Asymmetrical natural hybridization between Populus deltoides and P. balsamifera (Salicaceae)

Mona Hamzeh, Christina Sawchyn, Pierre Pé rinet, and Selvadurai Dayanandan

Abstract: Natural hybridization has long been recognized as a means for gene flow between species and has important evolutionary consequences. Although hybridization is generally considered to be symmetrical, with both hybridizing species being equally likely to be the male or female parent, several studies have demonstrated the presence of asymmetrical hybridization and introgression from one species to the other. We investigated the direction of natural hybridization between two sympatric forest tree species in North America (Populus deltoides Bartr. ex Marsh. and Populus balsamifera L.) using species-specific single nucleotide polymorphism (SNP) markers in both the nuclear and chloroplast genomes. All natural hybrid individuals, identified from morphological traits, had nuclear alleles corresponding to both parental species, while the chloroplast genotypes showed similarity to P. deltoides, indicating asymmetrical hybridization with P. deltoides as the maternal and P. balsamifera as the paternal donor species. This observed asymmetrical hybridization may be attributable to cytonuclear interactions.

Key words: Populus, natural hybridization, asymmetry, SNP markers.

Introduction

Natural hybridization among species influences the evolution of biological diversity in a variety of ways including an increase in intraspecific genetic diversity (Anderson 1948), altering genetic adaptations (Anderson 1948; Stebbins 1950), the origin of new ecotypes or species (Grant 1981; Whitham et al. 1994), and the reinforcement or breakdown of reproductive barriers (Ellstrand and Elam 1993; Rieseberg and Gerber 1995; Levin et al. 1996). Introgression, or the transfer of genetic material from one species to another, has been documented in a wide variety of plant and animal taxa (Arnold 1992; Rieseberg and Wendel 1993) and may serve as a source of genetic variation for adaptive evolution (Lewontin and Birch 1966; Grant and Grant 1994, 1996; Wang et al. 1997). Hybridization between species has traditionally been examined for its role in finalizing the speciation process through reinforcement of reproductive barriers (Dobzhansky 1940; Mayr 1963; Howard 1993). The evolution of barriers to interspecies gene flow plays significant roles in the process of speciation. Therefore, understanding the patterns of reproductive isolation between species is crucial for gaining insights into the mechanisms that control interspecific gene exchange or natural hybridization.

Interspecies hybridization is one of the common features among many members of the genus Populus L, a group of widely distributed forest trees in the northern hemisphere.
In North America, several species of the genus *Populus*, particularly species of the sections *Tacamahaca* Spach. and *Aigeiros* Duby, are broadly sympatric (Eckenwalder 1984a, 1984b), and known to hybridize extensively (Brayshaw 1965; Ronald et al. 1973a, 1973b; Eckenwalder 1984a, 1984b; Rood et al. 1986; Greenaway et al. 1991; Floate 2004).

Through multivariate analysis of leaf morphology of *Populus* species, Rood et al. (1986) showed continuous variation of characters and suggested the hybridization between *Populus deltoides* Bartr. ex Marsh., *Populus balsamifera* L., and *Populus angustifolia* James. These observations were further confirmed with gas chromatography – mass spectrometry data (Greenaway et al. 1991). Comprehensive analysis of leaf morphological characters of *P. balsamifera*, *P. angustifolia*, and *P. deltoides* in the hybrid zones in the riparian forests in southern Alberta suggested bidirectional hybridization between *P. angustifolia* and *P. balsamifera*, and unidirectional introgression between *P. balsamifera* and *P. deltoides* (Floate 2004). Molecular data has revealed a similar unidirectional introgression between *P. fremontii* and *P. angustifolia* in the Weber River drainage system in northern Utah (Martinsen et al. 2001). Although the hybrid nature and directionality of the hybridization has been successfully determined using morphological data, the maternal and paternal parental source of these natural hybrids remains unknown.

In this study, we used both nuclear- and chloroplast-based single nucleotide polymorphisms (SNPs), specific for *P. deltoides* and *P. balsamifera*, to test the hybrid status of morphologically identified hybrids, and determine the maternal and paternal parental species of the F1 hybrids between these two species (known as *Populus × jackii* Sarg.) collected from natural stands, to infer the parentage of hybrids in natural populations.

**Materials and methods**

**Plant material and DNA extraction**

Fresh leaf samples were collected from morphologically distinct *P. deltoides*, *P. balsamifera*, first generation progeny (F1) of controlled crosses between *P. deltoides* and *P. balsamifera*, and *P. × jackii* occurring in natural stands (see supplementary data3, Tables S1 and S2). These hybrids are usually recognised by their broadly ovate leaves with heart-shaped bases (see supplementary data3, Fig. S1). In this study, we used natural hybrids that can be morphologically distinguished from the parental species. Thus, complex backcross hybrids that are morphologically indistinguishable from the parental species may not have been sampled for the present study. However, the exclusion of such hybrids in our samples was not expected to affect the interpretation of our results. The collected leaf samples were stored at −80 °C prior to extraction of DNA. The total genomic DNA was extracted from frozen leaf tissue using the methods of Doyle and Doyle (1987) and Dayanandan et al. (1997).

**Selection and validation of SNPs**

Molecular markers represent a powerful tool for identifying hybrid taxa (Rieseberg and Wendel 1993). Species-specific molecular markers derived from both biparentally inherited nuclear and uniparentally inherited organelles such as the chloroplast, which is maternally inherited in *Populus* (Rajora and Dancik 1995), are invaluable for detecting the parentage of putative hybrids. In particular, SNPs or DNA sequence variations that occur at a specific site in the genome (Cho et al. 1999; Griffin and Smith 2000) are useful for detecting the hybrids. Based on nucleotide sequence matrices of two noncoding regions of the chloroplast DNA (intron of *trnL* and intergenic region of *trn-T-trnL*) and *ITS2* of the nuclear rDNA from a previous study of *Populus* (Hamzeh and Dayanandan 2004), four chloroplast and two nuclear SNPs specific to *P. balsamifera* and *P. deltoides* were identified (Table 1). The nrSNP1 and nrSNP2 were located within the *ITS2* region of the nuclear rDNA. The cpSNP1 was located within the intergenic region of *trnT-trnL*, and cpSNP2, cpSNP3, and cpSNP4 were located in the *trnl* intron of the chloroplast genome. The selected nuclear and chloroplast SNPs were further characterized for use as reliable molecular markers by genotyping 30 individuals of *P. deltoides* and 10 individuals of *P. balsamifera*. The inheritance pattern of SNPs was determined by genotyping 20 individuals selected from progenies of controlled crosses between *P. deltoides* and *P. balsamifera* and their parents.

**Template preparation**

Target genomic regions containing SNPs were amplified by the polymerase chain reaction (PCR). The oligonucleotide primers a, c, and d (Taberlet et al. 1991) and *trn*CR (Hamzeh and Dayanandan 2004) for the chloroplast genome and primers *ITS3* (Becerra and Venable 1999) and *ITS* 28*kJ* modified from Culling (1992) for the *ITS2* region were used for PCR amplification. Amplification reactions contained 230 µmol/L dNTP, 2.5 mmol/L MgCl₂, 0.2 µmol/L of each primer, 1 unit of *Taq* DNA polymerase, and 2.5 µL buffer (0.2 mol/L Tris PH. 9.5; 0.25 mol/L KCl; 1 mg/mL BSA, 5 µL/mL Tween 20) in a total volume of 25 µL. PCR amplification was performed in a Mastercycler gradient thermal cycler (Eppendorf, Westbury, N.Y.) at 94 °C for 60 s, 55 °C for 30 s, and 72 °C for 60 s for 35 cycles. The residual primers and dNTPs were dephosphorylated using 0.5 unit of shrimp alkaline phosphatase (SAP, Fermentas, Burlington, Ont.) and 20 units of exonuclease 1 from *E. coli* (Fermentas, Burlington, Ont.) at 37 °C for 2 h. Enzymes were inactivated at 80 °C for 20 min.

**Single nucleotide primer extension reaction and genotyping**

For each candidate SNP, an unlabeled oligonucleotide primer with its 3′ end adjacent to the SNP (Table 1) was

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3Supplementary data for this article are available on the journal Web site (http://cjb.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5232. For more information on obtaining material refer to http://cistd-icist.nrc-cnrc.gc.ca/trm/unpub_e.shtml.
The single nucleotide polymorphism (SNP) locus, genotypes in Populus deltoides, P. balsamifera, and P. × jackii, and genotyping primer sequences of nuclear and chloroplast SNPs used in the present study.

Table 1. The single nucleotide polymorphism (SNP) locus, genotypes in Populus deltoides, P. balsamifera, and P. × jackii, and genotyping primer sequences of nuclear and chloroplast SNPs used in the present study.

<table>
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<tr>
<th>Primer name and sequence</th>
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<th>Genotype Primer name and sequence</th>
<th>Genotype Primer name and sequence</th>
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<tbody>
<tr>
<td>SNPBLIT2R: 5'CGTGAGCCGAGGGGAG-3</td>
<td>P. deltoides</td>
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<tr>
<td>SNPBLIT3R: 5'GATGCCCGAGGGTCC-3</td>
<td>P. balsamifera</td>
<td>P. balsamifera</td>
<td>P. balsamifera</td>
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<tr>
<td>SNPDLTN1F: 5'TAAAGGAATCCTTCTGTTAAAGT-3</td>
<td>P. × jackii</td>
<td>P. × jackii</td>
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<tr>
<td>SNPBLTN1F: 5'ACCCTATAAACATAATACATAGGAAA-3</td>
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<tr>
<td>SNPBLTN2R: 5'ACGTACGTACGTACGTACGTACGATTCAAATCAAAGCAATTTT-3</td>
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<td>Note: The nrSNP1 and nrSNP2 are located within the ITS2 region of the nuclear rDNA. The cpSNP1 is located within the intergenic region of trnT-trnL, and cpSNP2, cpSNP3, and cpSNP4 are located in the intron of the chloroplast genome.</td>
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Validation of SNPs

The genotypes of all P. deltoides and P. balsamifera individuals were consistent with the expected genotypes for all nuclear and chloroplast SNPs tested in this study, as listed in Table 1, confirming the reliability and sensitivity of the SNPs for the present study. The nuclear and chloroplast SNP genotypes of the parental individuals used in the controlled crossing experiment were also consistent with the genotypes of the P. deltoides and P. balsamifera individuals tested in this study. The nuclear SNP genotypes of all P. × jackii from controlled crosses showed SNP alleles attributable to P. deltoides and P. balsamifera proving the Mendelian inheritance pattern of these SNPs. The chloroplast genotypes of all control-crossed P. × jackii were consistent with the chloroplast genotype of P. deltoides and this confirmed the maternal inheritance of the chloroplast genome in Populus.

Genotype of natural P. × jackii

The nuclear and chloroplast genotypes of all 23 natural P. × jackii individuals tested were consistent with the genotypes of control-crossed P. × jackii, confirming that these were hybrids of P. deltoides and P. balsamifera. The morphology (broadly ovate leaves with heart shaped bases; see supplementary data3, Fig. S1) and molecular identification of natural hybrids were congruent, suggesting that the natural hybrid P. × jackii could be reliably identified using morphological features. All putative natural hybrid individuals tested had nuclear alleles representing both P. deltoides and P. balsamifera as parental taxa, and the chloroplast genotype corresponded to that of P. deltoides, suggesting an asymmetrical pattern of hybridization in the natural hybrids. Thus, our results clearly show that P. deltoides and P. balsamifera serve as ovule and pollen donors, respectively, in the P. × jackii hybrid complex.

Discussion

All species of the genus Populus are dioecious and therefore cross-pollination through wind-dispersed pollen is needed for successful fruit set in P. balsamifera and P. deltoides (Dickmann and Stuart 1983). These two species
are somewhat reproductively isolated owing to different flowering times. Flowering of *P. balsamifera* generally occurs April–May and *P. deltoides* flowers from late February to April in the southern distribution range of the species in the United States (Schreiner 1974). In the Oldman river region of southern Alberta, both of these species and their hybrids are known to flower during May (Gom and Rood 1999). Flowering and pollen release may vary by as much as a month among trees in a stand (Farmer 1966; Gom and Rood 1999) and seasonal climatic fluctuations may shift the timing of flowering, leading to phenological overlap between these two species. Thus, some individuals of these two species can hybridize under natural conditions (Brayshaw 1965; Eckenwalder 1984b; Floate 2004).

The observed pattern of natural hybridization between *P. deltoides* and *P. balsamifera*, suggests an asymmetry in the reproductive isolation of these two species. The asymmetric reproductive barriers in general could be attributable to pre- and post-zygotic barriers. Various prezygotic mechanisms that may cause asymmetry in hybridization success include differences in style length (Kiang and Hamrick 1978; Sorensson and Brewbaker 1994), differential fruit abortion (Levin 1978; Howard et al. 1998), and self-incompatibility systems (Lewis and Crowe 1958). Controlled crosses involving female *P. deltoides* and male *P. balsamifera* showed a high success rate and produced viable seeds, whereas reciprocal crosses involving female *P. balsamifera* and male *P. deltoides* produced only a few seeds and the viability of these seeds was very low. For instance, in a crossing experiment performed in 1998, no seed set was observed in all 20 crossings between female *P. balsamifera* and male *P. deltoides*, whereas 5 out of 16 crosses between female *P. deltoides* and male *P. balsamifera* produced viable progeny. In another crossing experiment performed in 2004, only 2 out of 19 crosses between female *P. balsamifera* and male *P. deltoides* produced seeds, giving rise to only one seedling. Conversely, 4 out of 30 crosses between female *P. deltoides* and male *P. balsamifera* produced seeds and gave rise to over 600 seedlings. Similarly, the controlled crosses between female *P. trichocarpa*, and male *P. deltoides* yielded seeds with low viability, commonly associated with premature dehiscence of the capsule. Seedlings of these crosses are routinely produced through embryo rescue techniques (Dickmann 2001; Riemen Schneider et al. 2001) suggesting a postzygotic incompatibility between these two species. *Populus trichocarpa* is evolutionary closely related to *P. balsamifera* (Hamzeh and Dayanandan 2004) and, therefore, it is logical to assume that the asymmetric reproductive isolation observed in the *P. deltoides* and *P. balsamifera* complex may be mediated through postzygotic mechanisms. The evidence for asymmetric-cross incompatibility has also been found in a *P. angustifolia* and *Populus fremontii*. S. Wats. hybrid complex in Utah. Restriction fragment length polymorphisms (RFLP) analysis in this hybrid complex showed that the hybrid population consisted of either F1 hybrids or backcrosses to *P. angustifolia* (Keim et al. 1989). No trees attributable to crosses between F1, or between F1 and *P. fremontii* were found. The controlled backcrossing of F1 hybrids to *P. fremontii* resulted in early death of seedlings, suggesting a postzygotic developmental incongruity (Hogenboom 1984; Keim et al. 1989).

We speculate that the observed asymmetries in hybridization between *P. deltoides* and *P. balsamifera* may be due to cytonuclear incompatibility resulting from divergent evolution of nuclear and cytoplasmic genes coding for proteins interacting in photosynthesis or respiration that affect the viability of hybrids (Michaelis 1954; Levin 1978; Wu et al. 1999). Alternatively, the cytonuclear incompatibility may result from the activity of transposable elements, which are abundant in many plant genomes (Tiffin et al. 2001). The activity of some transposable elements can be suppressed by maternally inherited factors (Engels 1989). Therefore, hybrid progeny with a cytoplasmic background that has not evolved with a transposable element may experience greater transposon activity leading to cytonuclear genetic incompatibility and reduced hybrid fitness or viability. Further studies focused on cyto-nuclear interactions in the *P. × jackii* hybrid complex would be valuable to gain insights into mechanisms associated with asymmetric gene flow in forest trees.

In theoretical models of reproductive barrier reinforcement, the reciprocal crosses between lineages are assumed to be equally compatible (Felsenstein 1981; Liou and Price 1994; Kelly and Noor 1996). However, the asymmetries in gene flow between partially isolated taxa may greatly reduce the probability of evolution of reproductive barriers (Servedio and Kirkpatrick 1997) and are more likely to introgress. Under these circumstances introgression is likely to be directional, with genes moving mainly from one species to another, and to affect the phenotypic and genotypic variation in natural populations (Stebbins 1959). The proportion of parental genomes in hybrid species may serve as an indicator for assessing the directionality of introgression (Martinsen et al. 2001) and cytoplasmic and nuclear interactions in the hybrid zone (Rieseberg 1995).

In summary, our findings confirm the presence of asymmetric reproductive isolation in two interfertile plant species under natural conditions. Our data, based on both the biparentally inherited nuclear and the maternally inherited chloroplast genome, provide direct evidence for the long suspected presence of asymmetries in interspecific crossing barriers in *Populus* under natural conditions.

**Acknowledgments**

The authors thank Barbara Thomas of Alberta-Pacific Forest Industries (Alpac) and the University of Alberta, Alan Robertson (Alpac), David Kamelchuck (Alpac), Damase Khasa (Université Laval), Serge Morin (Ministère des Ressources naturelles et de la Faune, Québec) and François Caron (MRNFQ) for their help in collecting leaf samples; Chen Rae Hsu (Concordia University) for technical assistance; and Kevin Floate (Agriculture and Agri-Food Canada), Stewart Rood (University of Lethbridge), Paul Widden (Concordia University), and an anonymous reviewer for helpful comments that improved the manuscript. This research was supported by grants from Concordia University, Natural Sciences and Engineering Research Council of Canada, Le Fonds québécois de la recherche sur la nature et les technologies, and Canada Foundation for Innovation.
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