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Gender Determination in *Populus*

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Abstract

Gender, the expression of maleness or femaleness, in dioecious plants has been associated with changes in morphology, physiology, ecological position, and commercial importance of several species, including members of the Salicaceae family. Various mechanisms have been proposed to explain the expression of gender in Salicaceae, including sex chromosomes, simple Mendelian genes, quantitative genes, environment, and genotype-by-environment interactions. Published reports would favor a genetic basis for gender. The objective of this study was to identify molecular markers associated with gender in a segregating family of hybrid poplars. Bulked segregant analysis and chi-squared analysis were used to test for the occurrence of sex chromosomes, individual loci, and chromosome ratios (i.e., ploidy levels) as the mechanisms for gender determination. Examination of 2488 PCR based RAPD markers from 1219 primers revealed nine polymorphic bands between male and female bulked samples. However, linkage analysis indicated that none of these markers were significantly associated with gender. Chi-squared results for difference in male-to-female ratios between diploid and triploid genotypes also revealed no significant differences. These findings suggest gender is not controlled via sex chromosomes, simple Mendelian loci or ratios of autosome to gender-determining loci. It is possible that gender is determined genetically by regions of the genome not sampled by the tested markers or by a complex of loci operating in an additive threshold manner or in an epistatic manner. It is also possible that gender is determined environmentally at an early zygote stage, canalizing gender expression.

Key words: *Populus*, gender determination, polyploidy, RAPD markers, bulked segregant analysis, chi-squared analysis

Introduction

Genetic selection for desired traits (e.g. biomass production, disease resistance, or drought tolerance) in economically important crop species is an integral part of agricultural research and development. Plant species with a dioecious reproductive habit differ from other crops in that male and female reproductive structures occur on separate individuals. Traits may vary between the sexes in dioecious systems partially because males and females are under different reproductive constraints; i.e., males are typically involved in high gamete output and effective gene transfer, while females are typically committed to maximizing the success of individual

zygotes (Stebbins 1950). Consequently, morphological and/or physiological differences may exist between the sexes, differences which often have a genetic basis (Dellaporta and Calderon-Urrea 1993).

In plant breeding programs, attempts to select for a trait of interest can be complicated by gender since maleness or femaleness can only be determined at the onset of flowering in most dioecious plants species. If the trait of interest is gender ratio adjustment in different ecological settings or if the trait of interest is physiologically or morphologically associated with gender then trait evaluations can only be completed at reproductive maturity. The ability to identify gender in dioecious species at the zygote or seedling stage, would increase genetic gains per unit time within a breeding program can be increased.

Examples of gender associated traits in Salicaceae (the willow family - which contains two genera, *Salix* and *Populus*) have been identified where male clones have been shown to maintain higher turgor pressures at lower water contents (Dawson and Bliss 1989, Tschaplinski *et al.* 1994, Tschaplinski and Tuskan 1994). Female clones of intraamericana *Populus* hybrids tend to produce greater numbers of lateral branches (Tschaplinski *et al.* 1994). In *S. arctica*, Dawson and Bliss (1993) found that females have higher carbon assimilation rates than males. Grant and Mitton (1979), working with *P. tremuloides*, found that female clones produce higher mean annual growth than male clones. Female clones also tend to maintain higher basal area in vegetatively propagated stands (Sakai and Burris 1985). Alternatively, Farmer (1964) reported that male clones of *P. deltoides* were significantly taller than female clones, though there was no apparent male numerical advantage as evidenced by a 1:1 ratio of male to females in the examined native stands. In *P. trichocarpa*, the frequency of male clones was higher on more xeric sites (Reed 1994) and male clones tend to be more common in *P. tremuloides* (Pauley and Mennel 1957) and in *P. tremula* (Falinski 1980). Independent of gender ratio, male clones of *P. tremuloides* are the

preferred food source for ruffed grouse (Schladweiler 1968, Gullion and Svoboda 1972). And, Dannell *et al.* 1985 and Elmqvist *et al.* 1988 reported that male clones of *S. myrsinifolia x phylicifolia* are the preferred browse of voles. Insect damage has also been reported to be greater on male clones of *S. cinerea* (Alliende 1989). The occurrence of phenolic glucosides is thought to confer resistance to herbivory. Although no gender-based differences were detected in *S. sericea* (Nichols-Brians *et al.* 1993), Boecklen *et al.* (1990) and Elmqvist *et al.* (1991) were able to detect higher concentrations of phenolic glucosides in female clones of *S. lasiolepis* and *S. rigida*, respectively. Such differences have fueled interest in gender-determining mechanisms.

The gender-determining mechanism within Salicaceae is as yet unknown. Lester (1963) reported that 38% of the sampled *P. tremuloides* individuals (5.3% of the sampled flowers) expressed deviations from dioecy across environmental gradients. Heslop-Harrison (1924) noted that mite infestation causes female flower structures to arise on male flowers. Both of these reports suggest an environmental factor controlling gender in Salicaceae. Alternatively, Heslop-Harrison (1924) reported the identification of sex chromosomes in several *Salix* species and in *P. tremula*, though later researchers have not been able to confirm their existence (Mitton and Grant 1980 and ref. therein). Mosseler and Zsuffa (1989) reported female bias among progeny of intra- and interspecific crosses of numerous *Salix* species. Gender ratio was found to be genetically determined in *P. tremuloides* L. (Valentine 1975). Furthermore, Heslop-Harrison (1924) noted that in *Salix*, diploid species tend to have a 1:1 gender ratio while polyploids tend to be female biased. Several studies report no gender reversals, including Dawson and Bliss (1989) working with *S. arctica* over a four year period, Alliende and Harper (1989) working with *S. cinerea*, and Mitton and Grant (1980) working with *P. tremuloides*. These reports suggest that gender is genetically and not environmentally determined in this taxonomic family.

In addition to the applications noted above, basic research dealing with gender

differentiation will be facilitated through the use of a gender-related marker. Gender identification using molecular techniques has been met with some success at the level of isozymes (Maestri *et al.* 1991, Schnabel and Hamrick 1990) and DNA probes (Mulcahy *et al.* 1992). The primary objective of this study is to use DNA markers to test for the presence of genetic markers associated with gender; and secondly, to draw inferences regarding the importance of genetic vs. environmental factors controlling gender.

Materials and Methods

An F_1 hybrid pedigree consisting of six male and six female progeny, from a cross between a black cottonwood (*Populus trichocarpa* Torr. & Gray '93-968') female clone and eastern cottonwood (*P. deltoides* Bartr. 'ILL-129') male clone at the University of Washington, was used to obtain DNA for the male and female bulked samples. DNA characterization relied on a Polymerase Chain Reaction (PCR) procedure for generating randomly amplified polymorphic DNA (RAPD) markers (Welsh and McClelland 1990 and Williams *et al.* 1990). The RAPD reactions were conducted in a MJ Research PTC-100 thermocycler (MJ Research Inc., Chatham, NJ) with a 35 cycle protocol involving 5 s at 94°C denaturation, 30 s at 36°C annealing, and 1 min at 72°C polymerization steps within each cycle, followed by a 5 min final polymerization step. Each reaction contained 50mM KCl, 10 mM Tris-HCl pH 8.0, 2.5 mM $MgCl_2$, 0.1% (v/v) Triton X-100, 1.0 ng Bovine Serum Albumin, 0.5 units *Taq* polymerase, 200 *u*M each dNTP, 10 ng primer, and 1.0 ng DNA template, to a final volume of 10 *u*l. Bulked segregant analysis was used to identify polymorphic DNA markers (Michelmore *et al.* 1991). Similar techniques have been used to identify genetic markers associated with 1) differences between and within cultivars (Hu and Quiros 1991), 2) disease resistance (Martin *et al.* 1991, Paran *et al.* 1991), and 3) the Y chromosome in *Silene latifolia* (Mulcahy *et al.* 1992). This analysis involved screening male and female bulked samples of DNA from individuals originating from the above pedigree with

approximately 1219 different primers (539 primers from Operon Technologies inc., Alameda, CA and 680 primers from the University of British Columbia, Vancouver, B.C., Canada). DNA markers that were found to be polymorphic between the bulks were used to confirm the presence of gender-determining loci by separating individual plants that constituted the bulks and then testing for consistency within gender by means of linkage analysis. Logarithm of the odds ratio for linkage (LOD) scores and linkage estimates were provided Dr. Toby Bradshaw, University of Washington, through the use of MAPMAKER-QTL version 1.1 (Lander and Botstein 1989).

Chi-squared analysis was used to test for the possibility that the ratio of gender-determining loci to autosomal loci controls gender expression in *Populus*. Pedigree information regarding gender and ploidy level from the *Populus* breeding effort at the University of Washington (Stettler 1991) was used in this analysis. First, observed male-to-female ratios were tested against the expected 1:1 ratio for all diploid and triploid clones. Next, the gender ratio for the diploid clones was used as the expected ratio to test for deviations in the gender ratio expressed in the triploid clones. A total of 418 diploid progeny and 110 triploid progeny from 50 hybrid families were used in the analysis.

Results and Discussion

Of the 1219 random 10-mer primers examined, 381 revealed no amplified bands among the bulked samples. The remaining 838 primers yielded 2488 bands, with an average of 2.7 bands per primer for the Operon primers and 1.5 bands for the UBC primers. The majority of the bands were monomorphic between the male and female bulked samples. Seven primers did reveal nine polymorphisms between male and female bulks (Table 1). The segregation ratios among individual genotypes are presented in Table 1. None of the polymorphic bands occurred with 100% fidelity within a single gender. That is, the frequency of each polymorphic band was higher in one gender or the other, but no bands were found solely in all individuals of one gender and

absent in the other. Linkage analysis for these segregating bands indicated that none of the bands were significantly associated with gender (LOD scores ranged between .25 and 1.05, critical LOD score=3.5 at $P \leq 0.05$). The estimates of linkage distance ranged from 62.6 cM to 27.9 cM.

Of the 418 diploid progeny, 23.9% were flowering; 46 were male and 54 were female. Fifty-four percent of the triploid progeny were flowering, resulting in 28 male and 31 female progeny. Chi-squared values for testing deviations in gender ratios in diploid and triploid progeny revealed no significant departures from the expected 1:1 ratio ($X^2 = 0.51$ and 0.17, respectively, critical $X^2=3.841$ at $P \leq 0.05$). In addition, there were no significant differences in the observed male-to-female ratios between the diploid and triploid progeny.

It was anticipated that DNA markers could accurately identify the gender of the tested individuals. The bulked segregant analysis and subsequent linkage analysis failed to identify such markers. It is possible that different primers may probe a portion of the genome not sampled by the tested primers used in this study, thus revealing markers that are linked to gender-determining loci. This seems unlikely given the number of primers tested and the nature of the random 10-mers. Bulked segregant analysis has been used successfully to identify gender-related markers in *Silene latifolia* (Mulcahy *et al.* 1992). Here four markers were found after examining only 40 primers, although it was previously known the *Silene latifolia* contained sex chromosomes. The lack of gender-related markers detected in this study would argue against the presence of sex chromosomes in *Populus*. It is still possible that gender is controlled by separate loci operating in an additive, threshold manner or in an interacting epistatic manner (C. Alstrom, 1994, personnel communication, Dellaporta and Calderon-Urrea 1993). Bulked segregant analysis would not be effective in detecting such loci outside of a structured pedigree segregating for a single gender-determining locus.

The similarities between the male and female ratios for diploid and triploid progeny

suggest that it is unlikely that gender is determined via ratios of autosomes to gender-determining loci. In other words, the additional haploid set of chromosomes, contributed by the female parent (Bradshaw and Stettler 1992), did not bias the gender ratio in the triploid progeny. Additionally, across the 50 families used in this analysis, there appears to be no bias towards one gender or the other, though this ratio does vary on a within family basis. It is possible that as more individuals within these 50 families begin to flower there will be a shift towards female biased ratios, given that male clones tend to flower at an earlier age (Kaul and Kaul 1984).

As Karlin and Lessard (1986) indicate gender determination in dioecious species usually occurs at syngamy. It may be possible that in *Populus* gender is controlled by the environment (e.g. temperature, nutritional status, etc.) at the moment of syngamy and once determined it remains tightly canalized during ontogeny. Similar environmental gender determination mechanisms occur in nematodes, amphibians, fish, and reptiles (Bull and Vogt 1979, Charnov and Dawson 1989, Ross *et al.* 1983). Furthermore, Freeman *et al.* (1980) and Charnov and Dawson (1989) suggest environmental gender determination offers some advantages to perennial plant species that occupy patchy environments, as do members of the Salicaceae family.

Conclusions

Reported literature would suggest that gender is genetically controlled in *Populus* and that gender is consistently expressed across developmental age and environments. Bulked segregant analysis and chi-squared analysis failed to identify molecular markers associated with gender or relationships between gender and ploidy level, respectively. The genetic basis of gender determination in *Populus* must thus involve multiple interacting loci, or alternatively involve environmentally determined effects early in ontogeny.

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Table 1. The presence (+) or absence (-) of randomly amplified polymorphic DNA markers in a segregating F_1 hybrid poplar family for nine out of 1219 selected 10-mer primers.

Genotype	Gender	Random 10-mer Primer [1]								
		UBC523-575	UBC269-340	AG5-550	AG5-500	P9-850	P9-550	P14-650	R15-550	S11-280
50-180	male	+	+	-	-	+	+	-	+	-
50-181	male	-	-	+	-	+	-	+	+	-
50-183	male	+	+	+	-	+	+	+	+	-
50-186	male	-	-	-	-	+	+	-	-	+
50-190	male	+	-	+	-	+	-	+	-	+
50-194	male	+	+	+	-	-	+	-	+	+
50-185	female	-	-	-	+	-	+	-	-	+
50-188	female	-	-	+	-	-	-	-	+	+
50-191	female	-	-	-	+	-	-	-	-	+
50-192	female	-	-	-	+	+	+	-	-	+
50-195	female	-	-	-	-	-	-	-	+	+
50-196	female	-	-	+	-	+	-	-	-	-

[1] The UBC primers were purchased from the University of British Columbia, Vancouver, BC, Canada; the remaining primers were purchased from Operon Technologies Inc., Alameda, CA. Primer sequences are available upon request.