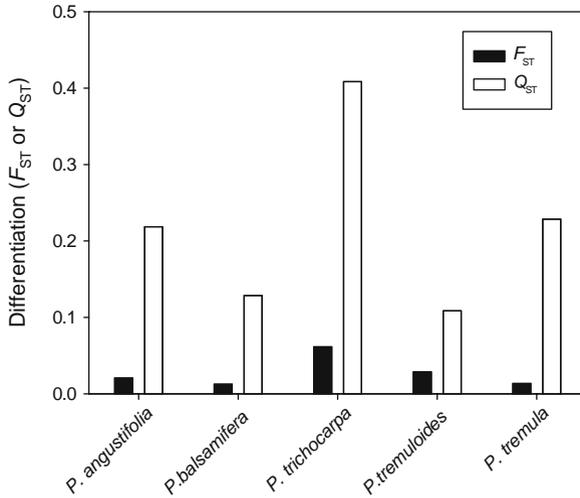


Fig. 2 Differentiation among populations for neutral genetic markers (F_{ST} ; Wright, 1965) and its equivalent for quantitative traits (Q_{ST} ; Whitlock, 2008) calculated for the timing of vegetative bud burst in five species of *Populus*. F_{ST} values are based on studies listed in Tables 3 and 4 (median values were used when multiple entries were available for a species). Q_{ST} values for *P. balsamifera* and *P. tremuloides* are from Table 1 in Howe et al. (2003), those for *P. tremula* were reported by Hall et al. (2007), and those for *P. angustifolia* and *P. trichocarpa* were calculated based on unpublished data (G.T. Slavov and S.P. DiFazio) and data from Dunlap and Stettler (1996), respectively, using Equation (1) in Howe et al. (2003)

4 Conclusions

1. *Populus* is comprised of morphologically and ecologically diverse species whose peculiar life history characteristics (e.g., dioecy, disturbance-dependent establishment, natural hybridization, clonality) and extensive neutral and adaptive genetic variation make it a unique model organism for basic and applied genetic research.
2. The outstanding genetic and genomic resources created over the last two decades have set the stage for a breakthrough in our understanding of the phylogenetics, population genetics, and molecular underpinnings of adaptation within and among species of *Populus*.

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