UNIVERSITÉ DE QUÉBEC EN ABITIBI-TÉMISCAMINGUE

EFFETS DES PERTURBATIONS NATURELLES ET DES COUPES FORESTIÈRES SUR LA STRUCTURE GÉNÉTIQUE ET CLONALE DU PEUPLIER FAUX-TREMBLE (*POPULUS TREMULOIDES* MICHX)

THÈSE

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A	Mean number of alleles per locus
AMOVA	Analysis of molecular variance
bp	Base pair
CC	Clear-cut
DBH	Diameter at breast height
G	Genet number
Ho	Heterozygosity observed
He	Heterozygosity expected
ha	Hectare
LDTRF	Lake Duparquet Research and Teaching Forest
N	Number of stems
PC1/3	One-third partial cut
SE	Standard error
SMM	Stepwise-mutation model
SND	Standard normal deviates analysis

RÉSUMÉ

Dans cette thèse, notre objectif était d'évaluer l'effet des perturbations naturelles (feux et trouées) sur la diversité génétique et clonale du peuplier faux-tremble et de le comparer avec celui des coupes de différentes intensités. Plus précisément, nous visions à: (1) suivre la structure génétique et clonale du tremble avec le temps suite à une série de perturbations majeures (le feu) et secondaires (trouées) à l'échelle du peuplement; (2) construire un modèle qui permet de suivre la structure clonale du tremble sur de longues échelles temporelles et sous des cycles de feux différents tant au niveau du peuplement qu'au niveau du paysage; (3) déterminer la distribution spatiale de la diversité génétique et clonale du tremble à l'échelle du peuplement (1 ha) en fonction de la distribution spatiale des cohortes de tremble issues de perturbations majeures (feu) et secondaires (trouées); et (4) évaluer l'effet sur la diversité clonale du tremble des coupes totales et des coupes partielles de 1/3 avec sélection des tiges. Quatre marqueurs microsatellitaires spécifiques ont été utilisés pour l'identification génétique des individus de tremble. Les analyses génétiques ont montré que le tremble maintient une grande diversité génétique et clonale le long du gradient successionel et ce malgré son mode de reproduction végétative. Les simulations ont démontré que la diversité clonale du tremble exprimée par le ratio génotype/effectif augmente progressivement durant les premières 150 années après feu à l'échelle du peuplement mais se stabilise autour de 0.83 par la suite. Ceci s'explique par la régénération d'un grand nombre de genets immédiatement après feu, le recrutement périodique de ces genets dans les trouées en absence de feu, et l'élimination intraclonale important avec le temps depuis le feu. Le pourcentage de genets éliminés à l'échelle du paysage augmente avec le temps depuis le feu ainsi qu'avec l'élongation du cycle de feu. Les pratiques d'aménagement équiennes à courtes rotations semblent avoir un effet similaire à celui des feux sur la survie des genets de tremble. En général, les ramets d'un seul genet sont agglomérés sur une distance d'environ 30 m, mais les différents genets sont étroitement entrecroisés. La distribution spatiale de la variabilité génétique, tout comme celle des arbres d'une même cohorte, tendent vers une structure plus aléatoire avec le temps depuis le feu, possiblement en relation avec la diminution de la densité du tremble et l'augmentation de la compétition végétative d'une cohorte à une autre. Un aménagement par coupes totales favorise la régénération d'un grand nombre de genets, alors que suite à des coupes partielles avec sélection, le drageonnement des genets est favorisé par les conditions environnementales.

Mots clés: peuplier faux-tremble, génétique, perturbations, cohorte, feu, distribution spatiale, coupes.

ABSTRACT

In this thesis, we evaluated the effect of natural disturbances (fire and gaps) on the genetic and clonal diversity of trembling aspen and to compare it with that of different logging intensities. More precisely, we attempted to: (1) follow aspen genetic and clonal structure with time at the stand level after major (fire) and secondary (canopy gaps) disturbances; (2) build a model to estimate the loss of aspen genets over long temporal scales and under different fire cycles at the stand and landscape levels; (3) determine the spatial distribution of aspen genetic and clonal diversity at the stand level (1 ha) in relation with the spatial distribution of aspen cohorts originating from major (fire) and secondary (gaps) disturbances; and (4) assess the effect of clear-cutting and one-third partial cutting with selection on aspen clonal diversity. We used four microsatellite loci specific for aspen for genetic identification. Genetic analysis indicated that aspen maintains a high genetic and clonal diversity along the successional gradient despite its vegetative reproduction mode. Simulations at the stand level showed that aspen genotypic diversity expressed by the ratio genets/samples increases progressively within the first 150 years after fire, but stabilizes at about 0.83 afterward. This was mainly linked to the recruitment of a large number of genets immediately after fire, to the recurrent recruitment of these genets in the gaps in the absence of fire, and to an important intraclonal competition with time since fire. At the landscape level, genet loss increases with time since fire and as the fire cycle gets longer. Management practices based on even-aged short forest rotations seem to have an effect similar to that of fires on aspen genet survival. Most ramets of one genet are aggregated within a distance of 30 m, but genets are closely intermixed. Both the spatial genetic structure and the spatial distribution of aspen trees within cohorts tend toward a more random spatial distribution with time since fire, most probably in relation with the decrease in aspen density and the increase of vegetation competition from one cohort to another. Management practices based on clear-cutting favor a strong regeneration of a large number of genets, while one-third partial cutting seem to reduce genet suckering with a stronger recruitment of genets favored by environmental conditions.

Keywords: trembling aspen, genetics, disturbances, cohort, fire, spatial distribution, logging.

INTRODUCTION

Le peuplier faux-tremble (*Populus tremuloides*) est une espèce d'arbre feuillue largement répandue dans la forêt boréale. Il se régénère massivement après perturbations majeures comme les feux et les coupes totales grâce à sa capacité de se reproduire par drageonnement racinaire. Bien que plusieurs études aient largement documenté sa régénération et sa structure clonale, les connaissances concernant l'évolution de cette structure à travers le temps et l'espace manquent toujours. Ces informations sont pourtant nécessaires pour bien saisir l'effet des perturbations naturelles sur la dynamique de cette espèce et, par la suite, suggérer des pratiques sylvicoles qui s'inspirent de la dynamique naturelle des perturbations pour l'aménagement des populations de tremble.

PROBLÉMATIQUE

Depuis quelques années, on observe un intérêt croissant à adopter des pratiques d'aménagement forestier qui s'inspirent de la dynamique des perturbations naturelles (Bergeron & Harvey, 1997; Harvey et al., 2002). Ceci consiste, non seulement à préserver la composition et la structure des peuplements, mais aussi à maintenir les processus écologiques essentiels de chaque écosystème. La mise en œuvre d'une telle approche pose des défis considérables à l'aménagiste forestier. D'une part, la dynamique naturelle de la forêt boréale du Québec favorise souvent le tremble en abondance dans le paysage. D'autre part, on estime dans certaines régions que les genets (i.e. un genet est l'ensemble de tous les individus ayant le même génotype, aussi appelé un clone) de tremble occupent plusieurs hectares et comptent plusieurs milliers de tiges (Kempermann & Barnes, 1976). Pourtant, les analyses génétiques démontrent que les grands genets représentent des événements rares. Également, la plupart des études rapportent des niveaux de diversité génétique et clonale élevée chez cette espèce (Cheliak & Dancik, 1982; Jelinski & Cheliak, 1992; Wyman et al., 2003), ce qui soulève de nombreuses interrogations quant aux facteurs qui affectent cette diversité et son maintien dans le temps et dans l'espace. Par exemple, est-t-il possible que cette diversité se maintienne

dans le temps dans un écosystème contrôlé par des perturbations naturelles qui varient d'un stade successionel à un autre? Le cas échéant, quel est le mécanisme qui permet de maintenir cette diversité. Même si la diversité génétique et clonale est élevée, comment cette diversité se traduit-t-elle au niveau de la structure clonale du tremble à petite échelle, et comment le recrutement des cohortes de tremble en absence de feu affecte-t-il la taille et la distribution spatiale des clones de tremble? Finalement, est-il possible de capitaliser sur cette diversité afin de suggérer des pratiques d'aménagement pour les peuplements de tremble qui permettraient de favoriser la régénération des génotypes à meilleur rendement ligneux, tout en respectant la dynamique des perturbations naturelles?

ÉTAT DE CONNAISSANCES

La régénération du tremble

Le peuplier faux-tremble est une espèce d'arbre clonale qui constitue une des principales composantes de la forêt boréale en Amérique du Nord. Il se régénère massivement après perturbations majeures comme les feux et les coupes totales, ce qui lui confère le nom d'une espèce pionnière. Sa densité après feu peut atteindre plus de 100,000 tiges à l'hectare (Perala, 1990; Brais et al., 2004). Il domine le couvert forestier pendant près de 100 ans avant de céder la place à d'autres espèces résineuses présentes en sous couvert. En absence de feux ou de perturbations majeures, le tremble se maintient dans le paysage, mais en plus faible densité en se régénérant dans les trouées formées par la mortalité des trembles issus du feu, les épidémies d'insectes ou les chablis. Dans certains cas, on rapporte la présence de deux ou même trois cohortes dans les peuplements mixtes ou âgés (Bergeron, 2000).

Le tremble se reproduit souvent par drageonnement racinaire. Le drageonnement est contrôlé par le rapport de deux hormones: l'auxine et la cytokinine. Une augmentation du taux d'auxine entraîne une réduction du drageonnement racinaire, alors qu'une diminution du rapport auxine/cytokinine stimule le drageonnement. Le processus par lequel le drageonnement des racines est inhibé par l'auxine est aussi appelé dominance apicale (Frey et

al., 2004). Lorsque la tige de l'arbre est éliminée, la dominance apicale est aussi éliminée, et la quantité d'auxine normalement transférée des parties aériennes vers les racines est réduite, stimulant ainsi le drageonnement des racines. L'élévation de la température du sol (20°C), l'exposition du sol minéral, et l'exposition à la lumière constituent également des éléments nécessaires pour la stimulation du drageonnement (Frey et al., 2004). Le tremble peut se reproduire par semis, mais les conditions favorables à sa germination et sa croissance sont rarement réunies. Les semis ont une très courte période de viabilité, leur germination peut être empêchée ou limitée par une température élevée à la surface du sol (> 20°C), une grande humidité (> – 4 bars), ou un humus épais à la surface du sol (Perala, 1990). Par conséquent, la reproduction par semis est considérée comme un événement rare dans les populations de tremble.

La variabilité génétique et clonale du tremble dans le temps

Plusieurs facteurs peuvent influencer la diversité génétique des espèces à travers le temps. Ceux-ci incluent les forces de l'évolution comme la sélection naturelle, la mutation, et la dérive génétique (Hartl & Clark, 1997; Suvanto & Latva-Karjanmaa, 2005). Ces forces affectent directement la richesse allélique à l'intérieur d'une population en favorisant ou en éliminant certains allèles, ce qui détermine la capacité des espèces à s'adapter à des gradients environnementaux stables. Chez les espèces se reproduisant de manière végétative et sexuée, la fréquence du recrutement sexué est aussi susceptible d'affecter la diversité génétique (Eriksson 1989; 1993); la diversité génétique des espèces qui se régénèrent de manière sexuée suite à des perturbations initiales seulement tendrait à diminuer dans le temps, alors que celle des espèces qui se reproduisent de manière sexuée de façon répétitive se maintiendrait avec le temps (Cronberg 2002). Certaines études suggèrent une relation entre le nombre et la taille des clones de certaines espèces clonales et l'ouverture du couvert forestier, la compétition végétative, et l'âge des clones (Kudoh et al., 1999; Suvanto & Latva-Karjanmaa, 2005), ce qui laisse entrevoir une interaction entre les perturbations naturelles et la structure génétique des espèces clonales.

Un des problèmes importants lié au suivi de la variation génétique à travers le temps est la difficulté de trouver des peuplements qui représentent la structure génétique et clonale à des phases successionelles différentes. Lorsque le cycle de feu s'allonge, les peuplements âgés sont souvent éliminés ou dégradés à cause de la mortalité naturelle, ce qui rend la comparaison avec les jeunes peuplements difficile. En outre, chez les espèces clonales, l'âge du ramet est beaucoup plus court que celui du genet, ce qui rend plus difficile l'évaluation de la diversité génétique à l'origine. Présentement, les études génétiques révèlent des résultats mixtes quant à la conservation de la diversité génétique et génotypique: certaines rapportent une accumulation de la variabilité génétique avec l'âge de la population et une corrélation entre l'âge de la forêt et la richesse en allèles rares (Cronberg 2002; Comps et al., 2001); d'autres observent une diminution de la diversité génétique avec le temps et une diminution graduelle du nombre des genets (plantes vasculaires; Maddox et al., 1989 et mycètes; Dahlberg & Stenlid, 1990). La question du suivi de la structure génétique chez les espèces clonales nécessite alors une approche qui permet le suivi de la structure génétique et clonale (i.e. ramet et genet) des peuplements sur de longues échelles temporelles (Chapitres 1 et 2).

La variabilité génétique et clonale dans l'espace

La distribution spatiale de la diversité génétique chez les espèces qui se reproduisent par voie végétative est souvent liée au mode de croissance clonale qui a été classé entre 2 types: le type phalanx qui correspond à une croissance des ramets autour d'un ramet central de façon agglomérée, et le type guérilla qui se caractérise par une interdigitation des ramets des clones différents (Little & Dale, 1999; Cronberg 2002; Maddox et al., 1989). Dans le cas du tremble, les études réalisées jusqu'à présent ne nous permettent pas d'établir le patron de la distribution spatiale des clones. Ceci est principalement dû au fait que la plupart des études ont évalué la structure clonale du tremble en se basant sur des caractères morphologiques ou en utilisant des échelles d'échantillonnage qui ne permettent pas de statuer sur le patron de distribution des clones à petite échelle (< 100m) (Hyun *et al.*, 1987; Jelinksi & Cheliak, 1992; Lund *et al.*, 1992). Par ailleurs, l'effet des perturbations naturelles comme les feux et les trouées sur la distribution de cette diversité demeure inconnu. Seules quelques études

fournissent quelques indices indirects sur une telle relation. Par exemple, Stevens et al. (1999) suggèrent l'apparition d'une variation génétique et d'une structure géographique entre les populations de tremble issues de semis avec le temps en parallèle avec le processus de transition de peuplements issus de semis vers des peuplements issus de drageonnement. Barnes (1966) suggère que la fréquence des feux et la compétition inter clonale jouent un rôle important dans la détermination du degré d'entrecroisement entre les différents clones de tremble. Par conséquent, une évaluation de l'importance relative des perturbations naturelles et de l'échelle à laquelle ces processus pourraient exercer un effet sur la distribution spatiale de la diversité clonale du tremble s'avère nécessaire afin de mieux comprendre la dynamique de la structure clonale du tremble (Chapitre 3).

Capitaliser sur la diversité génétique du tremble

Depuis quelques décennies, on observe une augmentation significative de la demande commerciale pour le tremble, notamment pour la fabrication des panneaux à particule, des panneaux à fibres, et la décoration. Un des moyens pour répondre à cette demande consiste à augmenter la productivité des peuplements de tremble en favorisant la régénération des génotypes les plus prometteurs. Cependant, l'efficacité de cette stratégie demeure inconnue et se heurte à plusieurs obstacles tel que: (1) le manque d'information sur la répartition spatiale des clones de tremble à petites échelles, ce qui ne facilite pas la sélection des génotypes car les différents clones peuvent être étroitement entrecroisés (Wyman et al., 2003) ou avoir une grande similarité morphologique (Suvanto & Latva-Karjanmaa, 2005); 2) le manque d'information quant à l'effet des coupes partielles dans les peuplements de tremble et leur effet sur la régénération de certains génotypes; et (3) la grande variabilité génétique et clonale observée à l'intérieur des populations de tremble qui implique que le processus de sélection ne doit pas compromettre la diversité génétique de cette espèce à long terme. Jusqu'à présent, les coupes totales des peuplements de tremble demeurent la pratique sylvicole la plus répandue. Notre approche consistait à faire une évaluation de l'effet des perturbations naturelles sur la structure clonale du tremble à l'échelle du peuplement (trois premiers chapitres). Une meilleure compréhension des processus naturels nous permettront de mieux

adapter ces stratégies afin de concilier l'objectif de la préservation de la dynamique naturelle avec celui de l'augmentation du rendement ligneux dans les peuplements de tremble (Chapitre 4).

MÉTHODOLOGIE

Pour l'identification génétique, nous avons utilisé quatre marqueurs moléculaires très variables appelés microsatellites. Ces marqueurs ont été développés spécifiquement pour le tremble par Dayanandan et al. (1998). Dans le premier chapitre, nous avons sélectionné un échantillon de 30 arbres dans le même site, représentant trois cohortes successives qui se sont développées suite aux feux et aux perturbations secondaires. Ces cohortes se sont régénérées sur 180 ans après feu. Dans le deuxième chapitre, nous avons opté pour une approche de chronoséquence et choisi 7 sites ayant brûlé à différentes dates. Les échantillons ont été récoltés à des intervalles réguliers le long de transects de 300 m à 1200 m. Ces données ont été ensuite projetées pour construire un modèle qui simule la perte de genets en fonction du temps écoulé depuis le dernier feu ainsi qu'en fonction du cycle de feu à l'échelle du peuplement et du paysage. Dans le troisième chapitre, une cartographie complète des trembles présents dans deux hectares séparés a été effectuée afin d'analyser la distribution spatiale de la diversité génétique et clonale du tremble à l'échelle du peuplement. Finalement dans le quatrième chapitre, nous avons utilisé un dispositif expérimental établi à la forêt d'enseignement et de recherche du lac Duparquet (FERLD) pour comparer l'effet de différents traitements sylvicoles sur la diversité clonale du tremble. Ces traitements incluaient un témoin, une coupe totale, et une coupe 1/3 avec sélection des tiges à bonne croissance. Les échantillons ont été sélectionnés selon deux stratégies: une à l'échelle des parcelles de 20 m x 20 m, et l'autre en échantillonnant le long de transects de 40 m dans chaque traitement.

OBJECTIFS ET APPROCHE EXPÉRIMENTALE DE L' ÉTUDE

L'objectif général de cette thèse vise à mieux comprendre la structure génétique et clonale du tremble suite aux perturbations naturelles et de la comparer avec celle obtenue suite à des coupes de différentes intensités. Pour le faire, nous avons bénéficié de sites localisés à la FERLD où l'historique des perturbations naturelles est très documenté et où un dispositif expérimental de différentes coupes a été établi en 1999. Les chapitres 1, 2, et 3 de la thèse ont été publiés sous forme d'articles dans des revues avec comité de lecture. Le quatrième est présentement en évaluation.

Chapitre 1: MC Namroud, F Tremblay, Y Bergeron (2005). Temporal variation in quaking. Aspen (*Populus tremuloïdes*) genetic and clonal structures in the mixedwood boreal forest of eastern Canada. Ecoscience 12, 82-91.

Dans ce chapitre, nous avons suivi l'évolution de la structure génétique et clonale du tremble avec le temps depuis le feu à travers trois cohortes issues de perturbations majeures (feu) et secondaires (trouées) au niveau du peuplement. MC. Namroud a fait l'échantillonnage sur le terrain, les analyses génétiques en laboratoire, les analyses statistiques et a rédigé le manuscrit. Dr. Tremblay et Dr. Bergeron ont contribué à la définiton de la problématique de recherche, de l'approche expérimentale et à la rédaction du manuscrit.

Chapitre 2: MC Namroud, A Leduc, F Tremblay, Y Bergeron (2005). Simulations of clonal species genotypic diversity - trembling aspen (*Populus tremuloïdes*) as a case study. Genetics Conservation, sous presse.

Dans ce chapitre, nous avons tenté de construire un modèle qui permet de suivre la structure clonale du tremble sur de longues échelles temporelles (500 ans) et sous des cycles de feu différents au niveau du peuplement et du paysage. Ces observations ont été ensuite comparées avec celles obtenues sous différentes rotations forestières. MC. Namroud a fait l'échantillonnage sur le terrain, les analyses génétiques en laboratoire, les analyses statistiques, les simulations, et a rédigé le manuscrit. Dr. Tremblay et Dr. Bergeron ont contribué à la définition de la problématique de recherche et de l'approche expérimentale

ainsi qu'à la rédaction du manuscrit. Dr. Leduc a contribué à la construction des modèles de simulation et à la correction du texte.

Chapitre 3: MC Namroud, A Park, F Tremblay, Y Bergeron (2005). Clonal and spatial genetic structure of aspen (*Populus tremuloïdes* Michx.). Molecular Ecology, 14: 2969- 2980.

Dans ce chapitre, nous avons évalué la distribution de la diversité génétique et clonale du tremble à petites échelles spatiales (1 ha), et ceci en fonction des perturbations naturelles et de la distribution spatiale des cohortes de tremble. Nous avons également dressé un portrait de la taille et de la distribution spatiale des clones de tremble. MC Namroud a fait l'échantillonnage sur le terrain, une partie des analyses génétiques en laboratoire, les analyses spatiales et statistiques et a rédigé le manuscrit. Dr. Tremblay et Dr. Bergeron ont contribué à la définition de la problématique de recherche et de l'approche expérimentale ainsi qu'à la rédaction du manuscrit. Dr. Park a contribué aux analyses spatiales et à la correction du texte du manuscrit.

Chapitre 4: MC Namroud, F Tremblay, Y Bergeron. Effect of clear and selective one-third partial cutting on aspen genotypic diversity in Quebec's boreal forest. À soumettre à Forest Ecology and Management.

Dans ce chapitre, nous avons évalué l'effet des coupes totales et des coupes partielles sélectives sur la régénération clonale du tremble afin de déterminer la meilleure stratégie capable de capitaliser sur la diversité de cette espèce. MC Namroud a fait une partie de l'échantillonnage sur le terrain, les analyses statistiques et a rédigé le manuscrit. Dr. Tremblay et Dr. Bergeron ont contribué à la définition de la problématique de recherche et de l'approche expérimentale ainsi qu'à la rédaction du manuscrit.

CHAPITRE I

TEMPORAL VARIATION IN TREMBLING ASPEN (*POPULUS TREMULOÏDES*) GENETIC AND CLONAL STRUCTURES IN THE MIXEDWOOD BOREAL FOREST OF EASTERN CANADA

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1.1 RÉSUMÉ

Deux sites de la forêt boréale mixte du Québec, ayant brûlé respectivement 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 à l'aide de la dendrochronologie. Une trentaine d'arbres ont été choisis aléatoirement dans chaque cohorte pour le génotypage avec quatre loci microsatellitaires. Les premières cohortes se sont installées suite au passage du feu, alors que les deuxièmes et troisièmes cohortes se sont régénérées dans les trouées crées par la mortalité des peupliers établis immédiatement après feu et par les conifères affectés par l'épidémie de tordeuses de bourgeons de l'épinette. L'hétérozygotie 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 se retrouve à l'intérieur des cohortes, alors qu'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 dans trois cohortes successives, ce qui indique une faible sélection des genets spécifiques susceptibles de dominer les peuplements de peupliers avec le temps. La diversité génétique et clonale varient peu entre les cohortes provenant des feux et celles issues de trouées. La perte de la dominance apicale a probablement favorisé la production de drageons chez les genets qui existaient dans les cohortes établies immédiatement après feu mais qui ont été ensuite éliminés par mortalité naturelle.

1.2 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 postfire 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. The release of apical dominance might have favoured the suckering of genets that existed in the post fire cohorts and that were later eliminated by natural mortality.

1.3 INTRODUCTION

Temporal variation in population genetic and clonal diversity is a complex phenomenon that is strongly influenced by the timing of initial seedling recruitment (Eriksson, 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 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 disturbances history affect this recruitment.

Trembling aspen (*Populus tremuloïdes*) 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 Kilometers (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 abundant source of water during seed germination are rarely met (McDonough, 1979; Mitton & Grant, 1996), and also because suckers generally outcompete seedlings. Some even asserted that broad scale seedling establishment has not occurred since the last glaciation, 10,000 years ago in the Western United States (Einsphar & Winton, 1976), while others suggested only "windows of opportunities" 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 *et al.,* 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 *et al.,* 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. They advocated that the development of canopy gaps before aspen stands reach maturity (as early as 40 y after stand initiation), allow these stands to evolve towards an uneven-age structure and develop into self-maintaining old growth stands (Cumming *et al.,* 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 *et al.,* 2000). A partial opening in the canopy may result in restraining sucker growth as apical dominance of the intact stems is maintained, and as 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 *et al.*, 1987; Jelinksi & Cheliak, 1992; Lund *et al.*, 1992; Stevens *et al.,* 1999, Wyman *et al.,* 2003), but its change 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 we demonstrated recently by using molecular markers that the number of genets sampled in aspen stands in northwestern Quebec could be quite high, thus reflecting the

presence of many small clones (Wyman *et al.*, 2003). 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 they were performed in different populations at different geographical locations. One way to overcome this problem is by using 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 change with time of aspen genetic and clonal structures across several cohorts. More specifically, our aims were 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 disturbances (fire, insect outbreak, windthrow, or death of large aspen) on aspen genet and ramet dynamics with time.

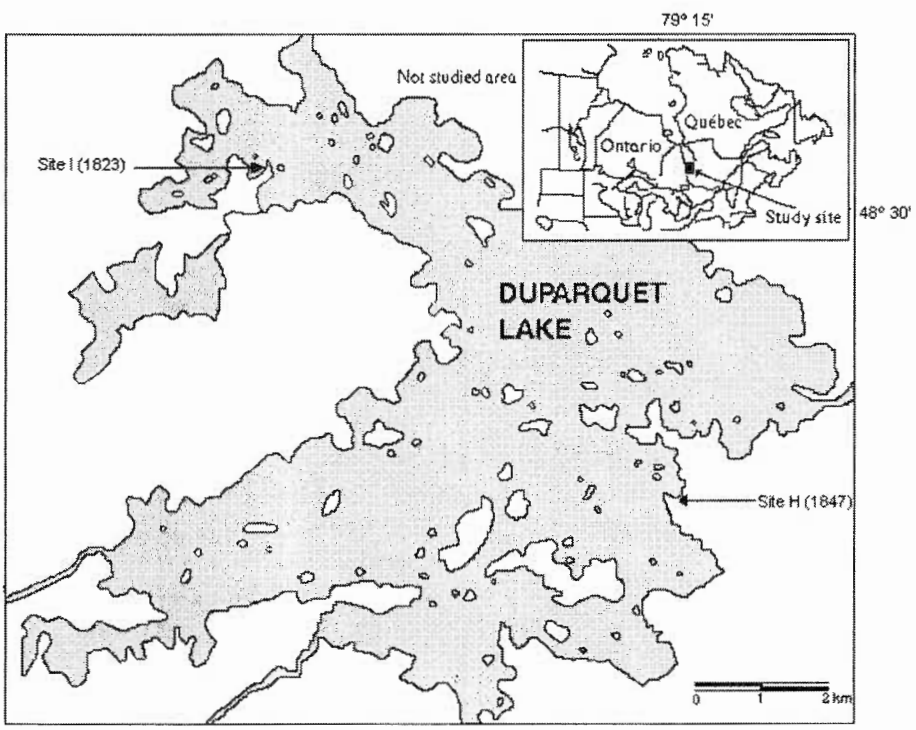
1.4 MATERIALS AND METHODS

1.4.1 Study area

The study sites were located in Lake duparquet Research and Teaching Forest (LDRTF) in northwestern Quebec (79°1'W-48°30'N) (Figure 1.1). 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 Quebec 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 (Anonymous, 1982). Vegetation types vary in relation to soil deposits and successional stages (Bergeron & Dubuc, 1989). Young successional stages (<100 y) are dominated by

trembling aspen (*Populus tremuloïdes*), intermediate stages (100-200 y) 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, 2000).

Figure 1.1 Map of Lake Duparquet (Canada) showing the location of the two study sites H and I and their respective fire dates 1847 and 1823



1.4.2 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 per hectare in plots H and I, respectively. Diameter at breast height 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 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 about 30 trees that were randomly distributed in each plot for genetic analysis. 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 given to collect the samples at the base of the selected stem, and only the cambium tissue was used for DNA extraction.

1.4.3 DNA extraction and amplification

Samples were ground and genomic DNA was extracted using Doyle and Doyle standard procedure (Doyle & Doyle, 1987) with minor 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, California). Individuals were genotyped at four microsatellite loci, PTR1, PTR2, PTR3 and PTR4 as described in Dayanandan *et al.* (1998) (Table 1.1). Amplification was carried out using a 96-Well GeneAmp® PCR System 9700, from Applied Biosystems (California, USA) in a total volume of 25 µl containing 0.4 ng/µl of DNA, 0.5 pmoles/µl of primers, 0.2mM dNTP, 2.5 mM MgCl2 and 15mM 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. Each cycle lasted about 14 min and 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 ice. Amplified DNA was then analyzed using Gene Scan Software and ABI Prism 310 Genetic Analyzer from Applied Biosystems.

Table 1.1. Repeat pattern, primer sequence, annealing temperature (T), for four SSR loci in *Populus tremuloïdes* (from Dayanandan *et al.*, 1998)

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

1.4.4 Data analysis

Genetic diversity was calculated using four parameters: percentage of polymorphic loci, the mean number of alleles per locus, observed heterozygosity (Ho) and expected heterozygosity (He). Allelic frequencies were estimated using an expectation-Maximization (EM) algorithm with 5000 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 (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 difference between two alleles which is an analogue of Slatkin's R_{ST} (1995) 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 analysis were performed using Arlequin version 2.000, specialized software for population genetics (Schneider *et al.,* 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 $D = 1-\Sigma \{[n_i (n_i - 1)] / [N (N -1)]$ for corrected small samples, where n_i = number of ramets of the ith genet (Pielou, 1969). The number of genets per cohort and the number of ramets per genet were also numbered at each site.

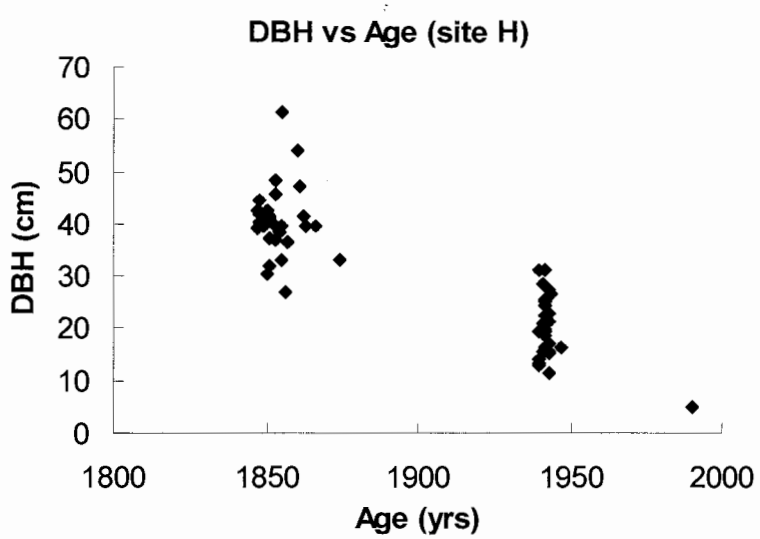
1.5 RESULTS

1.5.1 Age structure

In general, the dbh classes used to randomly select trees from each cohort were consistent with the results obtained by core dating (Figures 1.2 a,b). 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 fire of 1847 and 1823 at site H and I, respectively (Figures 1.3a, 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 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 1.3a), and from 31 to 65 y at site I (Figure 1.3b). 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.

Figure 1.2 Diameter at breast height (DBH) vs Age in site H (a) and I (b)

(a)



(b)

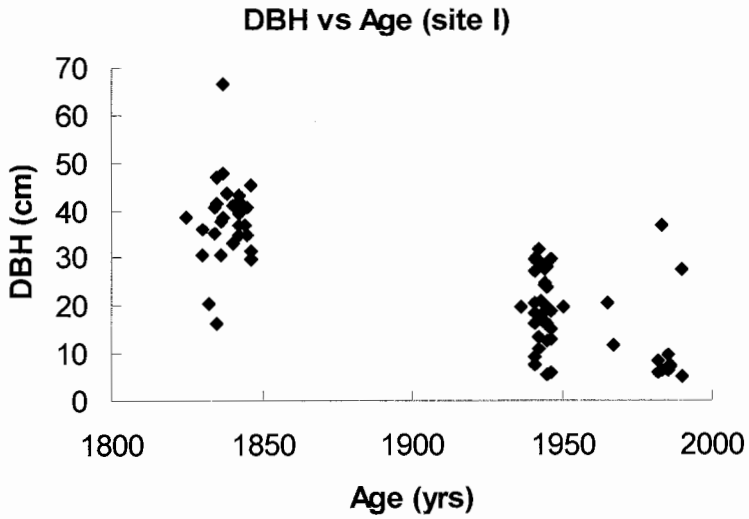
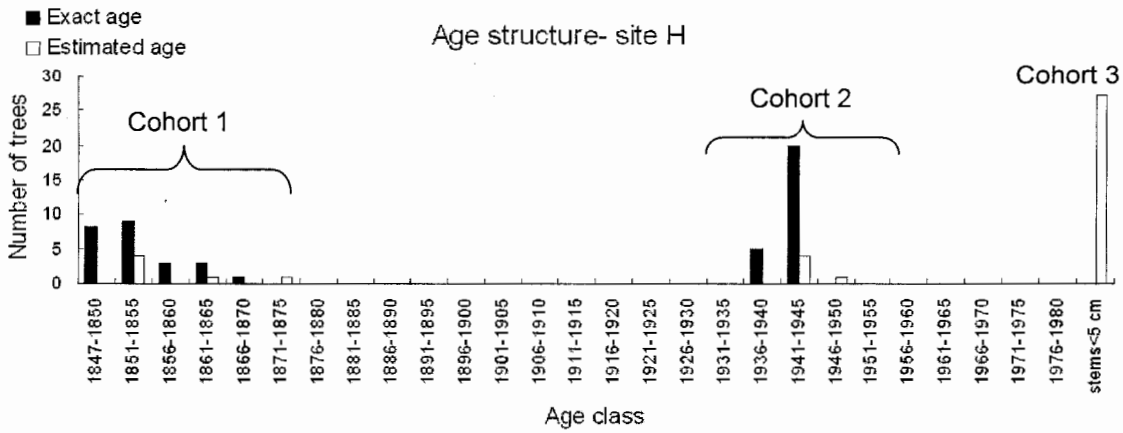


Figure 1.3. Aspen distribution per age class and per cohort (exact age in black; estimated age in white)

a)



b)

1.5.2 Genetic diversity

We detected a total of 49 different alleles over the four loci in the 168 samples analyzed. Although all loci were polymorphic, most alleles were shared between two cohorts but only few were shared by three cohorts: 18 at site H (1847) and 12 at site I (1823). When considering each locus separately, the number of alleles per locus 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 1.4). 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 1.2). 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 1.2 a), and from 0.37 to 0.64 at site I (Table 1.2 b). 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 = 0.000), while the pattern was variable for other loci and cohorts (Tables 1.2 a,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 1.3). Fixation indices were 0.02 and 0.03 revealing an extremely low differentiation at both sites between cohorts.

Figure 1.4. Average allele size frequencies and standard deviations per locus per cohort; Allele size is measured by the number of base pairs (bp); (a) locus1; (b) locus 2; (c) locus 3; (d) locus 4

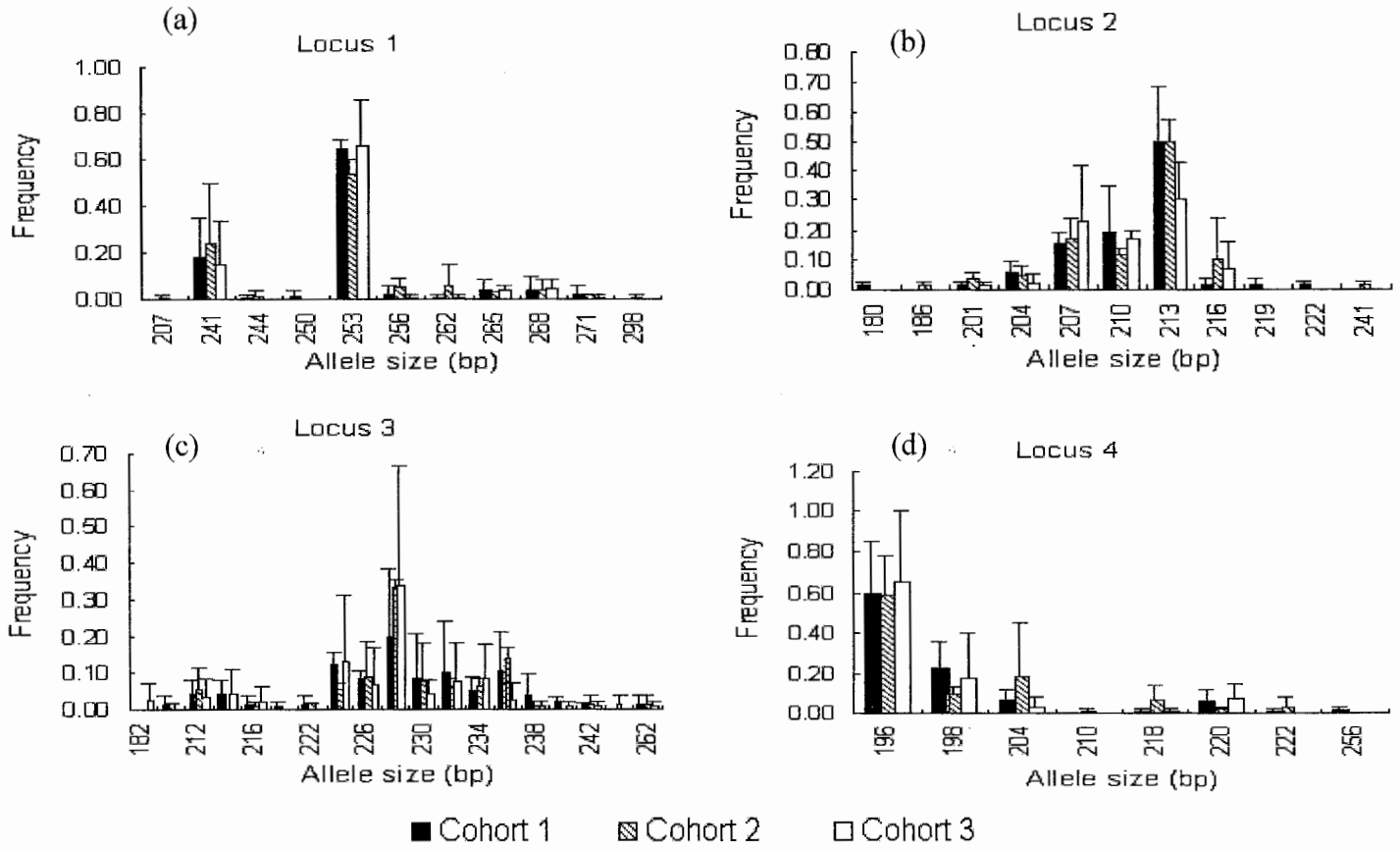


Table 1.2. Observed and expected heterozygosity per locus and per cohort at: (a) site H and (b) site I; Ho = observed heterozygosity; He = expected heterozygosity; Numbers between parentheses indicate the standard variations of heterozygosity; P is the probability that allele frequencies at a specific locus are in Hardy-Weinberg equilibrium.

(a)

Site H (1847)	**Cohort 1**			**Cohort 2**			**Cohort 3**			**Mean Ho/He**
Allele/locus	Ho	He	P	Ho	He	P	Ho	He	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 Ho/He**
Allele/locus	Ho	He	P	Ho	He	P	Ho	He	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**	**0.37 (0.28)**		

Table 1.3. Percentage of variation and fixation indices based on molecular and allelic variance analysis between and within cohorts at site H and I; * $P < 0.0025$.

	Site H (1847)		**Site I (1823)**	
Source of variation	Percentage of Molecular variation	Percentage of allelic variation	Percentage of Molecular variation	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*

1.5.3 Genotypic diversity

Genotypic diversity was very high at both sites and also did not change between cohorts. At site H (Table 1.4 a), 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 1.4 b), 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 (1847) and 62 at site I (1823) (Tables 1.4 a,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 1.5), but no consistent pattern was observed as to their regeneration for three successive cohorts (Table 1.5). At site H, the biggest clone contained three ramets, whereas at site I, one clone contained 10 ramets that suckered in the first and second cohort (Table 1.5).

Table 1.4. 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 > one ramet (clones)**	**Genet =one ramet**	**% Genet = 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 > one ramet (clones)**	**Genet = one ramet**	**% Genet = 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 1.5. Number of ramets per clone and per cohort observed in 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/cohort	5	5	8		10	11	5
clone/cohort	4	4	6		4	6	1

1.6 DISCUSSION

1.6.1 Aspen dynamics

The three cohorts identified at each site are congruent with the results of previous ecological studies that described aspen dynamics in Quebec'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 would allow the suckering of new aspen cohorts. These gap disturbance cohorts will regenerate even though shade tolerant species become progressively more abundant in the canopy (Bergeron & Dubuc, 1989). The 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 1940s-

1950s also enhanced this recruitment (Bergeron & Charron, 1994). At our sites, core dating indicated that this has 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 *et al.,* 1993). At this stage, outbreaks would not bring succession back to the deciduous stage dominated by hardwood species. On contrary, 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.

1.6.2 Genetic and genotypic diversity

The high levels of polymorphism (100%) observed in trembling aspen are comparable with values reported in the literature (Ellstrand & Roose, 1987; Liu & Fournier, 1993), and reflect the highly diverse genetic pool of aspen in Quebec'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 *et al.*, 2003), but exceed by two to three times the values reported in other studies using isozymes or RAPD (Hyun *et al.*, 1987; Jelinksi & Cheliak, 1992; Lund *et al.*, 1992; Yeh *et al.*, 1995; Stevens *et al.,* 1999). This was not unexpected since microsatellites are usually more variable than other markers as their mutation rate is estimated between 10^{-2} and 10^{-6} (Scrosati, 2002). No significant 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.

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 *et al.* (2003) who observed 11 aspen genotypes per 15 samples in Quebec's boreal forest. The general prediction about the decline in genotypic diversity in clonal species is therefore not supported in aspen.

1.6.3 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. Both genotypic diversity and the percentage of unique genets in postfire cohorts were not 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 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 some grass species are usually sufficient to form an insulating layer and inhibit aspen seedlings 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, and these 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 disturbances 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 65y), but favored the suckering of genets whose ramets have been eliminated from the post fire cohorts and whose apical dominance was removed. This was 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.

1.7 CONCLUSION

The results obtained in this study revealed 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 vs gap disturbances) does not allow to predict aspen genetic and clonal structure as these were 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 (>130y) that prevails in the mixedwood boreal forest in northwestern Quebec, in addition to the moist soil conditions that favour sexual reproduction immediately after fire, contribute 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 *et al.,* 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. The use of morphological characteristics or sampling in distant populations (several Kilometers) rather than molecular markers at a local scale might not reflect all genotypic variability present within aspen stands. This however remains to be examined.

1.8 ACKNOWLEDGEMENTS

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CHAPITRE II

SIMULATIONS OF CLONAL SPECIES GENOTYPIC DIVERSITY - TREMBLING ASPEN (*POPULUS TREMULOÏDES*) AS A CASE STUDY

Article sous presse dans Conservation Genetics

2.1 RÉSUMÉ

Deux modèles ont été construits afin de suivre la diversité génotypique (*G/N*) des espèces clonales sur de longues périodes de temps à l'échelle du peuplement ainsi qu'à l'échelle du paysage. Les modèles ont été ensuite validés avec des données empiriques provenant de peuplements de peuplier faux-tremble (*Populus tremuloides*) dans la forêt boréale du Québec. Les données ont été récoltées en utilisant une approche de chronoséquence dans 7 sites ayant brûlé en 1717, 1760, 1797, 1823, 1847, 1944, et 1916. L'identification génétique a été faite à l'aide de quatre marqueurs microsatellitaires. À l'échelle du peuplement, les simulations ont été répétées avec des genets composés de 5, 25, 50 et 100 ramets chaque. À l'échelle du paysage, nous avons simulé le taux cumulé de survie des genets sous différents cycles de feux (de 5 à 500 années) pour 500 ans après feu. Les simulations à l'échelle du peuplement indiquent que la mortalité des ramets parmi les genets plutôt que celle des genets explique l'augmentation du *G/N* avec le temps écoulé depuis le dernier feu. La taille initiale des genets et leur drageonnement périodique (ou recrutement des ramets) jouent un rôle important dans le maintien d'un niveau élevé du *G/N* quoique inférieur à un. À l'échelle du paysage, la perte de genets augmente avec l'élongation du cycle de feu. En forêt boréale, les pratiques d'aménagement équienne à courte rotation semblent favoriser un taux de survie de genets similaire à celui observé avec le régime de succession naturelle.

2.2 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 7 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 to 500 years) for 500 years after fire. Stand simulations indicate 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.

2.3 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. 2005), 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 retains 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 (Donough 1997, Mitton and Grant 1980). 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. 2005). 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.

2.4 MATERIALS AND METHODS

2.4.1 Study area

The study sites were located in the Lake Duparquet Research and Teaching Forest (LDRTF) in northwestern Quebec (79°1' W-48°30') (Figure 2.1). 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

1,062 m and the number of samples ranged from 19 to 28 (Table 2.1). 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 (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 2.1).

Figure 2.1. Location of the seven sampling sites in Quebec's boreal forest

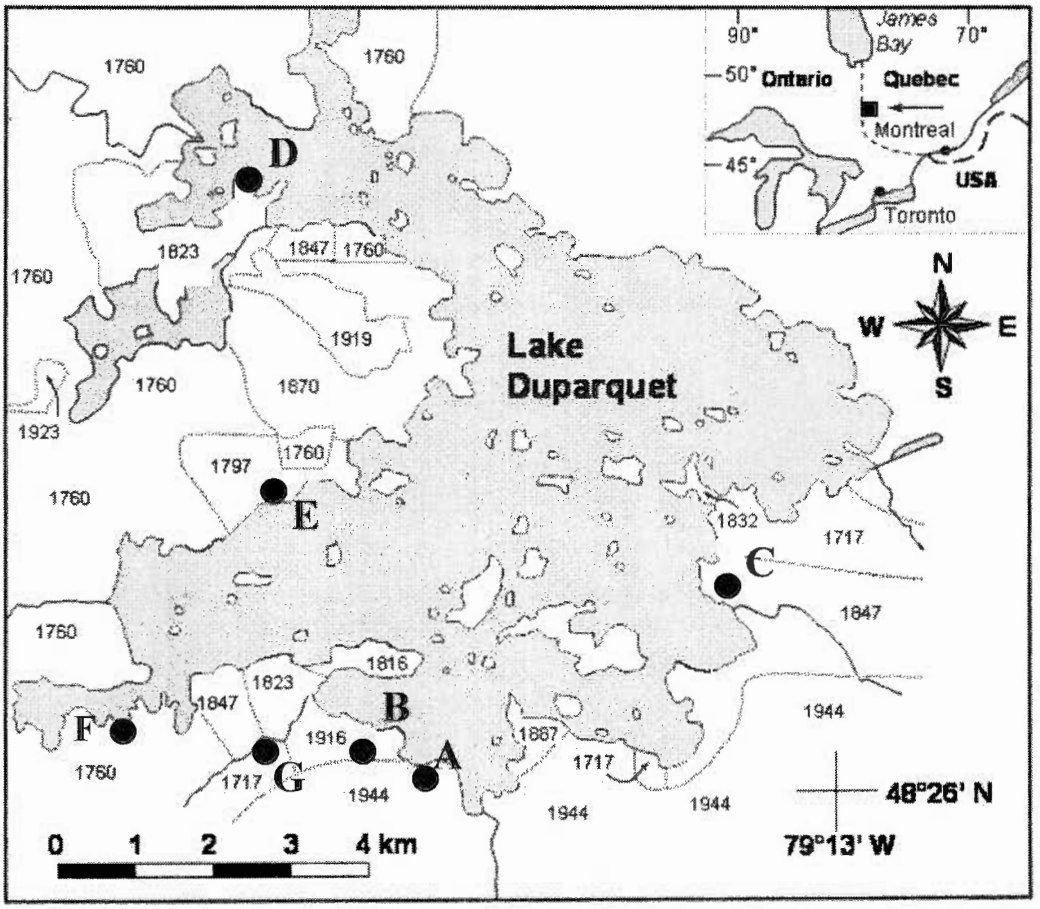


Table 2.1. Ecological characteristics of the sampling sites. The age of the stand is given in years (yrs), the transect length is measured in meters (m), density is expressed by the number of trees (nb) per hectare (ha), global density and global basal area are the density and basal area of all trees in the stand, respectively. Global basal area is expressed by the surface area (m^2) per hectare.

	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

2.4.2 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., 2005) 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: 4µl 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. Each cycle lasted about 14 minutes. At the end of each cycle, we added a final step of 72°C for 7 minutes 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).

2.4.3 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 one when each tree has a unique mutlilocus genotype. Genotypic diversity was also measured with 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 $D = 1\text{-}\Sigma \{[n_i (n_i - 1)] / [N (N-1)]$, 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) 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).

2.4.4 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 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).

$$CSR_t = D_t / 30{,}000 \quad (2)$$

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 five years. In the first year after fire, we assumed that the 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.

2.4.5 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 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 (equal to the average *G/N* over the 7 sites) 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:

$$GSR_t = G_t/G_{t-5} \quad (4)$$

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:

$$CA_t = e^{(-FC/t)} \qquad (5)$$

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:

$$NCA_t = CA_{(t-5)} - CA_t \qquad (6)$$

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_I 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.

2.4.6 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).

2.5 RESULTS

2.5.1 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 (±0.05) over the 7 sites. Expected heterozygosity was slightly higher and ranged from 0.55 to 0.69. It averaged 0.65 (±0.05). *G/N* ranged from 0.75 to 0.92, and averaged 0.83 (± 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 the 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% to 92%) single-ramet (Table 2.2).

Table 2.2. Genetic characteristics of the sampling sites. *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.

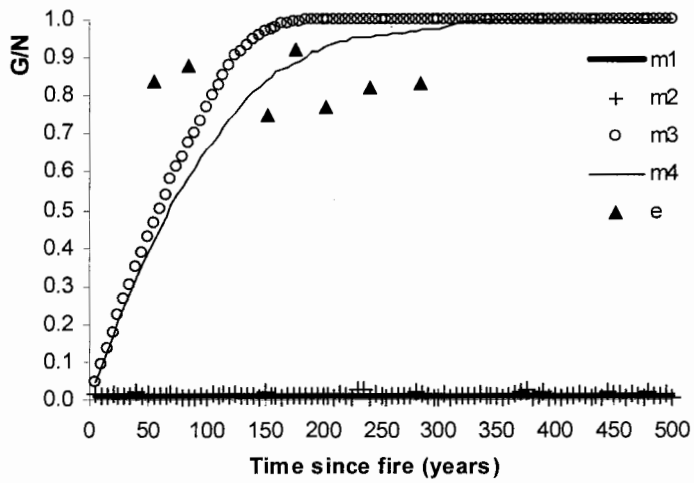
	A	**B**	**C**	**D**	**E**	**F**	**G**
Ho (SE)	0.61 (0.20)	0.53 (0.33)	0.64 (0.15)	0.51 (0.07)	0.54 (0.14)	0.55 (0.12)	0.54 (0.12)
He (SE)	0.67 (0.11)	0.55 (0.31)	0.64 (0.16)	0.66 (0.08)	0.66 (0.21)	0.69 (0.14)	0.68 (0.15)
Average number of alleles per locus	6.25	5.75	6.50	6.25	7.50	6.25	6.00
D	0.982	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

2.5.2 Stand model

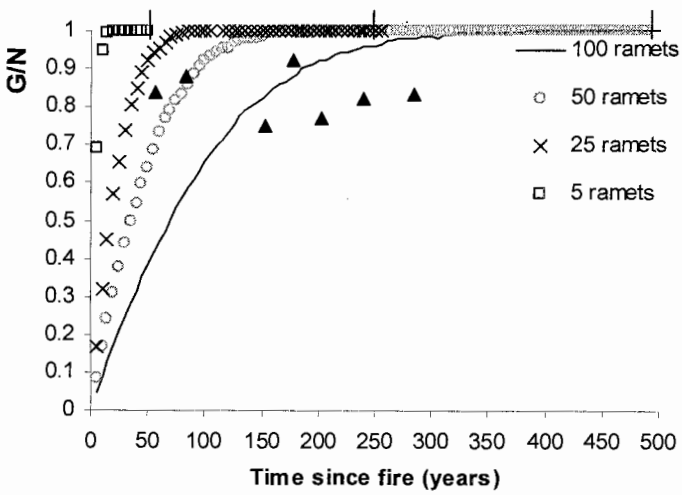
None of the four scenarios simulated at the stand level was completely congruent with the empirical data in our sampling sites. In the first two scenarios that simulated genet mortality, *G/N* maintained a constant value ranging from 0.01 to 0.20 depending on the original genet size. In the third and fourth scenarios that simulated ramet mortality, *G/N* increased gradually over time until it reached a maximum value of one, between 10 to 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 2.2a 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 2.2b). 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 five years after fire (Figure 2.2c).

Figure 2.2. 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.

(a)



(b)



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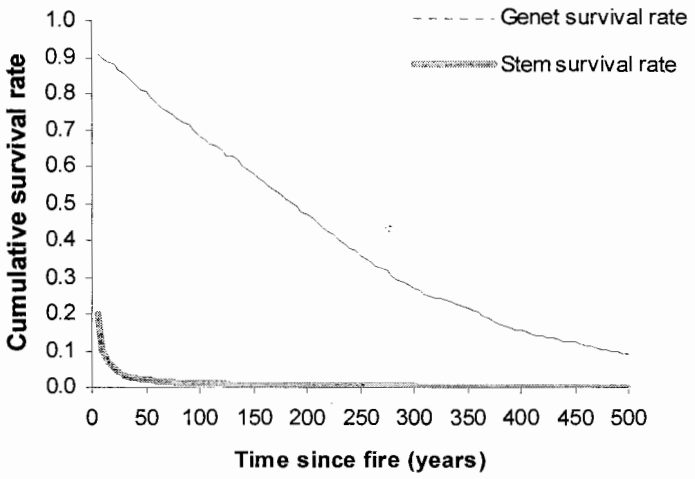


2.5.3 Landscape model

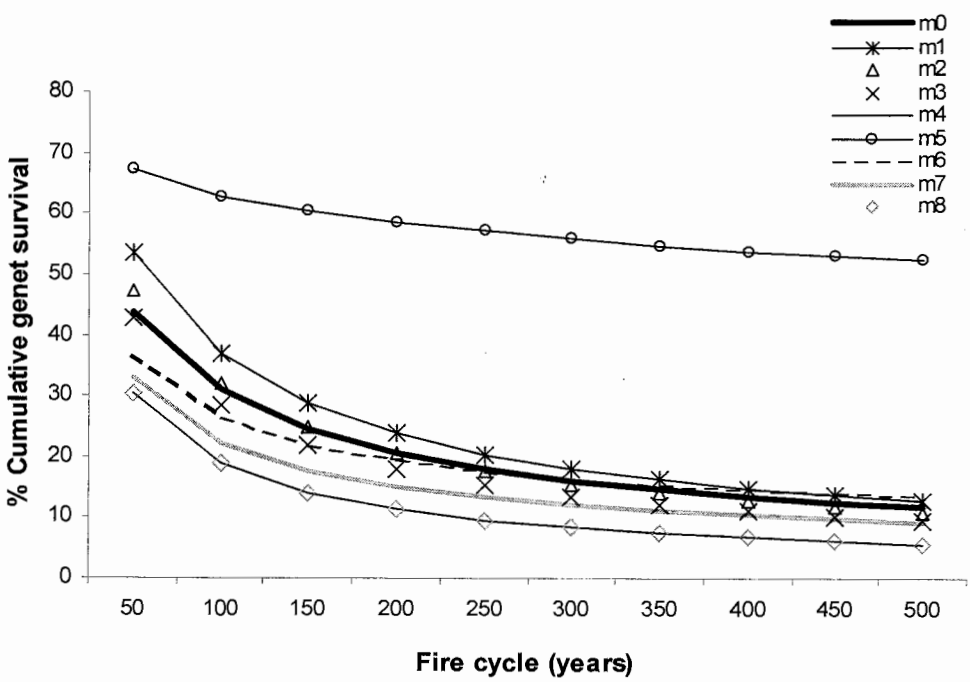
Simulations at the landscape level revealed important differences in genet survival under different fire cycles. The genet survival rate decreased when fire events became rare (i.e. under long fire cycles). It ranged from 11.7% under the longest fire cycle (500 years) to 43.6% under the shortest one (50 years) when assuming initial values of *G/N* and *T* equal to empirical values (Figure 2.3a(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 2.3a (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 2.3a (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 2.3b). Under a natural disturbance regime with a 100-year fire cycle, 31% of the genets were maintained in the landscape, while 35% survived under a 100-year rotation (Figure 2.3b).

Figure 2.3. 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.

(a)



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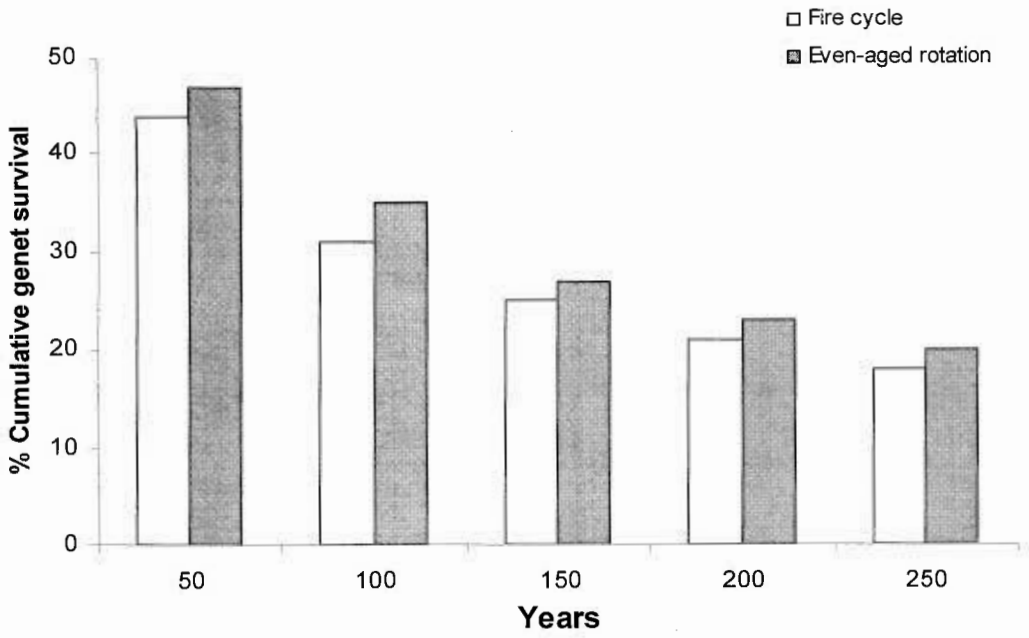

2.6 DISCUSSION

2.6.1 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 yr 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 to 65 years old stands, and with our previous observations following three cohorts based on a stand scale approach (Namroud et al. 2005). 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.

2.6.2 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 the 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 2.2c). 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 2.2c), 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 2.2), and after three successive cohorts in a previous local study (Namroud et al. 2005) makes 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.

2.6.3 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 2.3a) when the initial genotypic diversity fluctuates (low vs high *G/N* after fire; Figure 2.3a) 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. 2005). Under long fire cycles, sexual recruitment may become necessary to maintain aspen genotypic diversity at the landscape level.

2.7 ACKNOWLEDGEMENTS

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CHAPTER III

CLONAL AND SPATIAL GENETIC STRUCTURES OF ASPEN (*POPULUS TREMULOÏDES* MICHX.)

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3.1 RÉSUMÉ

Afin de suivre la distribution spatiale de la diversité génétique et clonale du tremble, nous avons cartographié et identifié le génotype de tous les arbres de tremble dans deux hectares. Chaque hectare comprenait trois cohortes de tremble qui se sont développées suite au passage du feu ou à des perturbations secondaires subséquentes. Nous avons utilisé quatre marqueurs satellitaires pour identifier les clones de tremble et une analyse dendrochonologique pour déterminer l'âge de chaque arbre. Les dimensions des clones ont été mesurées par la distance maximale entre deux ramets et le nombre de ramets par genet. Une analyse SND (standard deviation analysis) a été utilisée pour déterminer la distribution spatiale des genets et des clones de tremble, alors que des analyses multivariées d'autocorrélation génétique ont été utilisées pour déterminer la distribution spatiale de la diversité génétique. La plupart des genets de tremble sont formés d'un seul ramet (> 75%). La médiane de la taille des clones se situe entre 19 et 29 m (maxima de 74 et 102 m dans les deux sites). Aucune ségrégation spatiale n'a été observée entre les différents clones. Cependant, une ségrégation a été observée entre les différentes cohortes et une agrégation a été détectée entre les arbres des cohortes les plus âgées et de mi-succession. Les arbres dans les cohortes les plus jeunes avaient une distribution plutôt aléatoire. Ces patrons coïncident avec une autocorrélation génétique à petites échelles dans les cohortes les plus âgées et une distribution plus aléatoire dans les cohortes les plus jeunes. Nos résultats suggèrent que la distribution spatiale de la diversité génétique reflète les patrons crées par le recrutement des cohortes à différents stades de la succession. La reproduction asexuée semble être à l'origine de la structure génétique spatiale à petites échelles, au moins jusqu'à un stade de mi-succession. Cependant avec le vieillissement du peuplement, la distribution spatiale des arbres de tremble et leur diversité génétique évoluent vers une structure spatiale plus aléatoire contrôlée par un régime de perturbations par trouées.

3.2 Abstract

To portray aspen clonal and spatial genetic structure, we mapped and genotyped all trees in two one hectare plots, each containing three aspen cohorts originating from fire or subsequent secondary disturbances. We used four microsatellite loci to identify aspen clones and increment core analysis to determine tree age. Clonal dimensions were measured by the maximum distance between two ramets and the number of ramets per genet. Standard normal deviate (SND) was used to assess the spatial distribution of aspen genets and cohorts, and multivariate spatial genetic autocorrelations to assess the spatial distribution of aspen genetic variation. Most aspen genets consisted of only one ramet (> 75%). Median clonal dimensions were 19 m and 29 m (maxima: 104 m and 72 m in the two plots). No segregation was observed between clones. Aspen cohorts were spatially segregated but trees were spatially aggregated within old and medium-aged cohorts. In contrast, trees were more randomly distributed within the youngest cohort. This coincided with a spatial genetic autocorrelation at small scales (up to 30 m) in the older cohorts and a more random genetic distribution in the youngest ones. Our results suggest that aspen spatial genetic structuring reflects the spatial patterns produced by the regeneration of discrete cohorts at different stages of succession. Vegetative reproduction leads to aspen genetic spatial structuring at small scales (few meters) until mid succession. However, as the stand gets older, the spatial distribution of aspen trees and their genetic structure evolve from a structured pattern in to a more random one under a gap disturbances regime.

3.3 INTRODUCTION

In many clonal plant species, the spatial distribution of genetic variation is related to their growth forms (Birch, 2002; Kreher *et al.*, 2000). These are usually described in terms of the spatial patterns of genets (a single genetic individual, also called a clone) and ramets (individuals having the same genotype, thus belonging to the same genet). They are categorized to two extremes: the guerrilla (loose clustering of ramets) and the phalanx (distinct compact clumps of ramets), with most species having a growth form that falls between the two (Cheplik, 1997). Nonetheless, the growth forms of genets may also reflect processes that progress over time such as the processes of genet intermingling or exclosure (Barnes, 1966; Little & Dale, 1999). Under genet exclosure, old plant populations are expected to exhibit considerable local spatial genetic structuring (Epperson, 1993), even if the stand founder events were, originally, a non-structured random sample stemming from seed dispersal (Epperson, 1990). This, however, remains to be confirmed as some reports suggest that clonal growth does not necessarily lead to pronounced spatial genetic structuring in established populations (Chung & Epperson, 2000; Chung *et al.*, 2000; Erickson & Hamrick, 2003). Disturbances type, e.g. canopy openings, can also alter the effect of clonal growth and subsequently influence the spatial distribution of genetic variation in populations over time. Ramet distribution and clone size vary with disturbance frequency in herbs and angiosperms, (Kudoh *et al.*, 1999; Hämmerli & Reusch, 2003), but to date, this has not been investigated in clonal tree species. Understanding the interaction between the ecological and demographic processes that affect a species' spatial genetic structure over time will provide a tool for conservation biologists and foresters to choose management measures that will best capture and maintain the species genetic variation.

In this paper, we follow the spatial genetic structure of trembling aspen (*Populus tremuloïdes* Michx) during succession. Trembling aspen is an ecologically and commercially important tree of the Canadian boreal forest. It is a clonal species in which episodes of sexual reproduction are thought to be rare, and whose regeneration is closely related to disturbance dynamics (Mitton & Grant, 1996; Bergeron, 2000). Sexual regeneration from seed is scarce because the environmental conditions required for recruitment are rarely met (Mitton &

Grant, 1996; Romme *et al.,* 1997). Aspen suckering is stimulated by stand-replacing wild fires (here called primary disturbances) throughout most of its range (Mitton & Grant, 1996). In the absence of fire, natural senescence, insect outbreaks, and windthrow generate smaller (here called secondary) disturbances within stands (Bergeron & Dubuc, 1989; Bergeron & Charron, 1994; Bergeron, 2000; Cumming *et al.,* 2000). These lead to the recruitment of new but smaller aspen cohorts in older forests (Bergeron, 2000).

Aspen clones have been reported to cover large areas. The most spectacular example is a clone covering 43 hectare and containing more than 47,000 ramets (Kemperman & Barnes, 1976). In this case, estimation of clone size was based on morphological and phenological characteristics. In contrast, Yeh *et al.* (1995) suggested that a clone of that size is rare. Their RAPD-study showed that spatially aggregated aspen clones hardly exceed four hectares. Moreover, Wyman *et al.* (2003), who used microsatellites, reported high genetic diversity in young aspen populations, with most clones being composed of a single stem, and genets being intermingled within a few meters.

The goal of this study was to describe aspen genet ramet demography at the stand level (one hectare), by following the changes in the spatial distribution of aspen genets and cohorts after stand-initiating fire and mid-successional insect-mediated disturbances in Quebec's boreal forest. We mapped and genotyped all aspen trees on two 1 ha plots. Each plot contained three aspen cohorts originating from temporally distinct natural disturbances. These were stand-replacing fires in the 19th century (Dansereau & Bergeron, 1993), stand break-up in the mid 1940s enhanced by outbreaks of tent caterpillar (*Malacoma disstria;* Bergeron, 2000; Bergeron & Charron, 1994), and a regional spruce budworm (*Choristoneura fumiferana* (Clem.)) outbreak that affected balsam fir (*Abies balsamea* (L.) Mill) between 1970 and 1987 (Morin *et al.,* 1993). We hypothesised that (1) aspen genets within cohorts will be composed of few ramets and will be distributed at random, (2) aspen cohorts originating from different natural disturbances will be spatially segregated due to the spatial and temporal separation of sequential disturbances, and will be more aggregated at short distances within cohorts originating from local secondary disturbances, and (3) aspen genetic

variation based on allelic frequencies will be higher in cohorts originating from local secondary disturbances due to limited aspen suckering within canopy gaps.

3.4 MATERIALS AND METHODS

3.4.1 Study area

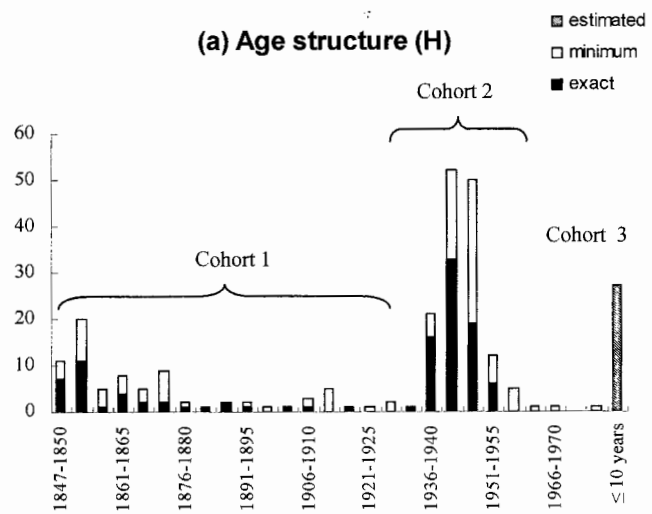
The study sites were located in the Lake Duparquet Research and Teaching Forest (LDRTF) in northwestern Quebec (79°1'W-48°30'N). Large portions of this forest are unaffected by human intervention (Bergeron, 2000). On mesic sites, such as those sampled in this study, young successional stages (< 100 years) are dominated by trembling aspen, intermediate stages (100-200 years) by balsam fir, trembling aspen, and white spruce, and old stages (> 200 years) are dominated by balsam fir and white cedar (Bergeron, 1991, 2000).

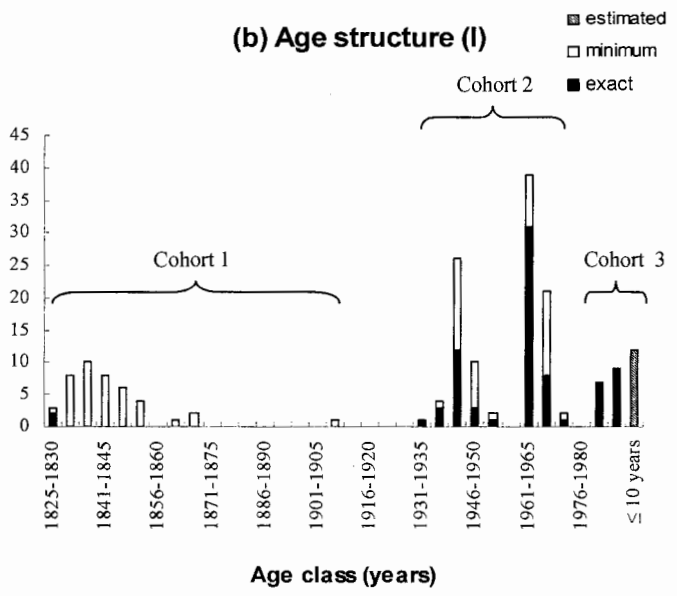
A one hectare plot was established at each of two sites H and I. Each plot was divided into a hundred 10 m ×10 m subplots, within which all aspen trees ≥ 5 cm in diameter at breast height (dbh) were tagged and mapped. Increment cores were taken from all mapped trees to determine their age. A total of 349 and 268 trees of *P. tremuloïdes* were sampled in plot H and I, respectively. Of these, age could not be determined (not estimated either) for 30 trees in H and 31 in I because cores were broken or presented indiscernible rings. These samples were eliminated from further analysis. Another 45 and 65 trees could not be successfully amplified in PCR (see below) and in sum, 247 and 172 samples were used for data analysis in plot H and I, respectively.

Increment core analysis confirmed the presence of three cohorts at each of the two sites (Figures 3.1a, b). The first cohorts included some very old trees, most of which had rotten cores. By taking the core at breast height, the true age of the trees was underestimated (minimum age). Estimation of the trees' minimum age did not affect our results, because age data were only used to roughly compare the relative ages of cohorts. Since no post-fire

disturbances were observed at either site until the mid 1930s (Bergeron, 2000), we considered that old trees had regenerated immediately after the 1847 and 1823 fires at site H and I, respectively. In site H, the large age span in cohort 1 (for trees with exact age) may be due to core analysis errors or to the recruitment of few new aspen trees after the 1847 fire under occasional canopy openings. In either case, these trees were very few and we assumed they belong to the first post-fire cohort. The second cohorts were composed of trees that originated between 1937 and 1966 in plot H, and between 1935 and 1969 in plot I. The average age of those trees was 55 and 44 years, which roughly coincides with the approximate periods during which tent caterpillar outbreaks occurred in 1944 and the 1950s (Bergeron & Charron, 1994). As for the third cohorts, these included young trees that originated following the most recent spruce budworm outbreak of 1970 to 1987. For trees whose dbh was less than 5 cm, we estimated their age to be ten years or less (Figure 3.1a, b).

Figure 3.1. Age distribution of the three aspen cohorts in plots H (a) and I (b). The term "minimum age" denotes the age of those trees whose core was broken or rotten. The "estimated age" is used for trees whose age was estimated with an error margin of a couple of years. The "exact age" is used for trees whose age was exactly determined by core analysis.





3.4.2 DNA extraction and amplification

Samples from root tissue or leaves were taken from all aspen trees and stored in the laboratory at -80°C. In the case of root samples, care was given to collect the cambium tissue from living roots. DNA was extracted using the GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich). DNA amplification was done using Taq polymerase (Gibco) and four SSR loci using dye-labelled oligonucleotide primers (*PTR1, PTR2, PTR3, PTR4*). Dayanandan *et al.* (1998), who originally identified these SSR, demonstrated that 89% of the aspen individuals can be uniquely identified by using only these four loci. In this study, we used the probability of identity $P_{(ID)}$ to estimate their resolution power. This consists in computing the probability that two individuals drawn at random from a population will have the same genotype at multiple loci. $P_{(ID)}$ was calculated by correcting for the sample size according to the following equation:

$$P_{(ID)}\ \text{unbiased} = \frac{n^3(2a_2^2-a_4) - 2n^2(a_3-2a_2) + n(9a_2+2) - 6}{(n-1)(n-2)(n-3)}$$

where n is the sample size, a_i equals Σp_j^i, and p_j is the frequency of the j^{th} allele (Paetkau *et al.*, 1998). $P_{(ID)}$ was calculated for each locus then multiplied across loci to give the overall theoretical $P_{(ID)}$. The observed and between sibs $P_{(ID)sib}$ were also calculated as suggested by Waits *et al.* (2001) to make sure that our values are below the conservative upper bound established by $P_{(ID)sib}$. In general, $P_{(ID)}$ values < 0.01 are considered to be sufficiently small to reject the null hypothesis of similar genotypes. Polymerase chain reaction (PCR) was done using a GeneAmp 9700 machine in a total volume of 10 µl containing 4µl of DNA extract, 0.625 pmol/µl of primers, 0.2 mM dNTPs, 3.125 mM $MgCl_2$, 1.4 µl BSA, and 12.5 mM Tris-HCl (pH= 8.0). Best results were obtained by performing a touch down PCR. The latter consists in decreasing the annealing temperature by 1°C every other cycle. We started with 10 minutes at 95°C to activate the enzyme and denature the DNA strands. We then used a series of annealing temperatures ranging from 60°C to 54°C over 33 cycles. At the end, a final extension of 72°C for seven minutes was added. 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-denatured and immediately placed on ice. Amplified DNA was analyzed using GENESCAN and an ABI 310 Genetic Analyzer.

3.4.3 Data analysis

3.4.3.1 Genetic diversity

Genetic diversity was measured by observed heterozygosity (*Ho*), expected heterozygosity (*He*), the average number of alleles in each cohort (*A*), and allelic frequencies per locus per cohort. Differences in allelic frequencies among cohorts were analyzed with a Chi-square test for each locus. Genetic differentiation within and between cohorts was assessed by F_{IS} and F_{ST}, respectively, according to Weir and Cockerham (1984). The F_{IS} statistics, also called inbreeding coefficient, measures the deviation from Hardy-Weinberg

(HW) equilibrium per plot or per cohort. The F_{ST} statistics allows a comparison of the distribution of genetic diversity between cohorts. The statistical significance of F_{IS} and F_{ST} were tested by performing 10,000 permutations of all alleles within cohorts and of all genotypes within the total population, respectively. All the above metrics were calculated with GENETIX, a software for population genetics developed by Belkhir *et al.* (2004). A parentage analysis was also performed with CERVUS 2.0 (Marshall *et al.*, 1998) in order to calculate the probability of a genetic relationship of descent between cohorts. This mainly consists in testing whether genets in cohort 2 can be the offspring of cohort 1, and whether genets in cohort 3 can be the offspring of cohorts 1 and 2. Significance (at $\alpha = 0.05$) was tested by performing 1,000 simulations for allelic frequencies.

3.4.3.2 Clonal structure

Genotypic diversity was determined as the *G/N* ratio, which is the proportion of different genotypes in a population, where G is the number of observed genets, and N is the total number of individuals analyzed (Pleasants & Wendel, 1989). We also calculated the Simpson's index D, which is the probability of sampling, without replacement, two successive individuals from different multilocus genotypes (Gregorius, 1987). It was corrected for small samples according to the following equation: $D = \Sigma \{[n_i (n_i - 1)] / [N (N - 1)]\}$, where n_i is the number of ramets of the *ith* genet (Pielou, 1969). Both indices have a range of 0 to 1, where 0 indicates that all trees have the same multilocus genotype, and 1 indicates a population in which each individual has a unique multilocus genotype. Clonal dimension was calculated as the maximum distance between two ramets belonging to the same genet. We also counted the number of genets, the number of single-ramet genets (genets composed of only one ramet), and the average number of ramets per genet for each cohort in both plots.

3.4.3.3 Spatial analysis

Mapping and joint-count statistics were used to describe and delimit the spatial distribution of aspen ramets within and between genets and aspen trees within and between cohorts. Joint-count statistics were calculated with standard normal deviates (SND) analysis (Sokal & Oden, 1978) using the PSAWIND v.1.1.1 (Takahashi, 2003). SND analysis compares the number of joins between clones or trees in different distance classes with the number of joins that would be expected if the population was a random sample from a normal distribution. Correlograms were first assessed globally at a Bonferroni-corrected significance level of $\alpha = 0.05$. In this analysis, only multiramet genets (genets composed of more than one ramet) were included, and 2 m distance classes were used to detect fine-scale spatial patterns between aspen genets or trees.

Spatial autocorrelation analyses were performed to calculate indices of spatial association among multiple alleles according to Smouse and Peakall (1999) using GENAIEX V5 (Peakall & Smouse, 2001). Distance classes were selected to have even sample sizes in each distance class to provide a consistent basis for correlation. Significance tests of the correlation coefficient r were computed for each distance class using 1,000 permutations of the analyzed samples. Spatial autocorrelation analyses were first conducted on the pooled populations of ramets from all three cohorts, and then on each cohort per plot separately to investigate differences in aspen spatial genetic structure at different stages of succession. These analyses were repeated using only the ramet with the largest diameter per genet. Differences in the patterns obtained when analysing all ramets and when using only one ramet per genet was used reflect the effect of clonal propagation on the spatial genetic structure of trembling aspen.

3.5 RESULTS

3.5.1 Microsatellite resolution power

Tests for estimating the probability of sampling identical genotypes revealed a high resolution power of the four SSR loci that were used to identify distinct genotypes. $P_{(ID)}$ values were lower than 0.001 (0.00001 in H and 0.00063 in I). They were also lower than observed $P_{(ID)}$ (0.0065 and 0.0207 in H and I, respectively) and the conservative upper bounds set by $P_{(ID)sib}$ (0.0226 and 0.554 in H and I, respectively).

3.5.2 Clonal structure

The number of aspen trees per cohort ranged from 27 and 21 in the third cohorts up to 140 and 106 in the second cohorts in H and I, respectively. We identified 158 and 108 genets in plot H and I, respectively. Genotypic diversity (*G/N*) ranged from 0.67 to 0.73 in cohorts 1 and 2 in both plots, and 0.92 to 0.76 in cohorts 3 and in H and I, respectively. Little differences in Simpson's Indices (*D*) were observed in both plots among cohorts (Table 3.1).

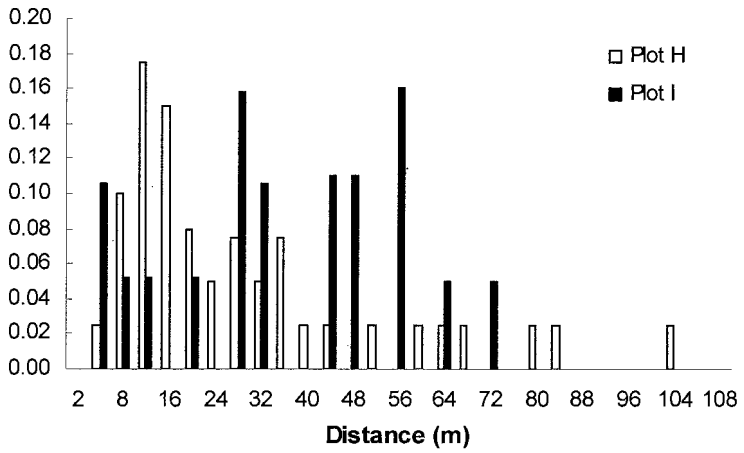
Table 3.1. Summary of genotypic and allelic variation for four microsatellite loci in three cohorts of aspen in plots H and I; The number of multi- and single-ramet genets are given. *G/N* is multilocus genotypic diversity; *D* Simpson's index of genotypic diversity; *Ho* observed heterozygosity and *He* expected heterozygosity. Numbers in parentheses are standard errors. *A* is the number alleles averaged over the four loci. F_{IS} is the inbreeding coefficient given per cohort and per plot.

Site	Cohort	Number of ramets	Number of genets	Number of multi-ramet genets	Number of single-ramet genets	*G/N*	*D*	*Ho (SE)*	*He (SE)*	*A*	F_{IS}
H	Cohort 1(1847)	80	57	25	32	0.71	0.986	0.43 (0.19)	0.68 (0.17)	10.25	0.37*
	Cohort 2 (1937-1966)	140	102	31	71	0.73	0.994	0.46 (0.13)	0.73 (0.08)	13.00	0.38*
	Cohort 3 (1990-2001)	27	25	10	15	0.92	0.994	0.41 (0.10)	0.64 (0.22)	7.00	0.37*
	Pooled cohorts	**247**	**158**	**40**	**118**	**0.64**	**0.993**	**0.45 (0.07)**	**0.73 (0.13)**	**10.08**	**0.39***
I	Cohort 1(1823)	45	32	9	23	0.71	0.956	0.51 (0.22)	0.56 (0.20)	8.50	0.11*
	Cohort 2 (1935-1969)	106	71	18	53	0.67	0.979	0.49 (0.13)	0.63 (0.18)	9.50	0.22*
	Cohort 3 (1990-2001)	21	16	3	13	0.76	0.948	0.37 (0.26)	0.47 (0.25)	4.75	0.24*
	Pooled cohorts	**172**	**108** †	**19** †	**89**	**0.63**	**0.979**	**0.48 (0.14)**	**0.61 (0.19)**	**7.58**	**0.21***

† Genets with more than one ramet may be shared by different cohorts. Their total number is different from the sum of genets identified in each cohort; * Significant deviation from 0: $P < 0.001$.

More than 75% of genets were composed of only one ramet in both plots. Multiramet genets were generally composed of few ramets. The number of ramets per genet averaged 3.26 (± 1.65) and 4.39 (± 4.15) in plots H and I, respectively. Maximum clonal dimensions were left-skewed in H, but not so in I (Figure 3.2). Median clonal dimensions were 19 m in H and 29 m in I. The maximum clonal dimension was 104 m in H and 72 m in I.

Figure 3.2. Maximum clonal dimension of aspen in plots H and I



Multiramet genets were mostly found in the second cohorts. In plot H, eleven multiramet genets (27%) formed ramets in both the first and the second cohort 120 years after fire, but only five (12.5%) also had suckers in the third cohort 154 years after fire. In plot I, seven multiramet genets (36%) built ramets in the first two cohorts, while only one suckered in all successive cohorts over 178 years of succession. The number of ramets per genet, the number of multiramet genets in the third cohort, and the absolute number of aspen ramets recruited in the third cohorts were lower than that in the first and second cohorts in both plots (results not shown).

All cohorts had a relatively high genetic diversity. Observed heterozygosity ranged from 0.37 to 0.51, with an average of 0.45 and 0.48 in plot H and I, respectively. Average expected

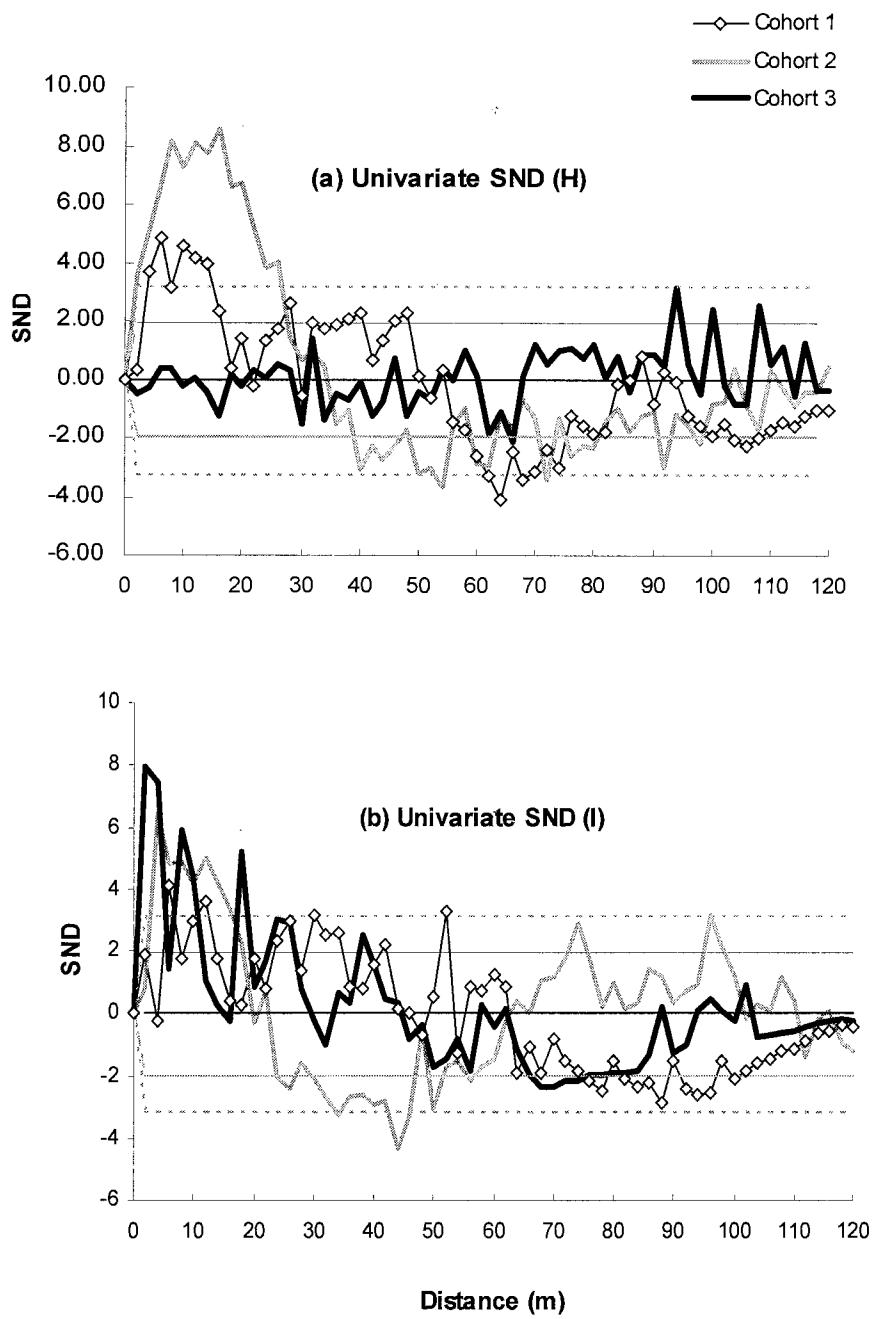
heterozygosity was 0.73 and 0.61 in plot H and I, respectively. It ranged from 0.47 to 0.73, but showed no clear pattern across cohorts. The mean number of alleles per locus (A) was lower in younger cohorts in both plots. It ranged from 7.0 to 13.0 in plot H and from 4.8 to 9.5 in plot I. Repeating heterozygosity calculations using only one ramet per genet showed minor differences with the above results.

Most of the genetic variation was within rather than between cohorts (F_{ST} = 0.04 and 0.03 in H and I, respectively). These findings were congruent with the non significant differences in mean allelic frequencies between cohorts (Chi-square test per locus, $P > 0.05$; *Table A.1*). F_{IS} was significantly ($P < 0.0001$) higher than that expected under HW equilibrium in each cohort. It averaged 0.39 and 0.21 in plot H and I, respectively (Table 3.1). In both plots, parentage analysis revealed no significant parentage relationship between the genets of the first two cohorts and those of cohort 3 or between cohort 1 and cohort 2.

3.5.3 Spatial patterns within cohorts

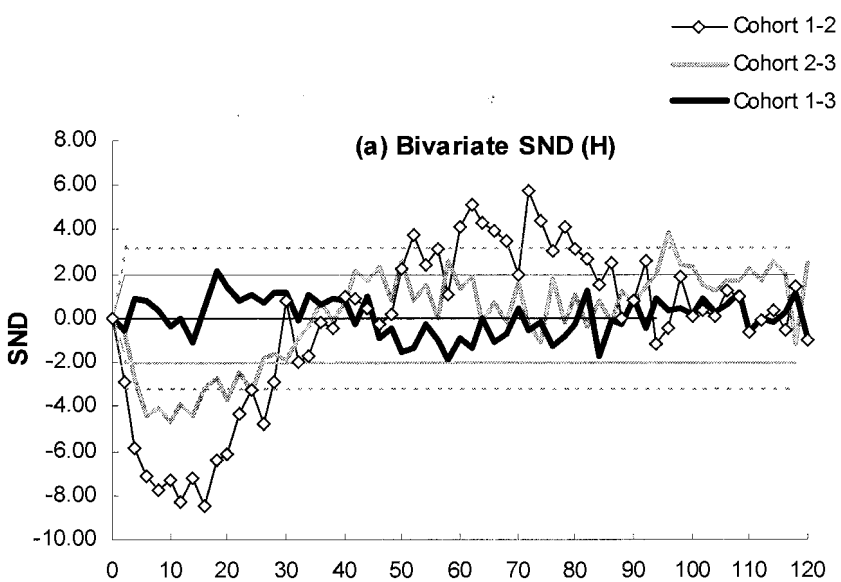
Univariate SND analysis revealed a significant spatial aggregation (positive SNDs) among aspen trees within all cohorts over small distances of 4 to 16 m ($p \leq 0.05$) except within cohort 3 in H (Figure 3.3a, b). The first cohorts showed intermittent aggregation at distances of 4 to 48 m in H and 6 to 52 m in I. The second cohorts exhibited the greatest aggregation at scales of up to 26 and 18 m in H and I, respectively. In plot I, SND distributions for the second cohorts suggested the possible occurrence of a patch structure with a periodicity of approximately 50 m. Contrasting patterns were observed in the third cohorts in plots H and I. In H, cohort 3 stems were randomly distributed in almost every distance class, but showed intermittent strong aggregations in several distance classes (2-12, 18-20, and 22-28 m) in plot I (Figure 3.3a, b).

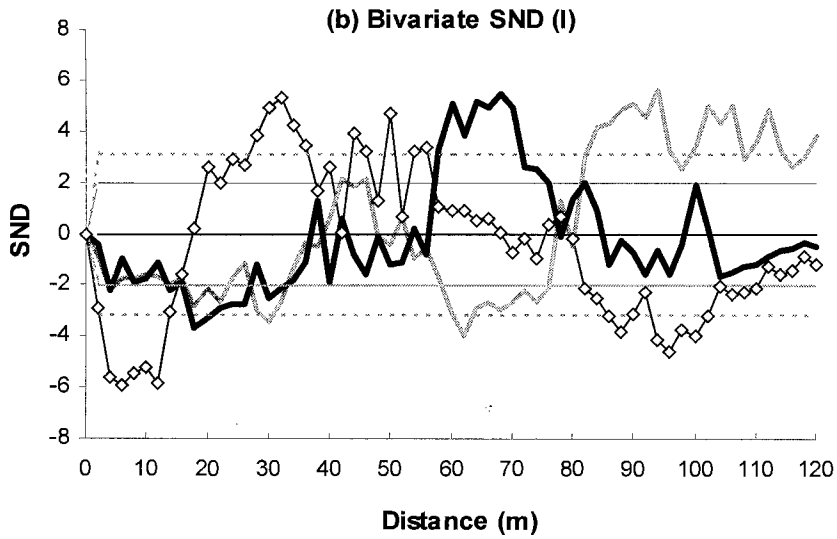
Figure 3.3. Standard normal deviates analysis (SND) within aspen cohorts in plots H (a) and I (b). Solid lines represent the upper and lower significance levels for SND values (± 1.96) at a confidence level $\alpha = 0.05$. Dashed lines represent the upper and lower significance levels of the whole correlogram according to Bonferroni-corrected α value (= 0.0008).



Bivariate SNDs revealed different patterns between cohorts at small distances, although not consistent between the two plots. In H, cohort pairs 1 - 2 and cohorts 2 - 3 were significantly ($p \leq 0.05$) dispersed (negative SNDs) at 2 – 28 m and 4 – 24 m, respectively (Figures 3.4a). In I, cohort pairs 1 - 2 and 2 - 3 had two zones of dispersal. Cohorts 1 and 2 were dispersed at 2 – 14 m and 82 – 110 m, and cohorts 2 and 3 were dispersed at 18 – 32 m and 60 – 76 m. The only aggregations between cohort pairs 1 and 2 were observed at 50 to 82 m in H and 20 to 56 m in I (Figure 3.4b). Cohorts 1 and 3 were distributed at random with respect to each other over all distance classes in H, but were weakly segregated at distances of 18 - 28 m and aggregated from 58 to 78 m in I (Figure 3.4a, b).

Figure 3.4. Standard normal deviates analysis (SND) between aspen cohorts in plots H (a) and I (b). Solid lines represent the upper and lower significance levels for SND values (±1.96) at a confidence level $\alpha = 0.05$. Dashed lines represent the upper and lower significance levels of the whole correlogram according to Bonferroni-corrected α value (= 0.0008).





3.5.4 Clone demography

In both plots, univariate SND analysis revealed a significant aggregation (> +1.96) between ramets belonging to the same genet. Most of this aggregation occurred at small scales of 5 to 30 m, and only few genets showed a spatial aggregation between ramets at longer distances up to 104 m in H and 54 m in I. The only significant negative value (< -1.96) was observed in plot I at 68 m. When comparing the spatial distribution of multiramet genets relative to each other (bivariate SND), one common thing observed between all correlograms was the complete absence of any significant segregation between clones, indicating the absence of a phalanx growth type in the studied populations (results not shown).

Stem maps of cohort and genet distribution provided qualitative support for the spatial analysis (results not shown). The spatial separation between patches of cohort 1 and cohort 2 trees was clearly visible in both plots. Cohort 3 trees were scattered among cohorts 1 and 2 in

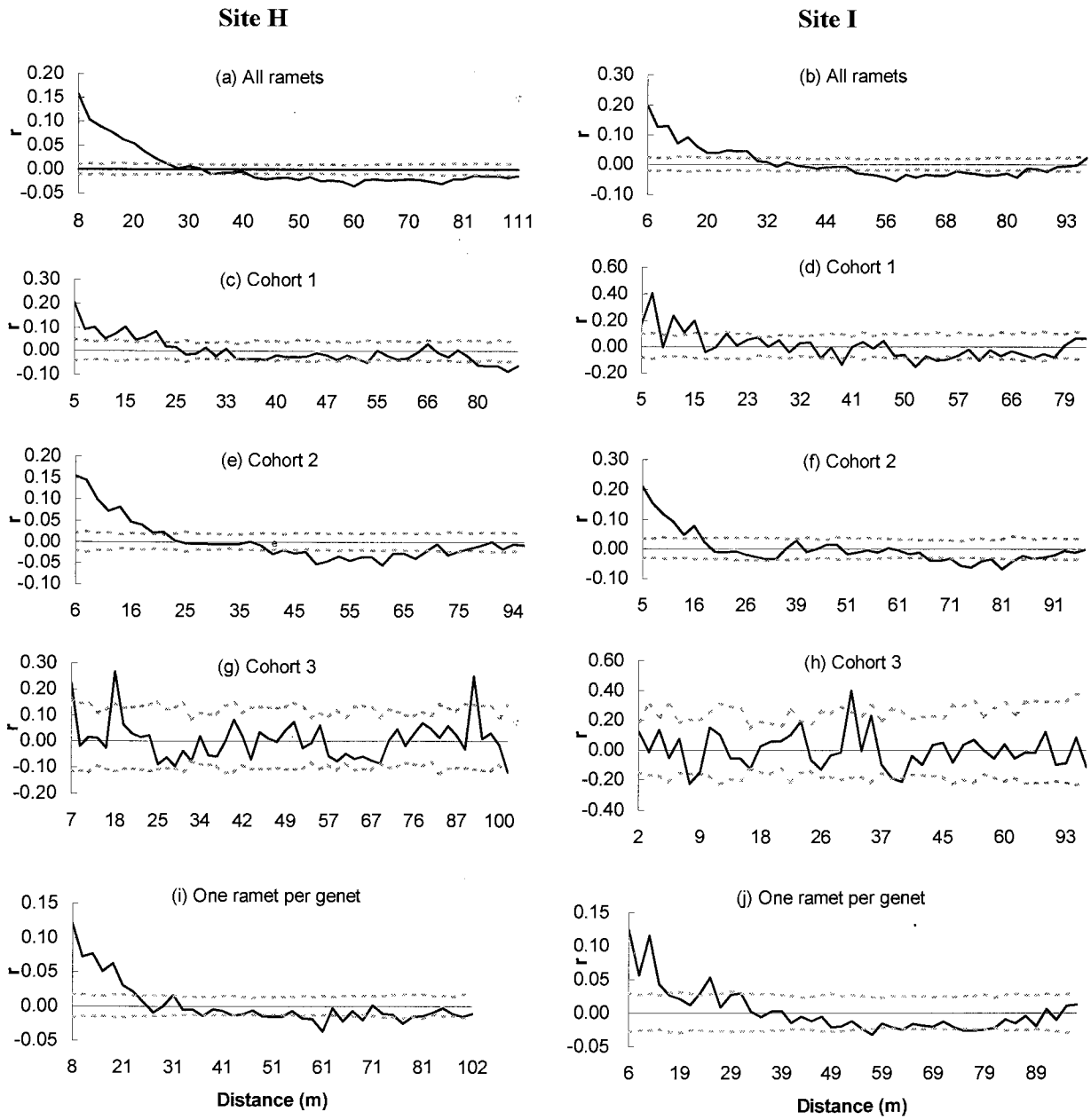
plot H, but were sequestered in several tight clusters on the west side of plot I. In addition, no clear boundaries could be seen between genets in either plot.

3.5.5 Spatial genetic structure

Positive spatial autocorrelation in allele frequencies was highest (0.16 in H and 0.20 in I) at distances of 6 - 8 m when using all ramets (Figure 3.5a, b). It did not exceed 30 m in either plot, and a significant negative multivariate allelic spatial autocorrelation occurred in H at 42 – 88 m (Figure 3.5a), and between 52 and 84 m in I (Figure 3.5b).

The scale of spatial autocorrelation was similar in cohorts 1 and 2. Allele frequencies in these cohorts were positively autocorrelated at distances up to 23 and 18 m in H and I, respectively, but were distributed at random or were negatively autocorrelated at larger distances (Figure 3.5c, d, e, f). In the case of cohort 3, significant allelic autocorrelations occurred in certain distance classes in an inconsistent way extending over a maximum of two distance classes (Figure 3.5g, h). Patterns of autocorrelation observed using one ramet per genet did not differ from those observed using all ramets in each plot (Figure 3.5i, j). Both the autocorrelation level and the scale of positive spatial genetic structuring slightly decreased when using only one ramet per genet. It is less likely that the recurrence of two positive peaks after the decrease for a short interval (between 17 m and 23 m) in plot I reflects a repetition of the patchiness structure observed at shorter distance classes (<17 m). This is because no genetic patch width (i.e. the distance at which the autocorrelation coefficient first intercepts the x- axis) could be determined at that scale.

Figure 3.5 Correlograms of spatial genetic structure of aspen in plots H and I for: all ramets in the plot (a, b), cohort 1 (c, d), cohort 2 (e, f), cohort 3 (g, h), and for all genets represented by only one ramet each (i, j). Dashed lines indicate the upper and lower confidence levels at $\alpha = 0.05$.



3.6 DISCUSSION

3.6.1 Clonal structure

Our results confirmed our prediction about the limited number of ramets per genet. Most genets (>75%) were composed of one or a few ramets resulting in a high genotypic diversity. If we except the post-fire cohort (cohort 1), sexual recruitment cannot account for these findings. Seedling establishment is possible after a fire due to the complete canopy opening, but is less likely to occur following secondary disturbances because ecological conditions in canopy gaps (soil temperature, light, etc.) do not favour seed germination and survival. In fact, no aspen seedlings have been observed in other studies that described species succession in the same sites (Bergeron & Charron, 1994; Kneeshaw & Bergeron, 1996; Bergeron, 2000). Also, monitoring sexual recruitment for five consecutive years in clear cut and control sites in the same sampling area did not detect any aspen seedling (Bergeron, in press). Instead, most studies report a continuous suckering in aspen populations, but due to insufficient canopy opening and browsing, only few of those suckers are found to survive for more than one year (Bergeron & Charron, 1994; Kneeshaw & Bergeron, 1996). This would explain the recruitment of many single-ramet genets in cohort 2 and 3 and therefore the high genotypic diversity observed.

The low *Ho* values relative to *He* and the significantly high F_{IS} indicate a deficiency of heterozygotes in all cohorts. Inbreeding often invoked to explain heterozygote deficiencies, has to be excluded as no parentage relationship and no significant differences in allele frequencies were observed between cohorts. Alternatively, the high levels of genetic diversity in all cohorts and the low differentiation among them suggest that the three cohorts originated from a common and diverse genetic pool that was established at the origin of the stand and that was maintained over time by periodic suckering.

The second part of the prediction of random spatial distribution of aspen genets was only partly confirmed. Ramets belonging to the same genet were mostly aggregated at small distances, but no segregation was observed between different genets, which would imply the absence of a phalanx type growth. Intermingling of genets is expected to occur in clonal species with time or in gap habitats (Kudoh *et al.*, 1999; Cronberg, 2002). In aspen, genet intermingling is believed to occur in stands where many seedlings were initially established followed by disturbance by one or more fires (Barnes, 1966; Wyman *et al.,* 2003).

Two potential pathways may have led to the small clonal dimensions and the spatial aggregation among ramets observed within genets. First, suckering is likely to occur close to parent stems (Barnes, 1966; Schier, 1975). Second, the increasing competition for light and nutrients in mid successional stages (second and third cohorts; Bergeron & Dubuc, 1989; Bergeron, 2000) may have limited the number of ramets per genet (mostly up to one ramet per genet) rather than the number of genets within the canopy gaps. In support of this, data collected by using a chronosequence approach and simulations over 500 years under different fire cycles indicated that mortality mostly affects ramets within genets as the stand gets older (Namroud *et al.*, unpubl. data).

3.6.2 Cohort spatial patterns

The prediction of a spatial segregation between cohorts was supported for cohorts 1 and 2 in both plots. The spatial patterns observed within and between them were consistent with the temporally separated, qualitatively different natural disturbances (Figure 3.4). The high percentage of single-ramet genets and the absence of distinct boundaries between multiramet genets indicate that the segregation pattern between cohorts can not simply be the result of the presence of distinct genet patches. Instead, it reflects primary and secondary disturbances. Following the fires in 1847 and 1823, massive recruitment of aspen trees is likely to have occurred due to the large canopy opening, which provides suitable conditions for root suckering (Perala, 1990). With time, natural mortality and insect outbreaks eliminated many

post-fire trees, resulting in the observed clustering of aspen trees within cohorts. In early and mid successional stages (78 and 124 years after fire) which correspond to the recruitment time of our second cohorts, the majority of the canopy gaps were supposedly small (25 m^2 to 250 m^2, Kneeshaw & Bergeron, 1998). This may have limited the recruitment of the second cohorts within these small isolated patches of canopy gaps, which would explain the higher level of aggregation observed at small spatial scales in the second cohorts. During later succession, both gap formation and inter and intra- specific competition may help to determine the spatial distribution of new trees (Park *et al.,* 2005). Aspen sprouts would generally receive enough light to grow into the canopy in fairly large treefall gaps. Since gap formation is often attributed to the random mortality of single trees or small groups of trees, gap formation can, therefore, be invoked to explain the random patterns of aspen trees in cohort 3.

Contrary to our expectations stating that genetic aggregation based on allelic frequencies will be more pronounced in cohorts originating from gap disturbances, spatial distribution of genetic variation occurred at almost similar scales in both the first and second cohort, and was more randomly distributed in cohort 3. The positive spatial genetic autocorrelation within the first two cohorts (at about 30 m) concurr with the clonal dimensions. Most multiramet genets were small (32-56 m) with 80% of the ramets growing within 16 m. These results fit to reports about aspen clonal extension up to 30 m from cut stumps (Mitton & Grant, 1996). The significant positive spatial allelic autocorrelation observed between genets after keeping only one ramet per genet in analysis (Figure 3.5i, j) is most probably related to the genetic relatedness of stems at short distances.

The decrease in the spatial genetic autocorrelation in cohort 2 compared to cohort 1 (established after fire), and the more random allelic distribution in cohorts 3 in both plots, mainly echo the spatial distribution of trees within cohorts. In the absence of sexual recruitment, we conclude that the original spatial patterns of aspen trees tend to gradually disappear in younger cohorts originating in smaller canopy gaps, although, as a whole, the stand still show a significant autocorrelation at small spatial scales (Figure 3.5a, b).

3.7 CONCLUSIONS

This work allowed for the first time to delineate aspen clone sizes at a fine scale. The distribution of individual aspen ramets, genets and their fine spatial genetic pattern make the transition from aggregated to more random patterns in our study area. The spatial scale at which structuring was detected is relatively small (within about 30 m) but is congruent with the extent of clonal propagation, at least until mid successional stages.

3.8 ACKNOWLEDGEMENTS

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CHAPITRE IV

EFFECT OF CLEAR- AND PARTIAL CUTTING ON ASPEN GENOTYPIC DIVERSITY IN QUEBEC'S BOREAL FOREST

Article à soumettre à Forest Ecology and Management

4.1 RÉSUMÉ

Nous avons évalué l'effet d'un modèle d'aménagement basé sur des coupes de différentes intensités sur la diversité génotypique du peuplier faux-tremble (*Populus tremuloides*) dans la forêt boréale du Québec. Trois traitements incluant une coupe totale, une récolte du 1/3 de la surface terrière totale et un témoin non coupé ont été appliqués à des peuplements de tremble qui ont brûlé en 1923. Dans la coupe 1/3, seulement les tiges de tremble à faible croissance ont été coupées. Les genets du tremble ont été identifiés en utilisant quatre marqueurs microsatellitaires. Les échantillons ont été récoltés dans des placettes de 20 m × 20 m dans les parcelles de coupe totale, coupe 1/3, et dans le témoin. Le *G/N* du site était en moyenne de 0.17, alors que le taux d'hétérozygotie observée et attendue était de 0,58. Dans les 2 traitements de coupe, la plupart des genets se sont régénérés à distance des souches et des arbres non-coupés. Dans la parcelle coupe totale, nous avons observé une forte régénération d'un grand nombre de genets. Par contre, seulement quelques genets se sont régénérés dans la parcelle coupe 1/3 avec au moins un arbre non-coupé parmi leurs ramets. La régénération des drageons était localisée en bordure de la parcelle coupe 1/3, indiquant que les conditions environnementales porraient avoir un impact plus important que la sélection des génotypes sur le potentiel de régénération des genets et leur distribution spatiale.

4.2 ABSTRACT

We have assessed the effect of a management model that uses different logging intensities on the genotypic diversity of quaking aspen (*Populus tremuloides*) in Quebec's boreal forest. Three treatments including a : (1) clear-cutting, (2) partial cutting of the one-third of the total basal area and (3) no-harvest were applied to aspen populations that burned in 1923. In the one-third partial cut treatment, the stand was also thinned from below and only slow-growing stems were removed. Aspen genets were identified by using four microsatellite loci. Data were collected within a 20 m × 20 m plot in the clear cut, the 1/3 partially cut, and the control plot. *G/N* averaged 0.17 at the site level, while observed and expected heterozygosity averaged 0.58. In the clear- and partial cutting treatments, most suckers regenerated away from stumps and non-harvested trees with a larger number of genets regenerating in the clear-cut plot. By contrast, only few genets suckered in the partially cut plot, with at least one non-harvested tree among their ramets. Most suckers (91%) were located near the border of the one-third partially cut plot which indicates that environmental factors might have a more important impact than genotype selection on genet regeneration potential and spatial distribution.

4.3 INTRODUCTION

Quaking aspen (*Populus tremuloides*) is an important clonal hardwood species in the boreal forest. It reproduces mainly by root suckering because conditions needed for seed germination are rarely met (Mitton & Grant, 1996; Romme *et al.*, 1997). In the past decade, aspen acquired an increasing commercial importance in Canada. For instance, its utilization increased by 800% in Alberta over the last 15 years, and the volume harvested reached 10.0 million m^3 and accounted for 43% of the total harvest in 1997. In British Columbia, its utilization increased to 2.9 million m^3 and accounted for 4.3 % of the total harvest (David et al., 2001). In Quebec, its production increased by about 700% since 1990, and accounted for 42% to 72% of the Canadian exportation values in terms of lumber manufacturing (Mercier, 2002). The greatest expansion was in its use for the production of engineering wood products such as oriented-strandboard and waferboard and for high quality pulp and paper products (David et al., 2001).

In parallel with the increasing demand for aspen, we also observe an increasing trend to advocate management practices that emulate the effect of natural disturbances (Bergeron & Harvey, 1997). In Quebec's boreal forest, this means introducing new practices to the silvicultural system in addition to the traditional clear-cutting currently used at large scales. The purpose of such practices is to maintain the stands biological diversity and ecological characteristics. To this effect, researchers in Quebec's boreal forest suggest using clear-cutting to emulate the effect of fire in regenerating aspen stands, and partial cutting with selection to emulate the role of a stand natural break up and that of gap dynamics in allowing the transition toward mixed and old-growth stands, respectively (Bergeron & Harvey, 1997).

To date, most studies evaluated the effect of silvicultural systems on aspen regeneration density, but none of them assessed the impact of the above proposed management practices on aspen clonal structure. Such information is nonetheless important for adjusting current practices in order to favour and collect vigorous aspen genets with a better commercial value. In this paper, we took advantage of an experimental set up in a young stand that burned in

1923 in Quebec's boreal forest to assess the impact of clear-cutting and selective one-third partial cutting on quaking aspen genotypic diversity. More specifically, we tried to answer the following questions: (1) how do clear- and partial cutting affect aspen clonal structure? (2) can a selective one-third partial cutting based on trees morphological characteristics be used to capitalize on aspen genetic diversity by favouring genets (i.e. an individual genotype) with a higher timber quality? And (3) do clear-cutting (primary disturbance such as fire) and selective partial cutting (secondary disrturbance such as gap) treatments emulate the effect of natural disturbances on aspen clonal structure?

4.4 MATERIALS AND METHODS

4.4.1 Study area

The study site was located in the Lake Duparquet Research and Teaching Forest (LDRTF) in northwestern Quebec (79°1' W - 48°30'N). Lake Duparquet is located in the southern limit of the boreal forest within the Missinaibi-Cabonga forest section (Rowe, 1972). The fire history of Lake Duparquet area has been reconstructed by using dendrochronological techniques and aerial photographs (Bergeron, 1991; Dansereau & Bergeron, 1993). The study site was of fire origin dating from 1923. The fire of 1923 was of high intensity and covered more than 1000 ha in the LDRTF (Dansereau & Bergeron, 1993). The site was healthy and not affected by insect outbreaks during the study period. On mesic sites in the mixedwood such as the one sampled in this study young successional stages (<100 years) are generally dominated by trembling aspen (Bergeron 1991, 2000), which makes our study site a representative of most postfire sites in the mixedwood boreal forests.

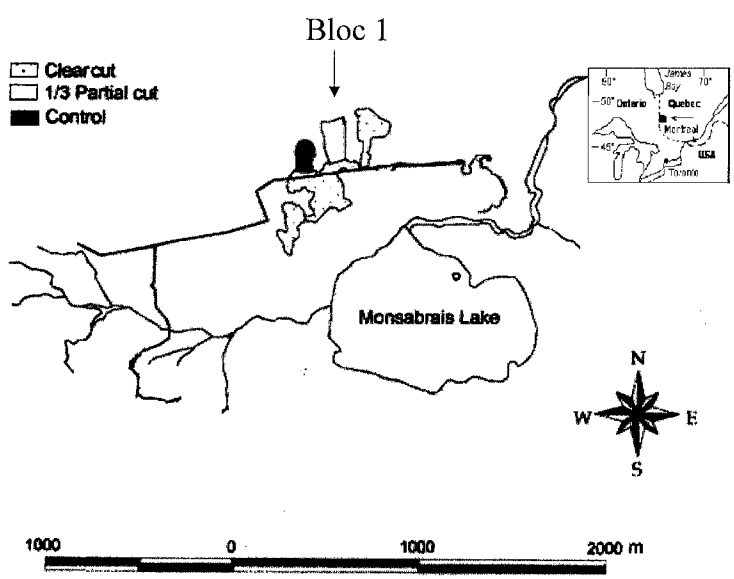
4.4.2 Experimental design and treatments

Sampling was performed in one of the replicated blocks (Bloc 1) of the first phase of an experimental set up called SAFE, the french acronym for "sylviculture et aménagement forestier écosystémique" or ecosystem management and silviculture (Figure 4.1). In each block, several levels of harvesting, including a clear-cutting (CC), a partial cutting with the removal of one third of the merchantable basal area of the stand (PC1/3), and a no-harvest (control) were randomly applied in the winter of 1998-1999 on 1 to 2.5 ha sections of the site. In the PC1/3 treatment, a thinning from below technique was used and only slow-growing stems were removed with some minor adjustments to have the partial cut treatment in areas where softwood understorey regeneration was present. Trees to be removed were marked prior to harvesting. Harvesting was done manually and a special care was taken to protect residual stems and advanced regeneration by hauling delimbed stems in the CC treatment. Harvested stems were bucked into 2.5 to 7.5 m lengths then hauled using small cable skidders. Skid trails averaged 4.5 m in width, and distance between trails averaged 30 m. At the time of harvesting, the ground was snow covered and physical soil disturbances (rutting or scarification) were minimal. Characteristics of the control stands in the experimental set up are summarized in table 4.1. Sky openness at 75 cm aboveground were 17.4 and 3.5 times higher in the CC and the PC1/3 than in the control, respectively. In general, no important differences were observed in air and soil temperatures and moisture between the CC, the PC1/3 and the control. However, more frequent differences in soil moisture were observed between the CT and the PC1/3 throughout the first year after the treatments. These characteristics are described in more details in Brais et al. (2004).

Table 4.1 Composition and characteristics of the control stands in the experimental set up (from Brais et al., 2004)

Species	**Mean density (stems/ha)**	**Mean dbh (cm)**	**Mean basal area (m^2/ha)**
Tree species (dbh > 2cm)			
Trembling aspen	848	24.17	39.89
White birch	172	9.08	1.20
Balsam poplar	5	39.20	0.61
Balsam fir	77	11.63	0.37
White spruce	113	9.41	0.80
Black spruce	5	13.13	0.09
Jack pine	3	24.20	0.16
Total	1223		43.12
High shrub species (dbh> 2cm)			
Mountain maple	1327	2.89	0.86
Speckled alder	97	3.49	0.08
Green alder	27	2.58	0.01
Beaked hazel	13	2.40	0.01
Pin cherry	3	6.60	0.01
American mountain ash	7	2.40	0.00
Total	1474		0.97

Figure 4.1. Location of the experimental set up in Lake Duparquet Forest in northern Quebec



We previously demonstrated that ramets belonging to the same genet mostly aggregate within 5 to 30 m (Namroud et al., 2005). For this, we established a plot of 20 m x 20 m in each treated section to assess the impact of various harvesting treatments on aspen clonal regeneration. In each plot, we counted the total number of aspen suckers, non-harvested trees, and stumps. We then divided each plot into hundred 4 m^2 quadrats in which we randomly sampled one aspen sucker per quadrat for genetic analysis. Aspen samples were also collected along 4 transects of 10 m each at an interval of 5 m in each treated section to follow the extension of the genotypes outside the 20 x 20 plots at the same time. Aspen stumps could not be genotyped because of their low DNA quantity and quality, but non-harvested trees were all genotyped. Samples for genetic analysis were taken from leaves or root tissue of aspen trees or suckers. They were stored in the laboratory at -80°C until analysis.

4.4.3 DNA extraction and amplification

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 latter were identified by Dayanandan et al. (1998). Previous study showed a very high resolving power of these microsatellites (Namroud et al., 2005). 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: 4µl 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 consisted 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 that decreased from 60°C to 54°C over 33 cycles. Each cycle lasted about 14 minutes and at the end of each

cycle, we added a final step of 72°C for 7 minutes 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).

4.4.4 Genetic analysis

The impact of the treatments on aspen clonal regeneration was assessed by counting the number of genets and the number of ramets (i.e. all stems having the same genotype) per genet in each treated plot. Genotypic diversity was measured by using the ratio *G/N*, where *G* is the number of observed genets, and *N* is the number of individuals genotyped (Pleasants & Wendel 1989). *G/N* approaches zero when the number of genotypes is very low, and reaches a maximum of 1 when each tree has a unique mutlilocus genotype. Genetic diversity was measured by observed heterozygosity (*Ho*), expected heterozygosity (*He*), the average number of alleles per locus (*A*), and the inbreeding coefficient (F_{IS}). These parameters were calculated by considering all the ramets in a genet as we observed little differences with the values observed when considering one ramet per genet. Genetic analysis was performed with *GENETIX* (Belkhir et al., 2004).

4.5 RESULTS

4.5.1 Genetic diversity

A total of 66 suckers was genotyped in the CC plot, compared to 48 suckers and 20 non-harvested trees in the PC1/3 plot, and 34 non-harvested trees in the control (Table 4.2).

Table 4.2. Number of suckers, non- harvested trees, stumps, logs and genets in the 20 m × 20 m plots following the three treatments: CC = clear-cutting, PC1/3 = one-third partial cutting with selection, and control. In the CC and PC1/3 plots, genets were identified by analysing all non-harvested trees and one sucker randomly sampled per quadrat of 2 m × 2 m.

Treatment	Composition	Total number	Number of stems sampled	Number of genets
CC	Suckers	601	66	20
CC	Stumps	20	20	_
PC 1/3	Suckers	247	48	4
PC 1/3	Trees	20	20	4
PC 1/3	Stumps/Snag	12/1	12/1	_
Control	Trees	34	34	4

Both genotypic diversity (*G/N*) and the mean number of alleles (*A*) were higher in the CC plot than in the other plots. By contrast, *Ho* and *He* were lowest in the CC plot and showed little differences between the PC1/3 and the control plots. This concurred with an excess of heterozygotes in the PC1/3 and the control plots. At the site level (when considering all treatments together) *G/N* was 0.17, *Ho* was 0.58, equal to *He,* and no significant deviation from equilibrium (F_{IS} <0.001) was observed (Table 4.3).

Table 4.3. Genetic characteristics of the plots following the treatments: CC = clear-cutting; PC1/3 = one-third partial cutting with selection; G = number of genets; N = number of stems; G/N = genotypic diversity; Ho = observed heterozygosity; He = expected heterozygosity; SE = standard error; Mean A = number of alleles averaged over four loci; F_{IS} = inbreeding coefficient.

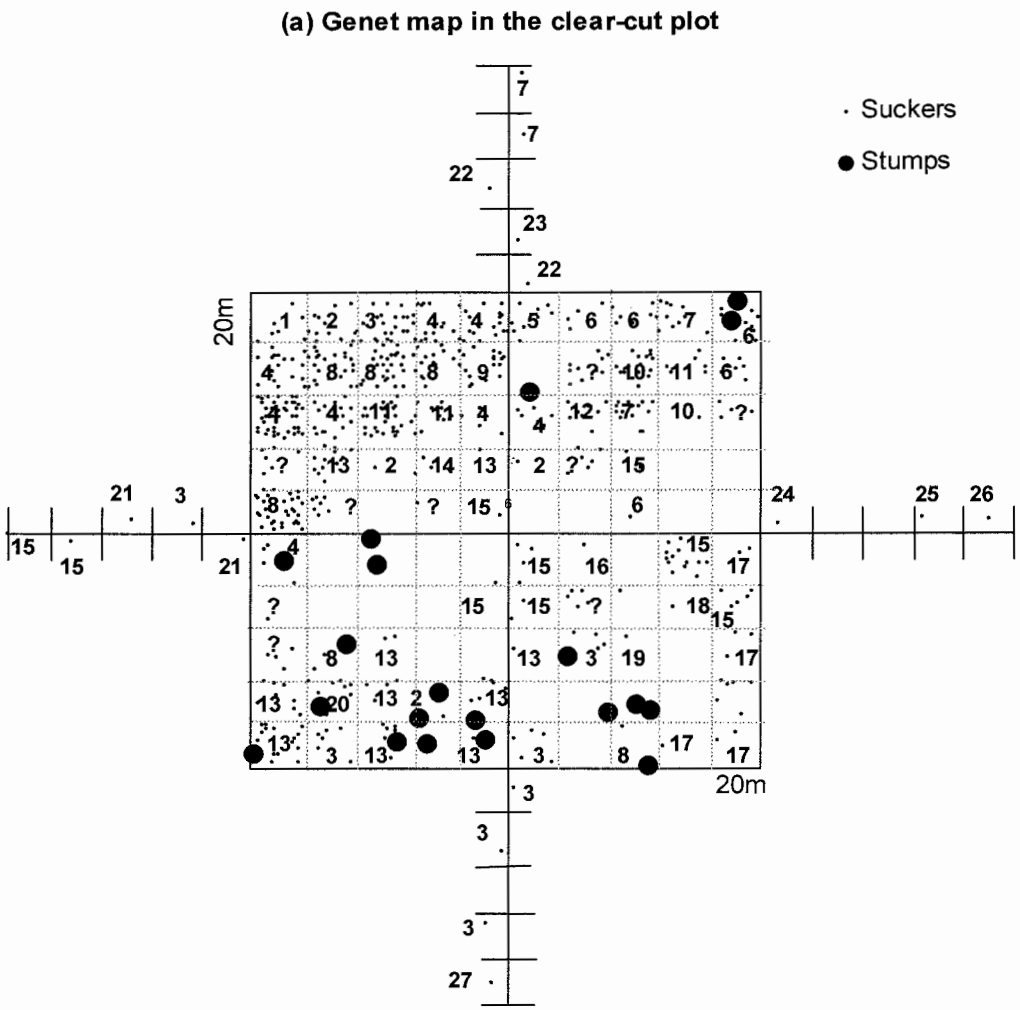
	Plot (20 m x 20 m)						
Treatment	**Number of stems sampled**	**Number of genets sampled**	***G/N***	***Ho* (*SE*)**	***He* (*SE*)**	**Mean A**	**F_{IS}**
CC	66	20	0.30	0.40 (0.41)	0.42 (0.27)	5.25	0.06
PC1/3	68	5	0.07	0.67 (0.42)	0.51 (0.30)	3.75	-0.31
Control	34	4	0.12	0.75 (0.50)	0.40 (0.27)	2.50	-0.86
Total	**168**	**29**	**0.17**	**0.58 (0.39)**	**0.58 (0.34)**	**7.5**	**0.002**

4.5.2 Suckers and clonal distribution within and outside the plots

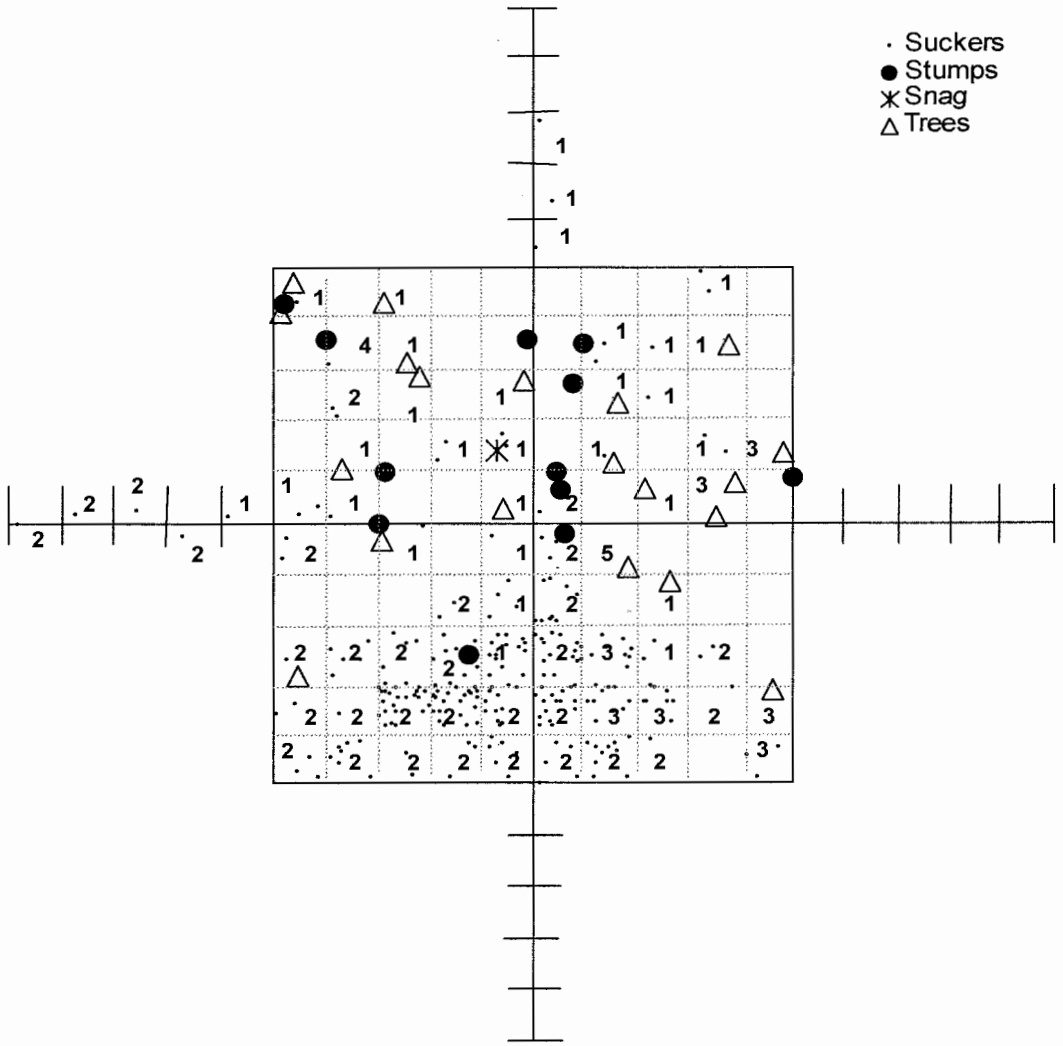
Different harvesting intensities resulted in different suckering responses but similar patterns for the spatial distribution of aspen suckers. In the 20 m x 20 m CC plot, we counted a total of 601 aspen suckers and 20 stumps compared with 247 suckers, 20 non-harvested trees, 12 stumps and one snag in the PC 1/3 plot; in the control, we counted only 34 non-harvested trees (Table 4.2). Among the genotyped suckers, 76% and 91 % regenerated away from stumps and non-harvested trees in the CC and PC1/3 plots, respectively. These suckers were mostly concentrated in the upper two quarters in the CC plot and in the two lower quarters in the PC1/3 plot.By contrast, 84 % of the stumps were located in the lower two quarters of the CC plot and in the upper quarters of the PC1/3 plot. Along the transects, we noticed that most genets (7/10) outside the CC plot were different from those within, all the ramets outside the PC1/3 plot were an extension of the genets within the plot, while only one genet of four had many ramets extending outside the control plot (Figure 4.2a, b, c).

The number of genets sampled in the CC plot exceeded that observed in the PC1/3 plot. In the former, we found 20 genets among the 66 suckers genotyped compared to 5 genets among the 48 suckers and the 20 non-harvested trees genotyped in the PC1/3 plot. In the CC plot, 9 genets were single-ramet (i.e. composed of one ramet) and the average genet size was 3.3 (± 2.9) ramets/genet. In the PC1/3 plot, two of the 5 genets were single-ramet, but only one suckered abundantly (c2) with 29 suckers and one non-harvested tree. The other largest genet sampled also contained 29 ramets but half of its ramets were non-harvested trees. In average, the genet size was 13.6 (±14.3) ramets/genet. In the control plot, only one genet was dominant with 30 ramets, the other three had one or two ramets, and the average genet size was 8.5 (±14.3) ramets/genet. These results are shown in table 4.4.

Figure 4.2. Sucker, non-harvested trees and stumps distribution within the 20 m x 20 m plots and along the 10 m transects following three different treatments: (a) clear-cutting (CC); (b) one-third partial cutting with selection (PC1/3); and (c) control. Only one sucker was genotyped in each 2 m x 2 m quadrat and each number indicates a different genotype. Interrogation marks (?) indicate that the selected stem could not be genotyped.



(b) Genet map in the one-third cut plot



(c) Genet map in the control plot

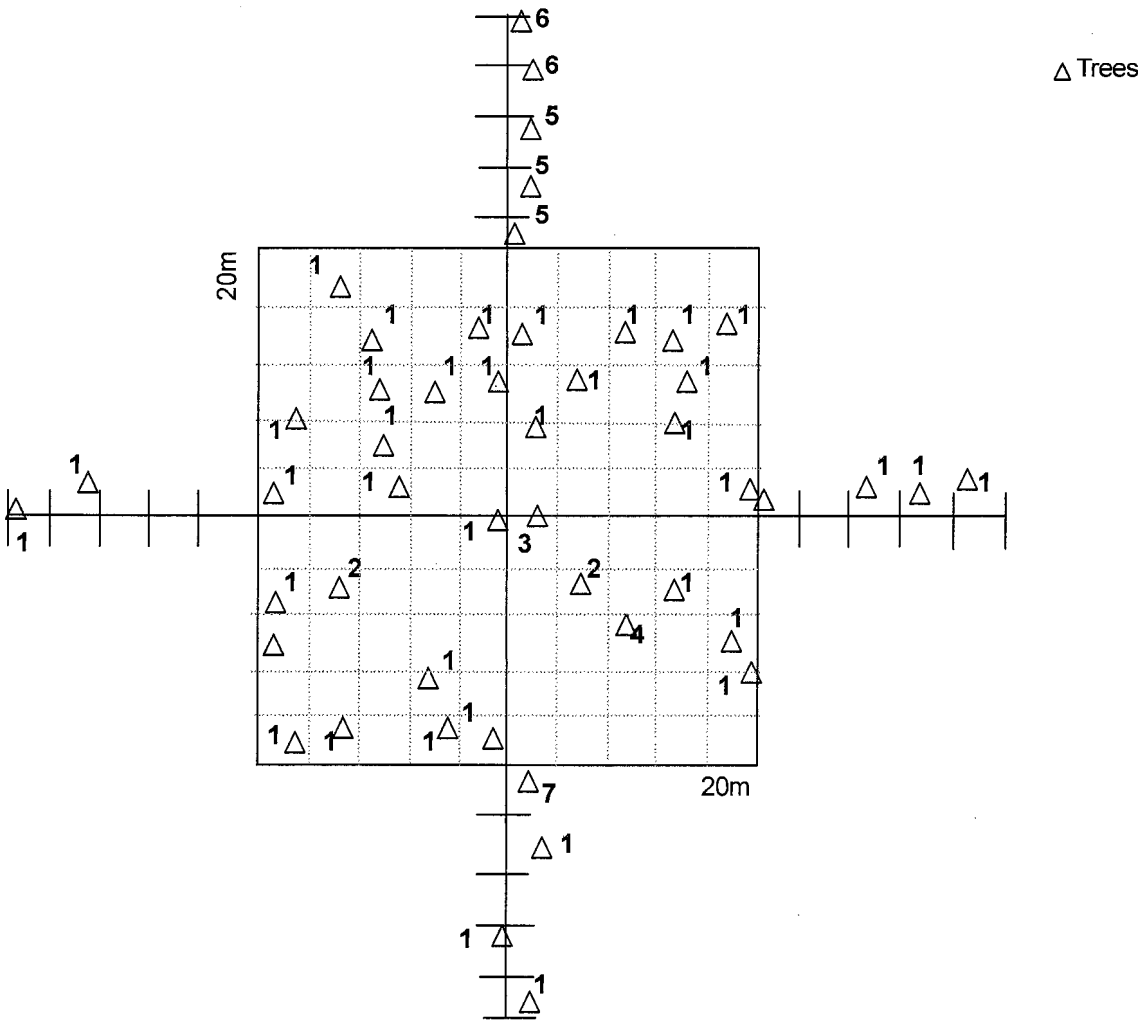


Table 4.4. Total number of ramets per genet within the 20 m x 20 m plots following the three treatments: CC = clear-cutting, PC1/3 = one-third partial cutting with selection, and control. The genet represented by the letter c followed by a number does not have the same genotype in all treatments. The total number of ramets per genet was calculated by randomly analysing one sucker per quadrat of 2 m x 2 m in addition to all non-harvested trees in the plot. The number of ramets with genotypes similar to non-harvested trees are indicated between parentheses.

	Total number of ramets per genet (non-harvested trees per genet)		
Genet	**CC**	**PC 1/3**	**Control**
c1	1	29 (14)	30 (30)
c2	4	29 (1)	1 (1)
c3	4	8 (4)	2 (2)
c4	8	1	1 (1)
c5	1	1 (1)	
c6	5		
c7	2		
c8	7		
c9	1		
c10	2		
c11	3		
c12	10		
c13	1		
c14	8		
c15	1		
c16	4		
c17	1		
c18	1		
c19	1		
c20	1		
Total	66	68	34
Average number of ramets/genet	3.3	13.6	8.5

4.6 DISCUSSION

Our results highlighted two important differences in the impact of CC and selective PC1/3 treatments on aspen regeneration: (1) CC induces a massive regeneration of aspen suckers compared to PC1/3; and (2) CC induces the regeneration of a proportionnally larger number of genets than the PC1/3.

Suckering following clear-cutting has often been reported to be higher than following partial cutting or other treatments (Maini & Horton, 1966; Steneker, 1974; Schier & Campbell, 1978). The concomitant increase in the number of genets with that of aspen density in the CC plot suggests that massive suckering after clear-cutting is related to the suckering of a large number of genets. In the absence of any report about aspen sexual recruitment in the sampling area (Bergeron, 2000), we deduce that these genets were present in the stand before the treatments, and that the increase in soil temperature combined with the elimination of vegetation competition following clear-cutting stimulated their suckering (Lavertu et al., 1994). Moreover, the relative abundance of ramets-per genet in the control plot, 80 years after fire, suggest that genets suckering after fire may contain a large number of ramets each, but most of these genets would be reduced to few ramets by self-thinning or stand break up with time since fire.

Several factors could have theoretically induced the suckering of a relatively large number of genets in the CC plot. These include the release of apical dominance (i.e. a hormonal process between the roots and the upper parts of the tree that prevents genets from suckering) (Steneker, 1974; Lavertu et al., 1994), the increase in soil temperature (Maini & Horton 1966; Zasada & Schier, 1973), the decrease in interspecific vegetation competition (Landhaüsser & Lieffer, 1998) and the increase in light intensity (Steneker, 1974; Frey et al., 2004). The release of apical dominance can only partly explain our observations: on one hand, most suckers (91%) in the PC1/3 plot (4 out of 5) were genetically different from the dominant genet that contained 14 non-harvested trees (c1). On the other hand, genets that were apparently not inhibited by apical dominance from non-harvested trees produced only

few suckers, and the largest suckering genet (c2) was eventually produced by a non-harvested tree in the lower quadrats of the plot. The impact of high soil temperature can not be clearly determined in our study because no significant differences in soil temperature were observed between the CC and the PC1/3 plots in the first two years after harvesting. This can be related to the increase in organic matter after clear-cutting (Brais et al., 2004). Similarly, the effect of light is not certain as a path analysis in the same experimental set up demonstrated that light intensity affects aspen biomass growth (96%) rather than aspen suckering (Brais et al., 2004). Alternatively, we estimate that physiological processes and vegetation competition played an important role in determining suckering potential and distribution in each treatment, in addition to apical dominance. In general, roots near the base of a mature tree are larger than lateral roots and often do not have suckers in their proximity (DesRochers & Lieffers, 2001). By contrast, root suckering occurs more abundantly on small lateral roots (less than 2 cm in diameter) (Kemperman, 1978; Schier & Campbell, 1978; DesRochers & Lieffers, 2001), located within 28 cm deep in the soil (Schier & Campbell, 1978), which would explain the abundant suckering (76% to 91% of suckers) away from stumps and non-harvested trees in both the CC and the PC1/3 plots. Moreover, the low overstory competition due to the absence of non-harvested trees may have favoured the abundant suckering of the dominant genet (c2). in the lower quadrats of the PC1/3 plot.

Although some studies suggest a role for genetic components in explaining differences in clonal suckering ability (Garret & Zahner, 1964; Steneker, 1974; Schier & Campbell, 1978), the relative importance of genetic selection during harvesting could not be clearly determined in the present study as we could not identify the genotype of harvested stems. In addition, most genets that suckered in the PC1/3 plot (4 out of 5) contained at least one non-harvested tree, eventually belonging to genotypes with vigorous growth (based on morphological selection during the treatment) and only the genet that existed in better environmental conditions (more light, less overstory competition) suckered abundantly in the PC1/3 plot, which suggest that physiological and ecological conditions, mostly the release of apical dominance, distance from stumps and canopy opening, have a greater impact on genet suckering ability than the genetic ones. The small sampling scale can not account for the selective suckering observed in the PC1/3 plot as genetic diversity observed at the site scale

was not much different from that observed at the stand scale in previous studies (Namroud et al., 2005).

MANAGEMENT IMPLICATIONS

One implication of our results is that clear-cutting enhances the suckering of a large number of suckers and genets compared to that observed at mid successional stages (the control is 80 years old) or after a partial cutting. Although no genetic analysis has been performed immediately after fire to compare it with the effect of clear-cutting, the maintenance of aspen genetic and genotypic diversity for hundreds and thousands of years under the natural regime controlled by fire suggests that clear-cutting has an impact similar to fire on aspen clonal and genetic diversity. Consequently, clear-cutting appears as an adequate practice when our objective is to maintain aspen regeneration and genetic diversity.

In the PC1/3 plot, aspen regeneration was less dense than in the CC plot, which can easily favour the transition toward mixed or coniferous dominated stands. In this context, the PC1/3 will be emulating the effect of gap dynamics in allowing the stand transition from one seral stage to another. In addition, genet suckering ability was better explained by environmental factors rather than by genetic selection during harvesting, which indicates that partial selective cutting treatments, with small adjustments to provide better environmental conditions for the growth of selected genets, is a promising approach that can be used to favour genets that meet industrial requirements while emulating the effect of gap dynamics in facilitating the transition toward non aspen dominated stands.

4.8 ACKNOWLEDGEMENTS

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CONCLUSION

Cette thèse s'inscrit dans le cadre d'une recherche interdisciplinaire qui joint deux domaines différents: l'écologie et la génétique. La méthode employée s'est appuyée sur l'utilisation de marqueurs moléculaires très variables (les microsatellites) pour identifier les clones de tremble, et sur une approche comparative entre des cohortes issues de perturbations naturelles, des sites à des stades successionnels différents, ou des populations soumises à différentes intensités de coupe. À ces fins, différentes stratégies d'échantillonnage ont été utilisées: (1) un échantillonnage aléatoire et limité à l'échelle du peuplement dans le premier chapitre; (2) une approche de chronoséquence et des simulations à l'échelle du peuplement et du paysage dans le deuxième chapitre; (3) une cartographie complète de tous les trembles à l'échelle d'un hectare dans le troisième chapitre; et (4) une cartographie des trembles dans dans une pracelle témoin et dans des parcelles coupées dans le quatrième chapitre.

De l'ensemble des résultats produits, quatre aspects majeurs émergent pour caractériser la dynamique de la diversité génétique et clonale du tremble: (1) les populations de tremble ont une structure génétique et clonale diversifiée qui s'établit immédiatement après feu; (2) cette diversité est maintenue dans le temps indépendamment de la nature des perturbations; (3) la majorité des genets de tremble sont constitués d'un seule tige et leur extension spatiale ne dépasse pas les quelques dizaines de mètres; (4) les coupes totales et partielles avec sélection engendrent des effets différents sur la structure clonale du tremble.

Contrairement à ce qu'on pourrait s'attendre d'un organisme qui se reproduit essentiellement par voie végétative, les deux premiers chapitres ont montré que le tremble maintient sa diversité génétique et clonale le long du gradient successionnel, indépendamment de la nature des perturbations. Dans un écosystème contrôlé par un régime de feu et par la succession de perturbations secondaires comme les trouées (causées par les épidémies d'insectes et le vent), il est normal que le maintien d'une telle diversité soulève des questions quant à l'origine de cette diversité qu'à l'efficacité de la stratégie d'échantillonnage et des analyses effectuées dans cette étude. Pourtant, l'absence de reproduction sexuée dans

la zone d'échantillonnage (à l'exception de certains cas possibles mais rares suite aux feux) et l'utilisation de marqueurs moléculaires avec une grande puissance de résolution (résolution testée) n'ont pas permis de retenir l'hypothèse de reproduction sexuée pour expliquer cette diversité. En fait, les différentes approches d'analyses utilisées dans les premiers trois chapitres (l'échantillonnage par sélection, l'approche de chronoséquence, et les simulations) ont tous amené à la même conclusion: le tremble maintient une grande diversité génétique et clonale aussi bien dans les cohortes issues après feux que dans celles régénérées dans les trouées où les conditions environnementales ne sont généralement pas favorables pour la germination des semis. Dans le futur, des études documentant la reproduction sexuée du tremble et ces effets sur la diversité génétique seraient d'une grande utilité pour confirmer nos observations.

Si l'effet des perturbations naturelles sur la diversité clonale reste minime, ceci est lié principalement à la diminution concomitante du nombre total des tiges et celui des clones. En effet, les simulations du deuxième chapitre montrent une diminution des ramets 4 à 5 fois plus importante que celle des clones avec le temps depuis le feu à l'échelle du peuplement et du paysage. Ces observations concordent avec les résultats du troisième chapitre, notamment la diminution du nombre des clones et du nombre total des tiges dans la troisième cohorte par rapport à la première et la deuxième cohorte. D'une part, cette diminution reflète une relation entre le taux de survie des clones et le temps depuis le feu ou le cycle de feu. D'autre part, elle démontre que certains clones sont maintenus en l'absence de feu, ce qui entraîne un questionnement sur les facteurs qui assurent le maintien de ces clones. En se basant sur les données de cette étude, il était difficile d'élaborer sur l'importance des facteurs physiologiques tel que ceux reliés au système racinaire, ou des facteurs écologiques tel que la lumière et la température du sol. Pourtant, les données ont permis de détecter un mécanisme intéressant par lequel le tremble réussit à maintenir sa diversité clonale longtemps après feu. Ceci se résume par: (1) le recrutement d'un grand nombre de clones immédiatement après feu; (2) le recrutement de clones composés d'un grand nombre de ramets (>100) après feu; (3) un processus d'élimination des ramets à l'intérieur des clones plutôt que l'élimination des clones, notamment durant les premières 100 à 150 années après feu; et (4) le recrutement périodique des clones dans les cohortes de tremble régénérées dans les trouées. Bien que ce

mécanisme ait été basé essentiellement sur des simulations, il a été partiellement confirmé par les données empiriques du troisième chapitre, notamment par l'observation d'un grand nombre de clones composés d'une seule tige et l'absence d'un clone dominant à travers trois cohortes successives dans le même site (Chapitre 3). Ces observations remettent en question l'hypothèse d'une sélection en faveur de certains génotypes, au moins sur un gradient de trois cohortes successives. Le recrutement d'un grand nombre de clones et d'un grand nombre de ramets par clone immédiatement après feu n'a pas pu être vérifié empiriquement due à l'absence de données génétiques immédiatement après feu. Les seules données génétiques disponibles jusqu'à présent sont celles obtenues dans le Parc National de Yellowstone suite au grand feu de 1988, mais ces données ne peuvent pas être considérées à cause de l'importante reproduction sexuée qui a eu lieu dans ces sites. Investir dans des études futures pour caractériser la structure génétique et clonale du tremble immédiatement après feu s'avère alors nécessaire pour élucider certains aspects encore inconnus de la structure clonale du tremble et pour suggérer des pratiques sylvicoles adéquates pour l'aménagement de cette espèce.

Un des résultats intéressants de cette thèse est qu'elle a permis de dresser, pour la première fois, un portrait clair de la distribution spatiale des clones de tremble et de leur taille dans la forêt boréale du Québec. La plupart des ramets appartenant au même clone étaient significativement agglomérés sur une distance de 30 m, mais les clones ne présentaient aucune indication d'une ségrégation spatiale. La croissance clonale suivait plutôt un patron du type «guérilla» avec le temps depuis le feu. À la lumière des résultats des simulations du deuxième chapitre de cette thèse, il est probable que les clones avaient une distribution plus compacte ou même du type « phalanx » après feu, mais avec le processus d'auto éclaircie (self thinning), la diminution du nombre de ramets par clone aurait entraîné un patron moins compact du type guérilla. L'autre hypothèse est que les clones présentaient le même patron spatial dès la première cohorte issue du feu, et que les ramets appartenant à différents clones étaient quand même entrecroisés dû à l'entrecroisement des racines appartenant aux différents clones. En l'absence de données empiriques pour confirmer l'une ou l'autre de ces deux hypothèses, une évaluation de la distribution spatiale des clones de tremble

immédiatement après feu et le suivi de cette distribution durant les premières 100 à 150 années après feu demeure une piste intéressante à explorer.

Parallèlement à la distribution clonale de tremble, les analyses spatiales ont montré une structuration génétique (basée sur la diversité allélique) spatiale sur une distance d'environ 30 m, qui s'atténue d'une cohorte à une autre. À première vue, ce patron semble être lié à la distribution spatiale des clones. Pourtant ce n'était pas le cas. Même en considérant un seul ramet par clone, les analyses d'autocorrélation spatiale continuaient à révéler une structure génétique spatiale similaire à celle observée quand tous les ramets des clones étaient considérés (30 m). En contrepartie, l'observation d'une structure spatiale entre les arbres de chaque cohorte et qui s'atténuait dans les nouvelles cohortes (elle était aléatoire dans la troisième cohorte) a permis d'avancer l'idée que la distribution spatiale de la diversité génétique du tremble dépend de la densité du tremble. Celle-ci dépend à son tour de la nature des perturbations naturelles et du stade successionel du peuplement. La similarité des analyses spatiales pour la distribution des clones en considérant tous ou un ramet par clone évoque plutôt des interrogations sur les possibilités que les différents clones provenaient du même pool génétique très diversifié. Des analyses phylogénétiques à travers plusieurs cohortes présenteraient fort possiblement des réponses plus précises à ces interrogations.

La comparaison de l'effet des coupes totales et des coupes partielles avec élimination d'un tiers de la surface terrière a permis d'établir une relation claire entre l'intensité des coupes et le nombre de clones régénérés: ces derniers augmentent avec l'augmentation de l'intensité des coupes, ce qui entraîne aussi une augmentation du nombre total de tiges régénérées. À cet effet, les coupes totales et les coupes partielles d'un tiers semblent avoir un effet similaire à celui des feux et des trouées, respectivement, sur la régénération du tremble. L'élimination de la dominance apicale, et l'élévation de la température du sol étaient toutefois incapables d'expliquer complètement ces différences, ce qui suggère un rôle plus important pour la compétition végétative.

Dans les parcelles coupées partiellement avec l'élimination des tiges à faible croissance, l'objectif de favoriser les génotypes à bonne croissance a été raisonnablement atteint. Ceci est essentiellement dû au fait que la majorité des clones régénérés comprenaient des arbres non coupés et qui sont en principe conservés à cause de leur bonne croissance. Cependant, le succès de cette pratique sylvicole visant à favoriser certains genets n'a pas pu être liée à la sélection génétique des clones avant la coupe. En revanche, la régénération abondante d'un seul clone et la faible régénération des autres clones suite aux coupes partielles avec sélection ont amené à avancer l'hypothèse de la compétition végétale pour la lumière et les nutriments sur celle de la sélection génétique des clones. Dès lors, il est possible d'envisager un scénario de coupes partielles qui vise essentiellement à assurer des conditions environnementales favorables au drageonnement à proximité des tiges qui présentent les caractéristiques recherchées.

APPENDICE A

Table A.1. Allele frequencies of aspen per locus and per cohort in plots H (a) and I (b).

(a)

Site H

Locus	Cohort 1	Cohort 2	Cohort 3
PTR 1			
238	_	0.008	_
241	0.048	0.050	0.019
244	0.029	0.008	_
247	_	0.008	_
250	0.058	0.164	_
253	0.721	0.567	0.846
256	0.029	0.021	_
259	_	0.034	_
262	_	0.059	0.019
265	0.010	0.046	0.019
268	0.067	0.025	0.077
271	0.029	0.004	0.019
274	0.010	_	_
298	_	0.004	_
PTR 2			
183	0.008	_	_
186	_	0.005	_
201	_	0.018	0.023
204	0.058	0.031	0.046
207	0.183	0.143	0.114
210	0.383	0.205	0.182
213	0.358	0.433	0.477
216	_	0.138	0.159
219	_	0.018	_
222	0.008	_	_
243	_	0.009	_
PTR 3			
182	_	_	0.058
184	0.032	0.019	_
198	_	0.004	_
210	0.006	_	_
212	0.045	0.015	0.077
214	0.026	0.011	_
216	_	0.015	_
222	0.019	0.004	_

(b)

Site I

Locus	Cohort 1	Cohort 2	Cohort 3
PTR 1			
241	0.302	0.240	0.316
244	_	0.005	_
253	0.628	0.659	0.579
256	_	0.010	0.026
262	0.012	0.010	_
265	0.058	0.043	0.053
268	_	0.010	0.026
271	_	0.024	_
PTR 2			
180	0.011	_	_
186	0.011	0.005	_
201	0.011	0.020	_
204	0.023	0.098	_
207	0.125	0.186	0.469
210	0.080	0.054	0.250
213	0.636	0.471	0.281
216	0.023	0.054	_
219	0.068	0.103	_
222	0.011	0.010	_
PTR 3			
184	_	0.005	_
212	0.011	0.043	_
214	0.011	0.019	0.100
216	0.067	0.010	0.050
220	0.011	_	_
222	0.022	0.019	_
224	0.067	0.135	_
226	0.111	0.115	0.150
228	0.344	0.298	0.600
230	_	_	0.025
232	_	0.053	_
234	0.033	0.096	0.025
236	0.178	0.154	_
238	0.067	0.014	_
240	0.033	0.029	0.025

Table A.1 (continued)

(a)

224	0.103	0.123	0.250
226	0.103	0.067	_
228	0.103	0.313	0.115
230	0.192	0.101	0.096
232	0.167	0.116	0.135
234	0.083	0.037	0.154
236	0.039	0.086	0.058
238	0.026	0.011	0.019
240	0.006	0.004	_
242	0.032	0.011	_
246	_	0.008	_
260	_	0.011	_
262	0.019	0.041	0.039
266	_	0.004	_
PTR 4			
196	0.423	0.389	0.423
198	0.377	0.091	0.346
204	0.062	0.337	0.077
216	0.015	0.008	_
218	0.015	0.040	0.019
220	0.085	0.095	0.135
222	0.008	0.040	_
256	0.015	_	_

(b)

242	0.022	0.010	0.025
338	0.011	_	_
350	0.011	_	_
PTR 4			
196	0.796	0.701	0.950
198	0.148	0.186	0.025
204	0.023	0.044	_
210	_	0.005	_
218	_	0.044	_
220	0.011	0.020	0.025
308	0.023	_	_

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