

The mating system in natural populations of western redcedar (*Thuja plicata*)

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Abstract: Outcrossing rates and the correlation of paternity were estimated in six natural populations of western redcedar (*Thuja plicata* Donn. ex D. Don) in southwestern British Columbia. Over 3000 offspring were assayed, as progeny arrays, for the only sufficiently polymorphic isozyme locus in this species, glucose-6-phosphate dehydrogenase. Estimates of population outcrossing rates ranged from 0.173 to 1.257 and averaged 0.715 ± 0.045 (mean \pm SD). Estimates of the correlation of paternity generally did not differ from zero. Six of the seven outcrossing estimates (one population was surveyed in two consecutive years) were higher than a previous seed orchard study. However, these outcrossing rates are still lower than those estimated for most other species of conifers. Population outcrossing rates also showed wide variation, and this variation is discussed in terms of ecological and phenological differences among populations.

Key words: Cupressaceae, conifers, isozymes, outcrossing rate, inbreeding.

Résumé : Les taux d'allofécondation et les corrélations de paternité ont été estimés pour six populations de thuya géant (*Thuja plicata* Donn. ex D. Don) du sud-ouest de la Colombie-Britannique. Trois mille individus ont été analysés, comme tableau de descendance, pour le seul marqueur isoenzymatique suffisamment polymorphe de cette espèce, glucose-6-phosphate déshydrogénase. Les taux d'allofécondation vont de 0,173 à 1,257, avec un taux moyen de $0,715 \pm 0,045$ (moyenne \pm écart type). Les estimations de corrélation de paternité n'étaient généralement pas différentes de zéro. Six des sept taux d'allofécondation (une population fut échantillonnée durant deux années consécutives) étaient plus élevés que celui d'une étude précédente dans un verger à graines. Cependant, ces taux étaient plus faibles que pour la plupart des autres espèces de conifères. La variation des taux d'allofécondation est discutée en terme de différences écologiques et phénologiques entre les populations.

Mots clés : Cupressacées, conifères, isozymes, taux d'allofécondation, consanguinité.

Introduction

Mating systems, inbreeding depression, and genetic diversity are inextricably linked (Harder and Barrett 1996; Holsinger 1996; Uyenoyama et al. 1993). Species with higher selfing rates tend to show lower genetic diversity and less inbreeding depression, and the evolution of these quantities involves their close interaction (Charlesworth and Charlesworth 1995; Charlesworth et al. 1990). One driving force in their evolution is the life-span of the organism. Long-lived plants are expected to accumulate a higher genetic load through somatic mutations, consequently leading to the evolution of outcrossing. The large size of trees also increases the chance of self-pollination through geitonogamy, and consequently, the evolution of mechanisms to prevent such accidental

selfing (Barrett et al. 1996). Indeed, studies have found that conifers are predominantly outcrossing (5–10% selfing; Adams and Birkes 1991), have high genetic variability (15–20% isozyme heterozygosity; Hamrick and Godt 1996) and high inbreeding depression (mean = 64%; Husband and Schemske 1996).

However, these conclusions are based upon studies restricted to mainly one family of conifers, the Pinaceae. In the Cupressaceae, genetic diversity has been measured in only a few genera and outcrossing rates have been estimated only in the genus *Thuja*. In conspicuous exception to other conifers, species of *Thuja* display high selfing and low gene diversity. *Thuja orientalis* L. showed 25% selfing (Xie et al. 1991) and 14% isozyme heterozygosity (Xie et al. 1992), while *Thuja occidentalis* L. showed 36–71% selfing (Lamy et al. 1999; Perry and Knowles 1990) and 3–13% heterozygosity (Lamy et al. 1999; Matthes-Sears et al. 1991; Perry et al. 1990). Likewise, western redcedar (*Thuja plicata* Donn ex D. Don) shows much reduced isozyme variation (4–6% heterozygosity), with only 1 of 21 loci analyzed being appreciably polymorphic (Copes 1981; El-Kassaby et al. 1994; Yeh 1988). In a seed orchard setting, *T. plicata* had a selfing rate of 68%, one of the highest measured in a conifer (El Kassaby et al. 1994).

In conifers, seed orchards are expected to exhibit higher outcrossing rates than their natural population counterparts (Muona 1988), or at least be similar to natural populations

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(Adams and Birkes 1991). In natural populations, western redcedar trees tend to occur at lower density and are larger, leading to more geitonogamy (Farris and Mitton 1984), and in contrast to seed orchards where neighbouring trees are unrelated, potential family structure within natural populations of *T. plicata* could further inflate natural levels of inbreeding. Knowledge of the rates of selfing in natural populations, and their variation among populations, will shed light on this puzzle of the evolution of selfing in predominately outbreeding conifers. In this study we estimated outcrossing rates in six natural populations of *T. plicata* in southwestern British Columbia using enzyme electrophoresis. This is the first study to document levels of selfing in natural populations of western redcedar.

Materials and methods

Cones were collected in the fall of 1996 from two natural populations of *T. plicata* near Vancouver, British Columbia: the Malcolm Knapp Research Forest in Maple Ridge and at Mount Seymour (Table 1). In 1997, cones were collected from three populations on southern Vancouver Island (Mount Brenton, Shawnigan, and Reinhart Lake) and from two populations on the mainland of British Columbia (Mount Seymour and Enderby) (Table 1). Populations consisted of trees less than 80 years old except at Enderby, where trees were over 100 years old. In 1996, cones were collected from the lower to middle crown of trees, while in 1997 cones were collected from the middle to upper crown of trees. We collected seeds from at least 12 trees from each population, but erratic germination reduced the number of families eventually assayed (Table 1).

Seeds were extracted from cones and stored at 4°C for up to 8 months. They were germinated on wet filter paper at room temperature for approximately 10 days. A few days after germination, seed tissue was ground using the buffer of Mitton et al. (1979), and extracts were stored at -80°C. Only the embryos from the 1996 collections were assayed, while tissues from the 1997 collections were assayed separately as embryos and megagametophytes. The latter tissue is haploid and is genetically identical to the maternal allele passed to the embryo. Assay of megagametophytes allows more accurate inference of maternal genotype in all progeny arrays, and more accurate inference of selfing rate and paternity in arrays descended from heterozygous mothers (Ritland and El-Kassaby 1985). Maternal genotypes from the 1996 collections were inferred from the progeny arrays.

Following Yeh and O'Malley (1980) and El-Kassaby et al. (1994), we assayed for the glucose-6-phosphate dehydrogenase (G6PD) isozyme locus on a morpholine-citrate buffer (pH 8.0). Other isozyme loci were known, from previous studies, to be monomorphic or not sufficiently informative (gene frequency $p < 0.05$) for mating system estimation (El-Kassaby et al. 1994; Yeh 1988). In the absence of more markers and to reduce statistical variance we increased the number of individuals per family assayed for outcrossing rates. We assayed from about 500 to 700 seed progeny per population, although the 1996 collections yielded fewer germinants for assay (Table 1).

Single-locus estimates of population outcrossing rates, allele frequencies, and correlated mating estimates (Ritland 1989) were obtained using a version of MLTR (Ritland 1990) that incorporated megagametophyte information. Because of the low number of trees sampled per population, parental fixation indices could not be estimated and, hence, were constrained by the estimation program to equal zero. As well, because there was only one marker locus, we could not jointly estimate both components of correlated matings (the correlation of paternity and the correlation of selfing). The latter was assumed to be zero. Errors of estimates were computed with the bootstrap method. Outcrossing and correlation of paternity

estimates were considered significantly different from one or zero when the 95th percentile of the bootstrap values did not overlap with these values. The mean outcrossing rate over all populations was obtained by weighting each estimate in proportion to the inverse of its statistical variance. A chi-square heterogeneity test was used to test whether outcrossing rates differed among populations.

Results

The gene frequency of the most common allele at the G6PD locus ranged within a remarkably narrow interval across all populations, from 0.52 to 0.59 (Table 1). By contrast, outcrossing rates (t) showed wide variation among populations, ranging from 0.173 to 1.257 (Table 1). Estimates of outcrossing were significantly lower than unity in three populations: Research Forest ($t = 0.173$), Mount Seymour ($t = 0.826$), and Enderby ($t = 0.771$). Estimates of population outcrossing rates differed significantly from each other ($\chi^2 = 40$, $df = 6$, $p < 0.001$). However, when the research forest population was excluded outcrossing rates did not differ among populations ($\chi^2 = 5.45$, $df = 5$, $p > 0.05$). The average weighted outcrossing rate for all populations was 0.715, while the unweighted average was 0.837. Estimates of the correlation of paternity generally did not differ from zero, except for the 1996 research forest population, which showed a high value of 0.917 but with a high attached error (it should be noted that estimates of correlated paternity are statistically independent from the estimates of selfing). The weighted average of correlated paternity estimates across populations was 0.025, which did not differ significantly from zero.

Discussion

The estimates of outcrossing we obtained for western redcedar are among the lowest in conifers. Estimates of outcrossing rates in most species of conifers are above 80% and exceptions include *Larix laricina* (Du Roi) K. Koch (mean $t = 72.9\%$, Knowles et al. 1987), *Pinus maximinoi* H.E. Moore ($t = 65\%$, Matheson 1989), *Picea glauca* (Moench) Voss (mean $t = 73\%$, Innes and Ringius 1990) and *Picea rubens* Sarg. (mean $t = 59.5\%$, Rajora et al. 2000). Low outcrossing rates occur in both *Picea chihuahuana* Martinez (0 and 15.3%; Ledig et al. 1997) and in *Picea martinezii* T.F. Patterson (mean 56%; Ledig et al. 2000), but these species are restricted to small, extremely isolated populations. The mean outcrossing rate for *T. plicata* in this study ($t = 71.5\%$) was similar to *T. orientalis* (mean $t = 75\%$, range 68–81%; Xie et al. 1991) and higher than in *T. occidentalis* (mean $t = 63\%$, range 51–75%; Perry and Knowles 1990; and mean $t = 30\%$, range 24–33%; Lamy et al. 1999). However, the outcrossing rates we found for natural populations of *T. plicata* were higher than we expected. In all populations, except the Research Forest, outcrossing rates were higher than in the seed orchard study ($t = 32\%$; El-Kassaby et al. 1994).

Our results, together with the high selfing rate found in the seed orchard (El-Kassaby et al. 1994), indicate that the mating system in western redcedar is quite labile, with marked among-population variation, and probably marked among-tree variation. A possible reason for the lower outcrossing rate in the western redcedar seed orchard is assortative mating, caused by asynchrony of receptivity to

Table 1. Locations, sample sizes (N), gene frequency of the most common allele at the G6PD locus, population outcrossing rates (t), and correlation of paternity (r_p) of *Thuja plicata* populations in southwestern British Columbia.

Population	Latitude (N)	Longitude (W)	Elevation (m)	Tree age (years)	No. of families	N	Gene frequency of G6PD*	t^*	r_p^*
1996									
Research forest	49°20'	122°75'	500	50–80	9	243	0.524 (0.113)	0.173 (0.095)	0.917 (0.488)
Mount Seymour	49°22'	122°57'	600	50–80	5	138	0.593 (0.082)	0.918 (0.252)	–0.028 (0.101)
1997									
Mount Seymour	49°22'	122°57'	600	15–30	12	718	0.535 (0.044)	0.826 (0.071)	0.083 (0.065)
Mount Brenton	48°53'	123°52'	750	15–50	8	505	0.568 (0.044)	0.968 (0.155)	<0.001 (0.093)
Reinhart Lake	48°58'	124°05'	680	15–50	8	459	0.538 (0.035)	0.946 (0.117)	0.007 (0.029)
Enderby	50°30'	118°47'	1100	>100	9	513	0.577 (0.083)	0.771 (0.133)	0.111 (0.085)
Shawnigan	48°45'	124°01'	725–775	7–9	9	556	0.584 (0.066)	1.257 (0.355)	0.059 (0.220)
Unweighted means							0.837 (0.064)	0.164 (0.058)	
Weighted means							0.715 (0.045)	0.025 (0.024)	

*Values are means with SDs given in parentheses.

pollen among trees from different geographical locations in the orchard. In a Douglas-fir seed orchard, El-Kassaby et al. (1988) found that trees that were receptive earlier or later had higher selfing than the remaining trees.

There are numerous factors that may affect the selfing rates of western redcedar, and of conifers in general, described as follows.

- (1) Clonal structure: Western redcedar probably exhibits clonal structure, because of extensive vegetative reproduction giving rise to groups of genetically identical ramets, which can increase the rate of inbreeding.
- (2) Family structure: Localized family structure due to limited dispersal can contribute to biparental inbreeding. More than one polymorphic locus is needed to differentiate between self-fertilization and mating between relatives.
- (3) Tree size: In the populations with the higher outcrossing estimates (Shawnigan, Mount Brenton, Reinhart), we collected seeds from young trees with larger, mature trees nearby while significant inbreeding was found in Enderby where seeds were collected from older trees only. A higher outcrossing rate in smaller trees is expected, because they produce less pollen, and therefore, their pollen constitutes a smaller proportion of the pollen cloud and increases the chance of being fertilized by outcross pollen.
- (4) Crown position: Outcrossing rates can also vary among different heights within a tree. Lower outcrossing rates have been found in lower crowns compared with upper crowns in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Chaisurisri et al. 1994; El-Kassaby et al. 1986; Omi and Adams 1985). In this study, cones were collected from the upper crown of trees in most populations, but seeds were collected from lower branches in the population with the lowest outcrossing rate, the research forest. However, seeds collected in the same way in the 1996 collection from Mount Seymour did not also show lower outcrossing.
- (5) Inbreeding depression: High inbreeding depression at the seed stage (mean = 0.59, Husband and Schemske 1996) can lead to the high rates of outcrossing observed in conifers. As seedlings are normally used to infer outcrossing rates, the selfed seeds that die are missed in the

outcrossing rate estimate. However, *T. plicata* seems to lack early depression at the seed stage (Husband and Schemske 1996, Owens et al. 1990), so our estimates of outcrossing are not as biased by this early acting inbreeding depression.

Very few mating system studies in conifers report correlation of paternity estimates. In *Pinus washoensis* Mason & Stockwell (Mitton et al. 1997) and *Tsuga mertensiana* (Bong.) Carr. (Ally et al. 2000), no significant correlation of paternity was found. In two species, *Picea martinezii* ($r_p = 0.389$; Ledig et al. 2000) and *Abies borisii regis* Mattfeld ($r_p = 0.990$; Fady and Westfall 1997), the high correlation of paternity estimates, were attributed to the low number of reproductive individuals in the population. In *Larix occidentalis* Nutt., correlation of paternity estimates were significant in high-density populations ($r_p = 0.062$ and 0.104) but not significant in low-density populations ($r_p = 0.001$ and 0.02 ; El-Kassaby and Jaquish 1996), probably because high tree density limited pollen movement. Overall our results showed no correlation of paternity in redcedar (mean weighted $r_p = 0.025$) indicating that the outcrossed seeds were fertilized by several different pollen parents.

In this study we have found significant amounts of inbreeding in natural populations of western redcedar and variation in outcrossing rates among populations. We have set up a framework for future studies on the mating system within natural populations with more polymorphic and informative genetic markers, microsatellites (O'Connell and Ritland 2000).

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