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Simulations of clonal species genotypic diversity – trembling aspen (*Populus tremuloïdes*) as a case study

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Abstract

We built two models to follow clonal species genotypic diversity (G/N) over long periods of time at the stand and landscape levels. The models were then validated with empirical data from trembling aspen (*Populus tremuloides*) populations in Quebec's boreal forest. Data was collected using a chronosequence approach in seven sites that burned in 1717, 1760, 1797, 1823, 1847, 1944, and 1916. Genetic identification was done by using four microsatellite loci. At the stand scale, simulations were repeated for a genet size of 5, 25, 50 and 100 ramets each. At the landscape level, we simulated the cumulative genet survival rate under different fire cycles (5–500 years) for 500 years after fire. Stand simulations indicated that ramet mortality within genets rather than genet mortality accounts for the increase in G/N with time since fire. Both the initial genet size and the recurrent suckering of some genets (or ramet recruitment) play an important role in maintaining high G/N levels for long periods of time. In general, the larger the number of ramets per genet, the longer the genet survives under a gap disturbance regime and a minimum of 100 ramets per genet is required to maintain aspen genet survival for 500 years. At the landscape level, genet loss increases as the fire cycle gets longer. In Quebec's boreal forest, short rotation even-aged management practices seem to maintain a genet survival rate similar to that produced by the natural succession regime.

Introduction

In the past few decades, conservation of plant genetic resources has become an important component of the total effort for a sustainable ecosystem management and biodiversity conservation (MIG 1998; Rogers 2002; Glaubitz and Moran 2003). Genetic diversity conservation is essential for the long-term stability and the short-term productivity of forest ecosystems (Young and Boyle 2003). It is also essential to maintain the evolutionary potential of populations by enabling them to adapt to new environmental conditions (Lande and Shannon 1996; FAO 2002).

In clonal species that can survive changing ecological conditions for thousands of years (Mitton and Grant 1996), genetic diversity has been extensively documented (Chung and Epperson 2000; Kreher et al. 2000; Pornon et al. 2000; Erickson and Hamrick 2003), but how this diversity is maintained over very long periods of time remains poorly understood. In an attempt to address this problem, several studies have compared measures of genetic variation in clonal species

across a range of sites representing different ages since regeneration. Some studies reported little or no genetic differences between different age classes (Erickson and Hamrick 2003; Namroud et al. 2005a), while others reported a decrease in genetic variation in younger ones (Cronberg 2002). A major challenge for addressing this question is that we can not be certain of the starting conditions. Once established, clonal species can maintain their presence in a stand for hundreds or thousands of years by reproducing vegetatively, i.e. by generating young individuals that have the same genotype as the parent ones. All individuals having the same genotype form together one genet or clone, and each of these individuals is called a ramet of this genet. The age of an individual ramet is typically much shorter than the age of the genet, and the composition of ramets and genets may change considerably over time. As a result, the populations of clonal species do not necessarily reflect the structure of ramets and genets established at the origin of the stand under different ecological conditions.

One way to overcome this difficulty is to use computer simulations that project ramet and genet composition at various stages of a stand development. To date, only few models have attempted to do so by simulating genotypic diversity, but they showed different results. For instance, Balloux et al. (2003) used analytical and stochastic simulation approaches to explore the consequences of variable rates of clonal reproduction on clonal population genetics. They suggested that genotypic diversity decreases at a constant rate with increasing rates of asexual reproduction. However, Bengtsson (2003) simulated the genotypic identity of clonal populations (i.e. the probability that two randomly sampled adult individuals from a population have the same genotype) and suggested that a population "retain its initial genotypic variation for a very long period of time even if it reproduces almost exclusively asexually later on. The differences between the predictions from computer simulations or empirical studies may reflect the need for a better adjustment of the model parameters with empirical data, the limitation of empirical studies to detect genetic effects over very long generation times, or simply a species specific behavior. Such discrepancies highlight our lack of information about clonal species and the need for long-term studies to understand their behavior. Collecting empirical data over long periods of time and using them to validate simulations may help overcome these problems and fill in the gaps between simulations and field data.

In this paper, we attempt to lay the frameworks for a model that simulates clonal species genotypic diversity over time by using trembling aspen (*Populus tremuloides*, Michx) as a case study. Aspen is a pioneer species in Quebec's boreal forest that regenerates massively after fire and dominates the stand for the first 100 years (Bergeron and Dubuc 1989). In the absence of fire, it continues to regenerate in the canopy openings, but in smaller cohorts due to increasing conifer competition; it is often reduced to small isolated patches in very old stands (Bergeron and Dubuc 1989; Bergeron 2000). Aspen mostly reproduces by suckering because conditions for seed germination are rarely met (Mitton and Grant 1980; Donough 1997). Many studies have documented its clonal diversity in early and mid successional stages, but we know little about this diversity in very late successional stages. The only other study that followed this diversity with time did it for only three successive cohorts for about 180 years (Namroud et al. 2005a). In the present study, we used a chronosequence approach in seven sites that burned at different times to collect empirical data and measure genotypic diversity over about 300 years. We then simulated aspen genotypic diversity expressed by the ratio G/N over a longer period of time (500 years after fire at the stand level) and under different fire cycles (up to 500 years at the landscape level). This provided a unique opportunity to: (1) validate simulations with empirical data; (2) identify some factors that affect aspen genotypic diversity over long periods of time; (3) determine the impact of various fire cycles on genet conservation at the landscape level; and (4) assess the level of genet conservation produced by even-aged management practices as currently used in Quebec and compare it with that produced under a natural succession regime.

Materials and methods

Study area

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

northwestern Quebec (79 °1′ W–48 °30′ N) (Figure 1). This forest was selected because it has been lightly disturbed by humans (Harvey and Bergeron 1989). On mesic sites, such as those sampled in this study, young successional stages (<100 years) are dominated by trembling aspen, intermediate stages (100–200 years) by balsam fir (*Abies balsamea*), trembling aspen, and white spruce (*Picea glauca*), while old stages (>200 years) are dominated by balsam fir and white cedar (*Thuja occidentalis*) (Bergeron 1991, 2000).

Seven sites that burned in 1717, 1760, 1797, 1823, 1847, 1916, and 1944 were selected for sampling. They were labelled A, B, C, D, E, F and G, respectively. In each site, we established a transect along which we collected samples at regular intervals. The number of samples varied between the sites depending on the transect length that, in turn, depended on the stand age and aspen density in each site. In sum, the transect length ranged from 208 m to 1062 m, and the number of samples ranged from 19 to 28 (Table 1). For each aspen tree, a core was taken at breast height to determine its age, the diameter at breast height was measured, and leaves or root tissue were collected for genetic analysis. In the case of root sampling, special care was given to collect the living cambium tissue from the roots of the tree sample. Along each transect, stand vegetation composition was determined using the point-centered quarter method according to Mueller-Dombois and Ellenberg (1974). Aspen density ranged from 726 trees/ha in the youngest site A to 154 trees/ha in the oldest site G. Global (of all tree species) density and basal area ranged from 1215 trees/ha and 56 m^2/ha, 65 years after fire, to 304 trees/ha and 16 m^2/ha, 240 years after fire, respectively (Table 1).

DNA extraction and amplification

In the lab, aspen root and leaf samples were ground, and genomic DNA was extracted using the GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich Canada Ltd, Oakville, Canada). DNA amplification was performed using Taq polymerase (Gibco from Invitrogen™ Life Technologies, Burlington, Canada) and four dye-labeled oligonucleotide primers (*PTR1*, *PTR2*, *PTR3*, and *PTR4*) at microsatellite loci complementary to Simple Sequence Repeat (SSR) flanking regions. The resolving power of these four microsatellites was tested in a previous study (Namroud et al., 2005b) and was found to be sufficiently high to reject the null hypothesis of similar genotypes ($P_{(ID)} < 0.001$).

Extracted genomic DNA was amplified by carrying out a Polymerase Chain Reaction (PCR) in a 96-Well GeneAmp® PCR System 9700 (Applied Biosystems, California, USA) with a total volume of 10 μl that contained: 4 μl of DNA extract, 0.625 pmol/μl of primers, 0.2 mM dNTP, 3.125 mM $MgCl_2$, 1.4 μl BSA, and 12.5 mM Tris–HCl (pH = 8.0). The best results were obtained by performing a touch down PCR that consists in decreasing the annealing temperature by 1 °C every other cycle. We started with 10 min at 95 °C to activate the enzyme and denature DNA strands. We then used a series of annealing temperatures ranging from 60 °C to 54 °C over 33 cycles. At the end of each cycle, we added a final step of 72 °C for 7 min for extension. 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 ice. Amplified DNA was analyzed using Gene Scan Software and ABI Prism 310 Genetic Analyzer from Applied Biosystems (California, USA).

Genetic analysis – empirical data

Aspen genetic diversity was measured by observed heterozygosity (*Ho*), expected heterozygosity (*He*), the average number of alleles per locus, and genotypic diversity expressed by the ratio G/N, where G is the number of observed genets, and N is the total number of individuals analyzed (Pleasants and Wendel 1989). G/N tends to zero when the number of genotypes is very low, and can reach a maximum of 1 when each tree has a unique mutlilocus genotype. Genotypic diversity was also measured with the Simpson's index D corrected for small samples to ensure that G/N values are not biased by the sample sizes. The Simpson's index was calculated with the formula $D = 1 - \Sigma\{[n_i(n_i-1)]/[N(N-1)]$, where n_i = number of ramets of the ith genet (Pielou 1969). For the purposes of this study, only genotypic diversity expressed by G/N was simulated. G/N is an easier parameter to manage than allelic diversity in setting conservation management strategy. We also calculated the percentage of single-ramet genets (a genet composed of one ramet)

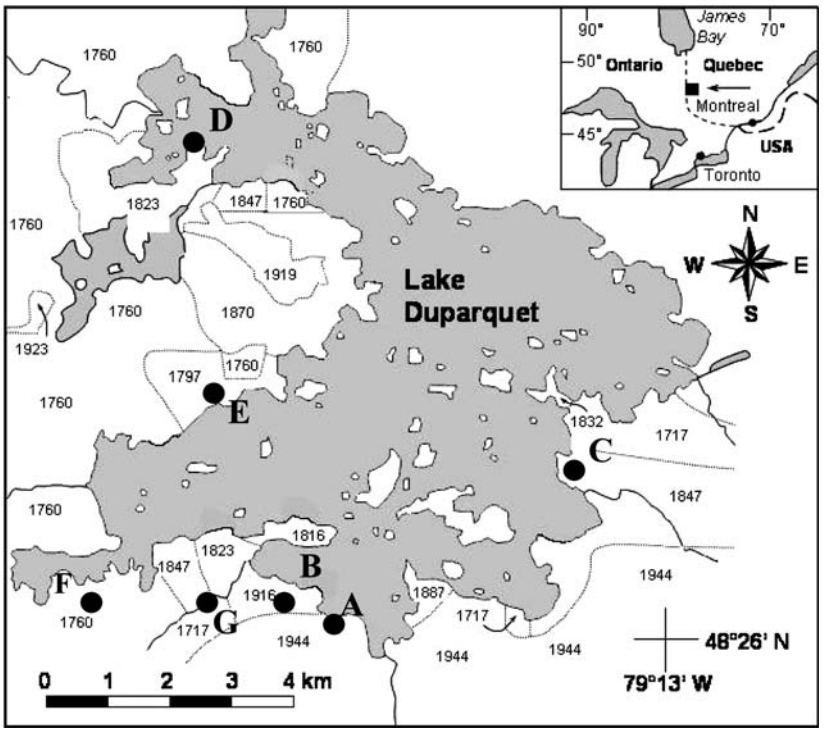

Figure 1. Location of the seven sampling sites in Quebec's boreal forest.

Table 1. Ecological characteristics of the sampling sites

	A	B	C	D	E	F	G
Mean stand age (yrs)	56	84	153	177	203	240	283
Transect length (m)	208	290	310	312	667	1062	987
Number of samples	19	26	28	26	22	28	23
Average distance between sampled trees (m)	10	10	11	12	30	38	43
Aspen density (nb/ha)	726.2	286.5	361.3	174.9	87.5	158.5	154.2
Global density (nb/ha)	1215	719	1296	398	304	408	397
Global basal area (m^2/ha)	55.8	33.3	42.1	47.8	17.9	15.7	24.8

The age of the stand is given in years (yrs), the transect length is measured in meters (m), density is expressed by the number of trees (nb) per hectare (ha), global density and global basal area are the density and basal area of all trees in the stand, respectively. Global basal area is expressed by the surface area (m^2) per hectare.

and the number of multiramet genets (a genet composed of more than one ramet – also called clones). Genetic analysis was carried out with *GENETIX*, a specialized software for population genetics (Belkhir et al. 2004).

Simulations at the stand level

To follow aspen genotypic diversity variation over time, we built two models: the first one to monitor G/N with time since fire at the stand scale; the second to determine the rate of genet survival G_t/G_1 under various fire cycles at the landscape level. G_1 and G_t are the number of genets in the first year after fire and after t years, respectively.

To build the stand model, we first used the empirical data to calculate aspen density per hectare. This was obtained by multiplying aspen relative density by the global density per hectare then dividing it by 100. These data allowed us to

build a regression model that derives aspen density per hectare in function of time since fire according to the following equation:

$$D_t = 31092 \times t^{(-0.9839)} \quad (1)$$

where D_t = aspen density per hectare at time t, and t = time since fire expressed in years.

From this equation, we derived the cumulative survival rate of aspen trees (CSR) at each time interval assuming 30,000 trees regenerated in the first year after fire. This number was chosen because it falls within the range reported for aspen density after a clear cut (Perala 1990).

$$\mathrm{CSR}_t = D_t/30,000 \quad (2)$$

To simulate the ratio G/N, we considered four possible scenarios: in the first (m1) and second (m2) models, we assumed that genets have similar and different sizes (expressed by the number of ramets per genet), respectively, but mortality eliminates complete genets instead of some ramets within each genet; in the third (m3) and fourth (m4) models, we considered that genets have equal and different sizes, respectively, but mortality affects only the ramets within genets in a random manner. In the last two scenarios based on ramet mortality, we first built a matrix to simulate the ramet survival rate within 1220 genets over 500 years after fire at an interval of 5 years. In the first year after fire, we assumed that ramet survival rate follows a normal distribution among genets and we randomly assigned to each genet a ramet survival rate with a mean value equal to one and a variation coefficient of 0.58. This coefficient is characteristic of normal populations with a mean of 1. For subsequent time intervals (up to 500 years), we calculated the ramet survival rate for each genet by multiplying the randomly assigned ramet survival rate for each genet in the first year by the corresponding CSR_t calculated in Equation (2). In all scenarios, we repeated the simulations with different initial genet sizes: 5, 25, 50, and 100 ramets per genet. The initial genet size was assumed to include all the ramet stock each genet will have during the simulation period. In the first two scenarios (based on genet mortality), the number of genets varied depending on the genet size, but we started the simulations with 30,000 trees in the first year after fire as explained above. In the last two scenarios, simulations were run on 1220 genets (equivalent to 30,500 stems with 25 ramets/genet), but the total number of stems varied depending on the genet size. For scenarios with different genet sizes, we assumed that the genet size follows a normal distribution with an average of 5, 25, 50 or 100 ramets/genet and a variation coefficient of 0.58.

Simulations at the landscape level

To build the second model (at the landscape level), we first calculated the genet survival rate with time since fire, up to 500 years at an interval of 5 years. For this, we made three assumptions based on the empirical data and simulations at the stand level: (1) clonal diversity expressed by G/N is equal to 0.2 immediately after fire; this is the highest G/N value observed at the beginning of the stand simulations; (2) G/N increases with time since fire until it reaches a stable level (plateau) equal to 0.83 after a certain time (T); and (3) this stabilization time (T) is equal to 50 years, the age of our youngest site A. For the time intervals before stabilization, G/N was calculated according to the following equation:

$$G/N_t = G/N_{t-5} + (0.83 - G/N_1) \times (1/t) \times (1/5) \quad (3)$$

Subsequently, genet survival rate was calculated at each time interval as follows:

$$\mathrm{GSR}_t = G_t/G_{t-5} \quad (4)$$

To calculate the overall rate of genet survival at the landscape scale, we first used the Van Wagner (1972) negative exponential function to calculate the cumulated area percentage that is left without burning with time since fire (up to 500 years) and under different fire cycles ranging (from 50 to 500 years at an interval of 50 years) as shown below:

$$\mathrm{CA}_t = e^{(-\mathrm{FC/t})} \quad (5)$$

CA_t is the percentage of cumulated non-burned area t years after fire, and FC is the fire cycle expressed in years. From this equation, we deduced the percentage of non-cumulated non-burned area as follows:

$$\mathrm{NCA}_t = \mathrm{CA}_{(t-5)} - \mathrm{CA}_t \quad (6)$$

where NCA_t is equal to the percentage of non-cumulated, non-burned area, t years after fire. We

then multiplied the percentage of non-burned area left by the corresponding genet survival rate, which allowed us to calculate the percentage of aspen genets that survive under each fire cycle and at each time interval. The simulations were repeated with different values for G/N_1 and the stabilization time T. These ranged from 0.01 (equivalent to the lowest G/N observed in the genet mortality based model with 5 ramets per genet) to 0.83 (the stabilization level observed in empirical data), and from 15 years (minimum time interval needed for G/N stabilization, observed in the model based on ramet mortality with 5 ramets per genet) to 370 years (the longest time interval needed for G/N stabilization, observed in the model based on ramet mortality with 100 ramets per genet), respectively.

Even-aged management practices

To calculate the genet survival rate under even-aged management practices, we first divided the landscape into equally divided areas, each corresponding to a different age class following the methodology described in Bergeron et al. (1999). The area proportion of each age class as well as the number of age classes varied depending on the rotation period considered in even-aged management practices. This is because in even-aged management practices, no stands will be left older than the rotation period used (i.e. the landscape is composed of age classes younger than the rotation period). Since the mean rotation period in Quebec's boreal forest is about 100 years, we repeated the simulations at the landscape level for even-aged management with a rotation of 50, 100, 150, 200 and 250 years. To calculate the percentage of aspen genets that survive under each rotation period and in each age class, we multiplied the percentage of each age class by the corresponding genet survival rate calculated in Equation (4).

Results

Empirical data

Little changes in aspen genetic and genotypic variation were observed between the sampling sites. Observed heterozygosity ranged from 0.51 to 0.64 and averaged 0.56 ($\pm$0.05) over the 7 sites. Expected heterozygosity was slightly higher and ranged from 0.55 to 0.69. It averaged 0.65 ($\pm$0.05). G/N ranged from 0.75 to 0.92, and averaged 0.83 ($\pm$0.06) over all the sites. Variance tests (within and between sites) for the number of alleles per locus and G-tests for allelic frequencies at each locus showed no significant differences in allelic diversity between the sites at $\alpha = 0.05$. The Simpson's index D was relatively high in all sites and ranged from 0.965 to 0.994. Among the 143 genets found in all sites, 25 were mutliramet genets (i.e. composed of two or more ramets each, also called clones). From these, only two clones had more than two ramets each: one with 5 ramets in site C and one with 3 ramets in site E. Genets were mostly (76.5–92%) single-ramet (Table 2).

Stand model

None of the four scenarios simulated at the stand level was completely congruent with the empirical data in our sampling sites. In the first two scenarios that simulated genet mortality, G/N main-

Table 2. Genetic characteristics of the sampling sites

	A	B	C	D	E	F	G
Ho (*SE*)	0.61 (0.20)	0.53 (0.33)	0.64 (0.15)	0.51 (0.07)	0.54 (0.14)	0.55 (0.12)	0.54 (0.12)
He (*SE*)	0.67 (0.11)	0.55 (0.31)	0.64 (0.16)	0.66 (0.08)	0.66 (0.21)	0.69 (0.14)	0.68 (0.15)
Average number of alleles per locus	6.25	5.75	6.50	6.25	7.50	6.25	6.00
D	0.982	0.991	0.966	0.994	0.974	0.987	0.984
G/*N*	0.84	0.88	0.75	0.92	0.77	0.82	0.83
% Single-ramet genets	81.25	86.95	80.95	91.67	59.09	78.26	78.95
Number of clones (multi-ramet genets)	3	3	4	2	4	5	4
Average number of ramets per clone	2.00	2.00	2.75	2.00	2.25	2.00	2.00

Ho = observed heterozygosity, *He* = expected heterozygosity; *SE* = standard errors; *D* = Simpson's index corrected for small sample sizes; G/N is the ratio of the number of genets (G) over the total number of stems (N). The term clone is used for multiramet genets i.e. composed of more than one ramet.

tained a constant value ranging from 0.01 to 0.20 depending on the original genet size. In the third and fourth scenarios that simulated ramet mortality, G/N increased gradually over time until it reached a maximum value of one, between 10 and 370 years depending on the genet size. At this stage, every single ramet was unique genetically. The pattern was similar when considering similar genet sizes. The only difference was the stabilization time (T) that was few years shorter than that observed with different genet sizes. These results are illustrated in Figure 2a by the model built with a genet initial size of 100 ramets. In the other models based on smaller genet sizes, the stabilization level of G/N at one was not maintained over the simulation period of 500 years. This is because all ramets were eliminated after 30, 150 and 325 years in models built with a genet size of 5, 25, and 75 ramets, respectively (Figure 2b). In all ramet based models, mortality occurred mostly within genets. The genet survival rate was four times higher than the total stem survival during the first 5 years after fire (Figure 2c).

Landscape model

Simulations at the landscape level revealed important differences in genet survival under different fire cycles. The genet survival rate decreased when fire events became rare (i.e. under long fire cycles). It ranged from 11.7% under the longest fire cycle (500 years) to 43.6% under the shortest

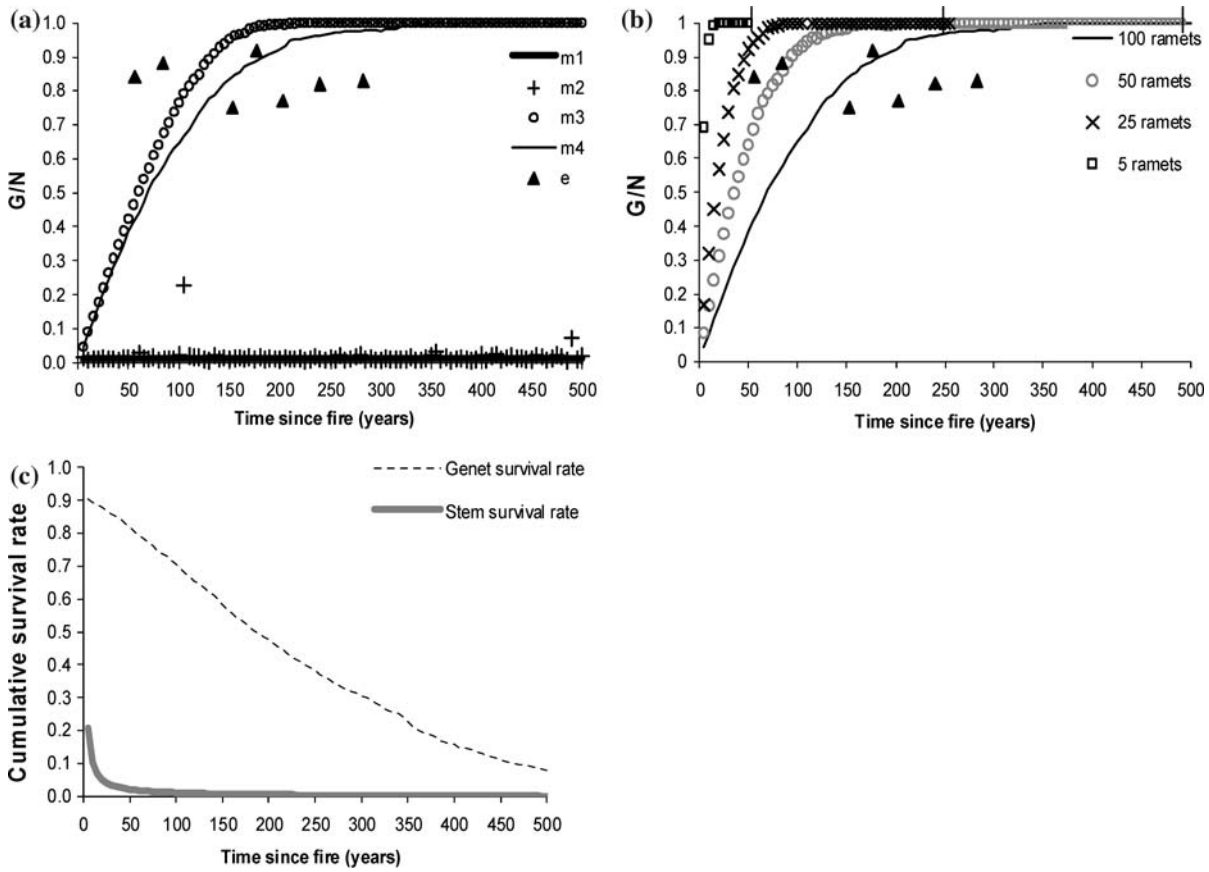

Figure 2. Simulations at the stand level of: (a) G/N with time since fire assuming: (m1) mortality eliminates complete genets and all genets are composed of 100 ramets each, (m2) mortality eliminates complete genets and genets have different sizes with an average of 100 ramets each, (m3) mortality eliminates ramets within genets and all genets are composed of 100 ramets each, (m4) mortality eliminates ramets within genets and genets have different sizes with an average of 100 ramets each, and (e) empirical G/N in seven sampling sites; (b) G/N with time since fire assuming a genet size of 5, 25, 50 and 100 ramets; (c) genet and stem survival rates with time since fire assuming all genets are composed of 100 ramets each.

one (50 years) when assuming initial values of G/N and T equal to empirical values (Figure 3a (m0)). Similar patterns were observed when simulating different sets of values for G/N and T. The genet survival rate ranged from 5.7% under the longest fire cycle (500 years with $G/N=0.83$) to 53.7% under the shortest one (50 years with $G/N=0.01$ and $T=15$ years) (Figure 3a (m1, m2, m3, m4, m6, m7, m8)). Only the model with a very low G/N value (0.01) combined with a very long stabilization time ($T=370$ years) showed a remarkable difference with the other models: the genet survival rate was relatively high under all fire cycles (up to 67.3%; Figure 3a (m5)).

Even-aged management practices revealed little differences in the level of genet survivorship compared to natural disturbance regimes. The cumulative genet survival percentage decreased from 47% to 20% as the rotation period increased from 50 to 250 years, and from 44% to 18% as the fire cycle increased from 50 to 250 years. In general, short rotations maintained a higher level of genet survival than longer ones (Figure 3b). Under a natural disturbance regime with a 100-year fire

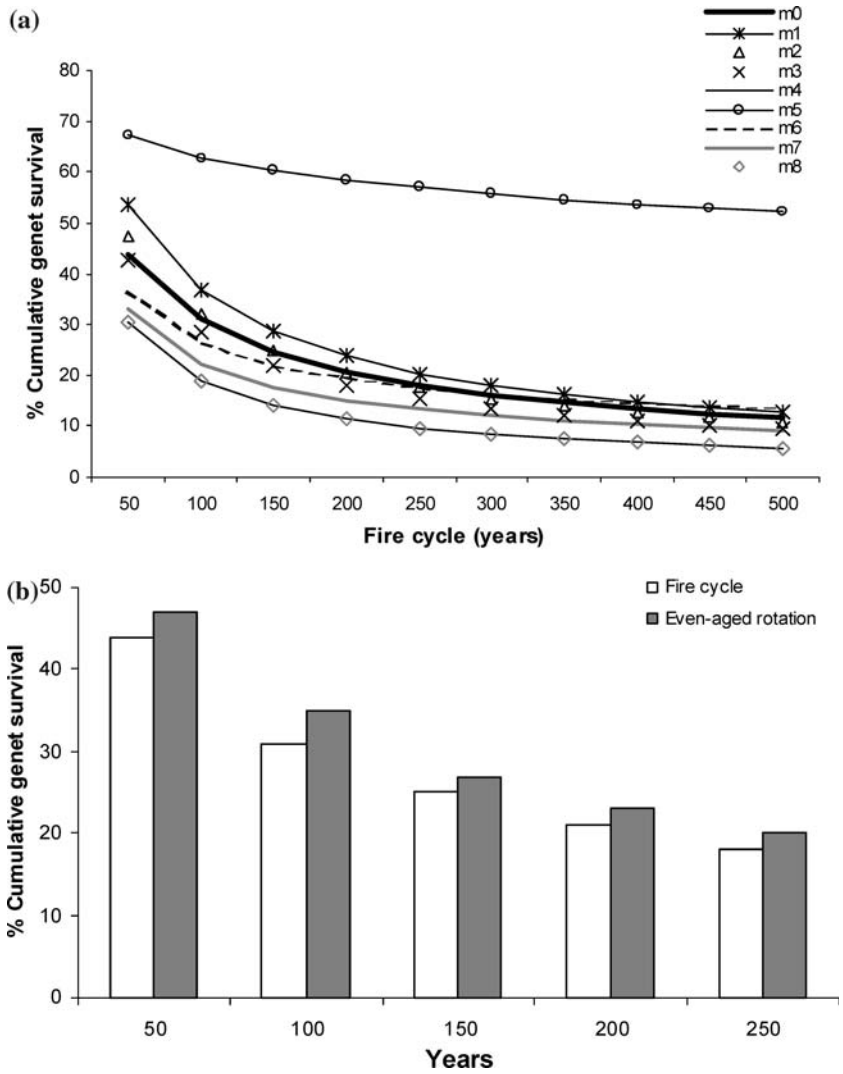

Figure 3. Simulations at the landscape level of: (a) the cumulative survival rate of genets under different fire cycles, assuming: (m0) $G/N=0.2$ and stabilization time $T=50$ years, (m1) $G/N=0.01$ and $T=15$ years, (m2) $G/N=0.1$ and $T=15$ years, (m3) $G/N=0.2$ and $T=15$ years, (m4) $G/N=0.83$ and $T=15$ years, (m5) $G/N=0.01$ and $T=370$ years, (m6) $G/N=0.1$ and $T=370$ years, (m7) $G/N=0.2$ and $T=370$ years, and (m8) $G/N=0.83$ and $T=370$ years; (b) the cumulative genet survival percentage under different fire cycles and various even-aged management rotations.

cycle, 31% of the genets were maintained in the landscape, while 35% survived under a 100-year rotation (Figure 3b).

Discussion

Empirical data: evolution of aspen genetic and genotypic diversity with time since fire

We detected little differences in the pattern of genetic diversity across our sites that spanned 300-year period since fire. This indicates that aspen genetic diversity does not significantly decrease over time in Quebec's boreal forest. In the absence of any evidence for sexual recruitment in the study area (Bergeron 2000), we suggest that the dismissal of the post-fire aspen cohort (aspen stand breakup) and the recruitment of subsequent cohorts of aspen maintain aspen high genetic and genotypic diversity. Apparently, the increasing vegetation competition and the decrease in aspen population size (mortality) with time since fire do not induce a genetic erosion in aspen populations in Quebec's boreal forest.

Our findings, mainly the high genotypic diversity levels observed in old growth stands, are comparable with those reported in the same sampling area by Wyman et al. (2003) in 40–65 years old stands, and with our previous observations following three cohorts based on a stand scale approach (Namroud et al. 2004). However, they can not be easily compared with values reported in Western Canada (Cheliak and Dancik 1982; Jelinski and Cheliak 1992). This is because of the differences in the sampling strategies and molecular markers used in the studies, as well as in the fire cycle length between the two regions.

Simulations at the stand level: genotypic diversity with time since fire

Simulations at the stand level revealed an important role of the genet size in maintaining aspen diversity for long periods of time: the larger the number of ramets per genet, the longer the genet survives under a gap disturbance regime. This was especially true for ramet based models that showed a high sensitivity to the number of ramets per genet. In general, a minimum number of 100 ramets per genet is required to maintain aspen genet survival for 500 years in Quebec's boreal forest.

Models based on genet mortality fell short from explaining the high levels of G/N observed in empirical data. In these models, G/N did not change with time since fire and maintained a value of 0.01 or 0.2, much lower than the average of 0.83 observed in the field. In contrast, all simulations based on ramet mortality (except those based on five ramets per genet), reflected a pattern that was closer to empirical data: genotypic diversity gradually increased with time since fire before it stabilized at a plateau of one, slightly higher than the average of 0.83 observed in the sampling sites. Theoretically, three possibilities can explain the increase in G/N in the first few years after fire: (1) mortality could have reduced the number of ramets within genets without affecting the number of genets (G); (2) the recurrent recruitment of genets (i.e. the periodic suckering of all or most genets) could have increased the number of genets (G) without changing the total number of stems (N); and (3) mortality could have affected both genets and ramets, but the proportion of the total ramet loss (N) remained higher than that of genets (G). The first hypothesis is not supported because simulations of genet survivorship at both the stand and landscape scales revealed a decrease in the percentage of genets with time since fire. For instance, genet loss can reach 9% within 75 years after fire when the genet size is 100 ramets (Figure 2c). The second hypothesis can be excluded because self-thinning, natural mortality, and insect outbreaks reduce aspen density in a stand with time since fire, thus inducing a gradual decrease in N (Greene et al. 1999; Bergeron 2000). Alternatively, the concomitant mortality of genets and ramets within genets with a higher rate of ramet mortality as suggested by the third hypothesis, is more likely to explain the increase in G/N. This is supported by the large difference (up to four fold in the first year after fire) in the genet and total stem loss at the same time interval after fire (Figure 2c), as well as by the large number of single-ramet genets.

In all simulations based on ramet mortality, G/N stabilized at a maximum value of one, higher than the average of 0.83 observed in the field. Differences between the stabilization levels of simulated G/N and those observed in the field may be related to the failure to consider in the model the recurrent suckering of some genets with time since fire. This parameter was not considered because more data

were required to quantify it and introduce it in the model. The observation of a few mutliramet genets in the old growth stands in this study (Table 2), and after three successive cohorts in a previous local study (Namroud et al. 2005a) make it reasonable to estimate that the gap fillers include at least few ramets of already existing genets, thus inhibiting G/N from reaching a level of one. In the absence of major disturbances that stimulate massive aspen suckering, we conclude that the recurrent suckering of some genets maintains the mutliclonal structure of aspen and increases the survival chances for many genets over long periods of time.

Simulations at the landscape: genetic variation under different fire regimes

Simulations at the landscape level clearly indicate an impact of the fire cycle on aspen genet survivorship: as the fire cycle becomes longer, aspen genet survival decreases. These findings can be related to the physiological processes of clonal functioning. Clonal viability closely depends on the regular regeneration of suckers that carry out photosynthetic activities to support the underground root system (Shepperd 1993; DesRochers and Lieffers 2001). Under long fire cycles, aspen massive suckering becomes less frequent. As a result, many genets would decay, especially those that are not regularly stimulated by fires to produce suckers, which would reduce the rate of genet survival in the landscape.

In Quebec's boreal forests controlled by a fire cycle of 100 years (Dansereau and Bergeron 1993), 37% of the landscape area are always kept unburned (Bergeron et al. 1999). This corresponds to 31% of aspen genets that will always survive in the landscape, which provides insufficient evidence to consider aspen genotypic diversity under an immediate threat. The absence of major differences in aspen genet survival (except with extreme values of G/N and T as shown in model m5, Figure 3a) when the initial genotypic diversity fluctuates (low vs. high G/N after fire; Figure 3a) further supports this conclusion. In addition, we expect the gap dynamics produced by insect outbreaks and natural mortality in the boreal ecosystems (Kneeshaw and Bergeron 1998; Bergeron 2000) to stimulate a periodic suckering of aspen genets, hence preventing their decay under the current fire cycle.

With even-aged management practices, the relatively high percentage of genet survival, especially with short rotations (100 years and less; 35% vs. 31%) can be related to the higher proportion of young stands that are preserved compared to those under the natural disturbance regime. Under even-aged management practices, only stands younger than the rotation period are maintained in the landscape (0% of the landscape will be older than the rotation period), while 37% of the landscape are composed of stands older than the length of the fire cycle under a natural disturbance regime (Bergeron et al. 1998). An increase in the proportion of old stands implies a decrease in aspen density and an increase in the number of genets eliminated by mortality, especially after 100 to 150 years. By contrast, a higher proportion of young stands under short rotations or fire cycles implies the conservation of a larger number of genets and, therefore, a higher level of genotypic diversity.

One of the most interesting results of the simulations was the detection of a mechanism by which clonal species succeed in conserving their genetic diversity. This mechanism consists in maintaining the largest possible number of genets by spreading the risk of elimination over ramets of several genets. Spreading the risk is important because it allows surviving suckers to keep supporting the underground root genets as long as possible, hence ensuring their viability over long periods of time. With time since disturbance, the above mechanism becomes less effective in maintaining the species genotypic diversity; the decrease in aspen density in late successional stages would progressively reduce genets to one ramet and, eventually, lead to genet loss. Which genets will be lost first or will keep suckering can not be predetermined as we found no selection for specific genets across three cohorts (Namroud et al. 2005a). Under long fire cycles, the addition of a new genetic load (through sexual recruitment) may become necessary to maintain aspen genotypic diversity at the landscape level.

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