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Figured grain in aspen is heritable and not affected by graft-transmissible signals

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Abstract Figure can add value to wood products, but its occurrence is unpredictable. A first step in reliably producing figured wood is determining whether it is faithfully transmitted to progeny via sexual and asexual reproduction. We describe a 26-year-old male aspen genotype, designated 'Curly Poplar', which was shown to be a *Populus* × *canescens* hybrid using microsatellite markers. All rooted cuttings of this genotype exhibited an undulating pattern on the radial surface that was not seen in the control trees, all of which showed a smooth radial surface and straight grain. We observed spiral grain with a magnitude of 2.77 ± 0.12°/cm from vertical in 11-month-old, field-grown rooted Curly Poplar cuttings, but spiral grain was not apparent in wood from the 26-year-old mature ortet that supplied these cuttings. Veneer cut from the mature tree exhibited a novel type of figure that we called 'Scattered Moiré'. Reciprocal grafts between Curly Poplar and various non-figured aspens showed that a graft-transmissible signals did not appear to be involved in figure formation in Curly Poplar or the induction of figure in straight-grained trees. Curly Poplar was crossed to a straight-grained clone to test the inheritance of the gene(s) responsible for figure. Samples from the resulting population revealed that 79 out of 377 seedlings exhibited figure. A Chi-square test led to the rejection of a 1:1 segregation ratio between figured and non-figured phenotypes ($p < 0.01$), but not of a 1:3 segregation ratio (p 0.0793). Overall, these analyses showed that figure in Curly Poplar is under genetic control, but its inheritance may not be simple.

Keywords Curly Poplar · Scattered Moiré · Figured wood · Heritability · *Populus* × *canescens* · Wood quality

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Introduction

Generally speaking, any design, pattern, or marking on the longitudinal surfaces of wood that can be used for decorative purposes is referred to as figure (Beals and Davis 1977; Panshin and Zeeuw 1980). Figured wood is widely used in furniture, paneling, musical instruments and other decorative applications. For centuries, people have tried to understand the mechanism of figure formation and to predictably produce figure in wood (Anonymous 1929; Robbins 1953; Rudolf 1954; Bragg and Stokke 1993; Bragg et al. 1997). To date, there are no reports of ways to reliably produce figured wood on a large scale (e.g., commercial plantations). Figure has been called the "most desirable, the least understood, and certainly the most complex gross characteristic of wood" (Beals and Davis 1977).

The occurrence of figure is rare, which makes wood possessing it more valuable than non-figured wood of the same species (Beals and Davis 1977; Harris 1989; Bragg 1999; Rioux et al. 2003). Despite the commercial value of figured wood, study of the mechanism responsible for its formation has been limited. One reason is the diversity of figure types. Some common types of figure include burl, Birdseye, curly, fiddleback, blister, and quilted. These types of figure have never been produced systematically for commercial purposes for two main reasons. First, attempts to identify causative factors of figure and to propagate trees with figured wood have been largely unsuccessful. Second, trees with figured wood are rare and often difficult, if not impossible, to recognize from their bark, except in the case of conspicuous burls (Pillow 1955; Beals and Davis 1977; Bragg and Stokke 1993; Bragg 1999), and possibly "ribbon curl" in koa (*Acacia koa* A. Gray) lumber (Eini Lowell, personal communication).

Figured wood is necessarily an emergent property of the size, distribution, type, abundance, and organization of the structural elements in wood. Two main theories have been proposed to explain figure initiation. One focuses on environmental factors, and can be considered an inducible model. For example, research has shown that wind affects spiral growth in Scots pine (*Pinus sylvestris* L.) and Norway spruce [*Picea abies* (L.) Karst.] (Eklund and Säll 2000). The second theory is one that invokes constitutive production of figure in the absence of dramatic external stimuli (e.g., strong winds) and presumably is solely controlled by genetics. The role of genetics was studied (Anonymous 1929; Righter 1934; Robbins 1953) and shown to be involved in the formation of certain types of figure in European birch (*Betula pendula* Roth) (Persson 1954; Václav 1969). Early attempts to study Birdseye maple using seedlings and cuttings failed to establish that this type of figure was heritable (Robbins 1953; Rudolf 1954). Research focused on transmissible signals revealed that ethylene regulates the severity of spiral grain angle in Norway spruce and balsam fir [*Abies balsamea* (L.) Mill.] (Eklund et al. 2003). However, there is mounting evidence that certain types of figure are best explained by a third theory: genetically controlled responses to specific environmental conditions (Bragg 1999). The diversity of figure and our relative ignorance of its initiation and formation stymies its commercial production.

The reliable production of figured wood of commercially important hardwood species, such as black walnut (*Juglans nigra* L.), is desirable. However, black walnut is difficult to propagate vegetatively, and breeders typically rely on grafts to propagate selected individuals. In an attempt to exploit this trait, breeders have grafted straight-grained black walnut onto curly black walnut rootstock, but the scions did not produce figured wood (MacDaniels 1953). Research at the Hardwood Tree Improvement and Regeneration Center (http://www.agriculture.purdue.edu/fnr/htirc/partners.html) has shown that over a 20-year period, scions of curly black walnut grafted onto straight-grained rootstock did not develop into trees with curly grain (HTIRC unpublished result). The recalcitrance of black walnut to genetic manipulation and vegetative propagation has limited our ability to investigate figure formation in this species. To understand figure in wood in general, we need a model tree that not only has figure but also is easily manipulated in the laboratory.

Poplars (*Populus* spp.) are considered important models because of the relative ease with which they can be clonally propagated ex vitro and transformed and regenerated in vitro (Chaffey 1999; Jansson and Douglas 2007; Taylor 2002; Wullschleger et al. 2002; Campbell et al. 2003). They have other advantages, such as rapid growth, a short juvenile period, and a relatively small genome (550 Mbp, only 4× larger than *Arabidopsis thaliana*, 40× smaller than pine) (Stettler et al. 1996; Mellerowicz et al. 2001; Lescot et al. 2004). Recently released genomic resources make poplars unique among tree species in their amenability to discovering genes and analyzing their functionality (Tuskan et al. 2006).

Figure has also been observed in poplar. Curly Poplar, thought to be "a hybrid between *P. alba* and *P. tremula* (*P.* × *canescens*), and possibly a back-cross of *P.* × *canescens* to *P. alba*", was first reported by Grober (1942), who recognized its economic importance for both its decorative wood and its potential for erosion control. More importantly, preliminary tests on Curly Poplar revealed figure in rooted cuttings derived from an ortet planted in Evanston, IL, which was a clone of another tree discovered in Maryland. In this study, we use Curly Poplar as a model for studying the heritability and repeatability of figure in wood and factors that may influence figure formation. Although poplar is not commonly used in the wood-products industry as a source of decorative veneer, the predictable appearance of figure in Curly Poplar may also point the way toward the use of this fast-growing, low-cost species for this purpose.

Materials and methods

Plant material and propagation

Curly Poplar plantlets were propagated using cuttings taken from a tree in Evanston, IL. This tree was established by Dr. Samuel Grober by rooting a cutting from a tree found in Maryland in 1981. It was suspected to have arisen from a cross between *P. alba* and *P. tremula*, a common hybrid between these two species that is designated

P. × *canescens*. For comparing grain and evaluating graft-transmissible signals, material from various straight-grained genotypes was used, including the following clones: '4877' (*P. alba*) 'Crandon' (*P. alba* × *P. grandidentata*) and 'Sherrill' (*P. alba* × *P. grandidentata*), all of which were provided by Dr. Richard Hall (Department of Natural Resource Ecology and Management, Iowa State University), and 'Ca-2-75' (*P.* × *canescens*), provided by Dr. Mike Cunningham (ArborGen, Inc., Ridgeville, SC, USA). Female clone 'A502' (*P. alba*), which was used to cross with Curly Poplar and as a straight-grain control, was provided by Patrick McGovern (Grand Rapids, MI, USA).

Plastic trays (50 × 24.5 × 6 cm) with drain holes were filled to a depth of about 4 cm with a 2:1 mixture (v/v) of fine perlite and vermiculite (coarse). Stem cuttings, which were approximately 8 cm in length and at least 0.4 cm in diameter, were arranged with about 4 cm between cuttings in all directions, resulting in about 20 cuttings per tray. Stem segments were trimmed to include two to three nodes. Before each cutting was inserted in potting mix, the basal leaf was removed; the upper leaf was cut in half to reduce transpirational loss during root formation, increase the number of cuttings per flat, and limit overlap of the remaining leaves while undergoing misting. The bottom 2 cm of each cutting was dipped into a commercial rooting agent (Rootone™; Gulfstream Home and Garden, Inc., Lexington, KY, USA). All treated cuttings were planted in the flats to a depth of approximately 3 cm. Filled flats were placed into a greenhouse with an air temperature of 25 °C during the daytime and 16 °C at night, and a relative humidity of 60 %. Sunlight was reduced to a nominal 50 % ambient with shade cloth from May to September; high-intensity discharge (HID) lamps extended the photoperiod to 16 h from September to May. Mist was provided initially for 4 s every 10 min from 1 h before sunrise until 1 h after sunset. After 2–3 weeks, ramets were transferred to D-40 tubes (Stuewe and Sons, Inc., Corvallis, OR, USA) filled with Premier PRO-MIX with Biofungicide (Premier Horticulture, Inc., Quakertown, PA, USA) potting mix, and then kept on the mist bench for 4–7 days, after which trays were transferred to the same settings but on a mist-free bench. Unless otherwise indicated, these greenhouse conditions were used for the duration of the project. Ten pellets of the slow-release fertilizer (Osmocote™; Scotts-Sierra Horticultural Products, Marysville, OH, USA) were added to the surface of the potting mix in the containers.

On 25 May 2005, a total of 350 rooted cuttings from Curly Poplar, 4877, Crandon, and Sherrill were planted at the Department of Forestry and Natural Resources (FNR) Farm (latitude 40°N25′47.45″, longitude 86°W57′13.46″), located 2.5 miles west of the Purdue campus (555 North Sharon Chapel Road, West Lafayette, IN, USA). The soil type at the FNR Farm is predominantly a Stark/Fincastle clay loam (http://websoilsurvey.nrcs.usda.gov/). To determine whether figure remained true to type in rooted cuttings, more than 100 Curly Poplar ramets were sampled at various times (7, 10, 11, 18, and 48 months) after growth in two environments (the field and greenhouse) and following two treatments (pruned and unpruned), which, presumably, could disrupt the balance of hormones in the plant body (Table 1). The 48-month-old trees were only tested under field conditions. Plants in both locations were established at the same time.

DNA isolation and genotyping

Genomic DNA from leaf tissues of propagated Curly Poplar were isolated using DNAzol® (Molecular Research Center, Inc., Cincinnati, OH, USA), following the protocol supplied by the manufacturer. Genotyping of 19 nuclear microsatellite loci was performed as described previously by Lexer et al. (2005) at University of Fribourg (Fribourg, Switzerland).

Figure identification

Figure was identified in the field and greenhouse using a simple split test—also called an axe-chip test—on branches or stems that were at least 10 cm in length, to determine the straightness of the grain and split-surface characteristics (Pillow 1955; Beals and Davis 1977). Clone 4877 was used as a non-figured control. Wood from a branch junction was avoided when selecting samples for splitting. Grafted trees were analyzed for figure after 3.5 years. The first branches

Table 1 Number of samples and experimental scheme to test figure reproducibility

Age[a] (months)	7		10		11	18		48	Total
Treatments	UP-GH	UP-F	P-GH	P-GH[b]	UP-F	P-GH	UP-F	UP-F	
Curly Poplar	6	8	12	38	20	10	10	3	107
4877	2	2	2	2	10	8	7	1	34

UP unpruned, *P* pruned, *GH* greenhouse, *F* field

[a] The age of the tree was calculated from the day the cuttings were taken

[b] 10-month growing season plus an extra 5 months at 4 °C

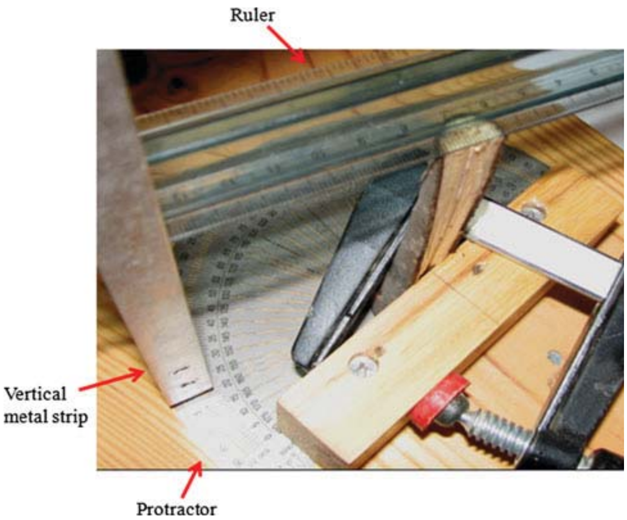

Fig. 1 Device used to measure stem spiral (for details and complete description, see "Materials and methods")

above and below the graft union were evaluated for grain pattern via the split test; all branches were at least 1.5 cm in diameter. The severity of spiral grain in 11-month-old, field-grown trees was measured using the device shown in Fig. 1. The bottom end of an evenly split stem segment was positioned on a protractor that was attached to a horizontal surface. The pith of the stem was placed on the center hole of the protractor, and the straight edge of protractor and the bottom edge of the split surface were aligned. A ruler was held parallel to the upper edge of the split stem segment. A vertical metallic straight edge was rotated to touch the edge of the horizontal ruler and the protractor. The acute angle indicated on the protractor from the straight edge defined the degree of twist (Fig. 1). The sampling scheme used to test for figure is shown in Table 1.

Veneer (0.6 mm thick) was sliced from the original 26-year-old Curly Poplar tree by the David R. Webb Co. (Edinburgh, IN, USA); the veneer-covered panels were laid up on a medium density fiberboard backing by the Select Veneer Company (Smithfield, KY, USA) and finished by Enviro Finishing of Indiana, Inc. (Richmond, IN, USA) using standard industrial methods (Fig. 3).

Scattered Moiré quantification

Because the figure produced by the 26-year-old Curly Poplar ortet appears similar to a fabric known as moiré, but with a more broken and irregular waving pattern, we called it 'Scattered Moiré'. To quantitatively describe Scattered Moiré figure, which showed up as unpatterned dark and light bands, the total number of dark and light bands (NDB and NLB, respectively) was counted on five pieces of radially sliced veneer from the ortet, each with an area of 300 cm^2 (30 × 10 cm). On these same samples, the number of dark and light bands touching a randomly drawn 30-cm line that was oriented vertically on the face of the veneer (NDBL and NLBL, respectively) was also counted. In addition, the widest part of randomly chosen 10 dark and 10 light bands that touched the vertical line was measured for each veneer (WDB and WLB, respectively).

Producing an F_1 population

Crosses were repeated three times between 2005 and 2007, using the same two parental trees. In early spring, prior to anthesis, branches bearing floral buds were collected from the mature, male Curly Poplar tree in Evanston, IL. Soon afterward, flowering branches were collected from a 13-year-old female of *P. alba*, A502. All branches were kept in tap water after collection and stored at 4 °C until use. After stigmas emerged, they were pollinated with Curly Poplar pollen using a small paint brush. Seeds were collected 24 days post-pollination and stored over Drierite (W.A. Hammond Drierite Co., Ltd., Xenia, OH, USA) at 4 °C. A total of 2,500 seedlings were produced and planted in the field.

Seed germination and field planting

Premier PRO-MIX FPX with Biofungicide (Premier Horticulture, Inc., Quakertown, PA, USA) potting mix was thoroughly wetted and mixed before being used to fill 288-cell flats (50 × 24.5 × 6 cm), which were placed in holding trays that were half-filled with distilled water. Once the soil was saturated, seeds were individually sown with tweezers. Trays were covered with a clear, plastic dome to slow evaporation. All flats were transferred to a greenhouse in which the conditions were the same as those described above. Ten days after seeds germinated, the plastic domes were removed and water was applied as needed. After 25 days, seedlings were transferred to D-40 tubes and acclimated in a lath house for 2 weeks before being planted at the FNR Farm.

Trees were planted in augered holes at a spacing of 3.05 m between and 2.29 m within plant-rows. Each spring, pre-emergent herbicide, Pendulum® (BASF, Ludwigshafen, Germany) was applied for weed control. Post-emergent herbicides included Transline® (Dow AgroSciences, Indianapolis, IN, USA) for broadleaf weeds; Envoy Plus™ (Valent USA Corporation, Walnut Creek, CA, USA) for grasses, and Roundup® (ScottsMiracle-Gro, Marysville, OH, USA) for all weeds. Herbicides were applied as needed and at rates specified on their labels. Alleys between the plant-rows were mowed regularly throughout the growing season. Trees were irrigated as needed using a T-tape drip-irrigation system (T-Systems International, Inc., San Diego, CA, USA).

Grafting

On 30 March 2006, more than 50 dormant branches were taken from each of the following genotypes: Curly Poplar, Crandon, Sherrill, and 4877, plants of which were established at the FNR Farm on 25 May 2005. Multiple cuttings could be taken from the same tree, and the cuttings were wrapped with black plastic bags and stored at 4 °C in a walk-in cooler until use. On 22 and 23 May 2006, 160 reciprocal grafts were performed, using a technique described by Hartmann et al. (2002). In short, Curly Poplar served as both rootstocks and scion and was grafted with all the other cultivars (Crandon, Sherrill, and 4877) and itself. Clone 4877 was also grafted onto itself, as a control. Due to the limited availability of certain rootstocks (4877, Crandon, and Sherrill), multiple scions were grafted onto a single tree, but scions of only one genotype were grafted onto an individual rootstock. After 2 months, graft survival was evaluated.

Statistical analyses

A Bayesian approach was employed to determine genetic admixture proportions (*Q*) using the software STRUCTURE (Pritchard et al. 2000), and populations of *P. alba* and *P. tremula* from northern Italy were used as controls. STRUCTURE analysis made use of an admixture model and of samples from reference populations of *P. alba* and *P. tremula* as described in Lexer et al. (2005). Genotype data for the reference populations were used to estimate the allele frequencies of each microsatellite locus in each parental species, which served as a basis for estimating admixture proportions in Curly Poplar. Chi-square tests, *t* tests, analysis of variance (ANOVA), and linear regression analyses were performed using SAS (Ver. 9.1; SAS Institute, Inc., Cary, NC, USA), as well as all basic descriptive statistics, such as means and variances.

Results

Parentage of Curly Poplar

Three genomic DNA samples were extracted from the propagated cuttings of Curly Poplar for pedigree analysis. Based on 19 microsatellite loci, it was determined that 51 % of the genome of Curly Poplar originated from *P. alba*; the rest was from *P. tremula*. The proportion of genetic admixture (*Q*) of Curly Poplar was 0.512, with a 95 % credible interval of 0.298–0.728. As neither 0.25 nor 0.75 was included in this interval, Curly Poplar does not appear to be a backcross with either *P. alba* or *P. tremula*. Therefore, Curly Poplar is a genetically intermediate genotype with roughly equal contributions from *P. alba* and *P. tremula*, commonly referred to as *P.* × *canescens*.

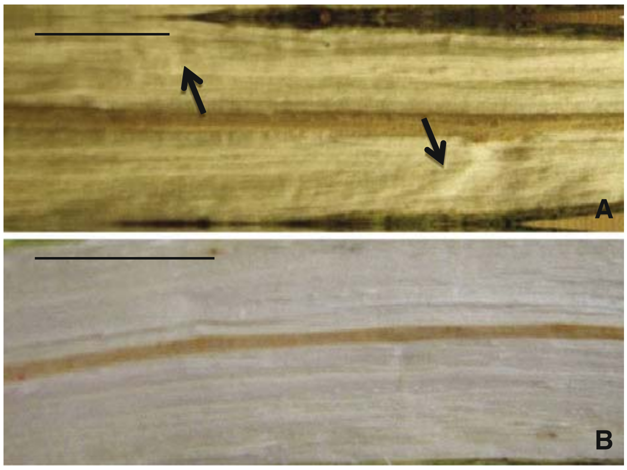

Fig. 2 Radial surfaces of xylem after a split test from **a** 11-month-old Curly Poplar, showing undulations (*arrows*), and **b** 11-month-old clone 4877, with straight grain and a smooth surface. *Scale bar* = 1 cm

Figure in Curly Poplar is reproducible and defined as Scattered Moiré

After splitting through the pith, Curly Poplar clearly exhibited undulations along its radial surfaces, whereas the split surfaces of the straight-grained, non-figured *P. alba* control (clone 4877) were smooth (Fig. 2). In addition, Curly Poplar was more difficult to split than 4877. Although figure was observed in both stems and branches of Curly Poplar, branches and stem less than 1.5 cm in diameter occasionally gave ambiguous results. In addition, when split, the tangential surface showed a minor undulation, which indicated that the Scattered Moiré in Curly Poplar not only has dominant radial undulation but also a weak tangential undulation.

Scattered Moiré has alternating light and dark bands in wing-like shapes (Fig. 3a), and the contrast between them was enhanced by staining (Fig. 3b). Light bands have a uniform width of 1.0 cm and are usually closely associated with a dark band, either on one side or both. Dark bands are of irregular size, having a bee-wing, boat, or block shape (Fig. 3). The width of the dark bands varied from a minimum 0.28 cm to a maximum 2.38 cm, with an average of 0.90 cm and a variance of 0.50 ($n = 50$) (Table 2). The *t* tests revealed that the total NDB was significantly less (*p* 0.026) than the NLB, based on five separate veneer samples, totaling 300 cm^2 area; there were more light than dark bands along a 30-cm length (*p* 0.023). All figure quantification data are summarized in Table 1. Analysis of variance revealed that there were no differences among the pieces of veneer for NDBL (*p* 1.0), NLBL (*p* 0.11), or

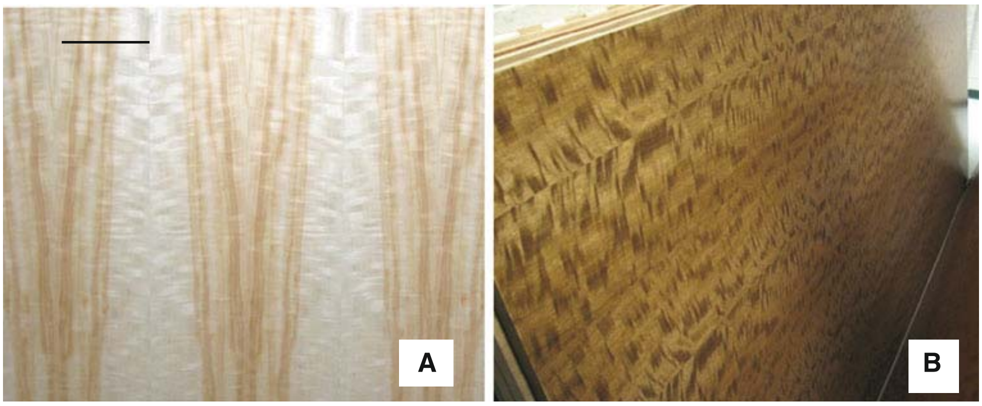

Fig. 3 Curly Poplar veneer mounted and with different finishes showing appearance of Scattered Moiré figure. Book-matched, radially sliced Curly Poplar veneer with **a** clear finish and **b** stained to look like black walnut. *Scale bar* = 14.5 cm

Table 2 Quantification of figure attributes in Curly Poplar veneer

Variable (cm)	*n*	Minimum	Maximum	Mean ± SD
NDB	5	44	56	49 ± 5
NLB	5	51	63	58 ± 5
NDBL	25	7	19	12 ± 3
NLBL	25	6	19	14 ± 3
WDB	50	0.28	2.38	0.90 ± 0.50

NDB number of dark bands, *NLB* number of light bands, *NDBL* number of dark bands in length of 30 cm, *NLBL* number of light bands in length of 30 cm, *WDB* width of dark bands, *n* number of total measurements

WDB (*p* 0.29). When compared with curly figure in other woods, which has a uniformly spaced light and dark pattern and is restricted to the radial surface (Beals and Davis 1977), Scattered Moiré showed not only an irregular pattern, but was present on both radial and tangential surfaces, though the banding pattern was seen most dramatically on the radial surface. Neither radial nor tangential surfaces displayed figure with regular periodicity, and thus the banding pattern could not be readily defined in terms of simple sine waves. The radial extent of figured areas in the wood clearly spanned multiple growth rings. The rift part of flat-sawn veneer most dramatically shows the figure; therefore, the wavy pattern on both the tangential and radial surfaces may be contributing to Scattered Moiré figure.

We examined Curly Poplar, Crandon, and Sherrill ramets grown in the greenhouse and the field for evidence of figure by searching for an undulating pattern on split radial surface. All Curly Poplar ramets ($n = 107$) exhibited figure, but none of the control ramets grown in the same location and examined at the same stage of growth showed figure (Table 1). Time and growth environment had no noticeable effect on figure development in Curly Poplar.

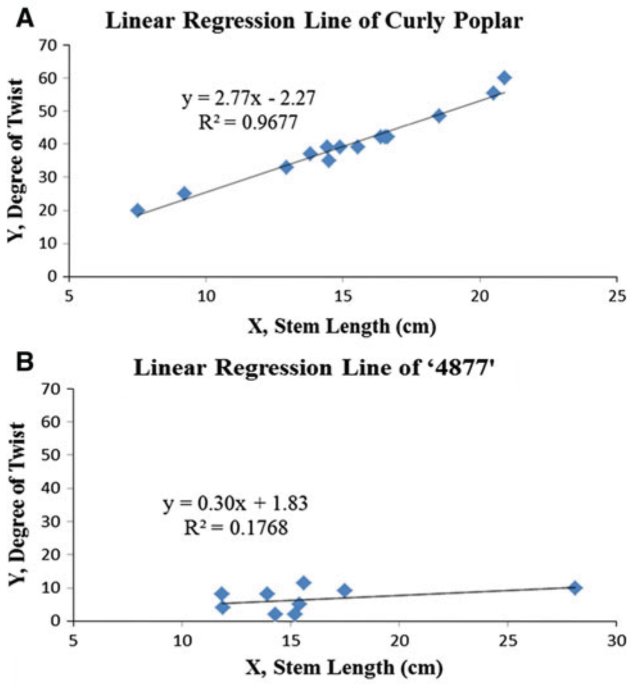

Fig. 5 Degree of twist in Curly Poplar and 4877. **a** Linear regression of Curly Poplar, based on 14 observations: $\beta_0 = -2.27$ (intercept, *p* 0.340), $\beta_1 = 2.77$ (slope, $p < 0.0001$). **b** Linear regression of 4877, based on nine observations: $\beta_0 = 1.83$ (*p* 0.665), $\beta_1 = 0.30$ (*p* 0.260)

Based on a split test, 11-month-old, field-grown Curly Poplar exhibited spiral growth with a pitch of 2.77 ± 0.12°/cm in the axial direction (Fig. 4). Linear regression showed that the degree of twist (slope) was significant ($\beta_1 = 2.77$, $p < 0.0001$) (Fig. 5a). In 4877, the linear regression indicated that both the intercept and slope were not different from "0", ($\beta_0 = 1.83$, *p* 0.665; $\beta_1 = 0.30$, *p* 0.260) (Fig. 5b). Spiral growth was not apparent in wood of the mature Curly Poplar tree from which cuttings were taken. In addition, spiral growth was not detected in 11-month-old, field-grown clone 4877.

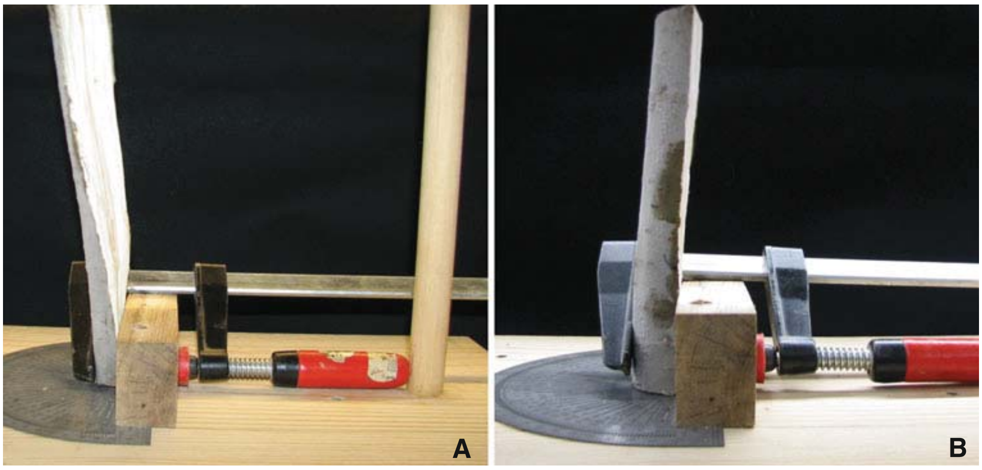

Fig. 4 Grain analyses of 11-month-old, field-grown Curly Poplar and 4877, and the linear regression analyses for spiral grain. **a** Spiral grain in Curly Poplar and **b** straight-grained 4877

We used a split test to determine the inheritance of figure from a randomly selected sub-population in an F_1 population ($n = 2{,}500$) resulting from the cross between A502 and Curly Poplar. We observed that 79 out of 377 of these saplings exhibited figure. Using a Chi-square test, it was determined that the segregation ratio between figured and non-figured trees differed significantly (at a 95 % confidence interval) from 1:1 ($\chi^2 = 127.22$, $df = 1$, $p < 0.001$), but not from 1:3 ($\chi^2 = 3.29$, $df = 1$, p 0.070). Based on these analyses, Scattered Moiré figure in Curly Poplar is assumed to be under genetic control, but is neither simply dominant nor recessive in its inheritance pattern.

Graft-transmissible signals did not affect figure formation in Curly Poplar

Two months after grafting, buds on 157 scions had flushed and the elongating branches displayed new leaves, while three scions died. Each of the multiple scions on an individual rootstock was considered an independent graft. Grafts were sampled after 3.5 years of growth in the field, when branch diameters were greater than 1.5 cm. To evaluate the figure in grafts, new axial growth from the scion and rootstock as well as the graft union were sampled in the preliminary test. The new growth of both the scion and rootstock had wood with a phenotype consistent with the plant from which it originated. Then, in 2009, only the new growth was used to evaluate the figure. The results from 48 grafts indicated that both scion wood and rootstock retained their original properties, indicating that graft-transmissible signals did not lead to or alter the formation of figure (Table 3). The rest of the grafts will be used for tracking the long-term effects of rootstock on figure formation.

Discussion

Genetic ancestry of Curly Poplar

The 95 % credible interval of the genetic admixture proportion of Curly Poplar was between 0.298 and 0.728. Because neither 0.25 nor 0.75 are included in this interval, Curly Poplar represents a genetically intermediate genotype of *P.* × *canescens*. Based on our data, we cannot exclude the possibility that Curly Poplar is an advanced recombinant hybrid, such as an F_2 between an F_1 individual and another *P.* × *canescens* (although there is no nomenclature to define this type of offspring), or an even higher-order hybrid. However, female *P.* × *canescens* are rare (Stace 1997), and *Populus* produce offspring with a highly distorted sex ratio, showing a preference for males (Farmer 1964; Grant and Mitton 1979; Rottenberg et al. 2000). Based on what is currently known about the population genetics of *P.* × *canescens* (Fussi et al. 2010; Lexer et al. 2005, 2010; van Loo et al. 2008), it is reasonable to consider Curly Poplar to be a *P.* × *canescens* genotype.

'Curly Aspen' is a misnomer

When Curly Poplar was discovered, it was called Curly Aspen, due to the appearance of its veneer (Grober 1942). However, the term 'curly' is used to describe wavy growth observed on radial but not tangential surfaces (Beals and Davis 1977). In addition, figure in Curly Poplar appears as small, broken, wavy patterns on the radial surfaces, along with rough undulations on tangential surfaces, but lacking spiral growth. This is not consistent with the accepted definition of curly (Beals and Davis 1977). We also did not observe a slower growth rate for Curly Poplar, as was reported in research on curly birch, which grows half as

Table 3 Summary of samples for each type of graft

Rootstock[a]	Scion	No. of sampled scions	No. of sampled rootstocks	Figure result[b]	
				Rootstock	Scion
Curly Poplar	4877	13	8	Yes	No
4877	Curly Poplar	12	6	No	Yes
4877	4877	9	3	No	No
Curly Poplar	Curly Poplar	4	2	Yes	Yes
Crandon	Curly Poplar	2	1	No	Yes
Curly Poplar	Crandon	2	1	Yes	No
Curly Poplar	Sherrill	2	1	Yes	No
Sherrill	Curly Poplar	4	2	No	Yes
Total		48	24		

[a] 4877, straight-grained *Populus alba*; Crandon, straight-grained *P. alba* × *P. grandidentata*; Curly Poplar, figured *Populus* × *canescens*; and Sherrill, straight-grained *P. alba* × *P. grandidentata*

[b] Split test was used to identify figure

fast as non-figured trees (Heinkinheimo 1940). Grober (1942) explored white poplar (*P. alba*) and its hybrids in Maryland for their botanic, erosion-control, and economic value, as well as its taxonomic and growth features. He divided the population into four categories: (1) *P. alba* female, (2) *P. alba* male, (3) *P. alba* × (*P.* × *canescens*) male, and (4) *P.* × *canescens* male. Although he did not mention the presence of *P.* × *canescens* females in his dissertation, Grober believed that female *P.* × *canescens* were present in eastern Maryland, but that none of them had figure. In his system, categories 3 and 4 were considered commercially valuable because of their sound, light-colored wood. In additional, some trees in categories 3 and 4 produced figure. He also observed spiral growth, especially interlocked grain, associated with figure formation in Curly Aspen. This is not consistent with what we observed in the 26-year-old Curly Poplar tree harvested in Evanston, IL. Spiral and interlocked grain were not observed in any portion of a 4-m-long panel made with veneer from the 26-year-old Curly Poplar tree. This may be because the cutting brought to Evanston by Grober, from his visit to Maryland in 1980s, was not one that he originally sampled in the 1930s. In addition, spiral grain can be age-dependent, although the details are unclear (Rauchfuss and Speer 2006).

We also discovered inconsistency in figure between the 11-month-old Curly Poplar rooted cuttings grown at the FNR Farm and the 26-year-old Curly Poplar in Evanston, IL, from which the cuttings were taken. All the 11-month-old Curly Poplar plants showed spiral growth (Fig. 3), whereas the 26-year-old Curly Poplar did not show any sign of spirality. As stated previously, different growing conditions can affect spiral growth, but it may be also due to differing properties of juvenile and mature wood, or affected by environmental factors that change throughout the year (Eklund and Säll 2000; Eklund et al. 2003).

The transition from juvenile to mature wood can be affected by environment (Matyas and Peszlen 1997), and usually happens during the first 10–20 years of a tree's life (Zobel and Sprague 1998). For example, the transition from juvenile to mature wood in Sitka spruce [*Picea sitchensis* (Bong.) Carr.] appears to occur at 12–13 years of age (Brazier and Mobbs 1993; Cameron et al. 2005). However, wood produced in rapidly growing 11-month-old trees would certainly be considered juvenile wood, presumably with attendant rapid changes in wood properties, whereas wood produced in the mature zone of 26-year-old poplar showed relatively constant wood properties. Generally speaking, juvenile and mature wood differ in their anatomical properties. For example, in poplar, fiber length is shorter in juvenile than in mature wood (Cheng and Bensend 1979), and fiber cell length was observed to increase rapidly in juvenile wood, leveling off as the tree matures (Boyce and Kaiser 1961). Differences in chemical properties also vary by species (Bao et al. 2001). There is no evidence, however, that juvenile wood is more likely to have spiral grain.

The gene(s) involved in figure formation

Based on an analysis of rooted cuttings and the F_1 population, it can be concluded that the figure in Curly Poplar, which is the only documented, reproducibly figured poplar, is heritable. Using a population size of more than 2,000 individuals and margin of error of 0.05, a minimum sample of 323 individuals is needed to estimate the whole population's segregation ratio with 95 % confidence (Kutner et al. 2004). If Curly Poplar is heterozygous with respect to figure and it is controlled by a single dominant gene, we would expect to see a 1:1 segregation ratio in an F_1 population. However, because 79 out of 377 individuals sampled exhibited figure (figure vs. non-figure, 1:3.77), a 1:1 segregation ratio was rejected, based on a Chi-square test ($p < 0.01$). If Curly Poplar is homozygous, and a single-gene controlling figure is either dominant or recessive, the F_1 population will be either all figured or all non-figured, respectively. Thus, neither a single-gene dominant nor a recessive model alone can explain the observed segregation ratio. *Populus* is a dioecious plant, and there is anecdotal evidence that only male trees produce figure in Curly Poplar (Grober 1942). In addition, male offspring are preferentially produced in *Populus* (Farmer 1964; Grant and Mitton 1979; Rottenberg et al. 2000). Thus, one possible explanation for these skewed results is sex-linkage, but this cannot be verified until the F_1 individuals mature.

It is possible the figure phenotype observed in Curly Poplar is under the control of a single gene with distorted segregation. Segregation distortion is commonly observed in interspecific crosses in general (Rieseberg and Carney 1998; Burke and Arnold 2001) and in *Populus* specifically (Yin et al. 2004; Macaya-Sanz et al. 2011). In addition, epigenetic phenomena such as genomic imprinting, in which certain genes are expressed in a manner specific to the parent of origin, have been observed in a variety of flowering plants (Gehring et al. 2004), and these phenomena are known to be pronounced in interspecific hybrids (Michalak 2009). As genetic imprinting is a process that operates independently of classic Mendelian inheritance, it could result in a 1:3.77 segregation ratio, as observed in our F_1 population.

Graft-transmissible signals

Graft-transmissible signals, as defined here, are molecules which can pass the graft union and affect plant growth. They include, but are not necessarily restricted to, plant hormones and RNAs (Turnbull et al. 2002; Harada 2010; Kasai et al. 2011). A study of Norway spruce and balsam fir showed that exogenous ethylene increased spiral growth (Eklund et al. 2003). Other plant hormones, such as auxin, which are required for tracheid differentiation and cell polarity (Kramer et al. 2008; Kramer 2009), and gibberellic acid, which is required for tracheid elongation (Kalev and Aloni 1999), may also contribute to certain types of figure. Curly black walnut has been observed (data not shown) to be more sensitive than wild-type to 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin commonly used as an herbicide to control broadleaf weeds. A mathematical model of auxin-mediated wood grain pattern formation was provided by Kramer (2006), but there is no direct evidence that links plant hormones to figure formation. Although hormones can cross the graft union, they did not cause the phenotype in our plants.

Conclusions

We provided evidence that figure in wood can be genetically heritable, but it may not be under simple genetic regulation, at least not for the new type of figure discovered in Curly Poplar, which we named Scattered Moiré. As a model for studying figure in wood, Scattered Moiré is reproducible by vegetative propagation and is amenable to genetic and molecular manipulation, unlike figured wood in other species. Characterizing the heritability and gross characteristics of a stably reproducible, commercially valuable figured wood is a first step toward a broader goal of understanding and producing figure in other woody species.

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Conflict of interest The authors declare that they have no conflict of interest. The use of trade names is for the information and convenience of the reader and does not imply official endorsement or approval by the United States Department of Agriculture or the Forest Service of any product to the exclusion of others that may be suitable.

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