ÉCOSCIENCE 12 (1): 82-91 (2005)

Temporal variation in quaking aspen (*Populus tremuloides*) genetic and clonal structures in the mixedwood boreal forest of eastern Canada[1]

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Abstract: Two sites that burned in 1847 (H) and 1823 (I) in the mixedwood boreal forest in Québec were selected to follow aspen genetic and clonal diversity over time. At each site, three cohorts were identified by core dating, and about 30 trees per cohort were randomly selected to compare tree genotypes using four microsatellite loci. The first cohorts were of post-fire origin (large disturbance), while the second and third cohorts were promoted by gap disturbances. These gaps were created by the natural mortality of post-fire aspen trees and a spruce budworm outbreak that attacked the coniferous species. Expected heterozygosity ranged from 0.37 to 0.72 across cohorts and averaged 0.66 and 0.54 in H and I, respectively. More than 99% and 96% of the genetic variability existed within cohorts, respectively. Genotypic diversity was high in all cohorts, and most genets were unique. Only two clones suckered for three successive cohorts, indicating little selection for specific genets to dominate aspen stands with time. High genetic and clonal diversity changed slightly between post-fire and gap disturbance cohorts. Apical dominance might have favoured the suckering of genets that existed in the post-fire cohorts but that were later eliminated by natural mortality.
Keywords: aspen, clonal structure, cohort, fire, gap disturbances, genetic diversity.

Résumé : Deux sites de la forêt mixte boréale du Québec, qui ont brûlé en 1847 (site H) et en 1823 (site I), ont été choisis pour suivre la diversité génétique et clonale du peuplier faux-tremble. Trois cohortes ont été identifiées dans chaque site par dendrochronologie. Une trentaine d'arbres ont été choisis de façon aléatoire dans chaque cohorte pour comparer les génotypes des arbres par l'utilisation de quatre loci microsatellitaires. Les premières cohortes se sont installées à la suite d'un feu, alors que les deuxièmes et troisièmes cohortes se sont développées à la faveur de trouées. C'est la mort des peupliers régénérés suite au feu ou celle de conifères causée par des tordeuses des bourgeons de l'épinette qui est responsable de la création des trouées. L'hétérozygosité attendue varie entre 0,37 et 0,72 chez les cohortes et atteint en moyenne 0,66 au site H et 0,54 au site I. Au site H, plus de 99% de la variabilité génétique existe à l'intérieur des cohortes. Au site I, cette valeur atteint 96%. La diversité génotypique est élevée dans toutes les cohortes et la plupart des genets sont uniques. Seulement deux clones ont produit des rejets de souche pour trois cohortes successives, ce qui indique la faible sélection des genets spécifiques qui dominent les peuplements de peupliers avec le temps. La haute diversité génétique et clonale a été légèrement modifiée entre les cohortes provenant des feux et celles issues de trouées. La dominance apicale a probablement favorisé la production de rejets de souche chez les genets qui existaient dans les cohortes après-feu, mais qui ont ensuite été éliminés par mortalité naturelle.
Mots-clés : cohorte, diversité génétique, feu, perturbations créant des trouées, peuplier faux-tremble, structure clonale.

Nomenclature: Ives & Wong, 1988; Morton & Venn, 1990.

Introduction

Temporal variation in population genetic and clonal diversity is a complex phenomenon that is strongly influenced by the timing of initial seedling recruitment (Erikson, 1989; 1993). It is predicted that when populations of clonal species experience limited seedling recruitment, their genetic and clonal diversity (number of genets) will decline with time (Shapcott, 1995; Pornon *et al.,* 2000; Moriguchi *et al.,* 2001), whereas those with repeated seedling recruitment will maintain high genetic and clonal diversity (Caron *et al.,* 2000; Chung *et al.,* 2000; Stehlik & Holderegger, 2000; Chung *et al.,* 2003; Ziegenhagen *et al.,* 2003). However, recent studies that addressed the effect of clonal reproduction showed little change in the genetic and clonal diversity between old and young populations of some species (Erickson & Hamrick, 2003), while others revealed that even a rare sexual

[1]Rec. 2004-06-14; acc. 2004-09-27.
Associate Editor: Josep Escarré.
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recruitment is sufficient to maintain high clonal diversity at small spatial scales (Persson & Gustavsson, 2001; Kjolner *et al.,* 2004). This suggests that more research is still needed to understand the effect of clonal reproduction on the genetic and clonal diversity of clonal species, especially when sexual recruitment is rare and when other factors such as the population age and disturbance history affect this recruitment.

Quaking aspen (*Populus tremuloides*) is a pioneer tree species in the Canadian boreal forest that regenerates immediately after fire. It is primarily dioecious and can reproduce both sexually and vegetatively. Sexual reproduction and dissemination is facilitated by wind pollen and seed dispersal over many kilometres (Strothmann & Zasada, 1965; Perala, 1990). However, seedling recruitment is a rare event at sites where aspen clones are already established, because conditions needed for successful seedling establishment, mainly the combination of an exposed mineral soil and an abundant source of water during seed germination, are rarely met (McDonough, 1979; Mitton & Grant, 1996), and also because suckers generally outcompete seedlings. Some have even asserted that broad-scale seedling establishment has not occurred since the last glaciation, 10,000 y ago in the western United States (Einsphar & Winton, 1976), while others have suggested only "windows of opportunity" for aspen sexual recruitment (Jelinski & Cheliak, 1992). Alternatively, root suckering is considered the main regeneration process triggering massive aspen recruitment following large disturbances such as fire (Mitton & Grant, 1996; Wang, 2003) and secondary disturbances such as gap openings in mature stands (Shepperd, Bartos & Steven, 2001).

Several studies have examined aspen dynamics in the boreal forest and established a close relationship between its recruitment and the forest natural disturbances, mainly fire and gap disturbances (Bergeron & Dubuc, 1989; Bergeron & Charron, 1994; Bergeron, 2000; Cumming, Schmiegelow & Burton, 2000). The traditional view of successional pattern in the mixedwood boreal forest was that in the absence of large disturbances, the even-aged post-fire aspen cohort will be replaced by more shade-tolerant coniferous species. Recent studies, however, have called this successional pattern into question. These studies have suggested that the development of canopy gaps before aspen stands reach maturity (as early as 40 y after stand initiation) allows these stands to evolve towards an uneven age structure and develop into self-maintaining old growth stands (Cumming, Schmiegelow & Burton, 2000). Disturbances of relatively minor extent (such as a gap created by the death of a large aspen) enhance the recruitment of aspen over several generations, even in the absence of major disturbances (Bergeron, 2000; Cumming, Schmiegelow & Burton, 2000). A partial opening in the canopy may result in restrained sucker growth, as apical dominance of the intact stems is maintained and soil temperature and light levels at the forest floor remain limiting (Perala, 1990; Groot *et al.,* 1997). Because of this, aspen density gradually decreases within the canopy and aspen recruitment is often limited to small isolated patches in old growth stands (Bergeron, 2000). So far, little information is available about the effect of this decrease on aspen clonal structure.

To date, aspen genetic diversity has been examined in several studies (Hyun, Rajora & Zsuffa, 1987; Jelinksi & Cheliak, 1992; Lund, Furnier & Mohn, 1992; Stevens *et al.,* 1999; Wyman, Bruneau & Tremblay, 2003), but its evolution with time and at the stand level has not been assessed. In addition, little is known about the mechanisms that control this genetic diversity along the successional gradient. Some ecological studies reported only one or a few clones in mature aspen stands due to hundreds and thousands of years of suckering (Kemperman & Barnes, 1976; Mitton & Grant, 1980), while Wyman, Bruneau, and Tremblay (2003) recently used molecular markers to demonstrate that the number of genets sampled in aspen stands in northwestern Québec could be quite high, reflecting the presence of many small clones. Inferring conclusions from these studies about the temporal evolution of aspen genetic and clonal structure is quite difficult, since they used different genet identification tools and were performed in different populations at different geographical locations. One way to overcome this problem is to use the same genetic markers to follow aspen genetic diversity with time in the same population.

In the present work, we benefit from a "natural experiment" set up that provided us the opportunity to examine for the first time the evolution of aspen genetic and clonal structures across several cohorts. More specifically, our aim was to 1) follow aspen genetic and clonal diversity at two sites that burned more than 180 y ago; 2) address the question of whether aspen clonal structure evolves toward a reduction in the number of genets or of ramets per genet, and 3) assess the impact of large (fire) and secondary (fire, insect outbreak, windthrow, or death of large aspen) disturbances on aspen genet and ramet dynamics with time.

Methods

STUDY AREA

The study sites were located in the Lake Duparquet Research and Teaching Forest (LDRTF) in northwestern

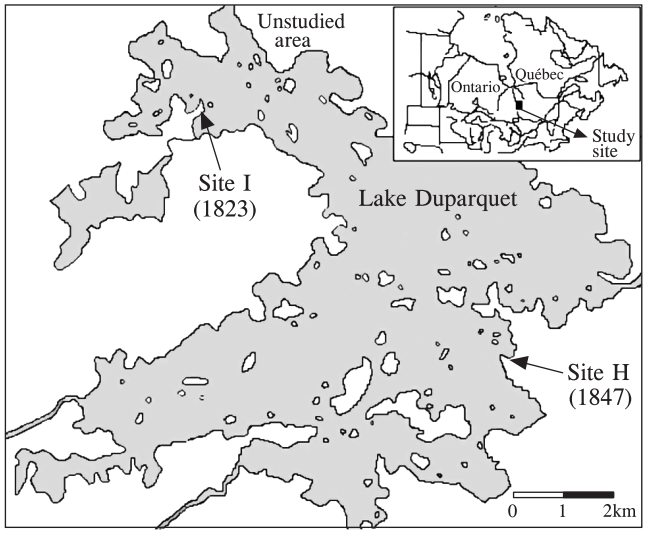

FIGURE 1. Map of Lake Duparquet (Canada), showing the location of the two study sites, H and I, and their respective fire dates, 1847 and 1823.

Québec (48° 30′ N, 79° 1′ W) (Figure 1). This forest was selected because human impact in this area has been minimal, with only a limited amount of mechanical forest harvesting that took place in 1978 (Harvey & Bergeron, 1989). It is part of the Northern Clay Belt of Québec and Ontario, a large region created by lacustrine deposits from the maximum post-Wisconsinian extension of postglacial lakes Barlow and Ojibway (Vincent & Hardy, 1977). The mean annual temperature is 0.6 °C, the mean annual precipitation is 822.7 mm, and the mean annual frost-free period is 64 d. Freezing temperature may occur throughout the year (Environnement Canada, 1982). Vegetation types vary in relation to soil deposits and successional stages (Bergeron & Dubuc, 1989). Young successional stages (< 100 y) are dominated by quaking aspen (*Populus tremuloides*), intermediate stages (100-200 y) by balsam fir (*Abies balsamea*), quaking aspen, and white spruce (*Picea glauca*), and old stages (> 200 years) are dominated by balsam fir and white cedar (*Thuja occidentalis*) (Bergeron, 2000).

SAMPLING STRATEGY

In 2001, aspen samples were collected at two sites, H and I, that burned in 1847 and 1823, respectively. These sites had similar historical traits that allowed us to consider them as replicate samples. The sites were selected because field observations revealed the presence of several cohorts at the same site (Bergeron, 2000). A sampling plot of 1 ha was established at each site, within which we tagged and measured the diameter at breast height (dbh) of all aspen trees. In sum, we counted a total of 352 and 237 trees·ha^{-1} in plots H and I, respectively. Diameter at breast height (DBH) measurements allowed us to detect three main diameter classes: the first one included trees of large dbh, from 30 to 62 cm, the second one included trees with dbh between 10 and 30 cm, and the third one included all young trees with 10 cm dbh and less. From each of these dbh classes, we selected for genetic analysis about 30 trees randomly distributed in each plot. This number was set in order to have rather similar sample sizes among the three size classes selected, and was limited by the small number of trees (< 30) with 10 cm dbh and less in each plot. A core was taken at breast height from the stem of each selected tree to determine its age. Some trees presented a rotten core, which made it impossible to determine their exact age. In this case, we only estimated their minimal age. In addition, root tissues or leaves were sampled from each selected aspen tree and stored in the laboratory at -80 °C for genetic analysis. For root sampling, special care was taken to collect the samples at the base of the selected stem, and only the cambium tissue was used for DNA extraction.

DNA EXTRACTION AND AMPLIFICATION

Samples were ground and genomic DNA was extracted using Doyle and Doyle's standard procedure (Doyle & Doyle, 1987) with small modifications. A total of 87 and 81 trees were analyzed at site H and I, respectively. DNA amplification was carried out using AmpliTaqGold® DNA Polymerase (Applied Biosystems, Foster City, California, USA). Individuals were genotyped at four microsatellite loci, PTR1, PTR2, PTR3, and PTR4, as described in Wyman, Bruneau, and Tremblay (2003) (Table I). Amplification was carried out using a 96-Well GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, California, USA), in a total volume of 25 µL containing 0.4 ng·µL^{-1} of DNA, 0.5 pmoles·µL^{-1} of primers, 0.2 mM dNTP, 2.5 mM MgCl2, and 15 mM Tris-HCl (pH = 8.0). The amplification conditions were as follows: 10 min at 95 °C to activate the enzyme and denature DNA strands, followed by a touch down PCR of 33 cycles with a temperature gradient varying from 60 °C to 54 °C. A final step of 72 °C for 7 min was added for extension during amplification. Prior to electrophoresis, 1.5 µL of PCR product was mixed with 0.25 µL of internal size standard (TAMRA; 500 base pairs) and 12 µL of deionized formamide. The loading product was then heat-denaturated and immediately placed on the ice. Amplified DNA was then analyzed using Gene Scan Software and an ABI Prism 310 Genetic Analyzer from Applied Biosystems (Foster City, California, USA).

DATA ANALYSIS

Genetic diversity was calculated using four parameters: percentage of polymorphic loci, the mean number of alleles per locus, observed heterozygosity (Hobs), and expected heterozygosity (Hexp). Allelic frequencies were estimated using an expectation-maximization algorithm with 5,000 iterations. This calculated maximum likelihood estimates for allele frequencies. Observed heterozygosity was calculated for each locus separately as well as averaged over all loci. Expected heterozygosity was computed for each locus and over all loci using average gene diversity according to Nei's (1987) formula. Departures from Hardy-Weinberg equilibrium were tested for each locus using more than 100,000 Markov chain steps for significance tests. The genetic structure in our populations was investigated by an analysis of molecular variance (AMOVA) using two genetic distances: the sum of the squared number of repeat differences between two alleles, which is an analogue of Slatkin's (1995) R_{ST}, and the number of different alleles, obtained by calculating the weighted average F_{ST} over all loci (Weir & Cockerham, 1984). Basically, R_{ST} is an analogue of F_{ST} assuming a stepwise-mutation model (SMM) (Slatkin, 1995), and the calculated average weighted F_{ST} is identical to the fixation index since the hierarchical structure was simple (Michalakis & Excoffier, 1996). AMOVA allowed us to calculate the covariance components within and between cohorts. All these analyses were performed using Arlequin

TABLE I. Repeat pattern, primer sequence, and annealing temperature (T) for four SSR loci in *Populus tremuloides* (from Dayanandan, Rajora & Bawa, 1998).

Locus	Repeat	Primer Sequence (5'-3')	T (°C)
PTR1	(CGT)5	AGCGCGTGCGGATTGCCATT	
	N45(AGG)9	TTAGTTTCCCGTCACCTCCTGTTAT	66
PTR2	(TGG)8	AAGAAGAACTCGAAGATGAAGAACT	
		ACTGACAAAACCCCTAATCTAACAA	63
PTR3	(TC)11	CACTCGTGTTGTCCTTTTCTTTTCT	
		AGGATCCCTTCCCTTTAGTAT	60
PTR4	(TC)17	AATGTCGGAGGCCTTTCTAAATGTCT	
		GCTTGAGCAACAAACACACCAGATG	60

version 2.000, specialized software for population genetics (Schneider, Roessli & Excoffier, 2000). Genotypic diversity was measured using the ratio G/N and Simpson's index D. G is the number of observed genets and N is the total number of individuals analyzed (Pleasants & Wendel, 1989). The Simpson's index is $D = 1 - \{[n_i(n_i - 1)]/[N(N - 1)]$ for corrected small samples, where n_i = number of ramets of the i^{th} genet (Pielou, 1969). The number of genets per cohort and the number of ramets per genet were also numbered at each site.

Results

AGE STRUCTURE

In general, the dbh classes used to randomly select trees from each cohort were consistent with the results obtained by core dating. Most trees having a dbh of 30 cm and more were very old and assumed to belong to the first post-fire cohorts that regenerated immediately after the fires of 1847 and 1823 at site H and I, respectively (Figures 2a,b). The relatively large age interval observed in these cohorts can be explained by the presence of many old trees that had a rotten or broken core, which made it impossible to determine their real age. The age of these trees was of necessity underestimated. The second dbh class (10 to 30 cm) was composed of trees whose age (exact and estimated) ranged from 54 to 65 y at site H (Figure 2a) and from 31 to 65 y at site I (Figure 2b). The third dbh class contained all young trees having a dbh of 10 cm or less. In this class, the oldest tree was 16 y old and many had a dbh less than 5 cm. For the latter, core analysis was not possible, and their age was estimated to be 10 y or less.

GENETIC DIVERSITY

We detected a total of 49 different alleles over the four loci in the 168 samples analyzed. Although all loci were polymorphic, few alleles were shared by three cohorts: 18 at site H and 12 at site I. When considering each locus separately, the number of alleles per locus

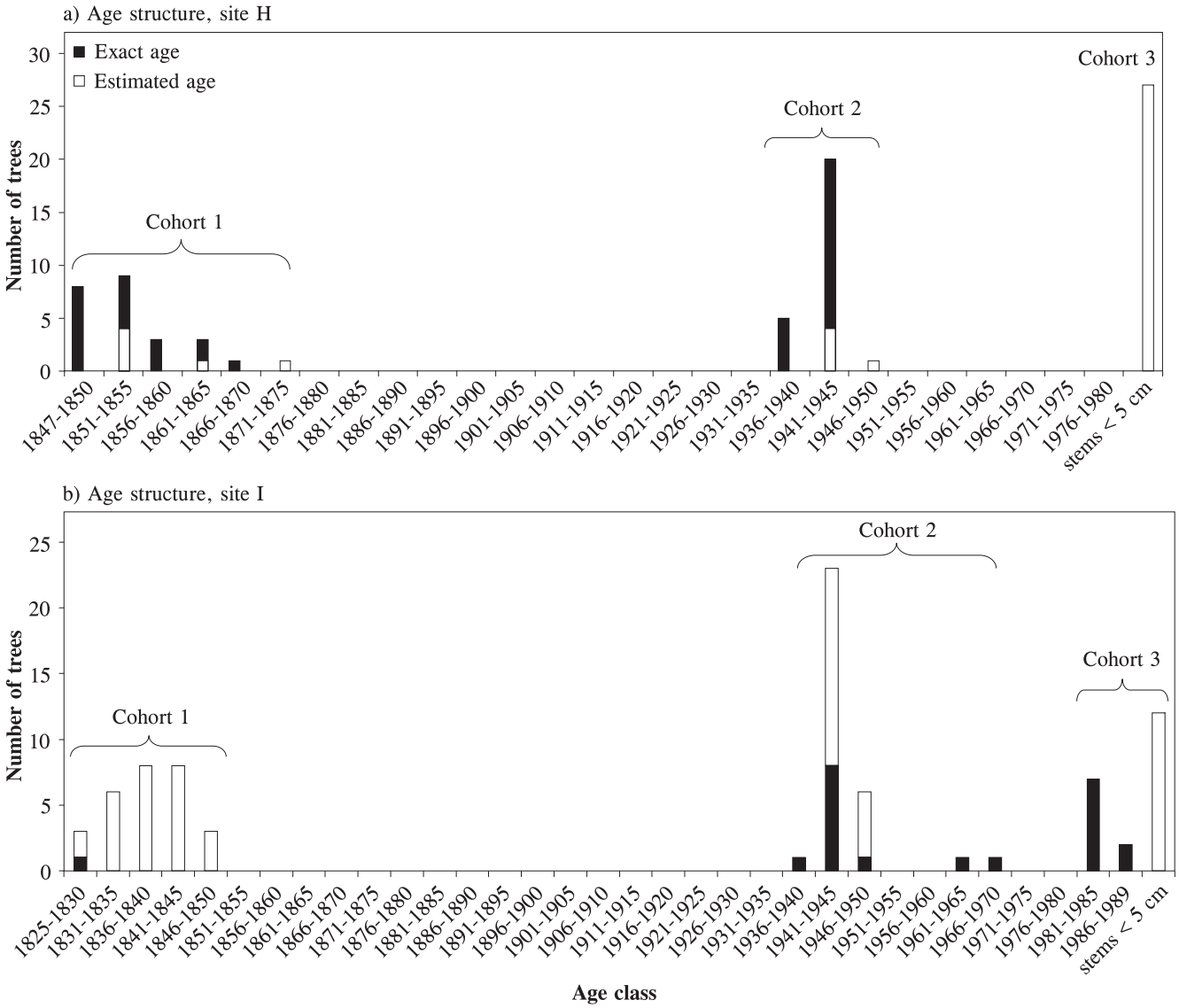

FIGURE 2. Aspen distribution per age class and per cohort (exact age in black; estimated age in white).

ranged from three to 14, and generally decreased in the third cohorts. Allele frequency varied between loci and cohorts, ranging from 0.02 to 0.90, but in all loci, one allele occurred more frequently than others. Alleles of low frequency (0.02) were lost after the first two cohorts, except allele size 214 and 242 in locus 3 (Figure 3). The same pattern was observed at both sites. Observed heterozygosity ranged from 0.10 to 0.80 at separate loci and averaged 0.46 and 0.47 at site H and I, respectively (Table II). The mean expected heterozygosity per cohort expressed by average gene diversity was relatively high and ranged from 0.59 to 0.72 at site H (Table IIa) and from 0.37 to 0.64 at site I (Table IIb). When considering all cohorts and loci, expected heterozygosity averaged 0.66 and 0.54 at site H and I, respectively. At both sites, locus 3 showed a consistent significant departure from Hardy-Weinberg equilibrium ($P < 0.001$), while the pattern was variable for other loci and cohorts (Tables IIa,b). Analysis of molecular variance based on R_{ST} and F_{ST} revealed that more than 99% and 97% of the genetic variability existed within cohorts, respectively (Table III). Fixation indices based on F_{ST} were 0.02 and 0.03, revealing an extremely low differentiation at both sites between cohorts.

GENOTYPIC DIVERSITY

Genotypic diversity was very high at both sites and also did not change between cohorts. At site H (Table IVa), the genotypic diversity was equal to 0.88 in the pooled population. When considering cohorts separately, G/N was even higher and ranged from 0.92 to 0.97. At site I (Table IVb), G/N was equal to 0.76 in the pooled population and ranged from 0.80 to 0.83 across cohorts. Simpson's index D exceeded 0.98 at both sites and also did not change considerably across cohorts. In sum, 77 different genets were identified at site H and 62 at site I (Tables IVa,b). Most of these genets—76% to 94% of all the genets in each cohort—were composed of one ramet (> 88%). Only eight (10.4%) and seven (11.2%) of them (at site H and I, respectively) were composed of more than one ramet, forming clones. Among these, 25% to 57% regenerated in the first two cohorts of site H and I, respectively (Table V), but no consistent pattern was observed as to their regeneration for three successive cohorts (Table V). At site H, the largest clone contained three ramets, whereas at site I, one clone contained 10 ramets that suckered in the first and second cohort (Table V).

Discussion

ASPEN DYNAMICS

Identification of three cohorts at each site is congruent with the results of previous ecological studies that have described aspen dynamics in Québec's boreal forests (Bergeron & Dubuc, 1989; Bergeron, 2000). Aspen is a pioneer species that regenerates massively after fire (Perala, 1990). The oldest trees found at both sites belong to the first post-fire cohort that regenerated following the fires in 1847 and 1823 at the sampled sites. This was not surprising, since aspen trees more than 150 y old were previously reported in the sampling area (Bergeron, 2000). As a stand ages, gaps created by the death of aspen trees allow the suckering of new aspen cohorts. These gap-disturbance cohorts regenerate even though shade-tolerant species become progressively more abundant in the canopy (Bergeron & Dubuc, 1989). The

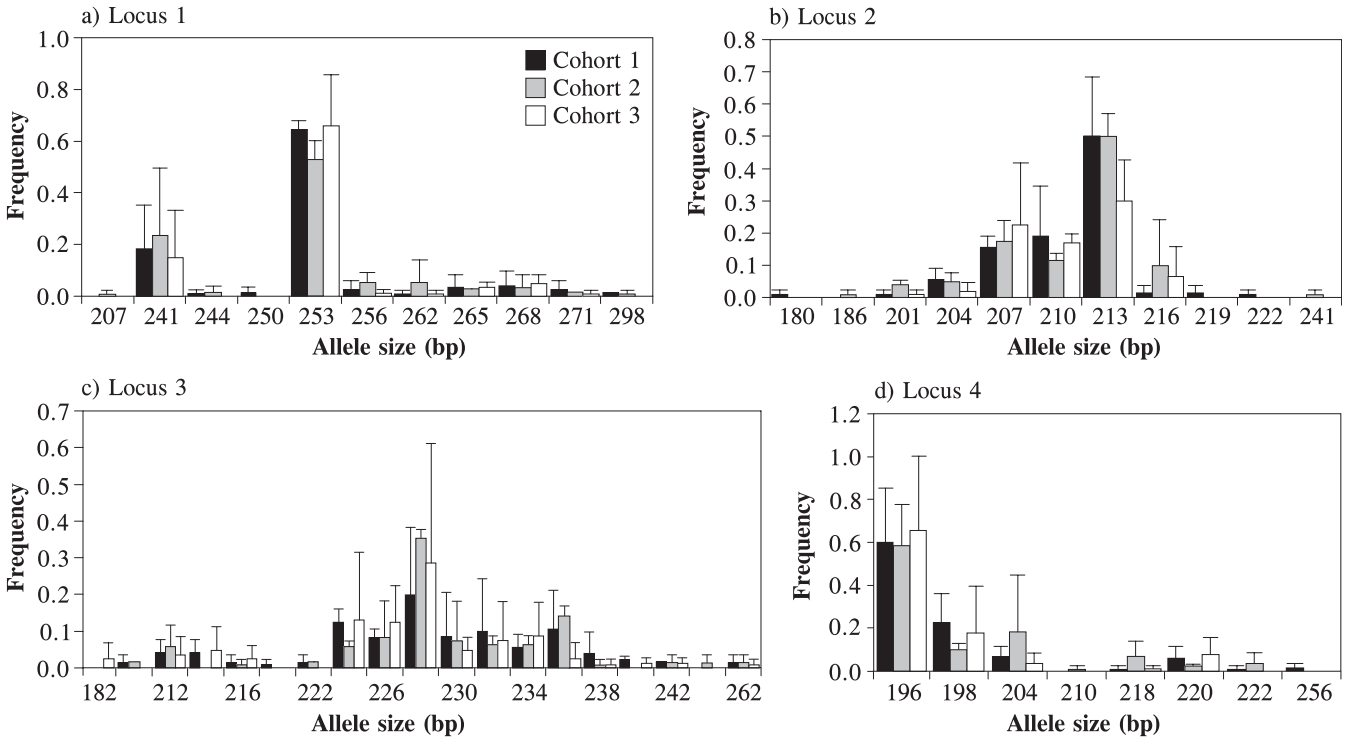

FIGURE 3. Average allele size frequencies and standard deviations per locus per cohort; allele size is measured by the number of base pairs (bp).

TABLE II. Observed and expected heterozygosity per locus and per cohort of aspen at a) site H and b) site I. Hobs = observed heterozygosity; Hexp = expected heterozygosity; numbers between parentheses indicate the standard variations of heterozygosity; *P* is the probability that the allele frequencies at a specific locus are in Hardy-Weinberg equilibrium.

a) SITE H (1847)

	Cohort 1			Cohort 2			Cohort 3			Mean Hobs/Hexp
Allele/locus	Hobs	Hexp	*P*	Hobs	Hexp	*P*	Hobs	Hexp	*P*	0.46 / 0.66
Loc 1	0.45	0.52	0.039	0.63	0.66	0.084	0.31	0.38	0.245	
Loc 2	0.61	0.71	0.025	0.33	0.74	<0.001	0.54	0.72	0.011	
Loc 3	0.47	0.91	<0.001	0.43	0.86	<0.001	0.38	0.89	<0.001	
Loc 4	0.23	0.74	<0.001	0.73	0.66	<0.001	0.42	0.71	<0.001	
Average gene diversity	0.44	0.68 (0.40)		0.53	0.72 (0.42)		0.41	0.59 (0.38)		

b) SITE I (1823)

	Cohort 1			Cohort 2			Cohort 3			Mean Hobs/Hexp
Allele/locus	Hobs	Hexp	*P*	Hobs	Hexp	*P*	Hobs	Hexp	*P*	0.47 / 0.54
Loc 1	0.73	0.53	0.077	0.77	0.60	0.003	0.63	0.57	0.447	
Loc 2	0.27	0.60	<0.001	0.30	0.66	<0.001	0.56	0.67	0.067	
Loc 3	0.80	0.83	<0.001	0.50	0.86	<0.001	0.20	0.66	<0.001	
Loc 4	0.34	0.32	0.434	0.43	0.49	0.094	0.10	0.15	1.000	
Average gene diversity	0.53	0.56 (0.35)		0.50	0.64 (0.38)		0.37 (0.28)	0.37		

TABLE III. Percentage of variation and fixation indices based on molecular and allelic variance analysis between and within cohorts of aspen at site H and I; * $P < 0.0025$.

Source of variation	Site H (1847) Percentage of molecular variation	Site H (1847) Percentage of allelic variation	Site I (1823) Percentage of molecular variation	Site I (1823) Percentage of allelic variation
Between cohorts	0.29	3.23	0.63	3.06
Within cohorts	99.71	96.77	99.37	96.94
Fixation index	0.0029 ($P = 0.439$)	0.032*	0.0063 ($P = 0.257$)	0.030*

absence of any report of major disturbances causing stand-level dieback of aspen in the sampling area between the fire dates and the mid-1930s indicates that the gradual dismissal of the post-fire cohort was the origin of the suckering of the second cohorts (Bergeron, 2000). A tent caterpillar (*Malacoma disstria*) outbreak that occurred in the 1940s-1950s also enhanced this recruitment (Bergeron & Charron, 1994). At our sites, core dating indicated that second-cohort recruitment started 89 and 113 y after the fire, which is consistent with the average longevity of aspen. As for the third cohorts, their recruitment was concurrent with a spruce budworm outbreak (*Choristoneura fumiferana*) that attacked coniferous species between 1972 and 1987 (Morin, Laprise & Bergeron, 1993). At this stage, outbreaks would not bring succession back to the deciduous stage dominated by hardwood species. Instead, aspen would regenerate in smaller numbers, and the canopy layer would be dominated by balsam fir and white cedar (Kneeshaw & Bergeron, 1998). This explains the limited number of aspen trees found in the third cohorts.

GENETIC AND GENOTYPIC DIVERSITY

The high levels of polymorphism (100%) observed in the quaking aspen are comparable with values reported in the literature (Ellstrand & Roose, 1987; Liu & Furnier, 1993) and reflect the highly diverse genetic pool of aspen in Québec's boreal forests. Given the relatively short temporal gradient covered by the three cohorts for this long-lived species, the decrease in the number of alleles per locus in the third cohorts could be attributed to the relatively small sample size used. Accordingly, Waples (1989) suggested that small sample sizes rather than genetic drift may be the primary reason for observed changes in allele frequencies. Heterozygosity was also very high in all cohorts, further reflecting the high genetic diversity of aspen. Expected heterozygosity values are consistent with our previous report using microsatellites (Wyman, Bruneau & Tremblay, 2003), but exceed by two to three times the values reported in other studies using isozymes or RAPD (Hyun, Rajora & Zsuffa, 1987; Jelinksi & Cheliak, 1992; Lund, Furnier & Mohn, 1992; Yeh, Chong & Yang, 1995; Stevens *et al.,* 1999). This was not unexpected since microsatellites are usually more variable than other markers, as their mutation rate is estimated to be between 10^{-2} and 10^{-6} (Scrosati, 2002). No decrease in aspen genetic diversity was detected across the three cohorts. It appears that aspen genetic diversity can be maintained for a long time, at least 180 y following fire.

TABLE IV. Aspen ramet and genet distribution per cohort, number of unique ramets and clonal diversity at sites H and I (clonal diversity G/N = number of genets / number of ramets; D = Simpson's index).

a) SITE H (1847)

	Ramets analyzed	Total genets	Genet with > one ramet (clones)	Genet with one ramet	% Genet with one ramet	G/N	D
Cohort 1 (1847)	30	29	4	25	86.21	0.97	0.991
Cohort 2 (1936-1947)	30	29	4	25	86.21	0.97	0.998
Cohort 3 (1990-2001)	27	25	6	19	76.00	0.92	0.994
Pooled cohorts	87	77	8	69	89.61	0.88	0.997

b) SITE I (1823)

	Ramets analyzed	Total genets	Genet with > one ramet (clones)	Genet with one ramet	% Genet with one ramet	G/N	D
Cohort 1 (1825)	30	24	4	20	83.33	0.80	0.963
Cohort 2 (1936-1970)	30	25	6	19	76.00	0.83	0.982
Cohort 3 (1981-2001)	21	17	1	16	94.12	0.81	0.952
Pooled cohorts	81	62	7	55	88.71	0.76	0.981

TABLE V. Number of ramets per clone and per cohort observed in aspen clones that suckered in the first (Coh1), second (Coh2), and third (Coh3) cohort.

Site H (1847)				Site I (1823)			
Clone	Coh1	Coh2	Coh3	Clone	Coh1	Coh2	Coh3
c4	1	1	1	c3	1	1	
c17	1	1	1	c5	6	4	
c18	1		1	c6	2	1	
c19			2	c9	1	1	
c22	2			c11			5
c25		1	1	c12		2	
c31			2	c13		2	
c37		2					
Ramet per cohort	5	5	8		10	11	5
Clone per cohort	4	4	6		4	6	1

Similarly to genetic diversity, no significant differences in genotypic diversity were observed between the three cohorts at both sites. The high levels of genotypic diversity and the large number of genets made of one ramet indicate that a highly multiclonal structure was maintained in the three cohorts. Our data contrast with reports of large and dominant clones within aspen populations in other regions based on morphological and phenological characteristics (Kempermann & Barnes, 1976), but are consistent with the results of Wyman, Bruneau, and Tremblay (2003), who observed 11 aspen genotypes per 15 samples in Québec's boreal forest. The general prediction about the decline in the genotypic diversity in clonal species is therefore not supported in aspen.

MECHANISMS CONTROLLING GENETIC AND CLONAL STRUCTURES ACROSS THREE COHORTS

No impact of disturbance type can be directly associated with aspen genetic and clonal diversity patterns across the three cohorts. Neither genotypic diversity nor the percentage of unique genets in post-fire cohorts was different from those observed in gap-disturbance cohorts, although aspen density decreased in the third ones. As the stand ages, and aspen recruitment is limited due to canopy gaps and increased vegetation competition, the genets in the new cohorts will be reduced to one or a few ramets and form isolated but highly multiclonal aspen patches.

Similarly, seedling recruitment is unable to explain the conservation of high levels of genetic and clonal diversity over time. Although conditions required for aspen seed germination (soil disturbance, exposed mineral soil, and moisture) are usually met after a fire (Barnes, 1966; Romme *et al.*, 1997), aspen seeds have a very short viability period that lasts 2 to 4 weeks (Perala, 1990), and a light layer of leaves or duff on the mineral soil or the presence of some grass species is usually sufficient to form an insulating layer and inhibit aspen seedling growth (Barnes, 1966; Landhäusser & Lieffers, 1998). Moreover, even if some seeds could have germinated, the coniferous species dominating the canopy in older seral stages would have prohibited them from acquiring enough light to grow and reach the canopy. This is particularly true since insect outbreaks would not immediately eliminate conifers from the canopy; trees can stay standing several years after being attacked by an epidemic (Kneeshaw & Bergeron, 1998).

Alternatively, an insight on the mechanism controlling aspen genet and ramet dynamics can be inferred from the observations of two concurrent processes. On one hand, a limited number of genets suckered in successive cohorts and most genets were unique (76% to 94%), indicating that no specific genet was selected to dominate the stand across time. On the other hand, very small genetic differentiation was observed between cohorts, indicating that the different genets they contain originated from the same gene pool, most probably established in the initial post-fire cohort. In the absence of significant seedling recruitment in the two gap-disturbance cohorts, apical dominance has to be invoked to explain these observations. Apical dominance is a process of hormonal control

that inhibits suckering (Frey *et al.,* 2003). Once the top of the tree is removed or cut, apical dominance is released, and suckering is promoted. We suggest that apical dominance inhibited living aspen stems in one cohort from suckering in the next one, especially at short time intervals such as between the second and third cohorts (45 to 65 y), but favoured the suckering of genets whose ramets were eliminated from the post-fire cohorts and whose apical dominance was removed. This conclusion is supported by the limited number of genets (2/77 and 4/62) that kept suckering for two or three successive cohorts. Since a large number of unique genets was continuously present in all cohorts without seedling recruitment (if we except the post-fire cohort), we deduce that the gene pool established at the origin of the stand is considerably large. In the absence of major disturbances and seedling recruitment, periodic suckering of genets in successive cohorts would contribute to the maintenance of aspen genetic variability across a long successional gradient.

Conclusion

The results obtained in this study reveal that vegetative reproduction does not necessarily reduce genetic and genotypic diversity in the boreal forest, at least 180 y after fire. Also, natural disturbance type (large *versus* gap disturbances) does not enable prediction of aspen genetic and clonal structure, as these are more related to the regeneration process. As time span between two major disturbances increases, aspen density will decrease progressively. Meanwhile, instead of regenerating few genets with several ramets each, regeneration of several genets with one or few ramets each will occur. We estimate that the long fire cycle (> 130 y) that prevails in the mixedwood boreal forest in northwestern Québec, in addition to the moist soil conditions that favour sexual reproduction immediately after fire, contributes to the conservation of highly diverse genetic and clonal structures within a stand. This scenario might, however, contrast with what is observed elsewhere, especially in the west, where larger aspen clones are reported (Kempermann & Barnes, 1976; Johnston & Hendzel, 1985). Two possible explanations may account for this difference. Firstly, western boreal mixedwoods are characterized by short fire cycles (Johnson, Miyanishi & Weir, 1998) and unfavourable conditions for seedling establishment (drier climate). Where fires are more frequent and severe, young dense aspen stands will burn regularly, which might eliminate some clones. Consequently, some genotypic diversity could be lost over time, leading to a monoclonal structure. The second may be related to clonal identification tools. Use of morphological characteristics or sampling in distant populations (several kilometres) rather than molecular markers at a local scale may fail to detect all genotypic variability present within aspen stands. This, however, remains to be examined.

Acknowledgements

We thank M. H. Longpré, K. Naydenov, and G. Souzede for their help in the fieldwork. We are also grateful to D. Charron for dendrochronological work and J. F. Bouffard for his help in DNA analysis. This study was funded by a doctoral scholarship to M.-C. Namroud (On-the-Job Research Scholarships). Other financial support was provided by the NSERC-UQAT-UQAM Chair in sustainable forest management and by an NSERC grant to F. Tremblay.

Literature cited

Barnes, B. V., 1966. The clonal growth habit of American aspens. Ecology, 47: 439-447.

Bergeron, Y., 2000. Species and stand dynamics in the mixed woods of Québec's southern boreal forest. Ecology, 81: 1500-1516.

Bergeron, Y. & D. Charron, 1994. Postfire stand dynamics in a southern boreal forest (Québec): A dendroecological approach. Écoscience, 1: 173-184.

Bergeron, Y. & M. Dubuc, 1989. Succession in the southern part of the Canadian boreal forest. Vegetatio, 79: 51-63.

Carcaillet, C., Y. Bergeron, P. J. H. Richard, B. Frechette, S. Gauthier & Y. T. Prairie, 2001. Change of fire frequency in the eastern Canadian boreal forests during the Holocene: Does vegetation composition or climate trigger the fire regime? Journal of Ecology, 89: 930-946.

Caron, H., G. Dumas, C. Marque, C. Messier, E. Bandou, R. J. Petit & A. Kremer, 2000. Spatial and temporal distribution of chloroplast DNA polymorphism in a tropical tree species. Molecular Ecology, 19: 1089-1098.

Chung, M. G., M. Y. Chung, G. S. Oh & B. K. Epperson, 2000. Spatial genetic structure in a *Neolitsea sericea* population (Lauraceae). Heredity, 85: 490-497.

Chung, M. Y., J. D. Nason, B. K. Epperson & M. G. Chung, 2003. Temporal aspects of the fine scale genetic structure in a population of *Cinnamomum insularimontanum* (Lauraceae). Heredity, 90: 98-106.

Cumming, S. G., F. K. A. Schmiegelow & P. J. Burton, 2000. Gap dynamics in boreal aspen stands: Is the forest older than we think? Ecological Applications, 10: 744-759.

Dayanandan, S., O. P. Rajora & K. S. Bawa, 1998. Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*). Theoretical Applied Genetics, 96: 950-956.

Doyle, J. I. & J. L. Doyle, 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. Phytochemistry Bulletin, 19: 11-15.

Einsphar, D. W. & L. L. Winton, 1976. Genetics of quaking aspen. US Department of Agriculture Forest Service Research Paper WO-25, Washington, DC.

Ellstrand, N. C. & M. L. Roose, 1987. Patterns of genotypic diversity in clonal plant species. American Journal of Botany, 74: 123-131.

Environnement Canada, 1982. Normales climatiques au Canada. Environnement Canada, Service de l'environnement atmosphérique, Ottawa, Ontario.

Erikson, O., 1989. Seedling dynamics and life histories of clonal plants. Oikos, 55: 231-238.

Erikson, O., 1993. Dynamics of genets in clonal plants. Trends in Ecology and Evolution, 8: 313-316.

Erickson, D. & J. Hamrick, 2003. Genetic and clonal diversity for *Myrica cerifera* along a spatiotemporal island chronosequence. Heredity, 90: 25-32.

Frey, R., V. J. Lieffers, S. M. Landhäusser, P. G. Comeau & K. J. Greenway, 2003. An analysis of sucker regeneration of trembling aspen. Canadian Journal of Forest Research, 33: 1169-1179.

Groot, A., D. W. Carlson, R. L. Fleming & J. E. Wood, 1997. Small openings in trembling aspen forest: Microclimate and regeneration of white spruce and trembling aspen. NODA/NFP Technical Report TR-47, Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario.

Harvey, B. D. & Y. Bergeron, 1989. Site patterns of natural regeneration following clear-cutting in northwestern Québec. Canadian Journal of Forest Research, 19: 1458-1469.

Hyun, J. O., O. P. Rajora & L. Zsuffa, 1987. Genetic variation in trembling aspen in Ontario based on isozyme studies. Canadian Journal of Forest Research, 17: 1134-1138.

Ives, W. G. H. & H. R. Wong, 1988. Tree and Shrub Insects of the Prairie Provinces. Information Report NOR-X-292, Northern Forestry Centre, Canadian Forestry Service, Edmonton, Alberta.

Jelinski, D. E. & W. M. Cheliak, 1992. Genetic diversity and spatial subdivision of *Populus tremuloides* (Salicaceae) in a heterogeneous landscape. American Journal of Botany, 79: 728-736.

Johnson, E. A., K. Miyanishi & J. M. H. Weir, 1998. Wildfires in western Canadian boreal forest: Landscape patterns and ecosystem management. Journal of Vegetation Science, 9: 603-610.

Johnston, B. C. & L. Hendzel, 1985. Examples of aspen treatment, succession, and management in western Colorado. US Department of Agriculture Forest Service, Rocky Mountain Region, Lakewood, Colorado.

Kemperman, J. A. & B. V. Barnes, 1976. Clone size in American aspens. Canadian Journal of Botany, 54: 2603-2607.

Kjølner, S., S. M. Såstad, P. Taberlet & C. Brochmann, 2004. Amplified fragment length polymorphism *versus* random amplified polymorphic DNA markers: Clonal diversity in *Saxifraga cernua*. Molecular Ecology, 13: 81-86.

Kneeshaw, D. & Y. Bergeron, 1998. Canopy gap characteristics and tree replacement in the southeastern boreal forest. Ecology, 79: 783-794.

Landhäusser, S. M. & V. J. Lieffers, 1998. Growth of *Populus tremuloides* in association with *Calamagrostis canadensis*. Canadian Journal of Forest Research, 28: 396-401.

Liu, Z. & G. R. Furnier, 1993. Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. Theoretical and Applied Genetics, 87: 97-105.

Lund, S. T., G. R. Furnier & C. A. Mohn, 1992. Isozyme variation in quaking aspen in Minnesota. Canadian Journal of Forest Research, 22: 521-524.

McDonough, W. T., 1979. Quaking aspen—seed germination and early seedling growth. US Department of Agriculture Forest Service Research Paper INT-234, Washington, DC.

Michalakis, Y. & L. Excoffier, 1996. A genetic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. Genetics, 142: 1061-1064.

Mitton, J. & M. Grant, 1980. Observations on the ecology and evolution of quaking aspen, *Populus tremuloides*, in the Colorado Front Range. American Journal of Botany, 67: 202-209.

Mitton, J. B. & M. C. Grant, 1996. Genetic variation and the natural history of quaking aspen. BioScience, 46: 25-31.

Moriguchi, Y., A. Matsumoto, M. Saito, Y. Tsumura & H. Taira, 2001. DNA analysis of clonal structure of an old growth, isolated forest of *Cryptomeria japonica* in a snowy region. Canadian Journal of Forest Research, 31: 377-383.

Morin, H., D. Laprise & Y. Bergeron, 1993. Chronology of spruce budworm outbreaks in the Lake Duparquet region, Abitibi, Québec. Canadian Journal of Forest Research, 23: 1497-1506.

Morton, J. K. & J. M. Venn, 1990. A Checklist of the Flora of Ontario. Vascular Plants. University of Waterloo Biology Series 34, Waterloo, Ontario.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, New York.

Perala, D. A., 1990. *Populus tremuloides* Michx. Quaking aspen. Pages 555-569 *in* R. M. Burns & B. H. Honkala (eds.). Silvics of North America. Volume 2. Hardwood. US Department of Agriculture Forest Service Agricultural Handbook 654, Washington, DC.

Persson, H. A. & B. A. Gustavsson, 2001. The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis. Molecular Ecology, 10: 1385-1397.

Pielou, E. C., 1969. An Introduction to Mathematical Ecology. Wiley-Interscience, New York, New York.

Pleasants, J. M. & J. F. Wendel, 1989. Genetic diversity in a clonal narrow endemic, *Erythronium propullans*, and in its widespread progenitor, *Erythronium albidium*. American Journal of Botany, 76: 1136-1151.

Pornon, A., N. Escaravage, O. Thomas & P. Taberlet, 2000. Dynamics of genotypic structure in clonal *Rhododendron ferrugineum* (Ericaceae) populations. Molecular Ecology, 9: 1099-1111.

Romme, W. H., M. G. Turner, R. H. Gardner, W. W. Hargrove, G. A. Tuskan, D. G. Despain & R. A. Renkin, 1997. A rare episode of sexual reproduction in aspen (*Populus tremuloides,* Michx) following the 1988 Yellowstone fires. Natural Areas Journal, 17: 17-25.

Schneider, S., D. Roessli & L. Excoffier, 2000. Arlequin ver. 2.000: A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Scrosati, R., 2002. An updated definition of genet applicable to clonal seaweeds, bryophytes, and vascular plants. Basic and Applied Ecology, 3: 97-99.

Shapcott, A., 1995. The spatial genetic structure in natural populations of the Australian temperate rainforest tree *Atherosperma moshatum* (Labill.) (Monimiaceae). Heredity, 74: 28-38.

Shepperd, D., D. L. Bartos & A. M. Stephen, 2001. Above- and below-ground effects of aspen clonal regeneration and succession to conifers. Canadian Journal of Forest Research, 31: 739-745.

Slatkin, M., 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics, 139: 457-462.

Stehlik, I. & R. Holdereger, 2000. Spatial genetic structure and clonal diversity of *Anemone nemorosa* in late successional deciduous woodlands of Central Europe. Journal of Ecology, 88: 424-435.

Stevens, M. T., M. G. Turner, G. A. Tuskan, W. H. Romme, L. E. Gunter & D. M. Waller, 1999. Genetic variation in postfire aspen seedlings in Yellowstone National Park. Molecular Ecology, 8: 1769-1780.

Strothmann, R. O. & Z. A. Zasada, 1965. Quaking aspen (*Populus tremuloides* Michx.). Pages 523-534 *in* H. A. Howells (ed.). US Department of Agriculture, Agriculture Handbook 271, Washington, DC.

Vincent, J. S. & L. Hardy, 1977. L'évolution et l'extinction des grands lacs glaciaires Barlow et Ojibway en territoire Québecois. Géographie Physique et Quaternaire, 31: 357-372.

Wang, G. G., 2003. Early regeneration and growth dynamics of *Populus tremuloides* suckers in relation to fire severity. Canadian Journal of Forest Research, 33: 1998-2006.

Waples, R. S., 1989. Temporal variation in allele frequencies: Testing the right hypothesis. Evolution, 43: 1236-1251.

Weir, B. S. & C. C. Cockerham, 1984. Estimating F-statistics with special regard to systems of mating. Evolution, 19: 395-420.

Wyman, J., A. Bruneau & M. F. Tremblay, 2003. Microsatellite analysis of genetic diversity in four populations of *Populus tremuloides* in Québec. Canadian Journal of Botany, 81: 360-367.

Yeh, F. C., K. X. Chong & R. C. Yang, 1995. RAPD variation within and among natural populations of trembling aspen (*Populus tremuloides*) from Alberta. Journal of Heredity, 86: 454-460.

Ziegenhagen, B., R. Bialozyt, V. Kuhlenkamp, I. Schulze, A. Ulrich & M. Wulf, 2003. Spatial patterns of maternal lineages and clones of *Galium odoratum* in a large ancient woodland: Inferences about seedling recruitment. Journal of Ecology, 91: 578-586.