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AVANT-PROPOS

Cette thèse est rédigée sous forme d'articles. Ainsi, en plus d'une introduction et d'une conclusion générale, trois chapitres sont présentés sous la forme d'articles scientifiques. Le style d'écriture varie légèrement d'un chapitre à l'autre puisqu'ils ont été soumis ou publiés dans des revues différentes et les répétitions d'un chapitre à l'autre sont inévitables. Le chapitre 3 est en préparation et les chapitres 2 et 4 ont déjà été publiés.

Chapitre 2 – Latutrie M., Bergeron Y. and Tremblay F. (2016) No genetic structure found after a fine scale assessment of genetic diversity of trembling aspen in northwestern North America. *BMC Evolutionary Biology* ,16 (1) 1-11

Chapitre 3 – Latutrie M., Bergeron Y. and Tremblay F. Genetic diversity and clonal structure of natural populations of trembling aspen in Canada: a transcontinental study. *En préparation*

Chapitre 4 – Latutrie M., Mérian P., Picq S., Bergeron Y. and Tremblay F. (2015) The effects of genetic diversity, climate and defoliation events on trembling aspen growth performance across Canada. *Tree Genetics & Genomes*, 11: 1-14

Je suis le premier auteur de chacun des chapitres de cette thèse, ayant réalisé l'ensemble du travail depuis la collecte des données jusqu'à la rédaction. Ma directrice Francine Tremblay ainsi que mon co-directeur Yves Bergeron ont suivi chaque étape de cette thèse et ont contribué à la rédaction des chapitres. Pour le chapitre 4, Pierre Mérian a effectué les analyses dendrochronologiques, et Sandrine Picq a participé au développement des protocoles pour une partie des marqueurs microsatellites utilisés. Tous deux ont également participé à la rédaction.

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RÉSUMÉ

La diversité et la structure génétique des espèces arborescentes sont influencées par des facteurs historiques comme les migrations et les flux géniques lors de la recolonisation postglaciaire ou encore des facteurs environnementaux comme les perturbations naturelles (climat, feux de forêts, sècheresse, insectes). Au cours du temps, ces facteurs ont modelé la répartition spatiale de la diversité génétique des espèces présentes dans les écosystèmes forestiers nord-américains comme le peuplier faux-tremble (ou tremble; *Populus tremuloïdes* Michx.). Ainsi la diversité génétique et la structure clonale du tremble peuvent varier en forêt boréale et dans la tremblaie-parc en fonction de facteurs tels que les conditions climatiques, les régimes de feux ou encore la fragmentation du paysage. De la même façon, la croissance radiale tend à être principalement influencée par les conditions environnementales propres à chaque site. Cependant, la composition génétique des arbres pourrait aussi jouer un rôle important dans les dynamiques de croissance, et aucune étude n'a encore évalué ces effets dans des peuplements naturels du peuplier faux-tremble. Cette thèse s'articule autour de trois chapitres dont les objectifs étaient: i) de comprendre les origines et les dynamiques de recolonisation du peuplier, ii) d'évaluer les variations régionales de diversité génétique et de structure clonale au Canada, et iii) de mesurer l'effet de la structure génétique des populations sur les variations de la réponse de croissance du peuplier.

Dans le chapitre 2, nous avons tenté s'identifier les zones refuges et les routes de la recolonisation postglaciaire à travers l'étude de la diversité et de la structure génétique des populations entre elles. En Amérique du nord, la dernière période glaciaire est l'évènement récent ayant eu le plus de conséquences sur la diversité génétique actuelle des espèces boréales. Un total de 28 populations a été échantillonné entre l'Alaska et la Saskatchewan. Nos résultats ont montré qu'il y avait peu à pas de différentiation génétique entre les populations de *P. tremuloides* sans signe d'isolation génétique avec la distance géographique dans la partie nord-ouest de son aire de répartition. Nous avons observé les valeurs les plus faibles d'hétérozygotie observée et les valeurs les plus élevées de richesse allélique au niveau du piémont des montagnes rocheuses de l'Alberta, une région qui pourrait être une zone d'*admixture*. Enfin, il n'y avait aucune évidence que la Béringie ou le *Ice-free corridor* aient été des zones refuges pour cette espèce.

Dans le chapitre 3, nous avons évalué les variations de diversité génétique et de structure clonale du tremble en relation avec les différences de conditions de site (aridité, feux de forêt, fragmentation). L'étude d'un réseau de 30 peuplements à travers la forêt boréale et la tremblaie-parc, nous a permis de montrer que le pourcentage de clones uniques et la diversité clonale élevée sont probablement causés par : i) la reproduction épisodique par graine en plus du drageonnement dans le cas où

le peuplier était déjà présent, et ii) une auto-éclaircie des ramets intra clone plus fort avec le temps écoulé depuis le dernier feux. Nous avons aussi montré que la fragmentation du paysage et des taux de brulage élevés influencent négativement les niveaux de diversité génétique. La diversité génétique et la structure clonale des peuplements de tremble au Canada semblent être plus similaires à l'échelle continentale qu'on aurait pu le supposer avec aucun effet observé du climat. Le tremble apparaît comme étant une espèce généraliste avec une grande capacité d'adaptation maintenant de fort niveau de diversité dans des environnements variés et hétérogènes.

Dans le chapitre 4, nous avons examiné la contribution relative de la génétique (c.-à-d. la diversité clonale, l'hétérozygotie observée) et de l'environnement (c.-à-d. insectes, climat) sur la croissance du peuplier (évaluée par la corrélation moyenne de la croissance entre les arbres (RBAR), l'accroissement en surface terrière des arbres (T BAI) et la variabilité interannuelle de croissance (MS)). Pour cela, nous avons échantillonné 440 arbres dans 22 peuplements naturels en forêt boréale canadienne. La croissance annuelle de peuplements multi-clonaux était moins homogène que celle de peuplements monoclonaux. Le maintien de peuplements de peupliers diversifiés dans le paysage pourrait assurer d'avoir une grande variabilité de la réponse de croissance face aux variations environnementales, qui au final pourrait permettre une plus forte résilience des tremblaies dans des conditions climatiques futures.

Le tremble est une espèce qui livre petit à petit ses secrets. Longtemps laissé de côté et peu étudié en raison de sa facilité de régénération et d'un intérêt économique moindre par rapport aux conifères, son écologie et sa dynamique ne sont pas si simples. Cette thèse a contribué à révéler les bases de l'organisation et des dynamiques génétiques chez cette espèce-clé. Ainsi, cette étude apporte des informations sur l'histoire démographique de cette espèce depuis la dernière glaciation mais aussi sur les différences génétiques observées à travers la forêt boréale et les liens avec la croissance. Dans le futur, il serait bon de développer et d'utiliser une approche génomique et plus particulièrement les SNPs afin de valoriser ce jeu de données riche de diversité et représentatif de l'ensemble de la forêt boréale nord-américaine (du Québec à l'Alaska). Cela pourrait permettre de répondre à des questions telles que : i) y a-t-il des SNPs sous sélection dans certaines zones où les changements environnementaux et le dépérissement sont déjà forts (zone sud de la tremblaie-parc) ; ii) existe-t-il des gènes d'adaptation propres à des conditions environnementales définies (conditions climatiques, résistance aux insectes). D'un point de vue plus pratique, il pourrait être important de comprendre plus précisément : i) quels facteurs gouvernent l'apparition et la persistance d'individus triploïdes (présence de 3 copie du génome) dans le paysage ; ii) qu'apporte la triploïdie comme avantage pour le tremble (physiologie, compétition, croissance) ; iii) quel est le rôle de la triploïdie dans l'adaptation aux changements climatiques. Il y a donc encore de nombreux axes de recherche qui mériteraient d'être développés et exploités dans le futur.

CHAPITRE 1 INTRODUCTION GÉNÉRALE

La diversité et la structure génétique des espèces arborescentes sont influencées par des facteurs historiques comme les migrations et les flux géniques lors de la dernière recolonisation postglaciaire (Hewitt 1996, 2000, 2004) ou encore par des facteurs environnement aux comme les perturbations naturelles (climat, feux de forêts, sècheresse, insectes; Turner *et al.* 2003; Namroud *et al.* 2006). Au cours du temps, ces facteurs ont modelé la répartition spatiale de la diversité génétique des espèces présentes dans les écosystèmes forestiers nord-américains telle que ceux dominés par le peuplier faux-tremble (ou tremble; *Populus tremuloïdes* Michx.).

Le climat et les perturbations naturelles telles que les cycles de feux et les attaques d'insectes varient dans l'espace et des différences régionales apparaissent. Au niveau climatique, les températures et les précipitations mensuelles varient entre l'Est et l'Ouest du Canada (Whitfield & Cannon 2000). On observe ainsi des différentes plus fortes en terme de précipitation qu'en terme de température à travers la forêt boréale canadienne ce qui affecte les dynamiques des écosystèmes forestiers (espèces présentes, régénération, dynamique des écosystèmes). Alors que les températures sont relativement homogènes en forêt boréale mixte, les précipitations et l'aridité sont des facteurs qui varient grandement. En effet, l'est du Canada (Ontario et Québec) est une zone relativement humide où on observe des précipitations de l'ordre de 600 à 1200 mm par an (Tableau 1.1; Bergeron *et al.* 2014) et des indices d'aridité variant de 20 à 70 en moyenne sur la période 1950-2010 (ici le *Climate Moisture Index*, CMI (Hogg, 1997) calculé à partir de données de BIOSIM 10 (Régnière *et al.* 2013)). En revanche dans l'ouest du Canada (Yukon, Territoire du nord-ouest, Colombie Britannique, Alberta, Saskatchewan et Manitoba), les précipitations annuelles sont de l'ordre de 400 à 600 mm par an (Tableau 1.1; Bergeron *et al.* 2014) et l'aridité est comprise entre -20 et 20 faisant de cette région une zone relativement sèche. Les variations de conditions climatiques ont aussi un effet indirect sur les régimes de perturbation comme les feux de forêt et les attaques d'insectes.

Les feux de forêt font partie de la dynamique naturelle en forêt boréale nord-américaine. Historiquement, les feux étaient plus fréquents qu'aujourd'hui dans l'hémisphère nord entre 6000 et 3000 BP avant de se stabiliser près des valeurs actuelles sur le continent nord- américain à partir de 3000 BP (Power *et al.* 2008). Il semble que l'Ouest de l'Amérique du Nord soit plus touché par les feux que la zone Est (Power *et al.* 2008). Ainsi, on observe de très grands feux se produisant avec un cycle court dans l'ouest, alors que dans l'est, ces feux sont grands et sont associés à un cycle long (Tableau 1.1; Bergeron *et al.* 2014), ce qui pourrait être un élément clé pour la compréhension de la structure clonale actuelle (diversité, taille et superficie clonale) des peuplements de tremble.

Tableau 1.1 Tiré du tableau 1 de Bergeron et al. 2002 résumant les différences clés entre l'est et l'ouest de la forêt boréale mixte canadienne.

Caractéristique	West (Yukon, Territoire du nord ouest, Colombie Britannique, Alberta, Saskatchewan et Manitoba)	Est (Ontario et Québec)
Températures	Similaires dans la zone de la forêt boréale mixte	
Précipitations annuelles	400 – 600 mm (sec)	600 – 1200 mm (humide)
Dépôt de surface principal	Argile (avec quelques alluvions)	Alluvions (sauf pour la ceinture d'argile)
Principale roche mère	Sédimentaire (calcaire – basique)	Intrusion métamorphique (granite – Acide)
Principales espèces d'arbre	Peuplier faux-tremble Peuplier baumier Bouleau à papier Epinette blanche	Bouleau à papier Peuplier faux-tremble Sapin baumier Peuplier faux-tremble Thuya occidental
Feux de forêt	Cycle court Très grand	Cycle long Grand
Perturbations secondaires	Sècheresse Livrée des forêts	Tordeuse des bourgeons

Figure 1.1 a) Peuplement de peupliers faux-tremble à l'automne au Québec; b) Reproduction végétative en Alberta dans la zone de la tremblaie-parc après ouverture de la canopée; c) Reproduction sexuée avec production de graines très facilement transportables par le vent (Crédit photo: Dave Hanson).

Les attaques d'insectes défoliateurs du peuplier faux-tremble comme la livrée des forêts (*Malacosoma disstria* Hübner; LDF) ou la tordeuse du tremble (*Choristoneura spp.*) peuvent engendrer de fortes réductions de croissance nécessitant 3 à 4 ans de rétablissement (MacKenzie 2010). Au Québec et en Ontario, les attaques de LDF sont des évènements récurrents ayant une périodicité de 9 à 13 ans (Cooke & Lorenzetti 2006). Ces attaques durent, en général, un à deux ans et localement elles peuvent persister jusqu'à 5 ans (Cooke *et al.* 2009). Au Manitoba, une étude dendrochronologique montre que la fréquence des attaques était de 20 ans (Sutton & Tardif, 2007). Pour l'est du Canada, les épidémies de livrée des forêts sont bien répertoriées dans la littérature (e.g. Cooke & Lorenzetti 2006; Cooke *et al.* 2009).

En revanche, il y a peu de données disponibles en ce qui concerne la zone ouest du Canada pour pouvoir comparer entre les deux zones. Les épidémies de LDF représentent un facteur important dans la formation de trouées dans les peuplements de peuplier faux-tremble (Moulinier *et al.* 2014).

Le tremble est une espèce écologiquement et économiquement importante pour la forêt boréale canadienne. En forêt boréale mixte, les peuplements de peuplier faux-tremble s'installent généralement après feu pour faire place dans la succession naturelle à des peuplements mixtes et conifériens (Figure 1.1a; Bergeron 2000; Bergeron *et al.* 2002; Bergeron *et al.* 2014). Il se régénère rapidement après perturbations (feux de forêt, coupes totales, trouées, chablis, défoliations) grâce à son mode de reproduction végétatif, par drageonnement racinaire (Figure 1.1b), et domine les premières étapes de la succession en forêt boréale mixte (Figure 1.2; Bergeron 2000; Cumming *et al.* 2000; MacKenzie 2010). La suppression de la dominance apicale, l'augmentation de la température du sol et la disponibilité en lumière stimulent le drageonnement racinaire (MacKenzie 2010).

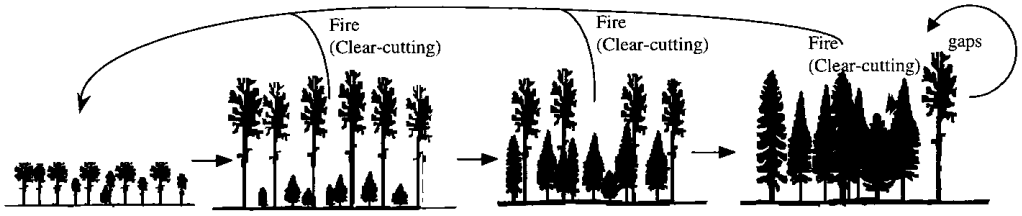

Figure 1.2 Dynamique naturelle et activités sylvicoles en forêt boréale mixte (Bergeron, 2002).

La reproduction par voie sexuée (Figure 1.1c) est généralement faible du fait que les conditions nécessaires pour permettre la germination des graines (humidité du sol adéquate, drainage, luminosité, compétition limitée avec d'autres espèces, ...) sont rarement rencontrées (Mitton & Grant 1996; Romme *et al.* 1997; Romme *et al.* 2005; MacKenzie 2010). Il est supposé que la reproduction sexuée domine chez le tremble

dans l'est où les conditions sont généralement humides et favorables pour la germination des graines alors que dans l'ouest, la reproduction végétative dominerait avec des conséquences sur la structure génétique et clonale des peuplements. La grande variabilité génétique observée chez le tremble qui lui confère le matériel nécessaire à son adaptation et son évolution (Cheliak & Dancik 1982; Grant *et al.* 1992; Jelinski & Cheliak 1992; Kanaga *et al.* 2008; Mock *et al.* 2008; Madritch *et al.* 2009) ainsi que de la capacité de cette espèce à utiliser les deux types de reproduction différent (sexuée et asexuée; MacKenzie 2010) expliquent la faculté de cette espèce à couvrir une aire de répartition aussi vaste (Little 1971).

1.1. Recolonisation postglaciaire du tremble

En Amérique du Nord, la dernière glaciation a forcé un grand nombre d'espèces à se retirer dans des zones refuges et représente sans conteste l'évènement climatique le plus récent dont les répercussions sur les espèces boréales ont été les plus sévères (Hewitt 1996). A l'intérieur de ces refuges, les populations se sont trouvées réduites et isolées, et se sont lentement différenciées au fil du temps. Lors de la débâcle glaciaire, les espèces ont commencé à recoloniser les territoires libérés par expansion de zones marginales conduisant à une réduction de la diversité génétique le long de cet axe d'expansion (Hewitt 2004). Par ailleurs, lorsque des lignées originaires de refuges différents se rencontrent, on observe généralement une augmentation de la diversité génétique dans les zones de contact dites d'*admixture* (Petit *et al.* 2003).

Selon Dyke *et al.* (2003), le dernier maximum glaciaire se situe il y a environ 21 000 ans. Ainsi la dernière expansion des espèces végétales et animales se serait produite à partir de refuges datant de cette période. L'évolution de la couverture forestière depuis la dernière glaciation permet de localiser des refuges glaciaires potentiels pour plusieurs espèces résineuses et feuillues (Williams 2003). Dans le cas du peuplier faux-tremble, la recolonisation postglaciaire sur le continent nord-américain s'est potentiellement effectuée à partir de six refuges (identifiés par Callahan *et al.* 2012

d'après les travaux de Beatty & Provan, 2010 et Shafer *et al.* 2010) deux situés au sud-est (grands lacs et Appalaches), deux au sud-ouest (montagnes Rocheuses et le *Ice-Free corridor*), un à l'est (Terre Neuve et Labrabor) et un dernier , en Alaska (Béringie). Plusieurs espèces de mammifères (Loehr *et al.* 2006), de plantes herbacées (Abbot *et al.* 2000; Beatty & Provan 2010) et d'arbres (Brubaker *et al.* 2005; Anderson *et al.* 2006; de Lafontaine *et al.* 2010; Anderson *et al.* 2011) sont présumées avoir trouvé refuge en Béringie durant la dernière période glaciaire.

En ce qui concerne le genre *Populus*, des études polliniques ou de macrorestes attestent de sa présence en Alaska rapidement après le dernier maximum glaciaire suggérant que cette zone ait bien été un refuge (Hu *et al.* 1996; Brubaker *et al.* 2005; Peros *et al.* 2008). Dans le cas de *Populus balsamifera*, une étude génétique récente n'a pas permis de confirmer que l'Alaska ait été un refuge glaciaire mais a cependant permis de confirmer l'existence de deux groupes distincts au nord-ouest de l'Amérique du Nord, soit: i) un premier au nord recouvrant l'Alaska et le Yukon, ii) un second sur la partie centrale de l'aire de répartition de l'espèce (Keller *et al.* 2010). Keller *et al.* (2010) ont donc conclus que le groupe central aurait pour origine un refuge principal situé au sud de l'aire de répartition actuelle pendant le Pléistocène et qu'une expansion rapide vers les zones marginales au nord s'est ensuite produite lors de la débâcle glaciaire. Enfin, Callahan *et al.* (2013) ont montré que les populations de peupliers faux-tremble situées dans le sud-ouest de son aire de répartition (des États-Unis au Mexique) appartiennent à un groupe différent du reste des populations situées plus au nord en raison d'un isolement prolongé pendant le dernier maximum glaciaire. Les autres refuges potentiels situés à l'ouest, n'ont pas pu être identifiés formellement dû à un échantillonnage qui ne couvrait pas toute la région (Callahan *et al.* 2013).

Le *Ice-free Corridor*, quant à lui, se situerait entre le glacier des Laurentides et le glacier de la Cordillère, cette région pourrait avoir été un refuge cryptique ou plus

probablement une zone d'*admixture* pour le tremble du fait qu'une forte diversité génétique ait été observée par Callahan *et al.* (2013). Dans cette zone les deux glaciers n'ont pas avancé durant la même période, laissant temporairement une zone libre de glace. De plus, de nombreuses zones de l'Est du piémont des montagnes rocheuses seraient restées sans glace durant le dernier maximum glaciaire (Mandryk *et al.* 2001; Arnold 2002; Jackson & Wilson 2004; Loehr *et al.* 2006) et le tremble aurait pu persister dans ces zones.

1.2. Climat, perturbations, diversité génétique et structure clonale

La régénération et l'organisation de la structure clonale et génétique des peuplements de peuplier faux-tremble pourraient donc varier en fonction du type et de la fréquence des perturbations (Stevens *et al.* 1999; MacKenzie 2010). L'utilisation de marqueurs moléculaires révèle toutefois que la structure clonale peut être complexe et des tiges de génotypes différents sont souvent mélangées dans les peuplements (Wyman *et al.* 2003; Mock *et al.* 2008). Ces outils ont amené une perspective nouvelle pour l'étude de la structure génétique et clonale du peuplier faux-tremble. Une étude de marqueurs microsatellites montre que la présence de trois allèles pour un même locus est fréquente suggérant des cas de triploïdie ou d'aneuploïdie (Mock *et al.* 2008; Mock *et al.* 2012). Mock *et al.* (2012) ont montré un effet significatif des conditions climatiques sur le taux de triploïdie avec une tendance des plus grands clones à être triploïdes. De forts niveaux de variation et des excès d'hétérozygotie sont rencontrés dans des conditions semi-arides (ouest de l'aire de répartition) alors qu'une plus faible variation et une d'hétérozygotie plus faible sont généralement observés en conditions relativement humides dans les forêts situées dans l'Est de l'aire de répartition (Cheliak & Dancik 1982; Hyun *et al.* 1987; Lund *et al.* 1992; Mitton & Grant 1996). Jelinski & Cheliak (1992) ont montré que dans des zones écologiquement différentes, toutes les populations étudiées, par une analyse isoenzymatique, conservent un haut niveau de diversité intra-populations. En ce qui

concerne la diversité génotypique, elle a tendance à diminuer avec une augmentation de la multiplication végétative chez les plantes clonales. Cependant, une forte diversité génotypique, comparable à celle d'une population d'origine entièrement sexuée, peut être maintenue par des événements occasionnels de reproduction sexuée qui créent de nouveaux génotypes (Balloux *et al.* 2003; Bengtsson 2003). Enfin, des taux élevés de multiplication végétative vont influencer positivement les niveaux d'hétérozygotie étant donné que deux fois plus d'allèles par locus peuvent être maintenus dans les populations purement clonales par rapport à celles purement d'origine sexuée (Balloux *et al.* 2003).

Turner *et al.* (2003) ont suggéré que les perturbations peuvent jouer un rôle clé dans la structure, la génétique et évolution des populations chez les espèces forestières. L'effet du temps depuis le dernier feu (TDF) sur la diversité clonale (génotypique) du tremble a été documenté au Québec (Namroud *et al.* 2006). La diversité clonale augmente progressivement durant les 150 premières années après feu à l'échelle du peuplement pour se stabiliser par la suite en forêt boréale mixte (Namroud *et al.* 2006). Namroud *et al.* (2006) ont suggéré que la mortalité des ramets intra-genets, plutôt que la mortalité des genets, contribuant à l'augmentation de la diversité clonale avec le TDF dans la forêt boréale mixte de l'Est du Canada.

Les différences régionales de climat, de régimes de feu, et au niveau de la fragmentation du paysage devraient influencer la diversité génétique et la structure clonale du tremble en forêt boréale canadienne. La structure clonale du tremble (richesse, équitabilité) devrait varier selon ces régimes de perturbations. Des conditions sèches dans l'ouest de la forêt boréale devraient limiter le recrutement par voie sexuée, tandis que les incendies plus fréquents devraient favoriser une augmentation de la taille relative des clones par multiplication végétative. A l'inverse, la diversité clonale et la proportion de clones composés d'un seul ramet devraient être plus élevées dans la partie Est en raison de la présence de conditions humides

(favorable au recrutement par graine) et de taux de brulage faible (cycle de feu long) favorisant l'auto-éclaircie des ramets (Namroud *et al.* 2006). Enfin, la diversité génétique (hétérozygotie, richesse allélique) devrait également varier d'une région à l'autre en raison de conditions environnementales et de structure clonale différentes.

1.3. Diversité génétique et croissance radiale

La dendrochronologie est un outil puissant pour mesurer la croissance des arbres et en particulier comprendre les effets de l'environnement sur cette dernière. Les techniques de dendrochronologie ont été utilisées pour l'étude des pertes de productivité ou de biomasse (Hogg *et al.* 2008) et pour évaluer les relations entre la croissance et le climat (Hogg 2001; Hogg & Bernier 2005; Leonelli *et al.* 2008), entre la croissance et la compétition (Drobyshev *et al.* 2013; Huang *et al.* 2013), ou les effets de perturbations sur la croissance (défoliateurs, vent, coupes partielles; Brais *et al.* 2004; Huang *et al.* 2008; Man *et al.* 2008; Gendreau-Berthiaume *et al.* 2012; Moulinier *et al.* 2014).

En forêt boréale mixte composée de peuplier faux-tremble et d'épinette noire (*Picea mariana*), la réponse de croissance aux variations climatiques diffère d'une espèce à l'autre et ne serait pas synchrone entre ces deux espèces (Drobyshev *et al.* 2013). Ceci pourrait donc conduire à des changements en ce qui concerne la structure et la composition des forêts mixtes dans le futur (Drobyshev *et al.* 2013). Les variations interannuelles de croissance chez le peuplier sont particulièrement sensibles aux évènements de défoliation par la livrée des forêts (LDF) et à l'aridité du sol (*Climate Moisture Index*, CMI; Hogg *et al.* 2002b). En revanche, dans la plupart des études, la composition génétique des populations étudiées n'est jamais évaluée et les variations de croissance sont principalement considérées comme étant seulement influencées par une réponse au climat sans contribution significative de la génétique (King *et al.* 2013).

Chez le peuplier, un seul génotype (ou clone) peut couvrir de grandes surfaces (e.g. le clone "Pando" dans le sud de l'Utah qui couvre 43 ha; Grant *et al.* 1992; DeWoody *et al.* 2008) et ces surfaces peuvent être affectées par un dépérissement rapide et soudain à la suite de changements environnementaux sévères, comme une sécheresse (e.g. Worrall et al. 2013). Ce dépérissement peut être dû à une mauvaise adaptation de certains génotypes aux nouvelles conditions environnementales. Il est tout aussi possible d'observer des génotypes qui seraient mieux adaptés aux nouvelles conditions climatiques. Par conséquent les performances de croissance d'une population pourraient être, en moyenne, similaires dans le temps si les peuplements sont constitués d'un assemblage d'une multitude de clones avec des tolérances climatiques différentes. Les plus grandes différences de croissance seraient donc observées au niveau du peuplement entre les génotypes plutôt qu'entre les populations.

Mitton & Grant (1996) ont émis l'hypothèse que les différences régionales en matière de génétique et de structure clonale des peuplements de peuplier reflèteraient l'aridité du climat, la capacité à se reproduire végétativement et le mode de reproduction dominant (sexuée ou végétative). Au niveau régional, une plus forte hétérozygotie individuelle pourrait permettre à l'arbre d'avoir une plus grande flexibilité dans sa réponse de croissance face aux changements environnementaux (Mitton & Grant 1984), conduisant à une plus forte croissance radiale (Jelinski & Cheliak 1992; Cole *et al.* 2010). Il à été montré qu'en moyenne 20% de la variation de croissance des arbres (valeurs variant entre 10 % et 40 % en fonction de l'espèce) est sous contrôle génétique (Cornelius 1994; Beaulieu & Bousquet 2010). L'étude des variations de croissance observées entre des peupliers d'origines géographiques différentes dans des tests de provenance ont montré que la croissance était en partie sous contrôle génétique (Gray *et al.* 2010; Li *et al.* 2010; Schreiber *et al.* 2013). L'héritabilité au sens large (h2 : proportion de variance phénotypique qui est sous contrôle génétique) pour la croissance en hauteur et le diamètre à hauteur de poitrine (DHP à 1,3m), sont

estimées à 0.45 et 0.43, respectivement, pour des clones de peuplier (Gylander *et al.* 2012). Il semble donc que la croissance du peuplier soit en partie influencée par des facteurs génétique ce qui n'a, jusqu'à présent, jamais été evalué dans des peuplements naturels de peupliers faux-tremble en forêt boréale.

1.4. Objectifs généraux

De nombreuses études génétiques ont été réalisées sur le tremble (e.g. Namroud *et al.* 2005a; Namroud *et al.* 2005b; Ally *et al.* 2008; Mock *et al.* 2008; Mock *et al.* 2012; Callahan *et al.* 2013), mais peu se sont intéressées aux dynamiques de la diversité génétique et de la structure clonale de cette espèce à l'échelle transcontinentale au Canada (Mock *et al.* 2012; Callahan *et al.* 2013). L'objectif principal de cette thèse était d'évaluer les différences régionales de diversité génétique et de structure clonale, de comprendre leurs origines (facteur historique, environnement, perturbations) et de quantifier l'effet de facteurs génétiques sur les variations de la réponse de croissance du peuplier. Plus précisément, cette thèse s'articule autour de trois chapitres:

- Refuge glaciaire et recolonisation : Déterminer les zones de refuges glaciaires et les voies de migration postglaciaire du peuplier faux tremble dans la partie ouest de l'Amérique du Nord.
- Structure génétique et clonale : Mesurer la diversité génétique et la structure clonale du peuplier dans les différentes régions de la forêt boréale canadienne et de la tremblaie-parc. Puis évaluer dans quelle mesure le climat, les feux de forêt et la fragmentation du paysage influencent les différences de diversité génétique et de structure clonale observées.
- Croissance : Evaluer l'influence de la diversité génétique et clonale des populations sur les variations de la réponse de croissance du peuplier.

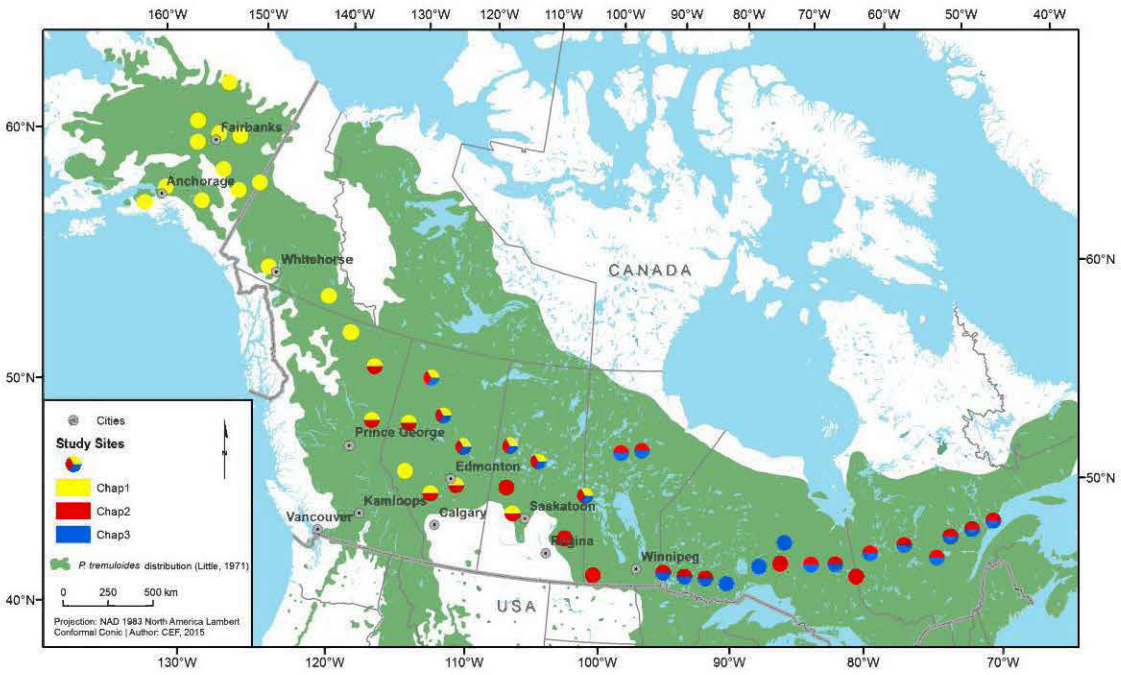

Figure 1.3 Localisation de l'ensemble des sites échantillonnés dans la forêt boréale et la tremblaie-parc. Les chapitres 1 (jaune), 2 (rouge) et 3 (bleu) sont respectivement composés de 27, 30 et 22 sites.

1.5. Zone d'étude

La zone d'étude couvre la forêt boréale mixte canadienne et la tremblaie-parc. La forêt boréale est continue et s'étend de l'Alaska au Labrador en traversant le Canada. La tremblaie-parc est fragmentée et forme la zone sud de transition entre la forêt boréale et les prairies canadiennes entre l'Alberta et le Manitoba. Les sites échantillonnés pour chaque chapitre de la thèse sont illustrés dans la Figure 1.3. En ce qui concerne les chapitres 2 et 3, l'ensemble des sites se trouve sur un sol mésique et dans des peuplements équiennes et matures (> 50 ans) où le peuplier est l'espèce dominante (> 75 % de surface terrière).

CHAPITRE 2 FINE SCALE ASSESSMENT OF GENETIC DIVERSITY AND GENETIC STRUCTURE OF TREMBLING ASPEN IN NORTHWESTERN NORTH AMERICA

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2.1. Abstract

Background: In North America, the last ice age is the most recent event with severe consequences on boreal species' ranges. Geographic patterns of range expansion in trembling aspen (*Populus tremuloides*) suggested that Beringia is likely to be a refugium and the "ice-free corridor" in Alberta may represent a region where small populations persisted during the last glacial maximum (LGM). The purpose of this study was to ascertain whether the origins of trembling aspen in western North America are reflected in the patterns of neutral genetic diversity and population structure. A total of 28 sites were sampled covering the northwestern part of aspen's distribution, from Manitoba to Alaska. Twelve microsatellite markers were used to describe patterns of genetic diversity. The genetic structure of trembling aspen populations was assessed by using multivariate analyses, Mantel correlograms, neighbor-joining trees and Bayesian analysis.

Results: Microsatellite markers revealed little to no neutral genetic structure of *P. tremuloides* populations in northwestern North America. Low differentiation among populations and small isolation by distance (IBD) were observed. The most probable number of clusters detected by STRUCTURE was $K = 3$ ($\Delta K = 5.9$). The individuals in the populations of the 3 clusters share a common gene pool and showed a high level of admixture. No evidence was found that either Beringia or the "ice-free corridor" were refugia. Highest allelic richness (AR) and lowest heterozygosity (Ho) were observed in Alberta foothills of the Rocky Mountains.

Conclusions: Contrary to our hypothesis, our results showed that microsatellite markers revealed little to no genetic structure in *P. tremuloides* populations. Consequently, no divergent populations were observed near supposed refugia. The lack of detectable refugia in Beringia and in the "ice-free corridor" was due to high levels of gene flow between trembling apsen populations. More favorable environmental conditions for sexual reproduction and successful trembling aspen

seedling establishment may have contributed to increase allelic richness through recombination in populations from the Albertan foothills of the Rocky Mountains.

Keywords: aspen, Beringia, genetic, Ice-Free Corridor, Last Glacial Maximum, microsatellites, northwestern North America, phylogeography.

2.2. Résumé

Contexte: En Amérique du Nord, la dernière période glaciaire est l'événement le plus récent ayant eu de fortes conséquences sur la répartition des espèces boréales. Les dynamiques d'expansion géographique du *Populus tremuloides* suggèrent que la Béringie serait susceptible d'avoir été un refuge et que le *Ice-free corridor* en Alberta pourrait représenter une région où de petites populations auraient persisté au cours du dernier maximum glaciaire. Le but de cette étude était de déterminer si l'origine du peuplier faux-tremble dans l'ouest de l'Amérique du Nord se reflète dans l'organisation de la diversité génétique neutre et dans la structure des populations. Un total de 28 populations a été échantillonné couvrant la partie nord-ouest de l'aire de répartition du tremble du Manitoba à l'Alaska. Douze marqueurs microsatellites ont été utilisés pour décrire les flux de gènes et la diversité génétique. Nous avons évalué la structure génétique en utilisant des analyses multivariées, des corrélogrammes de Mantel, des arbres phylogénétiques (*neighbour-joining)* et des analyses bayésiennes.

Résultats: Les marqueurs microsatellites ont révélé peu ou pas de structure génétique neutre des populations de P. tremuloides dans le nord-ouest de l'Amérique du Nord. Une faible différenciation entre les populations et un faible isolement par distance (IDB) ont été observés. Le nombre le plus probable de groupes détectés par STRUCTURE était K = 3 ($\Delta K = 5,9$). Les individus dans les populations des trois groupes partagent un pool génétique commun et ont montré un niveau élevé d'admixture. Aucune preuve n'a été trouvée que la Beringia ou le *Ice-free corridor* étaient des refuges. La plus grande richesse allélique (AR) et la plus faible hétérozygotie (*Ho*) ont été observées dans les contreforts des montagnes Rocheuses de l'Alberta.

Conclusions: Contrairement à notre hypothèse, nos résultats ont montré que les marqueurs microsatellites révélaient peu ou pas de structure génétique dans les populations de *P. tremuloides*. Par conséquent, aucune population divergente n'a été observée près des refuges supposés. Le manque de refuges détectables en Beringia et

dans le *Ice-free corridor* était dû à des niveaux élevés de flux de gènes entre les populations de peuplier faux-tremble. Des conditions environnementales plus favorables à la reproduction sexuée et des succès d'établissement de plantules de peuplier peuvent avoir contribué à augmenter la richesse allélique par recombinaison dans les populations des contreforts des montagnes Rocheuses de l'Alberta.

Keywords: Béringie, génétique, *Ice-Free Corridor*, Dernier Maximum Glaciaire, microsatellites, nord-ouest de l'Amérique du Nord, peuplier, phylogeographie.

2.3. Background

Earth has experienced several episodes of severe climatic variation that have led to a succession of ice ages during the Quaternary (2.58 Mya until present) [1]. During this period, many species have experienced a succession of range expansions and contractions that have affected their genetic structure and diversity [1,2,3]. In their review, Excoffier et al. [2] reported that the effects of these range expansions on genetic diversity could differ markedly from pure demographic expansions. Range expansions are characterized by a decrease in genetic diversity along the expansion axis, owing to recurrent bottlenecks and founder events [4].

In North America, the last ice age is unquestionably the most recent event to have had severe consequences for boreal species genetic diversity [5]. According to estimates by Dyke et al. [6], the Last Glacial Maximum (LGM) occurred 18–21 ky BP. It is therefore considered to be that point when the last massive expansion of plants from different glacial refugia was initiated. Williams [7] and Roberts and Hamann [8] have reconstructed the evolution of land covered by several tree species in North America since the LGM, thereby allowing the identification of potential refugia for those species. Also, molecular studies have shown evidence of genetic structure for several North American plant species [9,10,11,12,13,14].

Beatty and Provan [10] proposed a set of 10 potential glacial refugia for terrestrial plant and animals in North America. This set was subsequently reduced by Callahan [15] to 6 potential refugia for trembling aspen (*Populus tremuloides* Michaux), including: Beringia, the Grand Banks, the northeastern United States, the "Driftless Area" of the mid-western United States, the "ice-free Corridor" along the and eastern slopes of the Alberta Rocky Mountains, e.g., [16,17,18] and the Clearwater Refugium of northern Idaho, e.g., [19].

Beringia has been suspected of being a refugium for mammals [20], herbaceous plants [10,21], and trees [11,22,23,24] during the last ice age maximum. Simulated suitable habitat during the LGM for some boreal and sub-boreal species such as white spruce (*Picea glauca* [Moench] Voss), black spruce (*Picea mariana* [Mill.] BSP), lodgepole pine (*Pinus contorta* ssp. *latifolia* [Engelm.] Critchfield), and *P. tremuloides* were usually located along the northern Pacific coast and in Beringia, as well as their presence south of the ice sheet [8]. Paleoecological and palynological studies have revealed the presence of *Populus* in Alaska shortly after the beginning of the ice cap melting, suggesting that the genus has persisted in this area [8,24,25,26]. For balsam poplar (*Populus balsamifera* L.), recent molecular evidence does not support Alaska as glacial refugium but does confirm the existence of two distinct groups in northwestern North America, i.e., a northern group in Alaska and Yukon, and a central group in central distribution area [12]. Keller et al. [12] concluded that the central group descended from the main demographic refugium of *P. balsamifera* under Pleistocene range restrictions, with an expansion toward its margins during range expansion following LGM. For *P. tremuloides*, two models that were based on paleoecological data suggest the existence of refugial habitats in Beringia and was likely a true refugium for this species. Moreover, Callahan et al. [27] have shown the existence of two distinct groups for trembling aspen, namely, one in southwestern USA, and the other in Canada and Alaska. Within the second group, the higher allelic richness that was detected in aspen populations located in Alaska and in Alberta suggests that Beringia was likely to be a true refugium and that the presence of an "ice-free corridor" in Alberta could have permitted to *P. tremuloides* to persist in this area during the LGM [15].

The existence of an "ice-free Corridor" between the Laurentian and Cordilleran ice sheets is debated [16]. Since the ice sheets did not advance at the same time in this region, a temporally and geographically shifting ice-free zone could have existed [20,28]. The Laurentian and Cordilleran ice sheets only coalesced for a brief span of

time [16], while numerous isolated foothills of the Rocky Mountains also could have remained ice-free during the LGM [20,28], potentially leaving suitable habitats for *P. tremuloides*. Yet, suitable habitat conditions for *P. tremuloides* during the LGM were not found in this area according to the simulations of by Roberts and Hamann [8]. In contrast, Callahan et al. [27] reported a higher level of genetic diversity for *P. tremuloides* in this region. This area appears to be important either as a potentially cryptic refugium or more likely as an admixture zone.

The purpose of this study was to ascertain whether the origin of trembling aspen in western North America is reflected in the patterns of neutral genetic diversity and population structure. In the present study, the glacial origin and post-glacial migration route in the northwestern part of the range was uncovered by studying the area analyzed by Callahan et al. [27] at a finer scale. Our aim was to test whether Beringia and the “ice-free Corridor” that was situated between the Laurentian and Cordilleran ice sheets might have been the two glacial refugia for trembling aspen in northwestern North America during the Wisconsin Ice Age. The hypothesis were as follows: 1) aspen populations that were located near refugia (Beringia and the "ice-free corridor") should be highly divergent; 2) within-population diversity should decrease with distance from refugia, due to multiple founder events; 3) the “ice-free Corridor” was an admixture zone, where divergent lineages (from the south and from the Alaska) had converged.

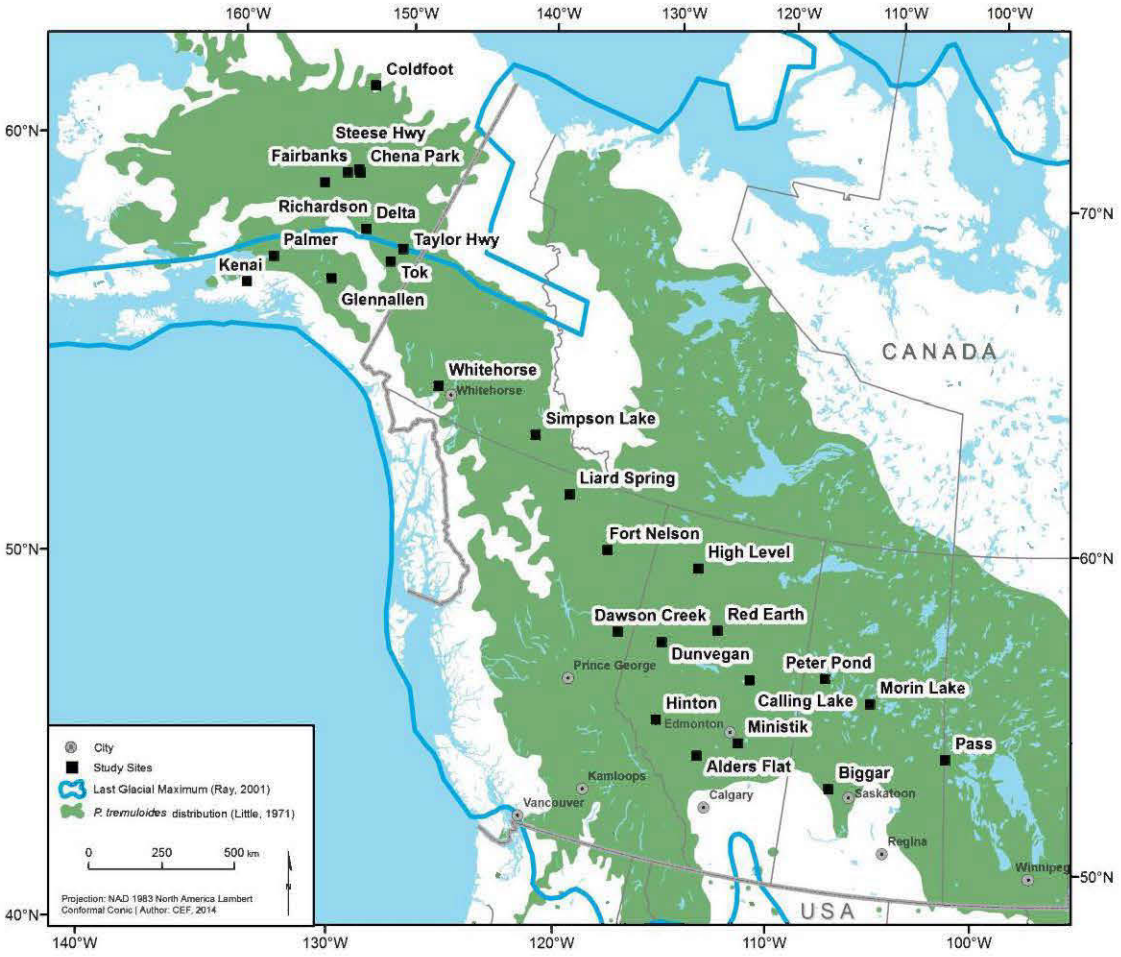

Figure 2.1 Study populations across the western part of *Populus tremuloides* distribution range

2.4. Materials and methods

2.4.1. Study area and sampling

Samples were collected from 28 geo-referenced sampling sites covering the northwestern part of trembling aspen's distribution from Manitoba to Alaska (51°18′40″ N to 67°25′30″ N; 101°40′37″ W to 150°08′38″ W; Figure 2.1), which resulted in a total of 879 trembling aspen trees being sampled. A minimum of 15 trees was sampled from each of 28 sampling sites. We used leaf samples that were collected by a collaborating team from the University of Saskatchewan (Chena Park,

Delta, Fairbanks, Richardson, Simpson Lake, Steese Hwy, Taylor Hwy, Tok and Whitehorse; 15 to 45 samples per location) and Utah State University/University of Alaska Fairbanks (Coldfoot, Glennallen, Hinton, Kenai, Liard Spring and Palmer; 30 samples per location). The rest of the samples were collected from root cambia (Alders Flat, Biggar, Calling Lake, Dawson Creek, Dunvegan, Fort Nelson, Glaslyn, High Level, Ministik, Morin Lake, Pass, Peter Pond and Red Earth; 40 samples per location). Within sampling sites, samples were collected to reduce the chance of resampling the same clone. The maximum distances between sampled trees could vary from hundreds of meters to few kilometers. Leaves and root cambia were collected, dried in silica gel, and maintained at room temperature prior to DNA extraction.

2.4.2. DNA extraction, amplification and sequencing

DNA was extracted using Extract-N-AmpTM Plant kit (Sigma-Aldrich, St Louis, MO, USA) using the manufacturer's protocol. To evaluate genetic diversity and structure, microsatellite markers were selected because they are rapidly evolving, powerful and economical tools for describing patterns of gene flow and diversity. Samples were amplified at 12 microsatellite loci: PTR1, PTR2, PTR3, PTR4, PTR6, PTR14, PMGC2571, WPMS14, WPMS15, WPMS16, WPMS17, and WPMS20 (Appendix 2.1) [27,29,30,31,32]. Each PCR was performed on a 10 µL total volume: 2 µL of a ten-fold diluted DNA extract (1:10 in ultra pure water); 5 µL QIAGEN® Multiplex PCR Kit (Qiagen, Venlo, Limburg, The Netherlands); and 1 µL H2O and 2 µL primer mix solution at 2 µM, for a final concentration of 0.4 µM. PCR was carried out separately for each primer. Reactions were performed in a Mastercycler® pro Thermal Cyclers (Eppendorf, Hamburg, Germany) with the following protocol: an initial denaturation step at 95 °C for 15 min, followed by 36 cycles of 94 °C for 30 s; a primer-specific annealing temperature for 90 s; 72 °C for 60 s; and a final extension at 60 °C for 30 min. Annealing temperatures were 60 °C for PTR1, PTR2,

PTR3, PTR4, WPMS14, WPMS17 and WPMS20 and 63 °C for PTR6, PMGC2571 and WPMS15. For PTR14 and VVPSM16, a touchdown PCR was applied with an annealing temperature ranging from 65 °C to 60 °C during the first ten cycles. PCR products were analyzed on an Automated Capillary DNA Sequencer (ABI 3730, Applied Biosystems, Foster City, CA, USA) using 2 µL of multiplexed PCR products, which were added to 8.4 µL of Hi-Di™ Formamide and 0.11 µL of the GeneScan-500 LIZ size standard (Applied Biosystems). Allele sizes were scored using GENEMAPPER version 5.0 (Applied Biosystems).

2.4.3. Data manipulation

The original set of 879 samples was then reduced: i) by removing loci with high presence of individuals with three alleles (i.e., > 20 %; that was the case for PTR1 and PTR3); ii) by removing any sample with three alleles at one loci (putative triploid; [33]); and iii) by removing duplicated genotypes (ramets) that were identical at all loci, to keep only one representative of each genotype. We used the software GENODIVE [34] to assign clone identities based on the stepwise mutation model (SMM). In a stepwise mutation model, alleles that differ only by a few repeats in length are thought to be of more recent common ancestry than alleles that differ by many repeats in length [34]. We considered that two individuals belonged to the same clone, if the total genetic distances (mutation frequency between two alleles) for all loci were lower than three mutations, to avoid identifying unique genotypes (genets) that had resulted from scoring errors and soma-clonal mutations (small genetic distances; [35]). Following this operation, the subset contained successively 879, 658 and 526 samples. From the 526 unique genotypes that were isolated from 10 microsatellite markers (without PTR1 and PTR3), populations with less than 7 unique trees were removed (the Glaslyn population) to maintain sufficiently high statistical power for the following analyses. The final dataset consisted of unique diploid genotypes for 10 loci, 523 genets for 27 populations.

2.4.4. Variation of genetic diversity

All descriptive genetic analyses were carried out with GenAlex v. 6.2 [36]. Allele frequency, allele number, private alleles (defined here as alleles found in a single population) and genetic estimates within populations, including the average number of alleles per locus (Na), average number of effective alleles per locus (Ne), observed heterozygosity (Ho), and expected heterozygosity (He), were calculated using GenAlex v. 6.2 [36]. To describe genetic diversity within sampling areas across the range, allelic richness (AR) across all ten loci were calculated using FSTAT v. 2.9.3 [37] with rarefaction, a method that was employed to account for differences in sample size. Correlations between AR and He were computed, while Hardy-Weinberg (HW) equilibrium was assessed by calculating the inbreeding coefficients (Fis) and their corresponding *P*-values for all sampling sites. We also ran a global test of HW equilibrium for all the samples pooled together. Bonferroni correction was applied when testing the significance of heterozygosity deficit and heterozygosity excess. All of the HW equilibrium tests were performed in FSTAT [37]. Finally, values of AR and Ho were interpolated using the inverse distance weighting (IDW) method to create maps of each variable, using ArcMap (Esri, California, USA).

Genetic structure analyses

Mantel tests and correlograms, together with multivariate analysis of spatial patterns of genetic divergence (PCoA and RDA) were performed with the package *ade4* [38] in the R statistical environment version 2.15.0 [39]. A global simple Mantel test was performed with the function *mantel.rtest* [38] to test for significant correlations between genetic (estimated by Fst; Additional file 2: Table S2) and geographical distances (in kilometers) between sites, as well as isolation-by-distance patterns. Assumptions of linearity and homoscedasticity were checked before interpreting the results of the Mantel test [40]. The definition of distance classes, both in terms of the total number of classes and their upper and lower limits, is somewhat arbitrary and

depends upon the spatial distribution of the populations [40]. A "rule-of-thumb" suggests four to five classes for 20 populations. The Mantel correlogram was constructed by plotting Mantel correlations between the genetic distances for 5 classes of geographical distances with the function *mantel.correlog* in the *vegan* package [41]. Particular care was taken to maintain a constant number of pairs of populations in each class creating unequal distance intervals. To complement the correlogram, we plotted the relationship between genetic and geographical distances, followed by plotting the genetic distance between two sites, which as estimated as Fst /(1-Fst), as a function of geographical distance. We finally performed an analysis of molecular variance (AMOVA) in GenoDive [34]. Pairwise Fst were calculated for each population, together with its corresponding *P*-value, after which Bonferroni corrections were applied (initial $P = 0.05$; number of pairwise tests = 351; adjusted critical $P = 0.05/351 = 0.00014$).

The results of the Mantel test and correlogram were confirmed by multivariate analysis, which was carried out using the functions *dudi.pco* and *pcaiv* to calculate the respective PCoA and RDA ordinations [38]. PCoA was first applied to the Fst matrix (Appendix 2.2), with the retention of the first five axes for further analyses. To analyze patterns in the genetic data, we performed RDA, using scores for the first five axes of the PCoA as the response variables, and longitudinal and latitudinal data as explanatory variables [42]. The output from the RDA, which was obtained with the function *summary*, provided the percentage of the unconstrained variation (PCoA axes representing the genetic differentiation) that was explained by the predictor (geographic location). The function *randtest* was used to evaluate the RDA significance by randomly permuting (Monte-Carlo test) the rows of the explanatory table [38].

To reveal genetic structure, and test whether the samples could be clustered according to their respective distribution zones, we used STRUCTURE v. 2.3.2 software [43].

The analyses were based on an admixture ancestral model. Correlated allele frequencies and a priori sampling locations were used as prior information (LOCPRIOR setting). LOCPRIOR was used to detect any further structures that could not be identified by standard settings [44]. Ten independent runs were performed for each value of K (1–27) with a burn-in of 100 000, followed by 200 000 MCMC iterations. The most likely value of K was determined using the ΔK criterion [45]. STRUCTURE HARVESTER version 0.6.93 was used to extract the results and created a graphical plot of the ΔK criterion [46]. The results were visualized for the best K, with DISTRUCT version 1.1 [47].

Finally, we computed a neighbour-joining tree (NJT) [48] to see how populations are genetically linked to one another, and whether clusters could be isolated similarly to the structuring that was found earlier. The NJT was constructed with POPTREE2 software [49] based on Nei's standard genetic distance, Ds [50]. The neighbour-joining tree was bootstrapped 1000 times.

Table 2.1 Descriptive genetic composition of 27 *Populus tremuloides* populations in northwestern North America

Population	Latitude	Longitude	N	G	AR*	Na	Ne	Ho	He	Fis
Pass	53.603	-101.677	20	9	4.48	4.9 ± 0.69	3.1 ± 0.68	0.58 ± 0.09	0.57 ± 0.07	0.01 ± 0.08
Morin Lake	55.143	-106.072	34	19	4.46	5.8 ± 0.71	3.4 ± 0.56	0.64 ± 0.07	0.65 ± 0.05	0.01 ± 0.1
Biggar	52.315	-107.768	36	18	4.97	7.2 ± 0.94	3.5 ± 0.6	0.62 ± 0.05	0.64 ± 0.05	0.02 ± 0.06
Peter Pond	55.744	-108.776	14	11	3.94	4.5 ± 0.69	3.0 ± 0.52	0.72 ± 0.07	0.61 ± 0.04	-0.2 ± 0.11
Calling Lake	55.292	-112.970	36	26	5.08	8.5 ± 1.28	4.1 ± 1.09	0.58 ± 0.07	0.63 ± 0.07	0.05 ± 0.04
Red Earth	56.607	-115.308	33	29	4.82	7.9 ± 0.98	3.5 ± 0.61	0.64 ± 0.06	0.66 ± 0.05	0.01 ± 0.06
High Level	58.338	-117.237	18	10	4.06	4.5 ± 0.48	2.9 ± 0.36	0.65 ± 0.06	0.6 ± 0.05	-0.1 ± 0.09
Ministik	53.276	-112.932	31	14	4.11	5 ± 0.89	3.2 ± 0.54	0.68 ± 0.09	0.59 ± 0.07	-0.18 ± 0.13
Alders Flat	52.602	-114.964	26	16	3.98	5.2 ± 0.76	2.9 ± 0.36	0.76 ± 0.07	0.61 ± 0.04	-0.26 ± 0.13
Hinton	53.429	-117.531	22	22	4.92	7.6 ± 1.15	3.9 ± 0.92	0.62 ± 0.07	0.64 ± 0.05	0.05 ± 0.05
Dunvegan	55.828	-118.263	33	27	4.56	7.3 ± 0.96	3.5 ± 0.92	0.6 ± 0.06	0.61 ± 0.05	0 ± 0.04
Dawson Creek	55.778	-120.816	8	7	4.7	4.7 ± 0.7	2.9 ± 0.59	0.56 ± 0.07	0.57 ± 0.05	0.03 ± 0.05
Fort Nelson	58.111	-122.746	37	20	4.29	6 ± 1.13	3.4 ± 0.93	0.66 ± 0.06	0.6 ± 0.05	-0.11 ± 0.07
Liard Spring	59.355	-125.957	24	24	4.76	7.3 ± 0.98	3.9 ± 0.83	0.63 ± 0.07	0.65 ± 0.06	0.04 ± 0.03
Simpson Lake	60.676	-129.222	12	11	4.81	5.6 ± 0.64	3.3 ± 0.6	0.64 ± 0.06	0.63 ± 0.05	-0.01 ± 0.06
Whitehorse	60.784	-136.025	17	13	4.46	5.7 ± 0.99	3.3 ± 0.74	0.58 ± 0.07	0.59 ± 0.06	-0.01 ± 0.09
Taylor Hwy	63.887	-142.243	15	15	4.79	6.3 ± 1.04	3.7 ± 0.76	0.63 ± 0.05	0.63 ± 0.06	-0.03 ± 0.07
Tok	63.370	-142.566	26	26	4.51	6.9 ± 0.91	3.6 ± 0.81	0.6 ± 0.05	0.63 ± 0.05	0.05 ± 0.05
Delta	63.809	-145.094	12	12	4.83	5.8 ± 0.95	3.7 ± 0.99	0.53 ± 0.06	0.63 ± 0.05	0.17 ± 0.06
Glennallen	62.005	-145.341	29	29	4.87	7.5 ± 1.16	3.8 ± 0.83	0.61 ± 0.06	0.65 ± 0.06	0.06 ± 0.03
Chena Park	65.105	-147.531	15	15	4.82	5.9 ± 0.69	3.7 ± 0.75	0.71 ± 0.07	0.66 ± 0.05	-0.07 ± 0.09
Steese Hwy	65.161	-147.743	58	56	4.89	9.5 ± 1.27	4.1 ± 1.04	0.65 ± 0.05	0.65 ± 0.05	0 ± 0.04
Fairbanks	64.902	-148.277	9	9	4.67	5.1 ± 0.43	3.3 ± 0.56	0.63 ± 0.05	0.63 ± 0.05	-0.02 ± 0.07
Richardson	64.267	-149.200	10	10	4.47	5.4 ± 0.52	2.9 ± 0.31	0.63 ± 0.05	0.6 ± 0.05	-0.05 ± 0.04
Palmer	61.581	-149.249	27	27	4.81	7.5 ± 1.28	3.9 ± 0.9	0.6 ± 0.06	0.66 ± 0.05	0.08 ± 0.05
Kenai	60.480	-149.724	17	17	5.22	7.3 ± 1.05	4.3 ± 0.96	0.65 ± 0.07	0.65 ± 0.07	-0.01 ± 0.06
Coldfoot	67.425	-150.144	31	31	4.85	8 ± 1.22	3.8 ± 0.91	0.6 ± 0.05	0.65 ± 0.05	0.07 ± 0.04

N, number of sampled trees genotyped; G, number of unique genotypes used in the analyses; AR, allelic richness; N_a, average number of alleles per locus; N_e, average number of effective alleles per locus; H_o, observed heterozygosity; H_e, expected heterozygosity; F_{is}, interbreeding coefficient

* Calcculated with rarefaction method based on the minimum number of unique genotypes

2.4.5. Population genetic bottleneck

M-ratios were estimated to detect historical bottlenecks at each site with the program MPval [51]. To interpret results of the M-ratios, we calculated the critical M-ratio (*Mc*) value for each population with the program M-crit, which was developed by JC Garza and EG Williamson [51]. To calculate *M*c and M-ratios we used a pre-bottleneck value ($\theta = 4\,Ne\,\mu = 10$; *Ne*, the effective population size; the mutation rate, μ) and the parameters were set as recommended by JC Garza and EG Williamson [51]. The settings were: a constant mutation rate (μ), which encompassed a range between 10−2 and 10−6 mutants/generation/locus; probability of changes greater than one step, $pg = 0.12$; and the size of non-one-step changes, $\Delta g = 2.8$. Each set of simulations consisted of 10 000 iterations with the same values of θ for all sites under a two-phase mutation model (TPM). We considered that a M-ratio below the critical value *Mc* was indicative of a population decline. To test for heterozygosity excess, Bottleneck version 1.2.02 [52] was used with a stepwise mutation model (SMM), an infinite allele model (IAM), and a two-phase model (TPM) with 12 % multistep mutations and variance = 0.36 [53]. Mode shifts and heterozygosity excess are transient [54]. To determine which sampling locations had a significant heterozygote excess across loci, a standardized differences test was used. We also used the graphical method to assess bottleneck-induced distortions of allele frequency distributions that cause alleles at low frequency (<0.025) to become less abundant than alleles in one or more intermediate allele frequency classes (e.g. 0.025–0.050) [54]. In this method, the probability (power) of detecting a recent historical bottleneck of fewer than 20 breeding individuals is estimated to be 80 % with eight to ten microsatellite loci [54].

Table 2.2 Results of analysis of molecular variance (AMOVA) for *Populus tremuloides* in northwestern North America (n = 523 genets), based on microsatellite allele frequencies

Source of variation	Sum of square	Variance component	% of variance
Within populations	2770.652	5.575	0.969
Among populations	233.945	0.178	0.031
Total	3004.597	5.753	

2.5. Results

2.5.1. Variation of genetic diversity

Over the entire population, the number of alleles that were observed per locus ranged from 10 (PTR2 and WPMS16) to 30 (PMGC2571; Appendix 2.1). Our results showed that all 12 loci were highly polymorphic and that PTR1 and PTR3 had a high rate of triallelic individuals (Appendix 2.3). These markers were therefore removed from further analysis. At the population level, AR averaged 4.63 and ranged from 3.94 (Peter Pond) to 5.22 (Kenai). Na ranged from 4.5 (Peter Pond and High Level) to 9.5 (Steese Hwy), with an average of 6.4 (Table 1). The mean Ne was 3.5, with lowest value being 2.9 (Richardson, High Level and Dawson Creek) and the highest being 4.3 (Kenai). Across all loci, only 34 individuals had one or more private alleles. Individuals with private alleles were spread across all sampled sites (data not shown). Ho had a mean value of 0.629 and was lowest in the Delta population (0.53) and highest in the Alders Flat population (0.76). The mean He was 0.625, ranging from 0.57 (Pass and Dawson Creek) to 0.66 (Chena Park, Palmer and Red Earth; Table 1). The variation in AR and Ho is represented on maps (Figure 2.2) to evaluate the spatial genetic variation visually. Higher genetic diversity was observed in Alberta and Alaska for AR, but only in Alberta for Ho. We found a positive correlation between AR and He (Pearson product-moment correlation: $r = 0.61$; $P < 0.001$).

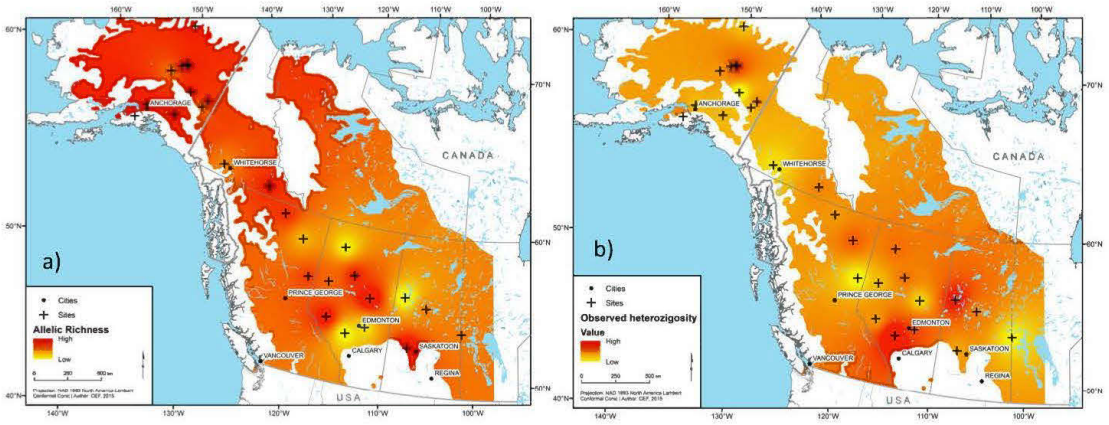

Figure 2.2 a) Interpolation of allelic richness (AR) and b) observed heterozygosity (H_o) across the range of *Populus tremuloides* based on average AR and H_o values at each sampling site. Across the range, AR and H_o respectively varied from 3.94 (Peter Pond) to 5.22 (Kenai) and from 0.542 (Delta) to 0.762 (Alders Flat). Red represents areas of higher values and yellow represents areas of lower values.

2.5.2. Patterns of genetic structure

The genetic and the geographical distance matrices were not strongly correlated (Mantel test rm = 0.134; $P = 0.024$; Figure 2.3). The results obtained with the Mantel test and the correlogram were confirmed by RDA ($R2 = 0.092$; data not shown). The AMOVA (Table 2.2) indicated that 3.1 % of the genetic variation (Fst = 0.031) was partitioned among populations and 96.9 % within populations ($P < 0.001$). No pairwise differences between populations, as estimated by Fst, appeared to be significant after applying a Bonferroni correction (adjusted critical $P = 0.00014$; Appendix 2.2).

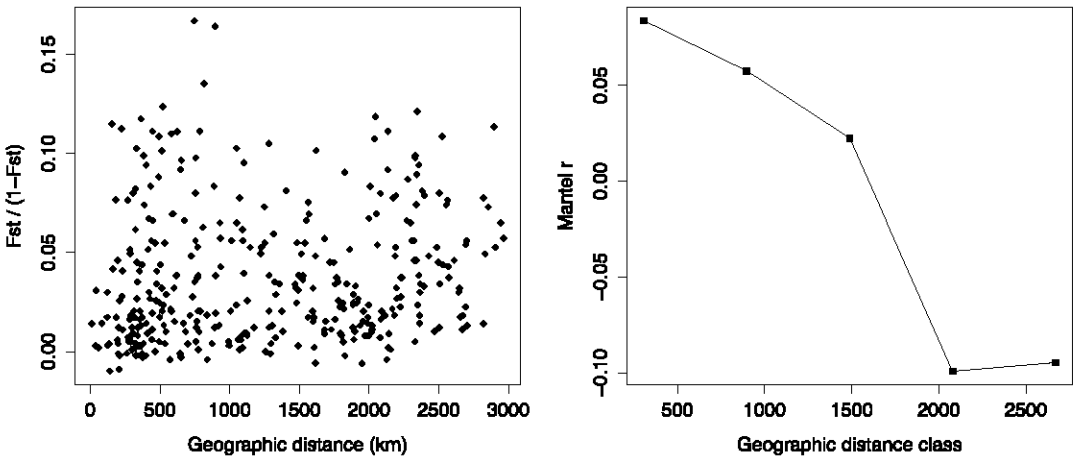

Figure 2.3 Isolation by distance patterns: (a) Each point represents the genetic distance Fst between two sites as a function of geographical distance (km); (b) Mantel correlogram for 8 geographic distance classes based on Fst genetic distances.

The results of the NJT (Figure 2.4), which were based on Nei's standard genetic distance, were consistent with the results of the Mantel test and RDA, showing low differentiation between sites. Populations that were genetically close to one another are not necessary spatially aggregated. Two clusters can be identified at increased confidence levels (bootstrap values > 50).

2.5.3. Population genetic bottlenecks

The M-ratio bottleneck test proved to be sensitive to the choice of θ. Significant M-ratio values were obtained for all sites using values of $\theta = 1$ (data not shown). Statistical significance to detected historical bottlenecks was maintained in 16 populations with higher values of θ (10), where M-ratio $< Mc$ (Alders Flat, Biggar, Calling Lake, Chena Park, Coldfoot, Fort Nelson, Glennallen, High Level, Liard Spring, Ministik, Morin Lake, Palmer, Red Earth, Simpson Lake, Tok and Whitehorse) [51]. Across all populations and loci, M-ratio varied from 0.583 (High Level) to 0.791 (Steese Hwy; Table 3).

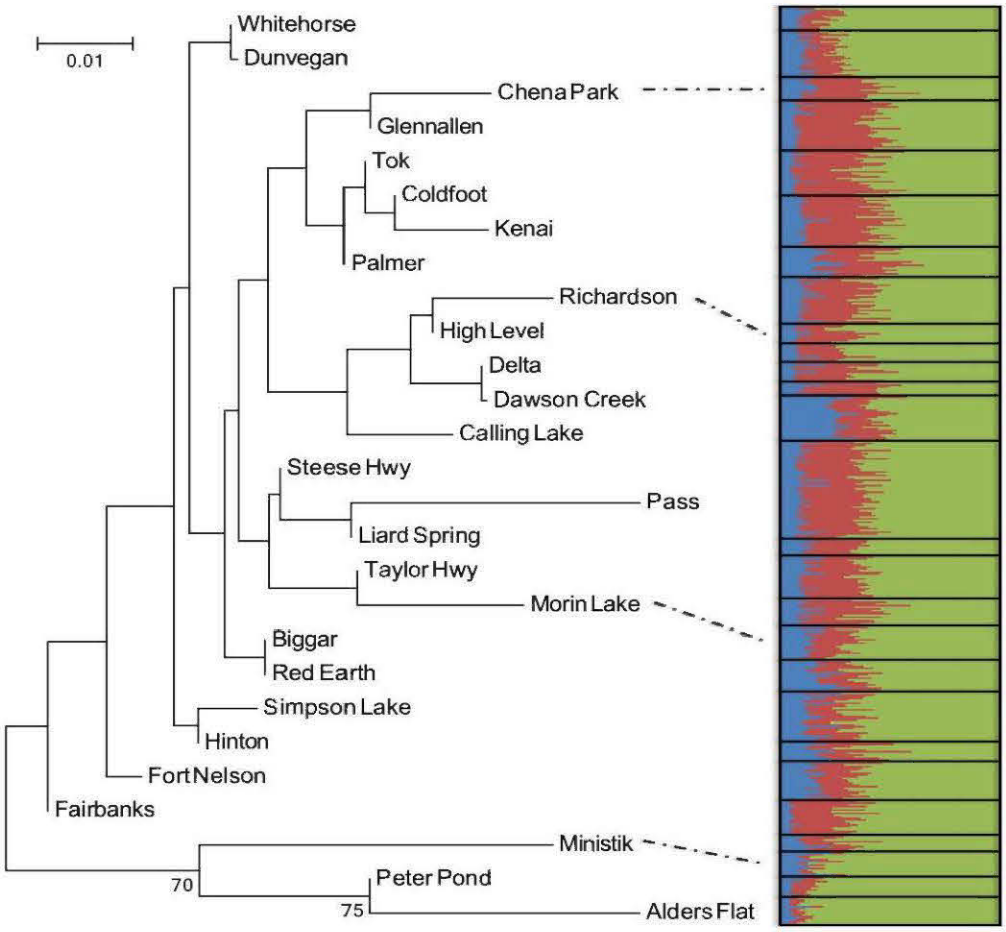

Figure 2.4 Neighbour-joining tree obtained using Nei's distance matrix for 27 populations of *Populus tremuloides* with data obtained from 10 polymorphic microsatellite loci. The numbers represent the bootstrap values as percentages. On the right side are the STRUCTURE results graphically displayed to show the likelihood of belonging to each of the 3 groups detected with structure for each sample.

Recent bottlenecks with heterozygote excess were detected in six populations (Biggar, Coldfoot, Dawson Creek, Dunvegan, Richardson and Tok; $P < 0.05$), using TPM (Table 2.3). The graphical method detected recent population bottlenecks in 6 populations (Biggar, Fairbanks, Pass, Morin Lake, Simpson Lake and Tailor Hwy; Appendix 2.3). Results from the heterozygote excess test and the graphical method were consistent only for 1 population (Biggar).

2.6. Discussion

The purpose of this study was to ascertain whether the origin of trembling aspen in northwestern North America is reflected in the patterns of genetic diversity and population structure. Contrary to our hypothesis, microsatellite markers revealed little to no genetic structure in *P. tremuloides* populations and indicated little isolation by distance (IBD). Consequently, no divergent populations were observed near supposed refugia suggesting no evidence that Beringia or the "ice-Free Corridor" were refugia for trembling aspen. Finally, favourable conditions for sexual reproduction and successful trembling aspen seedling establishment could have contributed to the highest AR and lowest Ho that were observed in Alberta foothills of the Rocky Mountains.

2.6.1. Genetic diversity and structure

Our results support the findings of Callahan et al. [27], who found no genetic structuring among populations in the northern part of aspen's range. Trees are known to have low differentiation at neutral molecular markers, indicating high levels of gene flow among populations [55,56]. We observed low levels of differentiation and variation in genetic distance between populations (Figsure 2.3 and 2.4). Specific aspen traits, such as outbreeding, wind pollination, aeolian seed dispersal, high seed production and/or longevity, can account for this observation. In addition, there was no pronounced pattern of IDB, even at great distances (Figure 2.3). We were not able to detect local genetic structure patterns. This indicates that aspen populations in the northern portion of the species range experience high levels of gene flow, making it difficult to identify refugia.

Table 2.3 Results of bottleneck analyses performed with the software Bottleneck version 1.2.02 (Cornuet & Luikart 1996) and the program MPval (Garza & Williamson 2001) to calculate the M-ratio and M-critical with θ = 10 for each of the 27 populations. Values in bold show a significant bottleneck detected.

Population	He	*P*-TPM	*P*-SMM	*P*-IAM	M-Ratio*	Mc**
Pass	0,607	0,393	0.372	0.436	0,641	0,624
Morin Lake	0,663	0,374	0.058	0.339	**0,615**	**0,696**
Biggar	0,658	**0,014**	**0.002**	0.176	**0,687**	**0,694**
Peter Pond	0,636	0,366	0.613	0.297	0,665	0,647
Calling Lake	0,638	0,058	**0.014**	0.170	**0,684**	**0,723**
Red Earth	0,667	0,063	**0.002**	0.378	**0,698**	**0,73**
High Level	0,632	0,596	0.386	0.304	**0,583**	**0,634**
Ministik	0,617	0,563	0.443	0.272	**0,642**	**0,671**
Alders Flat	0,63	0,183	0.066	0.308	**0,646**	**0,682**
Hinton	0,659	0,175	0.060	0.182	0,748	0,71
Dunvegan	0,618	**0,013**	**0.002**	0.380	0,754	0,722
Dawson Creek	0,611	**0,008**	**0.008**	**0.018**	0,637	0,597
Fort Nelson	0,613	0,386	0.376	0.573	**0,687**	**0,701**
Liard Spring	0,662	0,373	**0.002**	0.629	**0,705**	**0,714**
Simpson Lake	0,658	0,057	**0.012**	0.383	**0,6**	**0,647**
Whitehorse	0,613	0,055	0.058	0.608	**0,614**	**0,664**
Taylor Hwy	0,655	0,057	**0.013**	0.398	0,704	0,676
Tok	0,644	**0,014**	**0.002**	0.355	**0,653**	**0,723**
Delta	0,657	0,063	0.051	0.397	0,716	0,655
Glennallen	0,658	0,056	**0.000**	0.376	**0,723**	**0,73**
Chena Park	0,682	0,631	0.395	0.595	**0,656**	**0,676**
Steese Hwy	0,656	0,059	**0.015**	0.372	0,791	0,767
Fairbanks	0,67	0,167	0.058	0.611	0,663	0,624
Richardson	0,633	**0,017**	**0.002**	0.065	0,682	0,634
Palmer	0,668	0,06	**0.014**	0.365	**0,674**	**0,722**
Kenai	0,674	0,368	0.173	0.382	0,699	0,688
Coldfoot	0,659	**0,002**	**0.000**	0.375	**0,727**	**0,734**

For the heterozygosity excess test, we tested a stepwise mutation model, an infinite alleles model and a two-phase mutation model with 12 % multistep mutations and a variance = 0.36. M-ratio average was calculated across loci. Mc is the critical M-value calculated through the M-crit program developed by Garza and Williamson (2001). M-ratio test is significant if M-Ratio < Mc

*M-Ratio = number of alleles / range in allele size and range (size of largest allele - size of smallest allele + 1)

** Mc is defined such that ouly 5 % of the simulation values fall below.

The Rocky Mountains foothills of Alberta, which could have remained ice-free during the last glacial maximum (LGM; [20]), exhibited higher genetic diversity (AR), which is consistent with the observations of Callahan et al. [27]. Moreover, no divergent lineages or specific private alleles were found in this area or north of this

region in Beringia. The lack of detectable refugia in Beringia and in the "ice-free corridor" was due to high levels of gene flow between trembling apsen populations. We agree that the Alberta foothills were not an area of admixture because we don't have highly differentiated populations. More favourable environmental conditions for sexual reproduction and successful trembling aspen seedling establishment in this area [57,58] may have contributed to increase allelic richness through recombination in populations from the Albertan foothills of the Rocky Mountains. The existence of a refugium in Beringia during the LGM has been reported for *P. glauca* [9,23] and was consistent with the model simulation of suitable refugial habitats for this species (performed with the Community Climate Model and the Geophysical Fluid Dynamics Laboratory Model) [8], which suggested the presence of *P. glauca* in Beringia during the LMG. Moreover, those simulations also suggested the presence of suitable habitats for *P. contorta* and *P. tremuloides* in Beringia [8]. For the closely related species *P. balsamifera*, recent molecular evidence did not support Beringia as a glacial refugium, but confirmed the existence of two distinct clusters in our sample area of northwestern North America [12].

2.6.2. Population genetic bottlenecks

The M-ratio bottleneck test proved to be sensitive to the choice of θ, with significant M-ratio values being obtained for all sites using values of $\theta = 1$. The M-ratio detected persistent bottleneck signatures in 16 populations with $\theta = 10$. Low M-ratios that were detected indicate that these 16 populations might have suffered from demographic declines, although they were not severely reduced in their genetic potential. We did not detect strong evidence for excess heterozygosity. Consistent results were obtained only for 1 population. Indeed, wild populations are rarely completely closed and even small numbers of migrants can mask the genetic signature of bottlenecks [59,60]. Under TPM, the populations that were subject to a recent reduction in size (Biggar, Coldfoot, Dawson Creek, Dunvegan, Richardson and Tok) were not spatially

clustered and were present all over the sampled territory without showing any sign of spatial structure. Large effective population size implies that polymorphisms can persist during extended periods of time [56], even during reduction of the species distributional range. At low effective population sizes, asexual reproduction might better preserve heterozygosity than outcrossing at least in the short-term [56], hereby masking recent reductions in population size.

2.7. Conclusion

Most of the studies that have detected phylogeographic patterns in boreal tree species in western North America (reviewed by [13]) have used uniparental inherited cpDNA [23], mtDNA markers [61], or more recently genomic data (e.g., SNPs) [12]. For *P. glauca*, LL Anderson, FS Hu and KN Paige [9] suggested that the greater relative rate of mutation of nuclear microsatellites may allow finer scale resolution of the historic dynamics of populations (including the number, location, and population sizes of refugia), compared to chloroplast DNA that have extremely slow mutation rates (estimated to be $5.3 \times 10{-}9$ mutations per gene per generation). Their results with nuclear microsatellite markers support the idea that north-central Alaska served as a glacial refugium during the last glacial maximum for white spruce. Three genetic groups were detected: the first consisted of one population from north-central Alaska (the northern-most population sampled in Alaska, Dalton Highway); the second with one population from southern Manitoba; and the last group included the remaining 20 populations (ranging from Wisconsin and continuing a northwestwardly fashion into southern and central Alaska) forming the last group. These results revealed that there is not much structure and differentiation to be found for this boreal species, a result similar to what we found in trembling aspen. For *P. tremuloides*, Callahan et al. [27] found 2 distinct groups, with significant correlation of genetic and geographical distances and low AR, solely in the southwestern USA, but nothing in Beringia. Our study did not find any structuring in northwestern North America. The historic

dynamics of the populations vary from one species to another. In conclusion, future studies should combine different approaches and molecular analyses to elucidate the glacial origin and post-glacial migration route in the northwestern part of the species' range.

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2.9. Literature cited

1. Hewitt G. The genetic legacy of the Quaternary ice ages. Nature. 2000;405:907–13.

2. Excoffier L, Foll M, Petit RJ. Genetic consequences of range expansions. Annu Rev Ecol Evol Syst. 2009;40:481–501.

3. Taberlet P, Fumagalli L, Wust-Saucy A-G, Cosson J-F. Comparative phylogeography and postglacial colonization routes in Europe. Mol Ecol. 1998;7:453–64.

4. Austerlitz F, Jung-Muller B, Godelle B, Gouyon P-H. Evolution of coalescence

times, genetic diversity and structure during colonization. Theor Popul Biol. 1997;51:148–64.

5. Hewitt GM. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol J Linn Soc. 1996;58:247–76.

6. Dyke AS, Moore A, Robertson L. Deglaciation of North America. Ontario: Geological Survey of Canada Ottawa; 2003. Available at: http://geoscan.nrcan.gc.ca/starweb/geoscan/servlet.starweb?path=geoscan/fulle.web&search1=R=214399.

7. Williams JW. Variations in tree cover in North America since the last glacial maximum. Global Planet Change. 2003;35:1–23.

8. Roberts DR, Hamann A. Glacial refugia and modern genetic diversity of 22 western North American tree species. Proc R Soc Lond B Biol. 2015;282:20142903.

9. Anderson LL, Hu FS, Paige KN. Phylogeographic history of white spruce during the last glacial maximum: uncovering cryptic refugia. J Hered. 2011;102:207–16.

10. Beatty GE, Provan JIM. Refugial persistence and postglacial recolonization of North America by the cold-tolerant herbaceous plant Orthilia secunda. Mol Ecol. 2010;19:5009–21.

11. de Lafontaine G, Turgeon J, Payette S. Phylogeography of white spruce (*Picea glauca*) in eastern North America reveals contrasting ecological trajectories. J Biogeogr. 2010;37:741–51.

12. Keller SR, Olson MS, Silim S, Schroeder W, Tiffin P. Genomic diversity, population structure, and migration following rapid range expansion in the Balsam Poplar, *Populus balsamifera*. Mol Ecol. 2010;19:1212–26.

13. Shafer A, Cullingham CI, Cote SD, Coltman DW. Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. Mol Ecol. 2010;19:4589–621.

14. Marr KL, Allen GA, Hebda RJ. Refugia in the Cordilleran ice sheet of western North America: chloroplast DNA diversity in the Arctic-alpine plant Oxyria digyna. J Biogeogr. 2008;35:1323–34.

15. Callahan CM. Continental-scale characterization of molecular variation in quaking aspen. Logan: Utah State University; 2012.

16. Mandryk CAS, Josenhans H, Fedje DW, Mathewes RW. Late Quaternary paleoenvironments of Northwestern North America: implications for inland versus coastal migration routes. Quat Sci Rev. 2001;20:301–14.

17. Arnold TG. Radiocarbon dates from the ice-free corridor. Radiocarbon. 2002;44:437–54.

18. Jackson LE, Wilson MC. The ice-free corridor revisited. Geotimes. 2004;49:16–9.

19. Brunsfeld SJ, Sullivan J. A multi-compartmented glacial refugium in the northern Rocky Mountains: evidence from the phylogeography of *Cardamine constancei* (Brassicaceae). Conserv Genet. 2005;6:895–904.

20. Loehr J, Worley K, Grapputo A, Carey J, Veitch A, Coltman DW. Evidence for cryptic glacial refugia from North American mountain sheep mitochondrial DNA. J Evol Biol. 2006;19:419–30.

21. Abbot RJ, Smith LC, Milne RI, Crawford RMM, Wolff K, Balfour J. Molecular analysis of plant migration and refugia in the arctic. Science. 2000;289:1343–6.

22. Anderson CD, Epperson BK, Fortin M-J, Holderegger R, James PMA, Rosenberg MS, Scribner KT, Spear S. Considering spatial and temporal scale in landscape-genetic studies of gene flow. Mol Ecol. 2010;19:3565–75.

23. Anderson LL, Hu FS, Nelson DM, Petit RJ, Paige KN. Ice-age endurance: DNA evidence of a white spruce refugium in Alaska. Proc Natl Acad Sci U S A. 2006;103:12447–50.

24. Brubaker LB, Anderson PM, Edwards ME, Lozhkin AV. Beringia as a glacial refugium for boreal trees and shrubs: new perspectives from mapped pollen data. J Biogeogr. 2005;32:833–48.

25. Peros MC, Gajewski K, Viau AE. Continental-scale tree population response to rapid climate change, competition and disturbance. Glob Ecol Biogeogr. 2008;17:658–69.

26. Hu FS, Brubaker LB, Anderson PM. Postglacial vegetation and climate change in the northern Bristol bay region, southwestern Alaska. Quat Res. 1995;43:382–92.

27. Callahan CM, Rowe CA, Ryel RJ, Shaw JD, Madritch MD, Mock KE. Continental-scale assessment of genetic diversity and population structure in quaking aspen (*Populus tremuloides*). J Biogeogr. 2013;40:1780–91.

28. Levson VM, Rutter NW. Evidence of Cordilleran late wisconsinan glaciers in the 'ice-free corridor'. Quat Int. 1996;32:33–51.

29. Dayanandan S, Rajora OP, Bawa KS. Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*). Theor Appl Genet. 1998;96:950–6.

30. Rahman MH, Dayanandan S, Rajora OP. Microsatellite DNA markers in *Populus tremuloides*. Genome. 2000;43:293–7.

31. Smulders MJM, Van Der Schoot J, Arens P, Vosman B. Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L.). Mol Ecol Notes. 2001;1:188–90.

32. Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, et al. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science. 2006;313:1596–604.

33. Mock KE, Callahan CM, Islam-Faridi MN, Shaw JD, Rai HS, Sanderson SC, Rowe CA, Ryel RJ, Madritch MD, Gardner RS, et al. Widespread triploidy in western North American aspen (*Populus tremuloides*). PLoS One. 2012;7:e48406. doi: 10.1371/journal.pone.0048406 .

34. Meirmans PG, Van Tienderen PH. genotype and genodive: two programs for the analysis of genetic diversity of asexual organisms. Mol Ecol Notes. 2004;4:792–4.

35. Ally D, Ritland K, Otto SP. Can clone size serve as a proxy for clone age? An exploration using microsatellite divergence in *Populus tremuloides*. Mol Ecol. 2008;17:4897–911.

36. Peakall ROD, Smouse PE. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 2006;6:288–95.

37. Goudet J. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Heredity. 2001;97:418–26. Available at: http://www2.unil.ch/popgen/softwares/fstat.htm

38. Dray S, Dufour A-B. The ade4 package: implementing the duality diagram for ecologists. J Stat Softw. 2007;22:1–20.

39. R Development Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2013. Available at: http://www.R-project.org/.

40. Diniz-Filho JAF, Soares TN, Lima JS, Dobrovolski R, Landeiro VL, Telles MPC, Rangel TF, Bini LM. Mantel test in population genetics. Genet Mol Biol. 2013;36:475–85.

41. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. Package 'vegan'. In: Community ecology package. 2013. R package version 2.0-10. Available at: http://cran.r-project.org/web/packages/vegan/index.html.

42. Legendre, P. and Legendre, L. Numerical ecology, 3rd English edn., Elsevier, Amsterdam, The netherlands; 2012.

43. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155:945–59.

44. Hubisz MJ, Falush D, Stephens M, Pritchard JK. Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour. 2009;9:1322–32.

45. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 2005;14:2611–20.

46. Earl DA, vonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour. 2011;4:359–61.

47. Rosenberg NA. DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes. 2004;4:137–8.

48. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–25.

49. Takezaki N, Nei M, Tamura K. POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. Mol Biol Evol. 2010;27:747–52.

50. Nei M. Molecular evolutionary genetics. New York: Columbia University Press; 1987.

51. Garza JC, Williamson EG. Detection of reduction in population size using data from microsatellite loci. Mol Ecol. 2001;10:305–18.

52. Cornuet JM, Luikart G. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics. 1996;144:2001–14.

53. Piry S, Luikart G, Cornuet J-M. BOTTLENECK: a program for detecting recent effective population size reductions from allele data frequencies. J Hered. 1999;90:502–3.

54. Luikart G, Allendorf FW, Cornuet JM, Sherwin WB. Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered. 1998;89:238–47.

55. McKay JK, Latta RG. Adaptive population divergence: markers, QTL and traits. Trends Ecol Evol. 2002;17:285–91.

56. Petit RJ, Hampe A. Some evolutionary consequences of being a tree. Ann Rev Ecol Evol Syst. 2006;37:187–214.

57. Landhäusser SM, Deshaies D, Lieffers VJ. Disturbance facilitates rapid range expansion of aspen into higher elevations of the Rocky Mountains under a warming climate. J Biogeogr. 2010;37:68–76.

58. Ding C. Ecological and quantitative genetics of *Populus tremuloides* in western Canada. Edmonton: University of Alberta; 2015.

59. Peery MZ, Kirby R, Reid BN, Stoelting R, Doucet- Beer E, Robinson S, Vasquez- Carrillo C, Pauli JN, Palsboll PJ. Reliability of genetic bottleneck tests for detecting recent population declines. Mol Ecol. 2012;21:3403–18.

60. Keller LF, Jeffery KJ, Arcese P, Beaumont MA, Hochachka WM, Smith JNM, Bruford MW. Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. Proc R Soc Lond B Biol. 2001;268:1387–94.

61. Jaramillo- Correa JP, Beaulieu J, Bousquet J. Variation in mitochondrial DNA reveals multiple distant glacial refugia in black spruce (*Picea mariana*), a transcontinental North American conifer. Mol Ecol. 2004;13:2735–47.

2.10. Appendices

Appendix 2.1 Primer sequences, size range (in base pairs; bp) and number of alleles observed for 12 microsatellite loci of *Populus tremuloides*

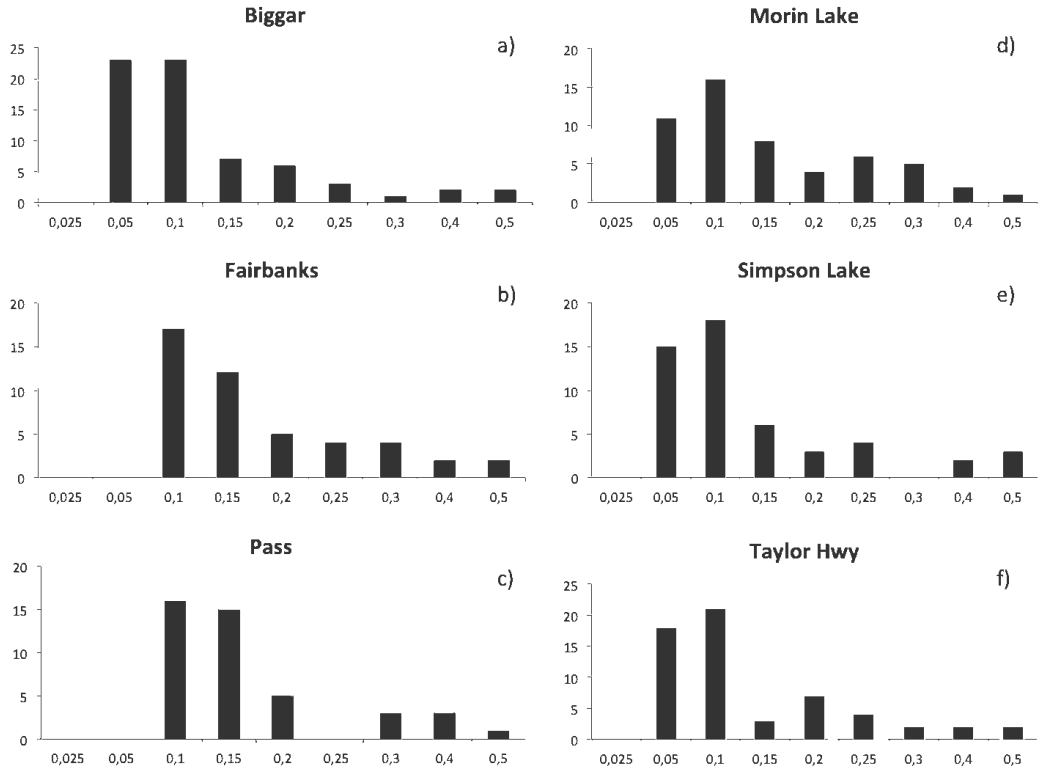
Locus	Repeat	Primer sequence (5'→3')	Dye colour	Size range (bp)	Number of alleles	Source
	(GGT)n	AGCGCGTGCGGATTGCCATT (F)				Dayanandan
PTR1	(AGG)n	TTAGTTTCCCGTCACCTCCTGTTAT (R)	FAM	239-278	12	et al., 1998
		AAGAAGAACTCGAAGATGAAGAACT (F)				Dayanandan
PTR2	(TGG)n	ACTGACAAAACCCCTAATCTAACAA (R)	VIC	201-229	10	et al., 1998
		CACTCGTGTTGTCCTTTTCTTTTCT (F)				Dayanandan
PTR3	(TC)n	AGGATCCCTTCCCTTTAGTAT (R)	NED	184-274	26	et al., 1998
		AATGTCGAGGCCTTTCTAAATGTCT (F)				Dayanandan
PTR4	(TC)n	GCTTGAGCAACAAACACACCAGATG (R)	PET	196-236	18	et al., 1998
		AGAAAAGCAGATTGAGAAAAGAC (F)				Rahman et
PTR6	(AT)n	CTAGTATAGAGAAAGAAGAAGCAGAAA (R)	VIC	184-217	12	al., 2000
		TCCGTTTTTGCATCTCAAGAATCAC (F)				Rahman et
PTR14	(TGG)n	ATACTCGCTTTATAACACCATTGTC (R)	NED	131-200	18	al., 2000
		CAGCCGCAGCCACTGAGAAATC (F)				Smulders et
WPMS14	(CGT)n	GCCTGCTGAGAAGACTGCCTTGAC (R)	PET	198-252	20	al., 2001
		CAACAAACCATCAATGAAGAAGAC (F)				Smulders et
WPMS15	(CCT)n	AGAGGGTGTTGGGGGTGACTA (R)	VIC	181-211	11	al., 2001
		CTCGTACTATTTCCGATGATGACC (F)				Smulders et
WPMS16	(GTC)n	AGATTATTAGGTGGGCCAAGGACT (R)	FAM	148-203	10	al., 2001
		ACATCCGCCAATGCTTCGGTGTTT (F)				Smulders et
WPMS17	(CAC)n	GTGACGGTGGTGGCGGATTTTCTT (R)	NED	115-157	14	al., 2001
		GTGCGCACATCTATGACTATCG (F)				Smulders et
WPMS20	(TTCTGG)n	ATCTTGTAATTCTCCGGGCATCT (R)	VIC	210-240	11	al., 2001
		TCTCGCAGATTCATGTAACCC (F)				Tuskan et al.,
PMGC2571	(GA)n	GACTGTATGTTGACCATGCCC (R)	PET	86-149	30	2006

Dye colours acronyms: FAM, Blue; VIC, Green; NED, Yellow; PET, Red

	Pass	Morin Lake	Biggar	Peter Pond	Calling Lake	Red Earth	High Level	Ministik	Alders Flat	Hinton	Dunvegan	Dawson Cree	Fort Nelson	Liard Spring	Simpson Lake	Whitehorse	Taylor Hwy	Tok	Delta	Glennallen	Chena Park	Steese Hwy	Fairbanks	Richardson	Palmer	Kenai	Coldfoot
Pass	--	**	ns	**	ns	ns	ns	**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Morin Lake	0.093	--	ns	**	**	**	ns	**	**	**	ns	ns	**	ns	ns	ns	ns	**	ns	**	**	**	**	**	**	**	**
Biggar	0.077	0.043	--	**	ns	ns	ns	**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Peter Pond	0.092	0.071	0.069	--	**	**	ns	**	ns	ns	**	**	**	**	**	ns	**	**	ns	**	ns	**	ns	**	**	ns	**
Calling Lake	0.074	0.053	0.033	0.071	--	**	ns	**	**	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Red Earth	0.061	0.031	0.010	0.049	0.025	--	ns	**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
High Level	0.087	0.050	0.037	0.065	0.026	0.027	--	**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ministik	0.143	0.098	0.105	0.090	0.101	0.086	0.100	--	**	**	**	**	**	**	**	**	**	**	ns	**	**	**	**	ns	**	**	**
Alders Flat	0.141	0.084	0.081	0.052	0.076	0.062	0.088	0.103	--	**	**	**	**	**	**	ns	**	**	**	**	**	**	ns	**	**	**	**
Hinton	0.061	0.036	0.014	0.037	0.013	0.004	0.028	0.074	0.044	--	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dunvegan	0.053	0.037	0.020	0.065	0.034	0.012	0.047	0.100	0.063	0.007	--	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dawson Creek	0.052	0.054	0.041	0.089	0.017	0.039	0.020	0.099	0.110	0.026	0.040	--	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fort Nelson	0.075	0.072	0.050	0.077	0.062	0.039	0.058	0.119	0.100	0.037	0.042	0.049	--	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
Liard Spring	0.046	0.037	0.020	0.038	0.019	0.004	0.031	0.093	0.053	-0.004	0.015	0.042	0.039	--	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Simpson Lake	0.083	0.037	0.017	0.056	0.025	0.013	0.046	0.095	0.068	0.001	0.018	0.027	0.052	0.011	--	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Whitehorse	0.073	0.049	0.015	0.054	0.052	0.020	0.058	0.092	0.065	0.010	0.012	0.061	0.059	0.020	0.017	--	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Taylor Hwy	0.069	0.027	0.018	0.065	0.012	0.009	0.033	0.106	0.077	-0.002	0.015	0.030	0.050	0.003	0.012	0.025	--	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tok	0.071	0.036	0.014	0.051	0.024	0.011	0.031	0.097	0.063	0.005	0.020	0.024	0.047	0.005	0.005	0.019	0.002	--	ns	ns	ns	ns	ns	ns	ns	ns	ns
Delta	0.051	0.029	0.012	0.050	0.010	0.005	0.002	0.072	0.084	-0.006	0.011	-0.006	0.033	0.008	0.012	0.021	-0.010	0.004	--	ns	ns	ns	ns	ns	ns	ns	ns
Glennallen	0.053	0.033	0.010	0.046	0.023	0.009	0.031	0.100	0.074	0.006	0.015	0.017	0.034	0.002	0.004	0.020	-0.001	-0.002	-0.001	--	ns	ns	ns	ns	ns	ns	ns
Chena Park	0.072	0.043	0.029	0.044	0.021	0.020	0.043	0.090	0.061	0.018	0.028	0.025	0.047	0.012	0.023	0.053	0.018	0.009	0.017	0.003	--	ns	ns	ns	ns	ns	ns
Steese Hwy	0.047	0.033	0.017	0.044	0.018	0.008	0.033	0.082	0.053	-0.004	0.010	0.022	0.037	-0.001	0.006	0.015	0.001	0.002	0.004	-0.001	0.014	--	ns	ns	ns	ns	ns
Fairbanks	0.068	0.042	0.018	0.037	0.031	0.014	0.034	0.086	0.053	0.002	0.024	0.008	0.052	0.013	0.007	0.020	0.020	0.004	0.006	0.003	0.030	0.003	--	ns	ns	ns	ns
Richardson	0.102	0.041	0.013	0.073	0.036	0.011	0.024	0.075	0.069	0.001	0.013	0.021	0.070	0.028	0.009	0.010	0.012	0.008	-0.009	0.017	0.029	0.017	0.014	--	ns	ns	ns
Palmer	0.050	0.036	0.011	0.032	0.026	0.008	0.022	0.089	0.062	0.007	0.023	0.016	0.035	0.004	0.006	0.018	0.006	-0.002	-0.002	-0.002	0.014	0.009	-0.003	0.012	--	ns	ns
Kenai	0.061	0.044	0.022	0.046	0.022	0.013	0.033	0.108	0.080	0.016	0.032	0.036	0.062	0.007	0.009	0.034	0.010	0.005	0.011	0.005	0.023	0.013	0.024	0.029	0.003	--	ns
Coldfoot	0.054	0.031	0.014	0.050	0.023	0.009	0.026	0.098	0.074	0.005	0.020	0.017	0.043	0.003	0.000	0.020	0.004	-0.004	-0.000	-0.001	0.016	0.005	0.008	0.016	-0.003	0.001	--

Appendix 2.2 Table of pairwise Fst and corresponding differentiation for the 27 populations sampled obtained with Genodive (Meirmans and Van Tienderen, 2004). The lower diagonal represents the Fst values and the upper diagonal represents the associated P-values obtained with 1000 iterations. ** represents the significant differences following Bonferoni correction (adjusted critical P = 0.00014) and "ns" means "not significant".

Appendix 2.3 Allele frequency distribution for the 6 populations that experienced bottlenecks Histograms were created based on 10 microsatellites loci. The X-axes represent the allele frequency classes and y-axis represent the number of alleles.



CHAPITRE 3 GENETIC DIVERSITY AND CLONAL STRUCTURE OF NATURAL POPULATIONS OF TREMBLING ASPEN IN CANADA: A TRANSCONTINENTAL STUDY

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3.1. Abstract

The aim of this study was to evaluate trembling aspen genetic diversity and clonal structure across the Canadian boreal forest in relation to regional differences in climate and the surrounding site conditions (aridity, fire cycle, fragmentation). A 30-site network with 40 sampled trees per location was established to cover the large amplitude of variation in the climate, fire regime and landscape fragmentation. Each site was categorized into three groups of clonal organization (clonal diversity-evenness ratio) and classes of Climate Moisture Index (CMI) and their effects on genetic diversity were assessed. Linear models tested the relationships between genetic diversity and structure and the surrounding site condition factors. The percentage of single ramet clones and clonal diversity were high and likely caused by: i) sexual reproduction events in all sites, together with suckering if aspen was already present on site, and ii) self-thinning of ramet intra-clones with long times since fire. No differences in genetic diversity and clonal structure were apparent among CMI classes, while allelic richness varied among clonal organization groups. Landscape fragmentation and higher burn rates negatively affected levels of genetic diversity. Genetic diversity and clonal structure of aspen stands across Canada were high and similar, with occasional sexual events being sufficient to introduce new genotypic variation into populations. Aspen is a generalist species with great capacity for adaptation and maintaining high levels of genetic diversity in diverse, heterogeneous environments.

Keywords: climate, disturbance, fire regime, heterozygosity, microsatellites, *Populus tremuloides*

3.2. Résumé

Le but de cette étude était d'évaluer la diversité génétique et la structure clonale du peuplier faux-tremble en forêt boréale canadienne en tenant compte des différences climatiques régionales et des conditions environnantes de chaque site (aridité, régime de feu, fragmentation). Un réseau de 30 placettes avec 40 arbres échantillonnés par site a été créé pour couvrir la grande amplitude de variation climatique, de régime de feux et de fragmentation du paysage. Chaque site a été classé dans un des trois groupes d'organisation clonale (rapport diversité clonale-équitabilité) et dans une des trois classes d'indice d'humidité du climat (CMI) préalablement créés et leurs effets sur la diversité génétique ont été évalués. Des modèles linéaires ont permis de tester les relations entre la diversité génétique et la structure clonale avec les facteurs de conditions des sites. Le pourcentage de clones à ramet unique et la diversité clonale étaient élevés et probablement causés par: i) des événements de reproduction sexuée présents dans tous les sites et accompagnés de drageonnement dans le cas où le peuplier était déjà présent sur le site, et ii) une auto-éclaircie des ramets intra clones plus forte avec le temps écoulé depuis le dernier feu. Aucune différence de diversité génétique et de structure clonale n'est apparue entre les classes de CMI, alors que la richesse allélique variait en fonction des groupes d'organisation clonale. La fragmentation du paysage et des taux de brûlage plus élevés affectent négativement les niveaux de diversité génétique. La diversité génétique et la structure clonale du tremble au Canada étaient élevées et similaires, avec des événements occasionnels de reproduction sexuée suffisants pour introduire une nouvelle variabilité génotypique dans les populations. Le peuplier est une espèce généraliste avec une grande capacité d'adaptation et de maintien de niveaux élevés de diversité génétique dans des environnements divers et hétérogènes.

Mots-clés : climat, heterozygotie, microsatellites, perturbations, *Populus tremuloides*, regime de feux.

3.3. Introduction

Many plant species are capable of extensive clonal reproduction, including aquatic sea grasses (Reusch 2006), terrestrial shrubs (Pornon *et al.* 2000; Erickson & Hamrick 2003) and trees (Mitton & Grant 1996; Chung & Epperson 2000; Douhovnikoff *et al.* 2005; Namroud *et al.* 2005a; Latutrie *et al.* 2015). In some species, the contribution to the recruitment of sexual and asexual reproduction is equal (Sun *et al.* 2001; Weppler *et al.* 2006) while in other species one mode of reproduction generally dominates (Ceplitis 2001). The relative contribution of asexual propagation to sexual recruitment can vary among populations of a single species across its range (Eckert 2002). The proportions of sexual and asexual recruitment within populations of clonal organisms may be influenced by biotic (e.g. genetic factors, including changes in ploidy and sterility mutations) and abiotic factors (e.g. climate, soil composition or natural and anthropogenic disturbances; Eckert 2002).

In the Canadian boreal forest, the main disturbances are wildfires, insect outbreaks and anthropogenic pressures (fragmentation, logging). The effects of climatic conditions on forest stands are diverse. They define the suitable conditions for seedling establishment, growth or dieback (consequences of extreme events such as drought), and drive local adaptation of trees to their environment (Parmesan 2006). In Canada, a strong climatic gradient with decreasing precipitation and increasing aridity is observed from east to west (Environment Canada 2014) conditioning regional differences (fire regime, secondary disturbances and stand dynamic) between eastern and western portions of the boreal forest (rewiewed by Bergeron *et al.* 2014). The frequency and severity of wildfire varies among ecozones (Bergeron *et al.* 2004b; Krezek-Hanes *et al.* 2011). The fire regime is long with large fires in the eastern boreal forest while it is short with very large fires in the western boreal forest (Bergeron *et al.* 2014) and depends upon the regional climatic conditions. Girardin

and Wotton (2009) were able to explain 63% of the observed variance observed in total annual area burned in Canada (fire > 200 ha) between 1959 and 1999 (Stocks *et al.* 2003) with a monthly aridity index (in this case the July Monthly Drought Code; Girardin *et al.* 2004).

Trembling aspen (*Populus tremuloides* Michaux) is a pioneer species of the boreal forest (Bergeron 2000; Bergeron *et al.* 2002). Aspen massively regenerates after fire and is often the most present species for the first 100 years following disturbance (Bergeron 2000; Cumming *et al.* 2000). Differences in mixedwood stand dynamics are observed between eastern and western Canada, depending upon the occurrence of secondary disturbances (i.e. drought and forest tent caterpillar outbreaks in the west and spruce budworm in the east) and fire regime (i.e. very large fires with short cycles in the west; and large fires with long cycles in the east) that usually reset the system to a younger condition (Bergeron *et al.* 2014).

In the absence of fire, aspen continues to regenerate in small cohorts (gap dynamics), together with the release of shade-tolerant conifers in the canopy openings (Bergeron 2000; Cumming *et al.* 2000; Bergeron *et al.* 2014). It has long been considered as a species that is easy to regenerate, mainly due to its habits of vegetative propagation (Long & Mock 2012; Lafleur *et al.* 2015). Successful seed reproduction events were considered rare, especially in dry regions (Lafleur *et al.* 2015). In the absence of sexual recruitment, one single genotype (also referred to as "clone" or "genet") can cover vast areas and be composed of hundreds or thousands of ramets (e.g. the 43 ha Pando clone in south-central Utah; Grant *et al.* 1992; DeWoody *et al.* 2008).

The genetic diversity and clonal structures of aspen populations could thus reflect the surrounding environmental conditions (aridity, fire regime), or the predominant mode of reproduction (sexual or asexual; Mitton & Grant 1996). Clone size (ramet numbers per genet) may depend upon the climate, the competition with adjacent clones for light and nutrients, disturbance regimes (e.g. fire, insects), the suckering ability of

each clone, and the frequency of successful seedling establishment (Suvanto & Latva-Karjanmaa 2005; MacKenzie 2010). Within aspen range, clonal diversity is generally assumed to decrease from east to west, while population heterozygosity would tend to increase (Cheliak & Dancik 1982; Hyun *et al.* 1987; Lund *et al.* 1992; Mitton & Grant 1996). However, few studies have examined geographical patterns of genetic and clonal structure in aspen at a continental scale (Keller *et al.* 2010; Callahan *et al.* 2013). For example, Keller *et al.* (2010) reported higher levels of expected heterozygosity (H_e) and percentage of polymorphic loci in the prairies and in the Rocky Mountain Foothills of Alberta for the related species balsam poplar (*P. balsamifera* L.). In the same region, Callahan *et al.* (2013) found the highest level of observed heterozygosity (H_o) for *P. tremuloides*. These studies have shown that regional variations in terms of genetic diversity exist within aspen's distributional range and that they might be driven by various underlying mechanisms such as post-glacial recolonization, climatic conditions or disturbances regimes.

The capacity of aspen to use the mixed mode of reproduction should influence the spatial genetic structure of populations especially when populations contain few clones. This mixed mode of reproduction contributes to skewed sex ratios in dioecious species and to potential problems in sexual recruitment (Ardehed *et al.* 2015). Consequently, asexual recruitment of new individuals from existing genotypes will dominate, and the ability to adapt to local environments can be reduced, thanks to recombination of existing clones (Ardehed *et al.* 2015). For an early successional species, asexual reproduction considerably increases population growth capacity and its ability to colonize new areas by outcompeting other species (MacKenzie 2010). Genotypic diversity in clonal plants steadily decreases with increasing rates of vegetative propagation. However, a high level of genotypic variation, comparable to that of a fully sexual population, can be maintained with occasional sexual reproductive events that create new genotypes (Balloux *et al.* 2003; Bengtsson 2003). High rates of vegetative propagation will positively affect the level of heterozygosity

given that twice as many alleles per locus can be maintained in purely clonal populations compared to purely sexual ones (Balloux *et al.* 2003).

Turner *et al.* (2003) suggested that episodic large-scale disturbances may also play a key role in the structure, genetics, and evolution of populations of long-lived, clonal plant species populations. The effect of time-since-fire (TSF) on aspen clonal (genotypic) diversity has been documented in Québec (Namroud *et al.* 2006). Namroud *et al.* (2006) suggested that ramets mortality within genets, rather than genet mortality, accounts for the increase in clonal diversity with TSF in the eastern boreal mixedwoods. Mock *et al.* (2012) have shown an effect of climatic conditions on the number of triploid aspen clones observed on their sampled populations. Within the species range there were a tendency of larger clones to be triploids.

This study evaluated the influence of climate, fire regimes, and fragmentation of the forest landscape on the regional differences in trembling aspen genetic diversity and clonal structure across the Canadian boreal forest. Our hypothesis is that aspen clonal structure, measured by the richness and evenness, should vary depending upon disturbance regimes in the different regions due to changes in climate, insect epidemics or forest fires. Drier weather conditions in the western boreal should limit sexual recruitment, while more frequent fires should promote an increase in the relative size of aspen clones by vegetative reproduction. Conversely, clonal diversity and the proportion of single ramet clones (SRC) should be higher in the eastern portion of aspen range as a consequence of moist conditions (driving more frequent sexual recruitment) and a low burn rate increasing the stand thinning of ramets (Namroud et al. 2006). Finally, we hypothesized that genetic diversity, measured as the heterozygosity and the allelic richness, will also vary regionally with the changing environmental conditions and aspen clonal structure.

3.4. Materials and methods

3.4.1. Study sites and sampling

3.4.1.1. Study sites

The study area included 30 sites, each measuring 7800 m^2 in area (50m radius plot), which had been established in pure (> 75 % basal area) and mature (> 50-years-old) trembling aspen stands. They were sampled in 2011 and 2012 across Canada (48°29'59" N to 58°20'18" N; 67°59'07" W to 122°44'45" W; Figure 3.1). The 30-site network included 13 CIPHA (Climate Change Impacts on Productivity and Health of Aspen) sites (Hogg *et al.* 2005; Michaelian *et al.* 2011) and was designed to cover a wide range of climatic conditions, fire regimes and fragmentation in aspen stands (Figure 3.1; Table 3.1). The main factor structuring this pan-Canadian transect is the east-west gradient of decreasing annual precipitation (from 1000 to 400 mm/year) and, therefore, a gradient of increasing aridity since we followed a isothermal 0°C line. In general, the topography was gently rolling, with elevations ranging from 233 to 1024 m asl (Table 3.1). Soil conditions were selected to be as similar as possible (data not shown). The study area was divided into 3 sub regions: BE, Boreal East; BW, Boreal West; and P, Aspen Parkland (Table 3.1).

3.4.1.2. Sampling

For each site, 50 trees were randomly selected and sampled. Sampling each tree consisted of measuring its height (m), DBH (diameter at breast height, cm), dominance status and canopy coverage (%) and removing root cambium tissue for molecular analysis. GPS coordinates of each tree were taken with a GEOExplorer 2008 Series unit (Trimble Navigation, Sunnyvale, CA, USA). The accuracy of measurements was 0.5 m; and the final coordinates were obtained by averaging at least 20 single GPS points per tree. Data were extracted from the GPS instrument with the software provided by Trimble (Trimble Navigation) and treated with

ArcMap (ESRI, Redlands, CA, USA) to transform the geographic coordinates (Latitude, Longitude) into a cartographic projection, first in WGS 84 (World Geodedic System 1984) and then in UTM (Universal Transfer Mercator) while taking into account the UTM zones for each site (from zone 19 in Quebec to zone 10 in British Columbia). From these data, the distance and azimut from the plot centre were calculated for each tree with ArcMap (ESRI, Redlands, CA, USA). For further analysis in R, these data were transformed as x/y coordinates for each tree in each plot with the plot centre at coordinate (0;0).

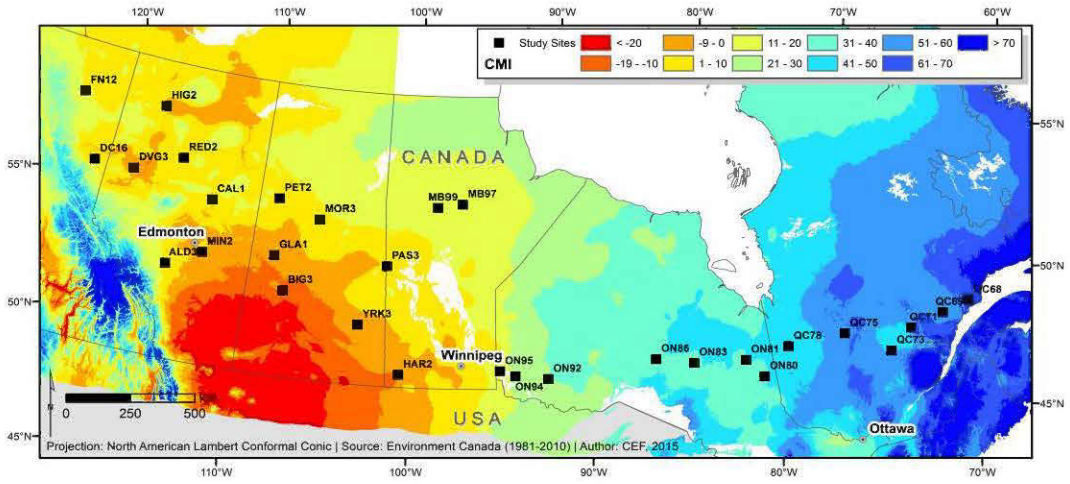

Figure 3.1 Locations of the 30 sites (filled squares) sampled across the Canadian boreal forest and Aspen Parkland. The background colours represent variation in the average Climate Moisture Index (CMI) for the period 1950-2010 (Régnière *et al.* 2013). Areas in red have the lowest CMI (arid) while those in dark blue have the highest CMI (moist).

3.4.2. Climatic data

All of the climatic data that were included in the dataset (e.g. Temperature, T; Precipitation, P; Potential Evapotranspiration, PET) were obtained on a monthly basis for all sites using with BIOSIM 10 (Régnière *et al.* 2013). The daily climate database for Canada for the period 1950-2010 was used to interpolate the climate variables for

each of the sites. The Climate Moisture Index (CMI; Hogg 1997; Hogg & Bernier 2005; Hogg *et al.* 2008; Lemprière *et al.* 2008; Michaelian *et al.* 2011) was then calculated, in centimetres, as follows: CMI = P – PET. CMI was estimated for the period between August and the following July to take into account possible winter moisture deficits that could affect next year's growth (Hogg 1997; Hogg *et al.* 2005, 2008). An averaged CMI value for the period 1950-2010 was later used. Sites were grouped into 3 classes of CMI so that each class represented different conditions of aridity. The three classes were defined as follows: the "moist" class was composed of sites with an averaged CMI value > 30; the "intermediate" class had site with values of averaged CMI comprised between 5 and 30; and the "dry" class regrouped sites with averaged values CMI < 5. These three classes were then used to analyze the regional differences in terms of genetic diversity and clonal structure.

3.4.3. Fire and fragmentation data

Area burned varies greatly within the Canadian boreal forest (Bergeron *et al.* 2004a; Girardin & Wotton 2009; Boulanger *et al.* 2012). Some researchers have tried to define homogeneous fire regime (HFR) zonation by using ecozones (Bergeron *et al.* 2004a) or completely new zonation boundaries (Boulanger *et al.* 2012). Using HFR zonation appeared not to be the best solution for our study since these zones can cover very large areas; moreover, many sites could be assigned the same value if they were present in the same zone. To avoid this, we calculated a specific value for the area that was burned and the corresponding annual burn rate for each site to use them in further analyses. Fire data were obtained from the National Fire Database (NFDB) fire polygon dataset between 1950 and 2010 (Canadian Forest Service 2010). Vegetation data were collected from the vegetation map that was prepared by Natural Resources Canada (2013). We used a circular buffer of 50 km radius that was centered on each site and all calculations were performed in ArcMap (ESRI, Redlands, CA, USA). For each site, we first calculated the total area of the buffer and

the vegetation composition was classified as forested, water, agriculture, urban, or wetland. From this, we calculated the degree of landscape fragmentation as the percentage of the area that was covered by agriculture, divided by the total area of the 50 km radius buffer. We then evaluated the total area that was burned within the 50 km radius buffer from the NFDB. For each site, the area that was burned was determined as the sum of all fire polygons that were present inside the buffer zone, regardless of whether the fire was completely or partly inside the buffer zone. All areas that were burned were counted even if fire repeatedly affected the exact same area. The annual burn rate was calculated as follows:

Annual burn rate (%) = (total area burned / total forested area) * (100/60)

where the proportion of area burned to total forested area is converted to an annual basis (dividing by 60 years, i.e. the period 1950-2010), and multiplied by 100 to yield a percentage value. These relative values were calculated to facilitate comparison among sites, given that our values of burn rate may differ among specific fire studies in Canada (e.g. Bergeron *et al.* 2004a; Girardin & Mudelsee 2008; Bergeron *et al.* 2010).

3.4.4. Spatial clonal structure and genetic diversity

DNA extraction, amplification and genotyping were performed on 40 randomly choose trees out of the 50 trees that had been sampled in the field (1200 samples) following the method that was described in Latutrie *et al.* (2015). Fourteen samples could not be used due to technical issues in the laboratory and, thus, the final dataset was composed of 1186 samples (35 to 40 trees per site) for the 30 sites.

3.4.4.1. Genetic diversity

We used GENODIVE (Meirmans & Van Tienderen 2004) to assign clone identities, based on the stepwise mutation model (SMM). In the SMM, alleles that differ only by

a few repeats in length are thought to be of more recent common ancestry than alleles that differ by many repeats in length (Meirmans & Van Tienderen 2004). We considered that 2 individuals belong to the same clone if the total genetic distances (differences in repeat lengths between 2 alleles) for all loci were lower than 3 mutations. We used this criterion to avoid identifying unique multilocus genotypes (MLG or genets or clones) that had resulted from scoring errors and somaclonal mutations (small genetic distances; Ally *et al.* 2008). Based on the dataset that contained only unique MLG, we calculated a set of genetic measures to evaluate genetic diversity: observed heterozygosity (H_o); expected heterozygosity (H_e); average number of alleles observe per locus (N_a) and the inbreeding coefficient (G_{is}). Triploid individuals were identified as the presence of three distinct alleles at two or more loci since the probability of detecting triploids at solely one locus was high (34% of all individuals genotyped) due to a higher frequency of triallelles that were observed at 2 loci (PTR1, 0.18; PTR3, 0.23; Appendix 3.1). The proportion of triploidy per site (Trip) was calculated as the proportion of total triploid clones that were observed, divided by the total number of observed clones (G_o). Triploidy was not confirmed with flow cytometry, since Mock *et al.* (2012) found that cytotype inconsistencies only affected 2.02% of the samples. Finally, a dataset with all unique diploid MLGs (all MLGs that have two alleles at all loci) was used to estimate allelic richness (AR) across all seven loci. AR was calculated using FSTAT v. 2.9.3 (Goudet 2001) with a rarefaction method; this method was employed to account for differences in sample size. Four sites with fewer than 5 unique diploid clones were not included in the calculation (QC71, GLA1, HIG2 and DC16).

Table 3.1 Summary data of the environmental characteristics of the 30 aspen plots sampled across Canada.

Population	Regions*	Latitude	Longitude	Elevation (m)	Age	Density	Mean T (°C)	P (cm)	PET	CMI (cm)	CMI class§	Buffer (km^2)	Forest (km^2)	Agri (km^2)	Frag (%)	Burned area (km^2)	Burn rate
QC68	BE	49.54	-67.99	337	53	1425	0.73	99.95	36.96	62.99	M	7808	6235	0	0	3	0,001
QC69	BE	49.39	-69.6	286	61	800	0.86	93.86	45.22	48.65	M	7801	6488	0	0	2050	0,527
QC71	BE	49.15	-71.54	304	62	1525	0.46	94.97	42.74	52.23	M	7791	6978	77	0.01	38	0,009
QC73	BE	48.5	-72.97	526	69	1525	0.03	87.38	38.3	49.08	M	7759	7170	188	0.02	1263	0,294
QC75	BE	49.57	-75.25	376	84	1075	0.05	97.95	37.45	60.5	M	7809	6003	0	0	223	0,062
QC78	BE	49.53	-78.1	306	90	850	0.29	87.4	40.11	47.3	M	7808	5375	9	0	55	0,017
ON80	BE	48.56	-80.17	360	86	1250	1.36	82.21	43.27	38.94	M	7762	5936	203	0.03	122	0,034
ON81	BE	49.31	-81.03	248	68	500	0.74	85.13	42.32	42.81	M	7797	5996	61	0.01	94	0,026
ON83	BE	49.49	-82.94	238	69	1050	0.68	84.8	42.97	41.84	M	7805	7457	0	0	17	0,004
ON86	BE	49.81	-86.04	293	83	875	0.46	77.92	40.93	37	M	7819	7183	0	0	267	0,062
ON92	BE	49.36	-92.22	434	83	600	1.94	72.33	44.53	27.79	I	7799	6586	0	0	78	0,02
ON94	BW	49.49	-94.06	385	79	1225	2.53	68.89	44.6	24.28	I	7805	5631	0	0	1122	0,332
ON95	BW	49.7	-94.94	358	58	900	2.09	63.4	45.93	17.47	I	7854	5364	0	0	426	0,132
MB97	BW	56.03	-97.16	233	79	1450	-3.31	50.24	34.73	15.51	I	8014	4216	0	0	4065	1,607
MB99	BW	55.89	-98.68	279	79	1200	-2.76	50.62	34.5	16.12	I	7889	4936	0	0	2498	0,843
PAS3	BW	53.6	-101.68	279	69	1125	0.09	43.68	42.96	0.72	D	7955	5853	283	0.04	630	0,179
MOR3	BW	55.14	-106.07	444	80	950	-0.33	47.22	39.82	7.39	I	7996	5351	0	0	1567	0,488
PET2	BW	55.74	-108.78	490	63	1100	0.08	45.52	37.46	8.06	I	8008	4786	0	0	2888	1,006
CAL1	BW	55.29	-112.97	675	62	1925	0.88	47.74	41.05	6.68	I	8000	6439	491	0.06	1443	0,373
RED2	BW	56.61	-115.31	521	59	1350	1.1	43.46	42.93	0.53	D	8024	6674	0	0	613	0,153
HIG2	BW	58.34	-117.24	343	69	1225	-0.92	40.89	42.64	-1.75	D	8046	6035	763	0.09	275	0,076
DC16	BW	55.45	-121.55	768	64	1000	2.38	59.12	43.99	15.13	I	8010	5428	2468	0.31	1852	0,569
FN12	BW	58.11	-122.75	562	49	1425	-0.37	50.88	40.12	10.76	I	8045	7206	3	0	1284	0,297
HAR2	P	49.49	-100.64	NA	86	375	2.87	46.78	56.55	-9.77	D	7805	224	5544	0.71	1	0,008
YRK3	P	51.31	-103.16	NA	48	550	1.39	46.37	45.76	0.61	D	7881	619	6478	0.82	5	0,013
BIG3	P	52.32	-107.77	713	69	625	1.62	43.28	50.18	-6.9	D	7915	224	6592	0.83	0	0,008
GLA1	P	53.6	-108.58	NA	81	325	0.37	41.76	44.27	-2.51	D	7955	3606	3863	0.49	37	0,017
MIN2	P	53.28	-112.93	785	74	525	2.56	48.7	44.91	3.8	D	7946	733	6544	0.82	102	0,233
ALD3	P	52.6	-114.96	1024	63	500	2.65	57.36	46.91	10.45	I	7925	5057	2637	0.33	351	0,116
DVG3	P	55,8	-118,26	566	58	1425	1,597	43,98	45,69	-1,71	D	8011	2362	5396	0.67	607	0,428

Regions, BE: Boreal East; BW: Boreal West; P: Aspen Parkland; § CMI class, M: Moist conditions (CMI > 30); I: Intermediate conditions (30 > CMI > 5); D: Dry conditions (5 > CMI); Mean T: Mean Temperature; P: Precipitations; PET: Potential Evapotranspiration; CMI: Climate Moisture Index; Agri: Agricutural area; Frag: Landscape Fragmentation

3.4.4.2. Clonal structure

Clonal structure was evaluated based upon the data set that contained all of the ramets that were sampled (i.e. 1186). Clonal heterogeneity (Arnaud-Haond *et al.* 2007) was estimated by calculating the clonal evenness (E). We estimated the clonal richness by calculating the observed clonal diversity (R_o), which is the proportion of different genotypes in a population, and is calculated as the following: $R_o = (G_o-1)/(N-1)$, where G_o is the number of observed genets and N is the total number of individuals that were analysed (Dorken & Eckert 2001). Effective clonal diversity (R_e) was calculated in the same way as clonal diversity (R_o) with G_o being replaced by G_e, the effective number of genotypes. Based upon the clonal richness and evenness, three groups of clonal organization were defined (G1, only unique clones with $R_o = 1$ and $E = 1$; G2, many small clones with intermediate values of R_o and E that ranged between 0.4-1 and 0.6-1, respectively; and G3, few large clones with relatively low values of $R < 0.4$ and $E < 0.6$) and used in further analyses. In each plot, each sampled tree had a unique GPS coordinates and a unique genotypic identity (genotype), which allowed us to calculate a series of measures using the function *pairdist* and *ppp* in the package *spatstat* (Baddeley & Turner 2005) in R (R Development Core Team 2013). We calculated the mean, minimum and maximum distances between trees that were sampled within a plot, regardless of their genotypic identity. We then we calculated also the mean, minimum and maximum distances between each ramets for each clone consisting of more than one ramet (or multi-ramets clones, MRC). We also calculated the mean clone size (ramet numbers per genet) and the percentage of single ramet clones (SRC).

3.4.5. Effects of climate and disturbances on the genetic diversity and clonal structure

Using the R function *aov*, we tested the differences in terms of genetic diversity and clonal structure between the defined classes of CMI. We also tested the effect of clonal structure (the 3 classes defined earlier) on the genetic diversity with the same method. We then carried out multiple linear regressions with the function *lm* on the continuous data to evaluate the relationship between genetic diversity (H_o, AR, N_a, Trip) or clonal structure (R_o, R_e, E, average distance between ramets of MRC, average and maximum clone size, percent of SRC) versus a set of environmental explanatory variables (CMI, temperature, burn rate, fragmentation, age and density), which were included in a saturated or global model. Model selection was performed using backward selection based on the Akaike Information Criterion (AIC) with the function *step* (R Development Core Team 2013). A maximum of 6 different explanatory variables could be included in the best final model to avoid over fitting, while maintaining high statistical power (a large number of parameters decrease precision of estimates; Verbeke & Molenberghs 2009; Zuur *et al.* 2009). Partial regression plots were created with the function *avPlots* in the *car* package (Fox & Weisberg 2011). Model assumptions of homogeneity of variance and normality were verified graphically and data were transformed where needed.

3.5. Results

3.5.1. Differences between regions in terms of climatic conditions, fire regimes and landscape fragmentation

The study area was divided into 3 sub regions (BE, Boreal East; BW, Boreal West; and P, Aspen Parkland), as presented in Table 3.1. CMI varied significantly (One-way ANOVA: $F_{2,27}$ = 79.49, P = 8.85e-12) from one sub-region to another with respective means of 46.3 in BE, 10.1 in BW and -0.9 in P. Within a 50 km radius

around site, landscape fragmentation (One-way ANOVA: $F_{2,27}$ = 96.58, P = 4.97e-13) was higher in P (0.67 %) compared to BE (0.006 %) and BW (0.04 %). Within the same sub regions, differences were observed in the fire regime that was assessed using the burn rate (One-way ANOVA: $F_{2,27}$ = 5.87, P = 0.0076), with greater values in BW (0.5) compared to BE (0.09) and P (0.12).

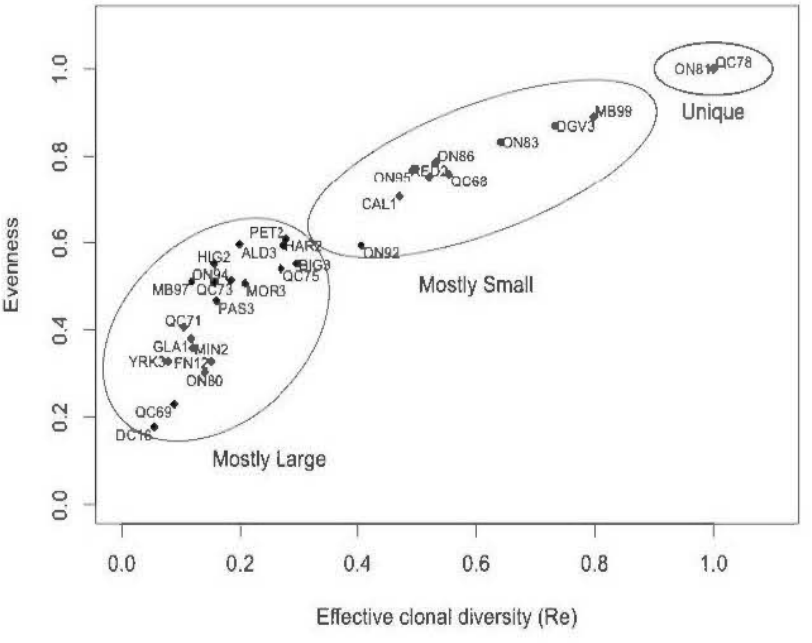

Figure 3.2 Clonal organization of *Populus tremuloides* populations across the Canadian boreal forest and Aspen Parkland. Based upon the relationship between effective genotypic diversity and evenness, the populations (n = 30) have been divided into 3 groups ranging from sites containing only unique genotypes (G1) to those containing few large clones (G3).

3.5.2. Clonal structure

Of the 1186 trees that were successfully genotyped, 629 different multi-locus genotypes (genets or clones) were identified. Clonal diversity R_o and effective clonal diversity R_e averaged 0.52 (range 0.23-1) and 0.34 (range 0.5-1), respectively. Average evenness (E) was 0.58, with a highest value of E = 1 and a lowest value of E = 0.177, where the differences between clone sizes within stands were large. Based

upon clonal diversity and evenness, three classes of clonal organization were determined (G1, only unique clones; G2, many small clones; G3, few large clones; Figures 3.2 and 3.3). In most sites, few large to many small multi-ramet clones were observed and two sites had unique clones (Figure 3.2). The spatial organization of ramets and genets that was observed in each group is presented in Figure 3.3. No relationship between the classes of clonal organization and any of the disturbances that were tested was found (One-way ANOVAs: CMI, $F_{2,27}$ = 1.62, P = 0.21; Landscape fragmentation, $F_{2,27}$ = 1.202, P = 0.31; Burn Rate, $F_{2,27}$ = 0.634, P = 0.54).

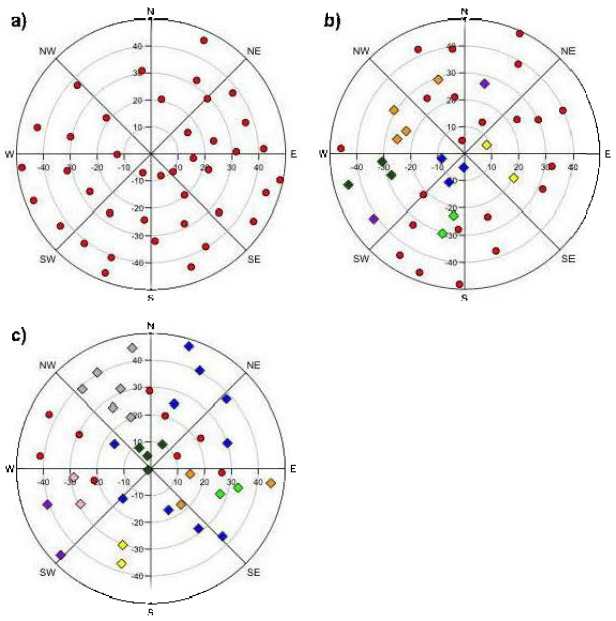

Figure 3.3 Polar plots of Populus tremuloides populations at three sites showing the distribution of ramets: a) QC78 (only unique clones); b) QC68 (many small clones); c) MOR3 (few large clones). Each symbol represents a sampled tree. Genets represented by single ramet are indicated with a red circle. A diamond shape represents multi-ramet genets, with individuals from the same genet indicated by the same colour. Radial axis represents distance in m; angular axis represents the orientation. Number of clones was 28, 17 and 13, respectively. Identified genets differed among sites. Radial increments are 10m intervals from the central location 0,0.

Table 3.2 Genetic diversity and clonal structure data of 30 aspen populations sampled across Canada

Population	N	Go*	Ro*	Ge*	Re*	Na*	AR§	Ho**	He**	Gis**	E*	Trip**	Mean clone size	Max clone size	SRC (%)	Tree dist (m)	Mean ramet dist (m)
QC68	38	28	0.73	21.24	0.55	7.71	3.8	0.62	0.69	0.1	0.76	0.11	1.36	4	0.79	39.61	20.83
QC69	40	16	0.38	3.67	0.09	5.86	2.86	0.6	0.61	0.02	0.23	0	2.5	20	0.75	40.66	17.62
QC71	40	11	0.26	4.47	0.1	5.86	NA	0.7	0.64	-0.09	0.41	0.09	3.64	17	0.36	40.33	12.79
QC73	40	13	0.31	6.61	0.16	6	2.64	0.67	0.63	-0.06	0.51	0.08	3.08	10	0.54	39.29	16.01
QC75	39	20	0.5	10.79	0.27	6.57	3.29	0.6	0.65	0.07	0.54	0.05	1.95	9	0.55	42.61	16.04
QC78	40	40	1	40	1	9.14	3.95	0.66	0.71	0.07	1	0.08	1	1	1	42.17	NA
ON80	40	19	0.46	5.76	0.14	6.86	3.17	0.6	0.64	0.06	0.3	0	2.11	15	0.79	40.08	17.54
ON81	40	40	1	40	1	8.86	3.82	0.63	0.65	0.03	1	0.03	1	1	1	44.32	NA
ON83	40	31	0.77	25.81	0.64	7.86	3.75	0.67	0.64	-0.04	0.83	0.06	1.29	3	0.77	44.6	16.37
ON86	35	24	0.68	18.85	0.53	7.57	3.68	0.58	0.65	0.11	0.79	0	1.46	4	0.67	44.36	13.19
ON92	39	27	0.68	16.01	0.41	8	3.5	0.64	0.64	0	0.59	0.07	1.44	6	0.85	43.25	18.81
ON94	40	15	0.36	7.69	0.18	6.43	2.81	0.53	0.62	0.14	0.51	0	2.67	10	0.6	40.98	17.43
ON95	40	26	0.64	20	0.49	7.71	3.8	0.68	0.69	0.01	0.77	0.19	1.54	4	0.65	44.27	20.34
MB97	40	10	0.23	5.1	0.12	5.43	3.06	0.56	0.64	0.13	0.51	0.6	4	14	0.4	42.36	19.41
MB99	40	36	0.9	32	0.8	7.71	3.81	0.64	0.68	0.05	0.89	0.03	1.11	3	0.92	43.16	8.86
PAS3	39	14	0.34	6.53	0.16	5.71	3.28	0.67	0.65	-0.03	0.47	0.21	2.79	12	0.5	41.25	19.64
MOR3	40	17	0.41	8.6	0.21	6	3.73	0.7	0.69	-0.02	0.51	0	2.35	10	0.53	37.94	16.27
PET2	38	18	0.46	10.94	0.28	6.43	3.28	0.6	0.7	0.14	0.61	0.06	2.11	7	0.56	42.65	22.18
CAL1	40	27	0.67	19.05	0.47	8.57	3.76	0.62	0.64	0.04	0.71	0.04	1.48	5	0.74	40.63	14.06
RED2	40	28	0.69	21.05	0.52	8.86	3.87	0.67	0.7	0.04	0.75	0.07	1.43	4	0.75	41.03	13.77
HIG2	40	12	0.28	6.61	0.16	6.29	NA	0.7	0.69	-0.02	0.55	0	3.33	11	0.33	46.55	22.69
DC16	40	13	0.31	2.31	0.05	5.71	NA	0.62	0.63	0.01	0.18	0.31	3.08	26	0.77	42.05	26.81
FN12	40	19	0.46	6.2	0.15	6.43	3.09	0.67	0.64	-0.04	0.33	0.05	2.11	14	0.74	42.87	23.5
HAR2	40	19	0.46	11.27	0.27	5.14	2.92	0.62	0.53	-0.17	0.59	0.11	2.11	6	0.63	41.4	31.87
YRK3	39	10	0.24	3.27	0.08	4.86	3.01	0.71	0.67	-0.05	0.33	0.3	3.9	19	0.7	35.83	25.44
BIG3	40	22	0.54	12.12	0.3	6.71	3.57	0.58	0.64	0.08	0.55	0.09	1.82	9	0.5	41.03	14.76
GLA1	40	13	0.31	4.94	0.12	5.14	NA	0.8	0.65	-0.24	0.38	0.23	3.08	16	0.54	40.82	29.63
MIN2	40	14	0.33	5	0.12	5.57	3.31	0.57	0.61	0.07	0.36	0.07	2.86	15	0.64	41.28	18.52
ALD3	40	14	0.33	8.33	0.2	6	3.45	0.7	0.66	-0.05	0.6	0.14	2.86	9	0.5	43.76	21.84
DGV3	39	33	0.84	28.7	0.73	8	3.95	0.61	0.65	0.06	0.87	0.06	1.18	3	0.85	43.91	10.97

N, number of individuals genotyped; G_o, observed number of unique genets; R_o, observed cloual diversity; G_e, effective number of unique genets; R_e, effective cloual diversity; N_a, average number of alleles per locus; AR, average allelic richness; H_o, observed heterozygosity; H_e, expected heterozygosity; G_{IS}, interbreeding coefficient; E, Evenness; Trip, Proportion of triploidy; SRC, Single Ramet Clones; * Calculated from the ramet dataset; ** Calculated from the genet dataset; § Calculated from the unique diploid clones

Between plots, the percentage of single ramet clones (SRC) averaged 0.66 and ranged between 0.36 and 1. Within plots, the average clone size (ramet numbers per genet) varied from 1 to 4, averaging 2.23 ramets per clone. Over all sites, clonal diversity was highly correlated with clone size (Pearson product-moment correlation: r = -0.93, $P < 0.01$) and the percentage of SRC (r = 0.79, $P < 0.01$). Average maximum clone size varied in a manner similar to that of average clone size. Large clones with more than 20 ramets represented less than 2% of all genotyped cloues. Mean distance observed between ramets of MRC varied from 8.8 m to 31.9 m and averaged 18.8 m among sites. Maximum distance observed between ramets of MRC ranged between 9.9 m and 55.9 m, with a mean value of 28.2 m among sites.

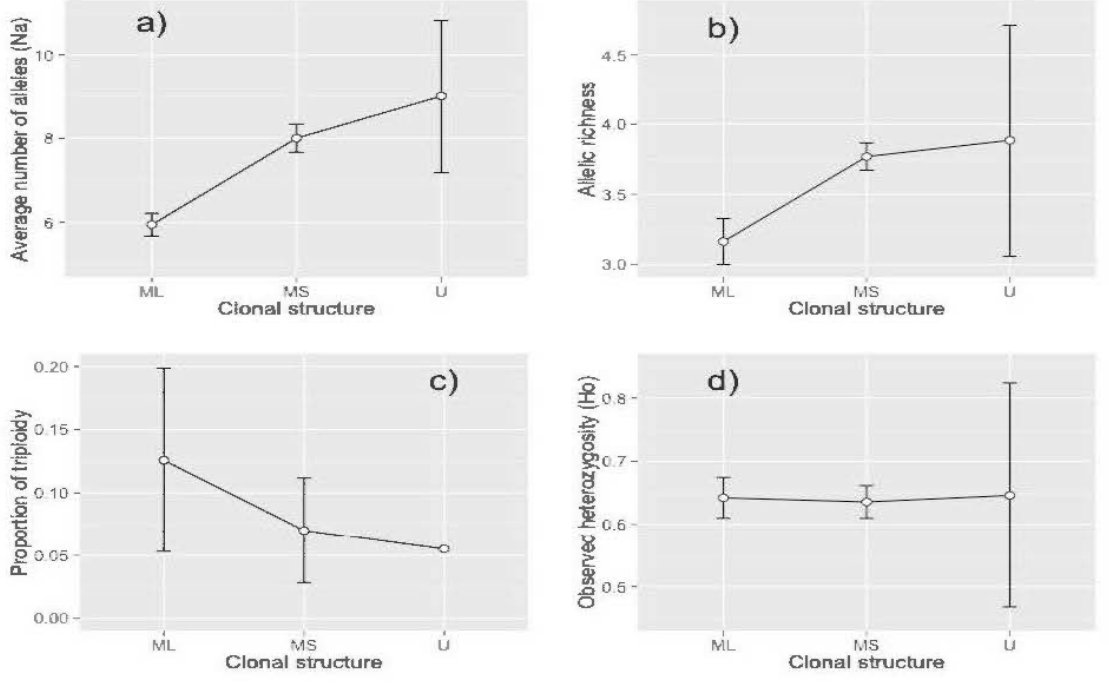

Figure 3.4 Comparisons of genetic measures between clone size classes: mostly large (ML); mostly small (MS); and unique (U). a) Mean number of alleles (N_a; ANOVA, $F_{2,27}$ = 68.35, P = 2.72e-11); b) Allelic richness (AR; ANOVA, $F_{2,23}$ = 21.19, P = 6.06e-06); c) Mean observed heterozygosity (H_o; ANOVA, $F_{2,27}$ = 0.048, P = 0.95); d) Proportion of triploidy (Trip; ANOVA, $F_{2,27}$ = 0.752, P = 0.48).

3.5.3. Genetic diversity

All descriptive genetic data are presented for the 30 sites across Canada in Table 3.2. All 7 loci were highly polymorphic and the average number of alleles per locus (N_a) ranged from 4.86 to 9.14 (average value across populations = 6.77 across populations). N_a varied significantly (One-way ANOVA: $F_{2,27} = 68.35$, $P = 2.72e\text{-}11$) among groups, with values of 9, 8 and 5.9 in G1, G2 and G3, respectively (Figure 3.4a). Allelic richness (AR) averaged 3.43 and varied from 2.81 to 3.95. AR varied significantly among groups (ANOVA: $F_{2,23} = 21.19$, $P = 6.06e\text{-}06$; Figure 3.4b): 3.9 (G1), but less so than N_a: 3.8 (G2), and 3.2 (G3). The proportion of triploid individuals (Trip) averaged 0.10, and ranged from 0 to 0.6. Trip did not vary significantly with the presence of a few large clones (One-way ANOVA: $F_{2,27} = 0.752$, $P = 0.48$), with respective values of 0.05, 0.07 and 0.13 for groups 1, 2 and 3 (Figure 3.4c). Observed heterozygosity (H_o) had a mean value of 0.64 and did not vary significantly between groups (ANOVA: $F_{2,27} = 0.048$, $P = 0.95$; range 0.53-0.80; Figure 3.4d). Mean H_e was 0.65, with values ranging from 0.53 to 0.71. The inbreeding coefficients were low with, an average G_{IS} of 0.013 (range: -0.242 to 0.143).

Table 3.3 Results of the best-fitting model (multiple linear regression) explaining the relationship between a) N_a, b) H_o, c) Mean ramet distance (for multi-ramet clones), and d) Proportion of triploidy and the explanatory variables (Landscape fragmentation, Burn rate, CMI, Density, Age). Backward selection was used to select the best-fitting model. Significant relationships are in bold.

Response	Predictor	Estimate	Std error	t-value	p-value
a) N_a	**Fragmentation**	**-25.332**	**10.082**	**-2.513**	**0.0183 ***
	Burn rate	-13.419	8.239	-1.629	0.1150
b) H_o	**Burn rate**	**-0.078**	**0.028**	**-2.751**	**0.0107 ***
	CMI	**-1.176 e-03**	**0.569 e-03**	**-2.066**	**0.0489 ***
	Fragmentation	-0.0681	0.0422	-1.615	0.1184
c) Mean ramet distance	**Density**	**-6.922 e-03**	**2.263 e-03**	**-3.058**	**0.00511 ****
d) Triploidy	**Fragmentation**	**0.216**	**0.090946**	**2.379**	**0.02500 ***
	Age	- 4.286e-03	2.334e-03	-1.836	0.07784

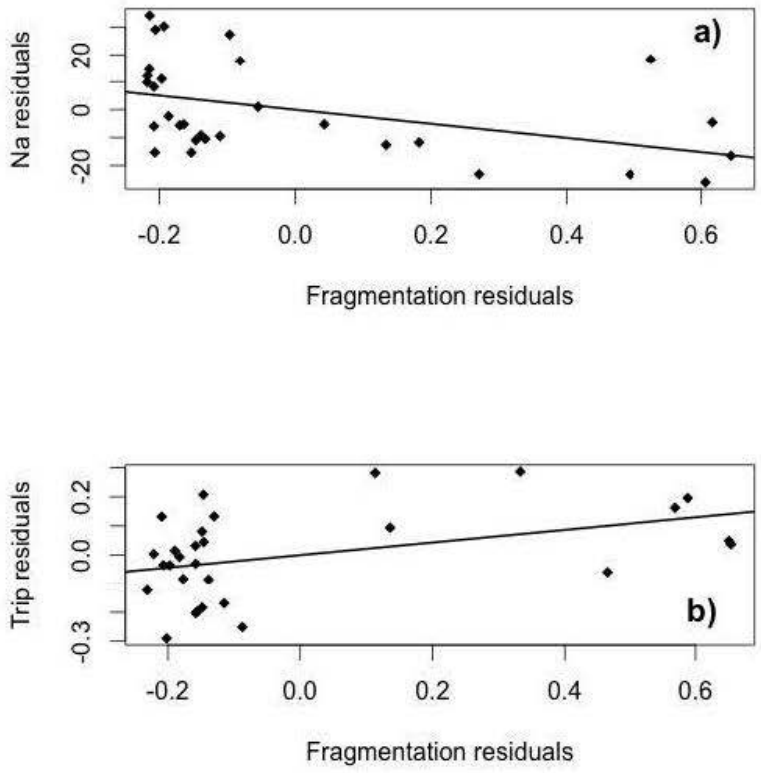

Figure 3.5 Partial regression plots showing the relationships between a) average number of alleles per locus (N_a) residuals and fragmentation residuals, and b) Trip residuals and fragmentation residuals for 30 populations of *Populus tremuloides* that were located across boreal Canada.

3.5.4. Effect of climate, fire regime and landscape fragmentation on aspen genetic diversity and clonal structure

No relationship between groups of clonal organization and CMI, burn rate or landscape fragmentation variables could be found (data not shown). Values of R_o were higher with high CMI (moist conditions; 0.61) and decreased with decreasing CMI, with values of 0.50 and 0.45, respectively, in intermediate and dry climatic conditions. Although there were trends among CMI classes, no significant differences

were apparent in terms of either observed clonal diversity R_o (One-way ANOVA: $F_{2,27} = 1.284$, $P = 0.29$) or evenness E (One-way ANOVA: $F_{2,27} = 0.502$, $P = 0.61$; Apendix 3.2). Other measures of clonal structure did not vary among the classes of CMI (One-way ANOVAs: Percentage of SRC, $F_{2,27} = 1.091$, $P = 0.35$; Mean clone size, $F_{2,27} = 0.953$, $P = 0.40$; Maximum clone size, $F_{2,27} = 0.283$, $P = 0.76$; Appendix 3.2). Likewise, mean genetic diversity indices were similar among CMI classes with no significant differences (One-way ANOVAs: N_a, $F_{2,27} = 1.553$, $P = 0.23$; AR, $F_{2,23} = 0.007$, $P = 0.99$; H_o, $F_{2,27} = 0.686$, $P = 0.51$; Trip, $F_{2,27} = 0.752$, $P = 0.48$; Appendix 3.2).

Among the candidate multiple linear regression models, the best-fitting model explaining the average number of alleles per locus (N_a) included both landscape fragmentation and the burn rate ($R^2 = 0.22$, $P < 0.05$). While burn rate alone exerted no significant effects, partial regression plots showed significantly higher N_a on sites with a lower degree of landscape fragmentation (Table 3.3; Figure 3.5a). Burn rate, CMI and landscape fragmentation ($R^2 = 0.26$, $P < 0.05$) influenced observed heterozygosity (H_o). The relationships between these explanatory variables and H_o are summarized in Table 3.3. H_o significantly increased with lower burn rates and CMI (Figure 3.6a,b). According to the AIC, the model that best explained the proportion of triploidy (Trip) included landscape fragmentation and stand age ($R^2 = 0.276$, $P < 0.05$; Table 3.3). Parameter estimates derived from multi-model inference revealed that age of the stand was not significant and that Trip increased with increasing landscape fragmentation (Table 3.3, Figure 3.5b). Finally, the distance between ramets of MRC decreased with increasing density of the stand ($R^2 = 0.265$, $P < 0.01$; Table 3.3). The response variables AR, R_o, E, average clone size, maximum clone size, and percentage of SRC were also tested against the set of explanatory variables (CMI, Temperature, Burn rate, Fragmentation, Age and Density), but no significant relationships were found (Data not shown).

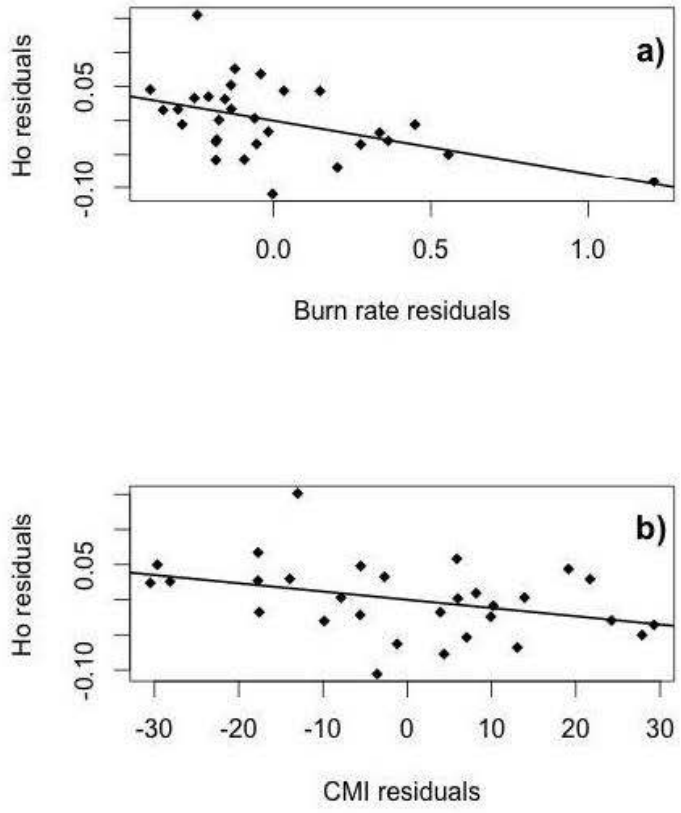

Figure 3.6 Partial regression plots showing the relationships between a) Observed heterozygosity (H_o) residuals and Burn rate residuals, and b) H_o residuals and CMI residuals for 30 populations of *Populus tremuloides* that were located across boreal Canada.

3.6. Discussion

Trembling aspen genetics have been increasingly studied over the last decade (e.g Namroud *et al.* 2005a,b; Ally *et al.* 2008; Mock *et al.* 2008; Callahan *et al.* 2013; Latutrie *et al.* 2015), yet no studies have evaluated variation in genetic diversity and clonal structure at a continental scale within the Canadian boreal forest. Our hypothesis was that climatic conditions (aridity), episodic large-scale disturbances (forest fire), and landscape fragmentation would influence aspen population clonal

and genetic structures. This study demonstrated no significant differences in genetic diversity (H_o, H_e, N_a) and clonal structure among the three classes of CMI that were tested (Moist, Intermediate and Dry conditions). However, our data showed that natural disturbances such as the burn rate, CMI and landscape fragmentation did influence observed heterozygosity (H_o), the average number of alleles per locus (N_a), and the proportion of triploidy (Trip). The genetic diversity (N_a, AR) of *P. tremuloides* varied across the continent, as did the groups of clonal organization. Stands where every clone was unique were likely to be sexually recruited and were found in the eastern part of the continent (Québec and Ontario), while stands with many small and few large clones were encountered throughout the continent. Our results showed that aspen clonal structure was similar regardless of the environmental conditions (climate, fire, fragmentation) that were encountered. Thus, in plant species that have a mixed mode of reproduction, occasional sexual events are sufficient to introduce new genotypic variation into a population, thereby maintaining a high level of genotypic diversity (Balloux *et al.* 2003; Bengtsson 2003).

3.6.1. Genetic diversity

Observed levels of genetic polymorphism within the set of 7 loci were as found by Latutrie *et al.* (2015). N_a and AR were lower in stands where few large clones were present. This contrasts sharply with an expected increase in the number of alleles per locus with increasing clonality (Balloux *et al.* 2003). Mean H_o (= 0.64) and H_e (= 0.63) were high for most sites, reflecting high levels of genetic diversity (Wyman *et al.* 2003; Namroud *et al.* 2005b). These values were comparable to what Callahan *et al.* (2013) found in their sites sampled in boreal forest (H_o = 0.773 and H_e = 0.779, respectively). The proportion of triploidy was similar among clonal organization groups. Mock *et al.* (2012) suggested that clonality might facilitate the persistence of triploids in the landscape, and that triploidy may be necessary to maintain large and long-lived clones. These authors (Mock *et al.* 2012) further showed that the

proportion of triploids was negligible in the eastern portion of the Canadian boreal forest and low (0 to 0.17) in their western Canadian sites, compared to what was observed in the southwestern USA (0.04 to 0.69). Finally, our results did not concur with those of Mock *et al.* (2012) who reported an increasing proportion of triploidy with drier climatic conditions (lower ombrothermic index).

3.6.2. Clonal structure

The clonal diversity (R_o) and the percentage of single ramet clones (SRC) were high across the continent. These results were consistent with those of Namroud *et al.* (2005a), who observed more than 75% SRC as well as high clonal diversity in aspen stands in Québec. This response suggested that: 1) sexual reproduction events had occurred in all sites, most probably after large-scale disturbances, *i.e.*, clear-cutting, fire and dieback, together with suckering if aspen was already present on the site; 2) the presence of thinning in ramet intra-clones (genets) with time-since-fire. Seedling establishment is possible following a fire with complete removal of the canopy. Yet, suckers usually outcompete seedlings when aspen is already present on a site. Seedling establishment could occur following secondary disturbances (*i.e.*, insect, wind, drought) but ecological conditions within canopy gaps (soil temperature, moisture, light) could not be optimal for seed germination and survival. Following secondary disturbance, suckering is stimulated by reduced hormonal suppression that is incurred by apical dominance, after which the production of new stems from root system starts (Mitton & Grant 1996). The continuous suckering in aspen stands that have not experienced disturbance (Bergeron & Charron 1994; Kneeshaw & Bergeron 1996) could also contribute to the maintenance of high genotypic diversity. One hypothesis is that the extensive root interconnectivity among aspen trees (between- and within-clones) could influence the recovery of genotypes that were dead for several years and preserved in the ground (Jelinkova *et al.* 2009). When aspen stems are eliminated from a stand through the self-thinning process, their roots can survive

as a part of a communal root system. This communal root system is important both for the survival of aspen clones (genotypes) and for maintaining the aspen component of many ecosystems (Jelinkova *et al.* 2009). This could explain why multi-ramets clones (MRC)) were observed in most sites together with single ramet clones. This pattern is similar to what Mock *et al.* (2008) observed in the Pando clone (south-central Utah) where small distinct genets were found within the larger clone.

Our results showed that stand density alone explained the average distance between ramets of MRC. The mean (8.8 to 31.9 m) and maximum (9.9 to 55.9 m) distances that were observed between ramets of MRC had the same range of variability between regions, as reported by Namroud *et al.* (2005a). In contrast, Mock *et al.* (2008) mapped several clones (including the Pando clone in Utah) and found that ramets were spaced as far as hundreds of metres apart. This later result suggests that the distances between ramets of MRC might depend upon the size of the sampling plot.

3.6.3. Effect of climate, fire regimes and landscape fragmentation on aspen genetic diversity and clonal structure

For all other clonal structure parameters, no effect was detected for any explanatory variables that had been tested (CMI, temperature, burn rate, fragmentation, age and density). Namroud *et al.* (2005b) have suggested that natural disturbance type (large vs gap disturbance) does not allow predictions to be made regarding aspen genetic and clonal structure; rather, it mainly influences regeneration processes (*e.g.,* density). Some documented disturbances, such as river flow or time-since-fire, influence the proportions of sexual and asexual recruitment and clonal structure in terrestrial populations (Douhovnikoff *et al.* 2005; Namroud *et al.* 2006). Clonal organization in our study was linked neither to climatic conditions nor to fire regimes. Consequently, no significant difference in terms of clonal diversity was observed with varying climatic conditions (*i.e.,* CMI, precipitation, temperature) or landscape

fragmentation. As previously mentioned, this response could be related to different mechanisms of aspen regeneration dynamics, including occasional sexual reproduction events, intra-clone thinning, or substantial root interconnectivity for genetically diverse units. It is also plausible that climatic conditions under study did not offer sufficient contrasts among sites to generate a specific clonal structure. Further, fire regimes (measured as the burn rate) did not explain variation in clonal diversity and do not support the results of Namroud *et al.* (2006), who suggested that ramet mortality within genets could account for the increase in aspen clonal diversity with time-since-fire.

Genetic diversity (H_o, N_a, Trip) varied among sites and was partly explained by the surrounding conditions. Observed heterozygosity (H_o) weakly increased with low burn rates and moist conditions (low CMI), showing a link with low disturbance environments. This partly supports Turner *et al.* (2003), who suggested that episodic large-scale disturbances may play a key role in the population structure, genetics, and evolution of long-lived clonal plants. In contrast to Balloux *et al.* (2003), we did not find any relationship between the presence of large clones and H_o or N_a. The average number of alleles per locus (N_a) must be interpreted cautiously since this measure is sample size-dependent (i.e. N_a increases with sample size; Leberg 2002). The average number of alleles per locus (N_a) and the proportion of triploidy (Trip) were lower and higher, respectively, with increasing landscape fragmentation. Reduction in N_a due to a fragmented landscape could be the consequence of lower gene flow between populations. However, aspen reproductive traits (wind pollination, outbreeding) should buffer tree populations against diversity loss resulting from fragmentation. It has been shown that black cottonwood (*Populus trichocarpa* Torr. & A.Gray) is capable of extensive gene flow between individuals located in populations that are kilometres apart (DiFazio *et al.* 2004). For *P. tremuloides*, seeds were found up to 15 km away from the nearest known clone in Yellowstone National Park, Wyoming (Turner *et al.* 2003). Finally, clonality may facilitate the persistence of triploids

(Mock *et al.* 2012), but we did not find any relationship between clonal structure and the fragmentation that would support this.

3.7. Conclusion

For the first time in a single study, aspen genetic diversity and clonal structure were characterized within the Canadian boreal forest at a continental scale. We anticipated that large monoclonal, highly heterozygote aspen stands would dominate the landscape in the western boreal forest and aspen parkland, while multi-clonal aspen stands would dominate in the eastern boreal forest. Contrary to these expectations, no difference in aspen clonal structure was observed between regions, fire regimes or landscape fragmentation. A total of 28 out of 30 sites were composed of multi-ramet clones. This illustrates that multi-clonal structure in aspen can be maintained within the landscape for several generations in a changing environment. Vegetative propagation appeared to dominate most sites, but sexual reproduction apparently occurred in all sites, even under drier conditions. Only landscape fragmentation and higher burn rate (short fire cycle) negatively influenced levels of genetic diversity (N_a and H_o). Aspen stands across Canada showed similar genetic structure, with no major climatic trends being observed. This species is a generalist with a great capacity for adaptation and the potential for maintaining high levels of genetic and clonal diversity in diverse, heterogeneous environments.

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3.9. References

Ally D, Ritland K, Otto SP (2008) Can clone size serve as a proxy for clone age? An exploration using microsatellite divergence in *Populus tremuloides*. *Molecular Ecology,* **17**, 4897-4911.

Ardehed A, Johansson D, Schagerström E, *et al.* (2015) Complex spatial clonal structure in the macroalgae Fucus radicans with both sexual and asexual recruitment. *Ecology and Evolution,* **5**, 4233-4245.

Arnaud-Haond S, Duarte CM, Alberto F, Serrão EA (2007) Standardizing methods to address clonality in population studies. *Molecular Ecology* **16**, 5115-5139.

Baddeley AJ, Turner R (2005) Spatstat: An R package for analyzing spatial point pattens. *Journal of Statistical Software,* **12**, 1-42.

Balloux F, Lehmann L, de Meeûs T (2003) The population genetics of clonal and partially clonal diploids. *Genetics,* **164**, 1635-1644.

Bengtsson BO (2003) Genetic variation in organisms with sexual and asexual reproduction. *Journal of Evolutionary Biology,* **16**, 189-199.

Bergeron Y (2000) Species and stand dynamics in the mixed woods of Quebec's southern boreal forest. *Ecolog,y* **81**, 1500-1516.

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

Bergeron Y, Chen HYH, Kenkel NC, Leduc AL, Macdonald SE (2014) Boreal mixedwood stand dynamics: Ecological processes underlying multiple pathways. *The Forestry Chronicle,* **90**, 202-213.

Bergeron Y, Cyr D, Girardin MP, Carcaillet C (2010) Will climate change drive 21st century burn rates in Canadian boreal forest outside of its natural variability: collating global climate model experiments with sedimentary charcoal data. *International Journal of Wildland Fire,* **19**, 1127-1139.

Bergeron Y, Flannigan M, Gauthier S, Leduc A, Lefort P (2004a) Past, current and future fire frequency in the Canadian boreal forest: implications for sustainable forest management. *Ambio,* **33**, 356-360.

Bergeron Y, Gauthier S, Flannigan M, Kafka V (2004b) Fire regimes at the transition between mixedwood and coniferous boreal forest in northwesternQuebec. *Ecology,* **85**, 1916-1932.

Bergeron Y, Leduc A, Harvey BD, Gauthier S (2002) Natural fire regime: a guide for sustainable management of the Canadian boreal forest. *Silva Fennica,* **36**, 81-95.

Boulanger Y, Gauthier S, Burton PJ, Vaillancourt M-A (2012) An alternative fire regime zonation for Canada. *International Journal of Wildland Fire* **21**, 1052-1064.

Callahan CM, Rowe CA, Ryel RJ, *et al.* (2013) Continental-scale assessment of genetic diversity and population structure in quaking aspen (*Populus tremuloides*). *Journal of Biogeography,* **40**, 1780-1791.

Canadian Forest Service (2010) Canadian National Fire Database – Agency Fire Data. Natural Resources Canada, Canadian Forest Service, Northern Forestry Centre, Edmonton, Alberta.

Ceplitis A (2001) The importance of sexual and asexual reproduction in the recent evolution of *Allium vineale. Evolution,* **55**, 1581-1591.

Cheliak WM, Dancik BP (1982) Genetic diversity of natural populations of a clone-forming tree *Populus tremuloides*. *Canadian Journal of Genetics and Cytology,* **24**, 611-616.

Chung MG, Epperson BK (2000) Clonal and spatial genetic structure in Eurya emarginata (Theaceae). *Heredity* **84**, 170-177.

Cumming SG, Schmiegelow FKA, Burton PJ (2000) Gap dynamics in boreal aspen stands: Is the forest older than we think? *Ecological Applications,* **10**, 744-759.

DeWoody J, Rowe CA, Hipkins VD, Mock KE (2008) "Pando" lives: molecular genetic evidence of a giant aspen clone in central Utah. *Western North American Naturalist,* **68**, 493-497.

DiFazio SP, Slavov GT, Burczyk J, Leonardi S, Strauss SH (2004) 23 Gene flow from tree plantations and implications for transgenic risk assessment (Chapter 23).pp. 406-422. *in* C Walter & M Carson (eds.). Plantation Forest Biotechnology for the 21st Century. Research Signpost, Kerala, India.

Dorken ME, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodonverticillatus* (Lythraceae). *Journal of Ecology,* **89**, 339-350.

Douhovnikoff V, McBride JR, Dodd RS (2005) *Salix exigua* clonal growth and population dynamics in relation to disturbance regime variation. *Ecology* **86**, 446-452.

Eckert C (2002) The loss of sex in clonal plants. pp. 279-298 *in* JF Stuefer, B Erschbamer, H Huber, and JI Suzuki, editors. Ecology and Evolutionary Biology of Clonal Plants. Springer Netherlands.

Environment Canada (2014) *Calculation of the 1971 to 2000 Climate Normals for Canada.*

http://climate.weather.gc.ca/climate_normals/normals_documentation_e.html?docID=1971.

Erickson DL, Hamrick JL (2003) Genetic and clonal diversity for Myrica cerifera along a spatiotemporal island chronosequence. *Heredity,* **90**, 25-32.

Fox J, Weisberg S (2011) An *R* Companion to Applied Regression, 2nd Ed. Thousand Oaks, CA, USA. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

Girardin M-P, Tardif J, Flannigan MD, Wotton BM, Bergeron Y (2004) Trends and periodicities in the Canadian Drought Code and their relationships with atmospheric circulation for the southern Canadian boreal forest. *Canadian Journal of Forest Research,* **34**, 103-119.

Girardin MP, Mudelsee M (2008) Past and future changes in Canadian boreal wildfire activity. *Ecological Applications* **18**, 391-406.

Girardin MP, Wotton BM (2009) Summer moisture and wildfire risks across Canada. *Journal of Applied Meteorology and Climatology,* **48**, 517-533.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www2.unil.ch/popgen/softwares/fstat.htm Updated from Goudet (1995).

Grant MC, Mitton JB, Linhart YB (1992) Even larger organisms. *Nature,* **360**, 216-216.

Hogg EH (1997) Temporal scaling of moisture and the forest-grassland boundary in western Canada. *Agricultural and Forest Meteorology,* **84**, 115-122.

Hogg EH, Bernier PY (2005) Climate change impacts on drought-prone forests in western Canada. *Forestry Chronicle,* **81**, 675-682.

Hogg EH, Brandt JP, Kochtubajda B (2005) Factors affecting interannual variation in growth of western Canadian aspen forests during 1951-2000. *Canadian Journal of Forest Research,* **35**, 610-622.

Hogg EH, Brandt JP, Michaelian M (2008) Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. *Canadian Journal of Forest Research,* **38**, 1373-1384.

Hyun JO, Rajora OP, Zsuffa L (1987) Genetic variation in trembling aspen in Ontario based on isozyme studies. *Canadian Journal of Forest Research,* **17**, 1134-1138.

Jelinkova H, Tremblay F, DesRochers A (2009) Molecular and dendrochronological analysis of natural root grafting in *Populus tremuloides* (Salicaceae). *American Journal of Botany,* **96**, 1500-1505.

Keller SR, Olson MS, Silim S, Schroeder W, Tiffin P (2010) Genomic diversity, population structure, and migration following rapid range expansion in the balsam poplar, *Populus balsamifera. Molecular Ecology,* **19**, 1212-1226.

Kneeshaw DD, Bergeron Y (1996) Ecological factors affecting the abondance of advance regeneration in Quebec's southwestern boreal forest. *Canadian Journal of Forest Research,* **26**, 888-898.

Krezek-Hanes CC, Ahern F, Cantin A, Flannigan MD (2011) Trends in large fires in Canada. Canadian Biodiversity: Ecosystem Status and Trends 2010, Technical Thematic Report No. 6. Canadian Councils of Resource Ministers, p. 48, Ottawa, ON, Canada.

Lafleur B, Cazal A, Leduc A, Bergeron Y (2015) Soil organic layer thickness influences the establishment and growth of trembling aspen (*Populus tremuloides*) in boreal forests. *Forest Ecology and Management,* **347**, 209-216.

Latutrie M, Mérian P, Picq S, Bergeron Y, Tremblay F (2015) The effects of genetic diversity, climate, and defoliation events on trembling aspen growth performance across Canada. *Tree Genetics & Genomes,* **11**, 1-14.

Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology,* **11**, 2445-2449.

Lemprière T, Bernier PY, Carroll AL, *et al.* (2008) The importance of forest sector adaptation to climate change. Natural Resouces Canada, Canadian Forest Service, Northern Forestry Centre, Edmonton, AB, Canada. Information Report NOR-X-416E.

Long JN, Mock K (2012) Changing perspectives on regeneration ecology and genetic diversity in western quaking aspen: implications for silviculture. *Canadian Journal of Forest Research,* **42**, 2011-2021.

Lund ST, Furnier GR, Mohn CA (1992) Isozyme variation in quaking aspen in Minnesota. *Canadian Journal of Forest Research,* **22**, 521-524.

MacKenzie N (2010) Ecology, Conservation and Management of Aspen: A Literature Review. Scottish Native Woods (Aberfeldy). 41 pp.

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes,* **4**, 792-794.

Michaelian M, Hogg EH, Hall RJ, Arsenault E (2011) Massive mortality of aspen following severe drought along the southern edge of the Canadian boreal forest. *Global Change Biology,* **17**, 2084-2094.

Mitton JB, Grant MC (1996) Genetic variation and the natural history of quaking aspen. *BioScience,* **46**, 25-31.

Mock KE, Callahan CM, Islam-Faridi MN, *et al.* (2012) Widespread triploidy in western North American aspen (*Populus tremuloides*). *PLoS One* 7, e48406. doi:48410.41371/journal.pone.0048406.

Mock KE, Rowe CA, Hooten MB, Dewoody J, Hipkins VD (2008) Clonal dynamics in western North American aspen (*Populus tremuloides*). *Molecular Ecology,* **17**, 4827-4844.

Namroud MC, Leduc A, Tremblay F, Bergeron Y (2006) Simulations of clonal species genotypic diversity - trembling aspen (*Populus tremuloides*) as a case study. *Conservation Genetics,* 7, 415-426.

Namroud MC, Park A, Tremblay F, Bergeron Y (2005a) Clonal and spatial genetic structures of aspen (*Populus tremuloides* Michx.). *Molecular Ecology,* **14**, 2969-2980.

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

Natural Resources Canada (2013) 2005 Land Cover of North America at 250 meters. Canada Centre for Remote Sensing (CCRS), Ottawa, ON, Canada.

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics,* **37**, 637-669.

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

R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.R-project.org/, Vienna, Austria.

Régnière J, St-Amant R, Béchard A (2013) BioSIM 10 User's manual. Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec, QC, Canada. Information Report LAU-X-137E. ftp://ftp.cfl.scf.rncan.gc.ca/regniere/software/BioSIM/

Reusch TBH (2006) Does disturbance enhance genotypic diversity in clonal organisms? A field test in the marine angiosperm *Zostera marina*. *Molecular Ecology,* **15**, 277-286.

Stocks BJ, Mason JA, Todd JB, *et al.* (2003) Large forest fires in Canada, 1959-1997. *Journal of Geophysical Research-Atmospheres,* **108**, FFR5, 1-12.

Sun S, Gao X, Cai Y (2001) Variations in sexual and asexual reproduction of *Scirpus mariqueter* along an elevational gradient. *Ecological Research,* **16**, 263-274.

Suvanto LI, Latva-Karjanmaa TB (2005) Clone identification and clonal structure of the European aspen (*Populus tremula*). *Molecular Ecology,* **14**, 2851-2860.

Turner MG, Romme WH, Tinker DB (2003) Surprises and lessons from the 1988 Yellowstone fires. *Frontiers in Ecology and the Environment,* **1**, 351-358.

Verbeke G, Molenberghs G (2009) Linear Mixed Models for Longitudinal Data. Springer.

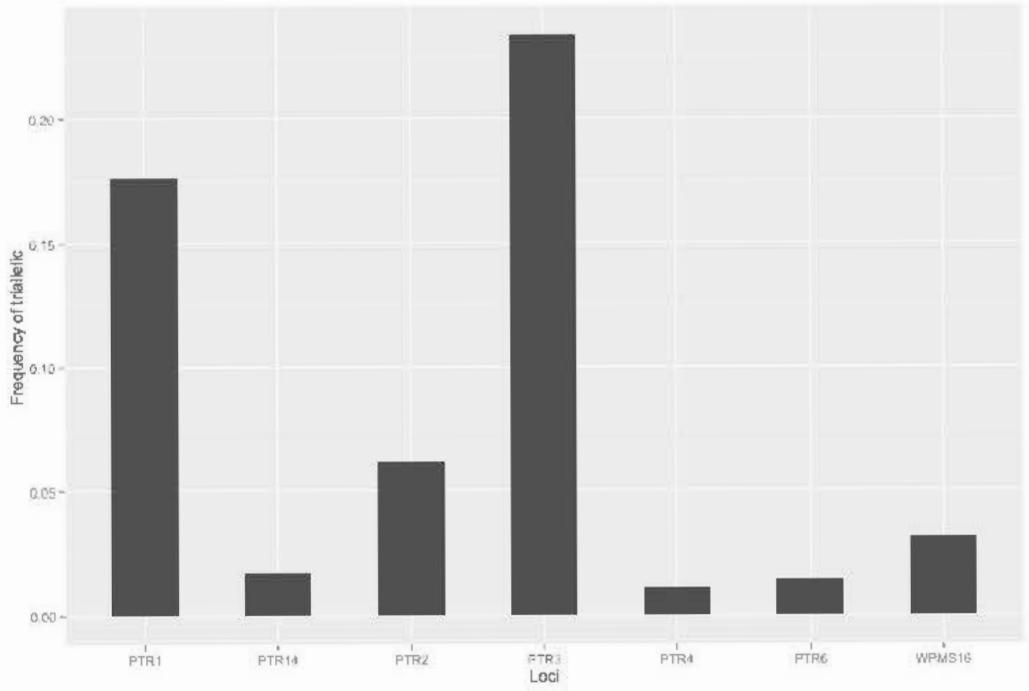
Weppler T, Stoll P, Stöcklin J (2006) The relative importance of sexual and clonal reproduction for population growth in the long - lived alpine plant *Geum reptans*. *Journal of Ecology,* **94**, 869-879.

Wyman J, Bruneau A, Tremblay MF (2003) Microsatellite analysis of genetic diversity in four populations of *Populus tremuloides* in Quebec. *Canadian Journal of Botany,* **81**, 360-367.

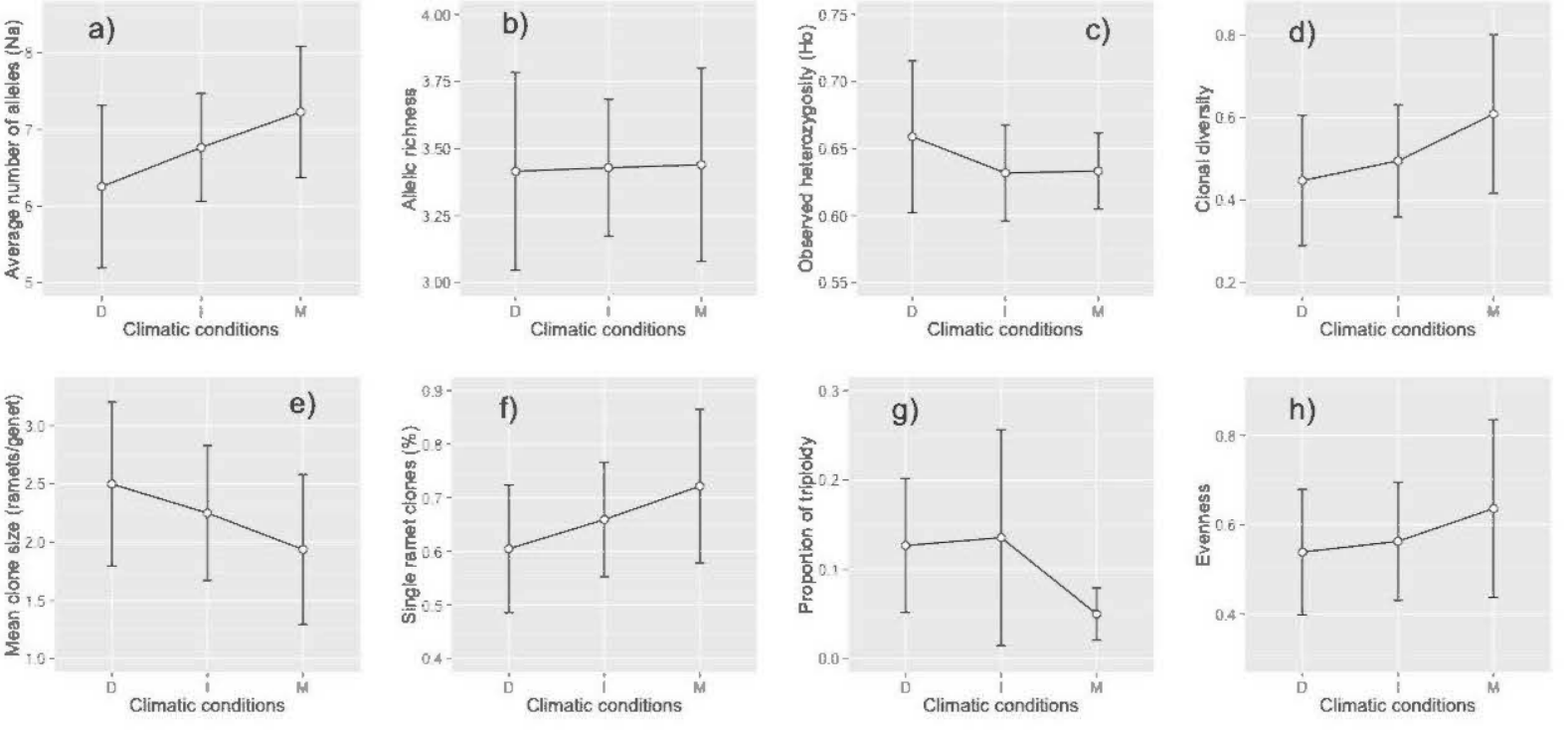
Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed Effects Models and Extensions in Ecology with R. Springer.

3.10. Appendices

Appendix 3.1 Frequencies of triallelic individuals that were observed per locus.



Appendix 3.2 Comparisons of mean site conditions between dry (D), intermediate (I) and moist (M) climatic conditions that were determined by CMI. a) Average number of alleles (N_a; ANOVA, $F_{2,27} = 1.553$, $P = 0.23$); b) Allelic richness (AR: AR: ANOVA, $F_{2,23} = 0.007$, $P = 0.99$); c) Mean observed heterozygosity (H_o; ANOVA, $F_{2,27} = 0.686$, $P = 0.51$); d) Clonal diversity (R; ANOVA, $F_{2,27} = 1.284$, $P = 0.29$); e) Mean clone size (ramets/genet; ANOVA, $F_{2,27} = 0.953$, $P = 0.40$); f) Percentage of single ramet clones (ANOVA, $F_{2,27} = 1.091$, $P = 0.35$); g) Proportion of triploidy (Trip: ANOVA, $F_{2,27} = 0.752$, $P = 0.48$); h) Evenness (ANOVA, $F_{2,27} = 0.502$, $P = 0.61$).



CHAPITRE 4 THE EFFECTS OF GENETIC DIVERSITY, CLIMATE AND DEFOLIATION EVENTS ON TREMBLING ASPEN GROWTH PERFORMANCE ACROSS CANADA

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4.1. Abstract

Tree genetic makeup may provide an important control of growth dynamics, however no studies have previously attempted to evaluate its effects in natural trembling aspen stands. In this study, we examined the relative contribution of genetics (i.e. clonal diversity, observed heterozygosity) and environmental conditions (i.e. insects, climate) on aspen growth as represented by mean inter-tree correlation (RBAR), tree basal area increment (TBAI) and inter-annual growth variability (MS). We sampled 440 trees in 22 even-aged natural stands dominated by aspen along an east-west continental gradient of decreasing annual precipitation in the Canadian boreal forest. Linear and mixed-effects models tested the relationships between tree growth, genetics and environmental factors. We showed that clonal diversity and number of years with forest tent caterpillar (FTC) defoliation (N_{FTC}) reduced and increased the level of growth synchronicity (RBAR), respectively. Clonal diversity explained 30 % of variation in RBAR among sites. TBAI was positively influenced by high moisture conditions while N_{FTC} and climate explained the variation in MS among trees for each site. No genetic effect could explain either TBAI or the MS variation. Climate and N_{FTC} drive annual growth variability in trembling aspen at stand and sub-continental scales. Tree genetic makeup contributed to these dynamics, the annual growth dynamics of multi-clonal stands being less homogeneous than those of monoclonal stands. Maintaining diverse aspen stands may ensure a wider range of growth responses to environmental variability, which in turn may help maintain resilience of aspen stands under future climate.

Keywords: *climate moisture index, forest tent caterpillar, genetic diversity, heterozygosity, radial growth, trembling aspen.*

4.2. Résumé

La composition génétique des arbres pourrait jouer un rôle important dans le contrôle des dynamiques de croissance, cependant aucune étude n'a estimé les effets dans des peuplements naturels de peuplier faux-tremble. Dans a présente étude, nous avons examiné la contribution relative de la génétique (c.-à-d. la diversité clonale, l'hétérozygotie observée) et de l'environnement (c.-à-d. insectes, climat) sur la croissance du peuplier évaluée par la corrélation moyenne de croissance diamétrale entre les arbres (RBAR), l'accroissement en surface terrière des arbres (TBAI) et la variabilité interannuelle de croissance (MS). Nous avons échantillonné 440 arbres dans 22 peuplements naturels équiens dominés par le peuplier le long d'un gradient continental Est-Ouest de diminution des précipitions annuelles en forêt boréale canadienne. Des modèles linéaires et à effets mixtes ont été utilisés pour tester les relations entre la croissance, la génétique et l'environnement. Nous avons montré que la diversité clonale et le nombre d'années de défoliation (N_{FTC}) par la livrée des forêts (FTC), respectivement, réduisait et augmentait la synchronicité de croissance (RBAR). La diversité clonale expliquait 30% de la variation du RBAR entre les sites. Le TBAI était positivement influencé par des conditions d'humidité élevée alors que N_{FTC} et les conditions climatiques expliquaient la variation du MS entre les arbres pour chaque site. Aucun effet de la génétique n'a permis d'expliquer la variation du MS. Le climat et N_{FTC} jouent un rôle dans la variabilité de la réponse de croissance du peuplier faux-tremble à l'échelle du peuplement et du continent. La composition génétique des arbres semble jouer un rôle important dans ces dynamiques, la réponse de la croissance annuelle de peuplement multi-clonaux étant moins homogène que celle de peuplements monoclonaux. Le maintien de peuplements de peupliers diversifiés dans le paysage pourrait assurer d'avoir une large variabilité de la réponse de croissance face aux variations environnementales et permettre une plus forte résilience des peuplements de peuplier dans les conditions climatiques futures.

Keywords: indice climatique d'humidité, livrée des forêts, diversité génétique, hétérozygotie, croissance radiale, peuplier faux-tremble

4.3. Introduction

Trembling aspen (*Populus tremuloides* Michaux) is the most widely distributed and abundant deciduous tree species in North America (Little 1971), particularly in Canadian boreal forests, where it covers 11.6% of the national forested land base (Peterson and Peterson 1992). Over the last two decades, widespread aspen decline and mortality has been observed mainly in western North America and, to a lesser extent, in eastern Canada. These observations have led to a growing interest in understanding the causes and underlying mechanisms of aspen growth variation (e.g. Frey et al. 2004; Hogg et al. 2008; Michaelian et al. 2011).

Species-specific responses to short-term environmental variation suggest that tree growth responses to climatic fluctuations would not likely be synchronized among species in a mixedwood boreal forest that was composed of aspen and spruce (Drobyshev et al. 2013). This could thus lead to changes in the structure and composition of future forest communities (Drobyshev et al. 2013). Tree-ring width analysis is a powerful proxy for estimating tree growth (Fritts 1976), and has been successfully used in trembling aspen to evaluate stand productivity (Hogg et al. 2008) and climate-growth relationships (Hogg 2001; Hogg and Bernier 2005; Leonelli et al. 2008). Variation in inter-annual aspen growth is especially sensitive to the effects of defoliation by the forest tent caterpillar (*Malacosoma disstria* Hübner; FTC), soil moisture availability (as measured using a climatic moisture index, CMI), accumulated growing degree-days, and snow depth (Hogg et al. 2002a). However, the genetic composition of populations under investigation is not explicitly considered in most studies, and the observed changes are assumed to be driven primarily by physiological responses to climatic change, without a significant genetic contribution (King et al. 2013).

In a clonal species such as trembling aspen one single genotype (also referred to as "clone") can cover vast areas (e.g. the 43 ha Pando clone in south-central Utah; Grant et al. 1992; DeWoody et al. 2008) that could be affected by sudden declines following severe environmental variations, such as drought (e.g. Worrall et al. 2013), due to maladaptation of certain genotypes to the new conditions. It is equally possible that either no response or a positive response would be observed for some genotypes that are better adapted to the changing conditions. The growth performance of a population could thus be, on average, similar over time if populations consist of multiple clones with different climatic tolerances. Most of the variation would then be observed between genotypes within populations.

Mitton and Grant (1996) hypothesized that regional differences in term of genetic and clonal structures in aspen populations reflect the aridity of the climate, the propensity for clonal reproduction, or the predominant mode of reproduction (sexual or asexual). Within its range, clonal diversity is generally assumed to decrease from West to East, while population heterozygosity would tend to increase. However, few studies have examined this source of variation at a continental scale (Callahan et al. 2013). Recent work has shown that regional differences in genetic variation among populations across aspen's distributional range are less important than was previously thought due to recent sexual reproduction events (Mock et al. 2008; Long and Mock 2012). However, at regional and local scales, Mitton and Grant (1984) made the hypothesis that higher individual heterozygosity could provide greater flexibility in their growth responses to environmental variation, resulting in higher average aspen radial growth (Jelinski and Cheliak 1992; Cole et al. 2010). An average 20% of tree growth variation (a value between 10% to 40%, depending upon the species) is under genetic control (Cornelius 1994; Beaulieu and Bousquet 2010). Geographic variation between aspen provenances that were tested in common garden experiments has been reported (Gray et al. 2010; Li et al. 2010; Schreiber et al. 2013), showing that growth is partly under genetic control. Broad-sense heritability (h_2: proportion of phenotypic

variance that is subject to genetic control) for height and diameter at breast height (DBH at 1.3m) respectively averaged 0.45 and 0.43 for aspen clones in common garden trials (Gylander et al. 2012).

In most studies, intra-population genetic structure has not been considered and was not expected to affect stand growth responses. In this study, our specific objective was to investigate whether the level of genetic variation in natural aspen stands is associated with differential growth responses to environmental conditions, viz, severe forest tent caterpillar (FTC) defoliation and water stress (estimated by the average climate moisture index (CMI)). Tree-growth was analysed using a dendrochonological approach (Fritts 1976) to quantify tree basal area increment (TBAI), the mean sensitivity of growth (MS), and the mean inter-tree correlation (RBAR). TBAI is the mean annual basal area increment calculated for each tree sampled and measures wood production in volume. MS quantifies the inter-annual variation in tree-ring widths (Fritts 1976), while RBAR is a measure of the strength of the common variation in radial growth between all possible pairs in tree-ring width chronologies (Cook and Pederson 2010). Higher values of MS are associated with high tree-growth sensitivity to inter-annual climatic variations, whereas variation in RBAR illustrates changes in the strength of common patterns of tree growth over time. More specifically we hypothesized that: i) higher aspen TBAI is correlated with higher genetic diversity of aspen stands; ii) and both growth synchronicity (RBAR) among trees within a site and year-to-year growth variability (MS) are negatively correlated with genetic diversity of aspen stands.

4.4. Materials and Methods

4.4.1. Study area and sampling

The study sites consisted of 22 plots, each measuring 400 m^2 (11.28 m radius), which had been established in dominant (> 75 % basal area), even-aged and mature (50-

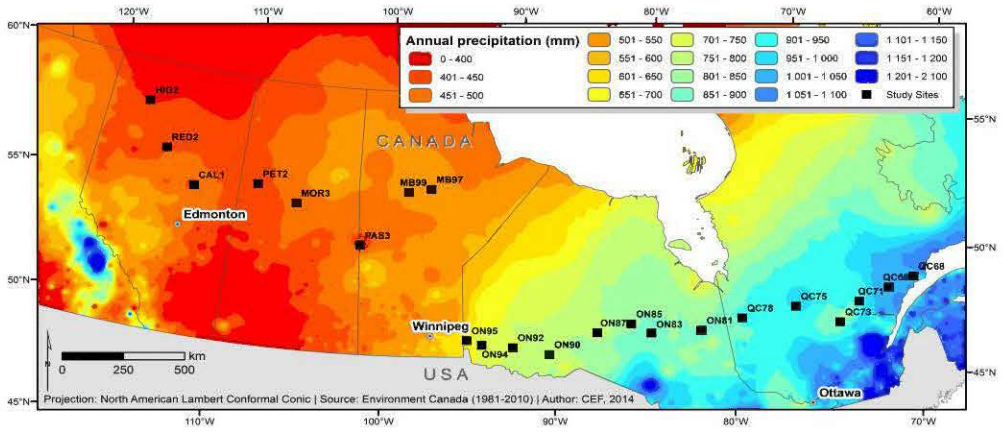

Figure 4.1 Study sites along the east-west gradient of decreasing annual precipitation (Environment Canada 2014) where 22 *Populus tremuloides* populations were sampled.

years-old) trembling aspen stands across Canada (48°33'36'' N to 58°20'01'' N; 67°59'07'' W to 117°14'13'' W; Figure 4.1). The 22 plot locations coincided with the 0 ± 1 °C isothermal envelope over the period 1971-2000 (Environment Canada 2014), except for two sites that were 2 °C above this value (ON95 and ON94) and two sites that were 2 °C below this value (MB97 and MB99). This pan-Canadian transect represents an East-West gradient of decreasing annual precipitation (from

1000 to 400 mm/year; Figure 4.1), which allowed a wide range of environmental conditions and genetic variability to be covered. The general topography was gently rolling, with elevations ranging from 195 to 675 m asl (Table 4.1). Soil conditions were selected to be as similar as possible (mesic clayey soils) to reduce variation between sites. Site index was obtained from stem analysis of three dominant trees (suppression-free trees with a normally formed canopy) per plot that were sampled in 2010 and 2011, as explained in Hanson et al. (2003). Cookies were taken every metre from the base to the top of each tree, and ring-widths were measured. We obtained age-height curves for these trees and computed a site index (SI) based on the average of the mean height at age 50 years in a given plot. Within each plot, the DBH and the height of every mature tree (DBH ≥ 10 cm) were measured. Among these individuals,

20 trees were selected per plot. Each tree was tagged, mapped and sampled (DNA from root cambium tissue and increment cores) for analysis. All analyses were performed based on ring-widths over the period between 1959-2009 (50 years).

4.4.2. Tree-ring width measurement and crossdating

Tree increment cores were mounted on wood and progressively polished with up to 600-grit sandpaper to permit clear recognition and differentiation of annual rings under the microscope (magnification 20x). Annual ring-widths were measured with + 0.01 mm accuracy using a LinTab measuring stage (RINNTECH Inc., St. Charles, IL, USA) and TSAP 3.0 software (Rinn 1996). For each plot, the individual ring-width series were visually compared during ongoing measurements to avoid any evident shifts between chronologies. The chronologies were then carefully cross-dated by progressively detecting regional pointer years using the method developed by Becker (1989). Methods and thresholds that were used to define pointer years were performed according to Lebourgeois et al. (2013). Pointer years were calculated in the R statistical environment (version 2.15.0, R Development Core Team 2013) using the function *pointer* in the package *dplR* (Bunn 2008). Absolute dating was checked with the application INTERDAT (Bunn 2008), which identifies locations within each ring series that may be subject to erroneous cross-dating (Becker 1989; Mérian and Lebourgeois 2011).

4.4.2.1. Tree basal area increment calculation

Tree basal area increment (TBAI; mm^2) was preferred to ring width because the former provides a better estimate of annual growth (Valentine 1985; LeBlanc 1990; Briffa et al. 1998; Yang et al. 2009). We calculated the TBAI at breast-height, following the equation $TBAI = \pi (R_t^2 - R_{t-1}^2)$, where R_t is the stem radius at the end of the annual increment, and R_{t-1} is the stem radius at the beginning of the annual increment. For cores with missing pith, the first radius R corresponding to the

distance between pith and the first recorded ring was estimated with the pith locator method (Applequist 1958). In some cases (107 cores, i.e., 12% of total cores), this method could not be used accurately due to a broken core or branch in the pith and thus, the TBAIs were calculated backwards with the radius of the last year of growth (2010; see Appendix 4.1). Eventually, the TBAI series were averaged to create a single basal area increment series for each site (40 cores for 20 trees) and the mean TBAI for the 1959-2009 period was calculated for each tree and then used in the later analysis. Due to incomplete core series, we were not able to calculate the TBAI for 23 trees over all sites. A final data set of 417 trees with complete TBAI values was then used to analyse relationships with selected variables

4.4.2.2. Inter-annual growth variability and growth synchronicity calculations

The raw tree-ring chronologies were computed and detrended to remove medium- and low-frequency signals, thereby emphasising inter-annual variation. The maximum period that was common to all chronologies within a dataset was used (1959–2009). Each series was detrended by fitting a cubic smoothing spline with 50 % frequency response cut-off and a rigidity of 33 % of series length (Cook and Peters 1981; Bunn 2008; Mérian 2012). For each tree (2 series per tree) and then each plot (20 trees per plot), the growth indices were averaged by year using a bi-weighted robust mean to develop a mean growth chronology, which represented the common high-frequency variation of the individual series (Cook 1985). To evaluate the effect of climate, insects and genetic diversity on inter-annual growth variability and inter-tree growth synchronicity across sites, the mean sensitivity of growth indices (MS; Briffa and Jones 1990; Cook and Pederson 2010) and the mean inter-tree correlation (RBAR; Cook and Pederson 2010) were respectively calculated on the growth indices. Mean sensitivity of growth indices (MS) is a measure of the relative change in ring-width index from one year to the next and reflects the proportion of high frequency variance (short period) in the chronology (Cook and Kariukstis 1990). MS

was calculated for each tree to measure year-to-year variability (Fritts 1976) as follows:

$$MS = \frac{1}{n-1}\sum_{i=2}^{n}\left|\frac{2(x_i - x_{i-1})}{(x_i - x_{i-1})}\right|$$

where x_i is the arithmetic mean for tree-ring series of length n. Bunn et al. (2013) showed that MS is nearly proportional to the standard deviation of a time series when using random data sets and we used this definition of MS in our study (standard deviation of detrended ring-widths). The mean inter-tree correlation (RBAR) is the arithmetic mean of the correlation coefficient $r_{jj'}$ between each possible pair of chronologies *j* and *j'* over the t trees calculated within each plot as follows:

$$RBAR = \frac{1}{2t(t-1)}\left(\sum_{j=1}^{t=1}\sum_{\substack{j'=1 \\ j'>j}}^{t} r_{jj'}\right)$$

This metric varies from 0 when series are strictly independent of one another, to 1 where the trees show identical growth patterns (Briffa and Jones 1990; Cook and Pederson 2010). RBAR also measures the proximity between the theoretical population chronology and the observed mean growth chronology (Wigley et al. 1984).

Table 4.1 Tree ring analysis statistics based on raw ring-widths per population.

Site	Longitude	Latitude	Elevation (m)	Slope (%)	Site index	Age	Density (t/ha)	P (cm)	CMI (cm)	P summer (cm)	Severe defoliation§ (years)	Mean DBH (cm)	Mean Height (m)	Basal area (m^2/ha)	MRW*§ (mm)	TBAI*§ (mm^2)	MS**§	RBAR**§
HIG2	-117.24	58.34	343	8.7	19.9	67	1225	40.89	-1.74	13.34	5	19.79	21.585	37.649	1.051	3.995	0.373	0.722
RED2	-115.31	56.61	521	12.1	24.6	57	1350	43.46	0.53	15.8	1	22.5	22.195	38.197	1.918	7.4	0.194	0.481
CAL1	-112.97	55.29	675	0.6	19.7	64	1925	47.74	6.68	17.14	6	18.135	19.645	36.343	1.363	3.861	0.332	0.676
PET2	-108.78	55.74	490	1.1	20.8	61	1100	45.52	8.06	14.54	13	20.79	23.11	36.972	1.462	5.45	0.476	0.646
MOR3	-106.07	55.14	444	10.8	13.4	72	950	47.22	7.39	15.75	10	21.75	21.485	29.725	1.078	4.775	0.375	0.666
PAS3	-101.68	53.6	279	1.6	17.9	65	1125	43.68	0.72	14.52	7	19.69	19.675	34.021	1.208	4.29	0.309	0.608
MB99	-98.68	55.89	279	3.8	14.8	78	1200	50.62	16.12	15.76	9	19.09	19.16	31.807	0.976	3.968	0.406	0.677
MB97	-97.16	56.03	233	2.5	12.9	80	1450	50.24	15.51	15.45	6	18.97	17.525	32.632	0.975	3.711	0.313	0.638
ON95	-94.94	49.7	358	7	20.8	56	900	63.40	17.47	22.91	6	23.64	21.035	36.596	1.798	6.609	0.286	0.654
ON94	-94.06	49.49	385	8.5	17.3	79	1200	68.89	24.28	24.87	9	25.96	22.215	40.786	1.151	6.243	0.405	0.735
ON92	-92.22	49.36	434	10	14.8	84	525	72.33	27.79	25.46	10	27.075	24.16	30.139	1.284	7.188	0.364	0.622
ON90	-90.08	48.99	496	5	18.1	83	925	76.18	28.79	25.89	5	23.54	23.44	32.029	1.126	5.461	0.196	0.442
ON87	-87.14	49.75	364	2.8	21.1	75	900	75.40	33.26	23.73	3	27.885	25.62	36.571	1.352	8.013	0.251	0.493
ON85	-85.06	49.97	195	7.8	15.8	93	1250	78.48	39.81	24.48	6	27.49	25.36	54.511	1.036	6.455	0.288	0.629
ON83	-83.94	49.49	238	3	19.2	69	875	84.80	41.84	25.34	9	31.185	23.795	44.701	1.734	10.035	0.341	0.701
ON81	-81.03	49.31	248	1.1	21	75	450	85.13	42.81	25.66	7	30.64	26.4	33.488	1.612	9.709	0.235	0.422
QC78	-78.52	49.53	306	1.1	13	88	825	87.40	47.30	26.28	4	24.995	23.521	39.298	1.054	5.928	0.198	0.341
QC75	-75.25	49.57	376	5	20.4	86	1050	97.95	60.50	28.27	5	30.645	24.56	55.929	1.009	6.712	0.21	0.453
QC73	-72.97	48.5	526	11.2	21.6	69	1550	87.38	49.08	26.35	5	21.2	21.585	45.542	1.233	5.055	0.253	0.468
QC71	-71.54	49.15	304	30.5	21.3	61	1525	94.97	52.23	27.14	4	21.11	22.585	41.281	1.465	5.993	0.232	0.652
QC69	-69.6	49.39	286	19.5	21.1	61	775	93.86	48.65	28.64	6	28.84	25.18	47.653	2.053	10.622	0.234	0.662
QC68	-67.99	49.54	337	31	20.4	53	1425	99.95	62.99	30.68	4	19.225	18.925	33.137	1.649	5.064	0.236	0.482

* Calculated from raw data; ** Calculated from detrended data; § Calculated for the past 50 years (1959-2009); P, precipitation; CMI, climate moisture index; DBH, diameter at breast height; MRW, mean ring-width; TBAI, tree basal areal increment; RBAR, mean inter-tree correlation.

4.4.3. Environmental data

4.4.3.1. Climatic data

The Climate Moisture Index (CMI) was selected as the climatic variable to estimate water stress (Hogg 1997; Hogg and Bernier 2005; Hogg et al. 2005; Lemprière et al. 2008; Michaelian et al. 2011). CMI was previously calculated from mean monthly precipitation (mm) and potential evapotranspiration (PET; mm) that were simulated for all sites using BIOSIM 10 (Régnière et al. 2013), based on the daily database for Canada covering the years 1901 to 2011. The CMI is the difference between precipitation and PET and is calculated for the period between August and the following July to take into account possible winter water deficits that could affect the next year's growth (Hogg 1997; Hogg et al. 2005; Hogg et al. 2008).

4.4.3.2. Forest Tent Caterpillar defoliation

Forest tent caterpillar defoliation causes the non-systematic presence of "white tree ring" or "missing ring" values, which are indicative of a severe FTC defoliation causing a reduction in growth and an increase in tree mortality (Hogg et al. 2002b; Moulinier et al. 2014). White tree rings are anomalously pale-coloured, low-density tree rings that are formed during severe early season defoliation in both young and mature aspen (Hogg et al. 2002b; Hogg et al. 2005). Years in which "white tree rings" occurred were recorded for each core and on three stems per site during stem analysis. For each plot and year during the 1959–2009 period, years with severe defoliation were calculated as described in the Appendix 4.2. Cumulative number of years with severe FTC defoliations (N_{FTC}) over the past fifty years (1959-2009) was then used as an explanatory variable in model building.

Table 4.2 Primer sequences, size range (in base pairs; bp) and number of alleles observed for 7 microsatellite loci of *Populus tremuloides*.

Locus	Repeat	Primer sequence (5'→3')	Dye colour	Size range (bp)	Number of alleles	Source
	(GGT)n	AGCGCGTGCGGATTGCCATT (F)	FAM			Dayanandan
PTR1	(AGG)n	TTAGTTTCCCGTCACCTCCTGTTAT (R)	(blue)	239-278	12	et al., 1998
		AAGAAGAACTCGAAGATGAAGAACT (F)	VIC			Dayanandan
PTR2	(TGG)n	ACTGACAAAACCCCTAATCTAACAA (R)	(green)	202-229	10	et al., 1998
		CACTCGTGTTGTCCTTTTCTTTTCT (F)	NED			Dayanandan
PTR3	(TC)n	AGGATCCCTTCCCTTTAGTAT (R)	(black)	186-260	19	et al., 1998
		AATGTCGAGGCCTTTCTAAATGTCT (F)	PET			Dayanandan
PTR4	(TC)n	GCTTGAGCAACAAACACACCAGATG (R)	(red)	200-234	11	et al., 1998
		AGAAAAGCAGATTGAGAAAAGAC (F)	VIC			Rahman et
PTR6	(AT)n	CTAGTATAGAGAAAGAAGAAGCAGAAA (R)	(green)	184-211	9	al., 2000
		TCCGTTTTTGCATCTCAAGAATCAC (F)	NED			Rahman et
PTR14	(TGG)n	ATACTCGCTTTATAACACCATTGTC (R)	(black)	140-200	14	al., 2000
	(GTC)n	CTCGTACTATTTCCGATGATGACC (F)	FAM			Smulders et
WPMS16	(ATCCTC)n	AGATTATTAGGTGGGCCAAGGACT (R)	(blue)	148-196	9	al., 2001

4.4.4. Clonal structure and genetic diversity

Root cambium tissue was collected, dried in silica gel and conserved at room temperature. DNA was extracted using Extract-N-AmpTM Plant kit (Sigma-Aldrich, St Louis, MO, USA). Individuals were genotyped at seven microsatellite loci (Table 4.2). Each PCR was performed upon a 10 µL total volume: 2 µL DNA extract, which was diluted to one-tenth; 5 µL QIAGEN® Multiplex PCR Kit (Qiagen, Venlo, Limburg, The Netherlands); and 1 µL H2O and 2 µL primer mix solution at 2 µM, for a final concentration of 0.4 µM. PCR was carried out separately for each primer. Reactions were performed in a Mastercycler® Pro Thermal Cycler (Eppendorf, Hamburg, Germany) with the following protocol: an initial denaturation step at 95 °C for 15 min, followed by 36 cycles of 94 °C for 30 s; primer specific annealing temperature for 90 s; 72 °C for 60 s; and a final extension at 60 °C for 30 min. PCR products were analysed on an ABI 3730 Automated Capillary DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Allele sizes were scored using GENEMAPPER version 5.0 (Applied Biosystems) and only 9 samples were not genotyped at all 7 loci. We used GENODIVE (Meirmans and Van Tienderen 2004) to

assign clone identities based on the stepwise mutation model. The ramet data set included all of the trees (440 sampled trees), while the genet set included the unique multi-locus genotype (118 unique genets). Ramet data were used to estimate clonal heterogeneity (Dorken and Eckert 2001), which was calculated as the Simpson diversity index (D) and evenness (E). Clonal diversity (R) was also estimated, which is the proportion of different genotypes in a population (Arnaud-Haond et al. 2007). Genet data were used to evaluate genetic diversity: observed heterozygosity (H_o); observed heterozygosity per individual (individual H_o), expected heterozygosity (H_e), average allelic richness (average number of alleles observed per loci: A), and the inbreeding coefficient G_{is}, (an analogue of F_{is}). All of these measures were estimated with the software GENODIVE (Meirmans and Van Tienderen 2004), except individual H_o, which was calculated as the sum of loci with more than one allele divided by the total number of loci that were used.

4.4.5. Statistical analysis

4.4.5.1. Annual growth and inter-tree growth variability

Mixed effects modelling was used to analyse the fixed effects of the genetics (H_o or individual H_o, A and D), the environment (moisture index CMI and N_{FTC}) and tree ages on TBAI and MS, while including random effects that were due to the experimental design (CloneID nested in SiteID). The random effects only consisted of the (random) intercepts. Nesting of CloneID within SiteID allowed us to take into account that each clone within a population responds differently to the explanatory variables for a given response variable. We considered a set of 10 candidate models to explain the variation in TBAI and MS (see results tables for the models list). The same set of models was tested with either H_o or individual H_o. Clonal diversity (R) was not included since R and D are highly correlated measures of clonal richness (Pearson product-moment correlation: $r = 0.86$, $P < 0.01$). Assumptions of homogeneity of variance and normality were verified graphically. TBAI and MS

were transformed with a log and a square root function, respectively, to meet normality and constant variance assumptions. Parameters in the candidate models were estimated by maximum likelihood with the *lme* function from the *nmle* package in R (Pinheiro et al. 2013; R Development Core Team 2013). We compared the models using the Akaike Information Criterion corrected for small sample sizes (AICc; Burnham and Anderson 2004) with the *aictab.lme* function from the AICcmodavg package (Mazerolle 2013). When the top-ranked model had an Akaike weight (ωi) < 0.9, we computed the model-averaged estimates of the explanatory variables and their 95% confidence intervals (Burnham and Anderson 2004) with the *modavg.lme* and *modavgpred* function of the AICcmodavg library (Mazerolle 2013). A confidence interval excluding 0 indicated that the response variable varied with the explanatory variable of interest. Multimodel predictions and figures were obtained with the *modavg* function of the *AICcmodavg* library (Mazerolle 2013).

4.4.5.2. Mean inter-tree correlation (RBAR)

Lastly, we carried out multiple linear regressions with the function *lm* to evaluate the relationship between RBAR and a set of 5 explanatory variables (CMI, FTC defoliation, R, A and H_0), which were combined in a set of 10 different models (see results table for the models list). A maximum of 3 different explanatory variables were included in each models to maintain high statistical power (a large number of parameters decrease precision of estimates; Kullback and Leibler 1951; Verbeke and Molenberghs 2009; Zuur et al. 2009). The variable D was not included because it was strongly correlated with R. We tested the set of 10 models with different combinations of the explanatory variables. We then compared the models using the Akaike information criterion with the functions described above. The model assumptions (homogeneity of variance and normality) were verified and partial regression plots were computed with the function avPlots of the car package (Fox and Weisberg 2011).

4.5. Results

4.5.1. Descriptive tree ring analysis

The variables of interest per site are presented in Table 4.1. Average tree basal area increment (TBAI) was 6.206 mm^2/year. The minimum TBAI per site was 3.711 mm^2/year, while the maximum was 10.622 mm^2/year. With respect to mean sensitivity (MS), its average value for all sites was 0.296; MS ranged from 0.21 to 0.658 for trees. We found higher values of MS (ANOVA, $F_{1,438} = 130.8$, $P < 0.001$) and lower values of TBAI (ANOVA, $F_{1,415} = 64.72$, $P < 0.01$) in western Canada (ON94 to HIG2) than in eastern Canada (QC68 to ON92). Mean inter-tree correlation (RBAR) averaged 0.585 and ranged from 0.341 to 0.735. The average total number of years with severe FTC defoliation (N_{FTC}) between 1959 and 2009 was 6.4 defoliations, with extreme values of 13 and 1, meaning that a severe defoliation occurred every 8 years on average. The climate moisture index (CMI) and N_{FTC} were not significantly correlated (Pearson product-moment correlation: $r = -0.26$, $P = 0.2415$).

4.5.2. Descriptive genetic statistics

All 7 loci were highly polymorphic and the number of alleles per locus ranged from 9 (PTR6 and WPMS16) to 19 (PTR3; Table 4.2). All descriptive genetic data are presented in Table 4.3.

4.5.2.1. Ramet data set

Clonal (genotypic) diversity and the Simpson diversity index (D), ranged between 0 (monoclonal sites) and 1, but did not vary significantly along the longitudinal gradient (Pearson product-moment correlation: $r = 0.0064$, $P = 0.7223$). The highest value for evenness (E) was E = 1 and the lowest value was E = 0.308, where the differences in the size of each clone within the stand were large.

Table 4.3 Genetic variability of 22 aspen populations sampled across Canada.

Population	N	G*	R*	A**	Ho**	He**	Gis**	D*	E*
HIG2	20	2	0.053	1.714	0.286	0.429	0.333	0.1	0.552
RED2	20	4	0.158	3.286	0.679	0.658	-0.031	0.432	0.424
CAL1	20	4	0.158	3.429	0.75	0.674	-0.113	0.553	0.526
PET2	20	11	0.526	4.429	0.656	0.642	-0.022	0.9	0.627
MOR3	20	3	0.105	2.143	0.571	0.438	-0.304	0.279	0.454
PAS3	20	4	0.158	3.429	0.607	0.594	-0.022	0.432	0.424
MB99	20	5	0.211	3.857	0.564	0.585	0.036	0.558	0.426
MB97	20	3	0.105	3	0.714	0.694	-0.029	0.279	0.454
ON95	20	5	0.211	4.143	0.857	0.686	-0.25	0.511	0.388
ON94	20	1	0.000	1.286	0.286	0.286	0	0	1
ON92	20	6	0.263	4.143	0.643	0.621	-0.035	0.674	0.463
ON90	20	8	0.368	4.429	0.633	0.62	-0.021	0.868	0.714
ON87	20	7	0.316	4.286	0.694	0.61	-0.137	0.679	0.402
ON85	20	5	0.211	3.429	0.543	0.566	0.04	0.368	0.308
ON83	20	8	0.368	5.714	0.768	0.677	-0.135	0.7	0.373
ON81	20	9	0.421	5.714	0.667	0.694	0.04	0.853	0.585
QC78	20	20	1	6.143	0.677	0.636	-0.065	1	1
QC75	20	4	0.158	3.143	0.607	0.6	-0.012	0.363	0.382
QC73	20	2	0.053	2.714	0.714	0.724	0.013	0.189	0.61
QC71	20	1	0	1.714	0.714	0.714	0	0	1
QC69	20	1	0	1.429	0.429	0.429	0	0	1
QC68	20	5	0.211	4.286	0.629	0.692	0.092	0.505	0.385

N, number of individuals genotyped; G, number of unique genets; R, clonal diversity; A, average allelic richness; H_o, observed heterozygosity; H_e, expected heterozygosity; G_{is}, interbreeding coefficient; D, Simpson diversity index; E, Evenness; * Calculated from the ramet data set; ** Calculated from the genet data set

4.5.2.2. Genet data set

Averaged allelic richness (A) ranged from 1.3 to 6.1, with an average value of 3.5 across populations. Observed heterozygosity (H_o) had a mean value of 0.622 and varied from 0.286 to 0.857. Mean H_e was 0.603, with values ranging from 0.429 to 0.724. Values of H_o were slightly higher than H_e, but these differences were not significant (Paired *t*-test: $t = 1.37$, $df = 21$, $P = 0.19$). H_o did not vary significantly along the longitudinal gradient (Pearson product-moment correlation: $r = 0.01896$, P

= 0.5412). Individual H_o varied from 0.143 to 1. For each locus, G_{is} varied from -0.092 (PTR14) to 0.038 (PTR3), with a global G_{is} value of -0.015.

Table 4.4 AICc candidate models for the log-transformed average tree basal area increment (TBAI) of *Populus tremuloides* in Canada, derived from raw ring-widths.

Candidate models	K[1]	AICc[2]	ΔAICc[3]	AICcWt[4]	Cumwt
TBAI ~ CMI + FTC + Age	7	487.23	0	0.52	0.52
TBAI ~ CMI	5	489.8	2.58	0.14	0.66
TBAI ~ Age	5	490.1	2.87	0.12	0.78
TBAI ~ Ho + A + D + Age	8	491.07	3.84	0.08	0.86
TBAI ~ CMI + FTC + Ho + A + D + Age (global model)	10	491.55	4.32	0.06	0.92
TBAI ~ CMI + FTC	6	491.75	4.52	0.05	0.97
TBAI ~ Ho + A + D	7	495.68	8.45	0.01	0.98
TBAI ~ D	5	496.56	9.33	0	0.99
TBAI ~ FTC	5	496.63	9.4	0	0.99
TBAI ~ Ho	5	496.8	9.57	0	1
TBAI ~ Ho	6	496.95	9.72	0	1

TBAI (ΔAICc<3) and therefore multimodel inference was performed
[1] Number of parameters
[2] AICc coefficient
[3] AIC relative to the best model
[4] AIC model weight (for more details see Burnham and Anderson 2004)

4.5.3. Modelling growth responses to genetics and environmental data

4.5.3.1. Annual growth and inter-tree growth variability

Many candidate models (ΔAICc < 3) could explain the differences in TBAI. Therefore, multi-model inference was used to calculate the averaged predictors for the different variables in these models (tree age, N_{FTC}, CMI, D, A and H_o or individual H_o; Table 4.1 & Table 4.6). TBAI was best explained in the mixed-effects models by CMI, N_{FTC} and tree age (estimated $R^2 = 0.45$, $P < 0.001$). On average, TBAI was significantly higher on sites with lower CMI (Figure 4.2a), while the other variables were not significant (Table 4.4 and Figure 4.2b-f). This analysis revealed no significant effects of the tested genetic parameters. Annual growth was only influenced by climatic conditions (Table 4.6).

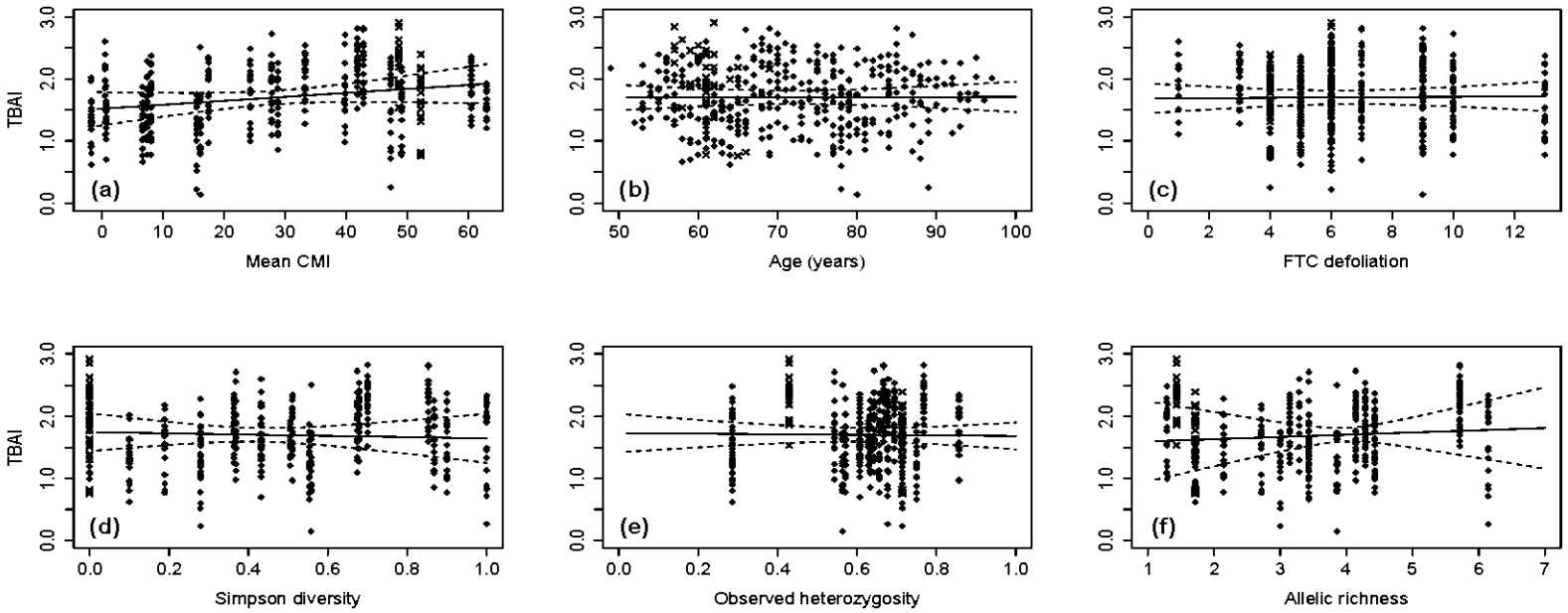

Figure 4.2 Observed (dots) and predicted logarithm of mean basal area increment (TBAI; solid line) in response to all explanatory variables that were used for multi-model averaging of the best candidate models (n = 417) in Canada. Dashed lines show 95 % confidence bands.

Concerning the inter-tree growth variability that was estimated by the MS, only one model had a ΔAICc < 3 and, therefore, multimodel inference was not used (Table 4.5). N_{FTC} and CMI were the most significant predictors explaining variation in MS (estimated $R^2 = 0.87$, $P < 0.001$). Our results showed that MS was not influenced by genetic diversity (neither H_o nor individual H_o, D and A) or tree ages. These variables were not included in the best-fitting models (Tavle 4.6). Model predictions suggested that an increasing N_{FTC} increased MS (Figure 4.3a), while a higher CMI (wetter conditions) tended to decrease MS (Figure 4.3b).

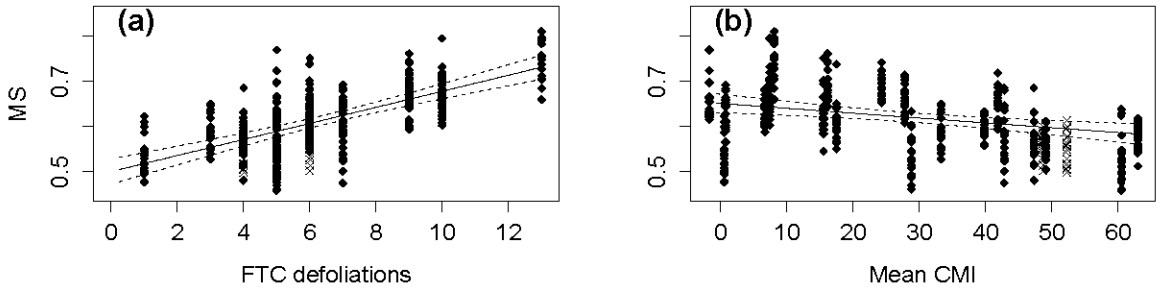

Figure 4.3 Observed (dots) and predicted square-root-transformed mean sensitivity (MS; solid line) in response to the explanatory variables present in the best candidate model (n = 440) in Canada. Dashed lines show 95 % confidence bands.

Table 4.5 AICc candidate models for the MS (square-root-transformed mean sensitivity) of *Populus tremuloides* growth in Canada, determined from detrended ring-widths.

Candidate models	K[1]	AICc[2]	ΔAICc[3]	AICcWt[4]	Cumwt
MS ~ CMI + FTC	6	-1617.48	0	0.99	0.99
MS ~ FTC	5	-1607.82	9.66	0.01	1
MS ~ CMI	5	-1585.58	31.9	0	1
MS ~ Ho	5	-1578.07	39.41	0	1
MS ~ D	5	-1577.32	40.16	0	1
MS ~ CMI + FTC + Age	7	-1576.64	40.84	0	1
MS ~ Ho + A	6	-1576.1	41.38	0	1
MS ~ Ho + A + D	7	-1575.75	41.73	0	1
MS ~ CMI + FTC + Ho + A + D + Age (global model)	10	-1573.89	43.59	0	1
MS ~ Age	5	-1539.31	78.17	0	1
MS ~ Ho + A + D + Age	8	-1535.58	81.9	0	1

Only one model explained √MS (ΔAICc<3) and no multimodel inference was performed
[1] Number of parameters
[2] AICc coefficient
[3] AIC relative to the best model
[4] AIC model weight (for more details see Burnham and Anderson 2004)

4.5.3.2. Mean inter-tree correlation (RBAR)

Only one model (R^2 = 0.683, P < 0.001; ΔAICc < 3), which included clonal diversity (R), N_{FTC}, and CMI explained the variability in RBAR across Canada. CMI was not a significant predictor (P = 0.083;Table 4.6) in the model. Therefore, multi-model inference was not used (Table 4.7). When taken separately, a total of 30 % of variation in RBAR was explained by clonal diversity (R^2 = 0.304, P = 0.007), while 28 % was explained by N_{FTC} (R^2 = 0.279, P = 0.012). Clonal diversity was negatively associated with RBAR (Figure 4.4a) and positively associated with N_{FTC} (Figure 4.4b). CMI had no significant effect (Table 4.6 & Figure 4.4c) on the RBAR even when this predictor was included in the best-fitting model.

Table 4.6 Results of the best-fitting models (linear mixed-effect [LME] and multiple linear regression) explaining the relationship between TBAI (a), MS (b) and RBAR (c), and the explanatory variables (Ho, R, A, D, Age, FTC and CMI). In the LME, each unique genotype within sites was treated as a random effect, nested within each of the 22 sites. The best model was selected according to AICc (Tables 4.4 and 4.5) for LME and multiple linear regression (Table 4.7).

Response	Predictor	Estimate	Lower 95% CI	Upper 95% CI
(a) TBAI	CMI	**0.0081**	**0.0023**	**0.014**
	FTC	0.0042	-0.0362	0.0447
	Age	0.0003	-0.0085	0.0091
	D	-0.7249	-1.8957	0.4458
	H_o	-0.3288	-1.4236	0.766
	A	0.2234	-0.0491	0.4958
(b) MS	FTC	**17.452e-03**	**13.641e-03**	**21.263e-03**
	CMI	**-1.064e-03**	**-1.625e-03**	**-0.503e-03**
(c) RBAR	R	**-0.2804**	**-0.4154**	**-0.1453**
	FTC	**0.02065**	**0.00939**	**0.03192**
	CMI	-1.422e-03	-2.942e-03	0.097e-03

4.6. Discussion

Variability in growth responses to climate for trembling aspen have been acknowledged in the literature (Hogg et al. 2005; Drobyshev et al. 2013; Huang et al. 2013), although few studies have attempted to quantify this variation in terms of genetic diversity. This study on aspen populations along an East-West Canadian transect demonstrated that higher levels of clonal diversity were associated with lower growth response synchronicity (low RBAR values). In contrast, environmental factors such as N_{FTC} and soil moisture conditions (CMI) were the major factors explaining variation in mean sensitivity (MS) between populations, while average tree basal area increment (TBAI) varied only with CMI.

Table 4.7 AICc candidate models for the RBAR (mean inter-tree correlation) of *Populus tremuloides* in Canada, determined from detrended ring-widths.

Candidate models	K[1]	AICc[2]	ΔAICc[3]	AICcWt[4]	Cumwt
RBAR ~ CMI + FTC + R	5	-49.53	0	0.8	0.8
RBAR ~ CMI + FTC + R + Ho + A (global model)	7	-46.03	3.5	0.14	0.94
RBAR ~ FTC + Ho + A	5	-44.2	5.33	0.06	0.99
RBAR ~ CMI + FTC	4	-37.18	12.35	0	1
RBAR ~ R	3	-36.25	13.28	0	1
RBAR ~ CMI + Ho + A	5	-36.14	13.39	0	1
RBAR ~ FTC	3	-35.46	14.06	0	1
RBAR ~CMI	3	-33.53	15.99	0	1
RBAR ~ R + Ho + A	4	-33.49	16.04	0	1
RBAR ~ CMI + Ho + A	5	-33.08	16.44	0	1
RBAR ~ Ho + A	3	-30.84	18.69	0	1

Only one model explained RBAR (ΔAICc<3) and no multimodel inference was performed
[1] Number of parameters
[2] AICc coefficient
[3] AIC relative to the best model
[4] AIC model weight (for more details see Burnham and Anderson 2004)

4.6.1. Descriptive genetic statistics

The level of genetic polymorphism that was observed with the set of 7 loci was similar to what has been found in previous studies and reflected the highly diverse genetic pool of aspen in Canada's boreal forest (Dayanandan et al. 1998; Rahman et al. 2000; Smulders et al. 2001; Namroud et al. 2005). Mean H_o and H_e were very high for most sites, reflecting substantial genetic diversity (Wyman et al. 2003; Namroud et al. 2005). For all loci that were considered, we observed slightly higher values of H_o compared to H_e, with a slight excess of heterozygotes. This trend was confirmed by negative G_{is} values for each locus. Generally, if heterozygotes have a fitness advantage, this tends to generate negative G values, although a negative G does not necessarily mean that there was heterozygote advantage (Jelinski and Cheliak 1992). Further, negative G_{is} are observed when a relatively small number of samples (unique genotype) are analysed per site (Meirmans and Van Tienderen 2004), which was the

case with the genet data set. No relationship between the level of genetic diversity and environmental conditions could be established. Clonal diversity (R) similarly varied along the climatic gradient, but no significant differences in this value were found between eastern and western Canada. Indeed, monoclonal and highly diverse polyclonal aspen stands were encountered throughout Canada.

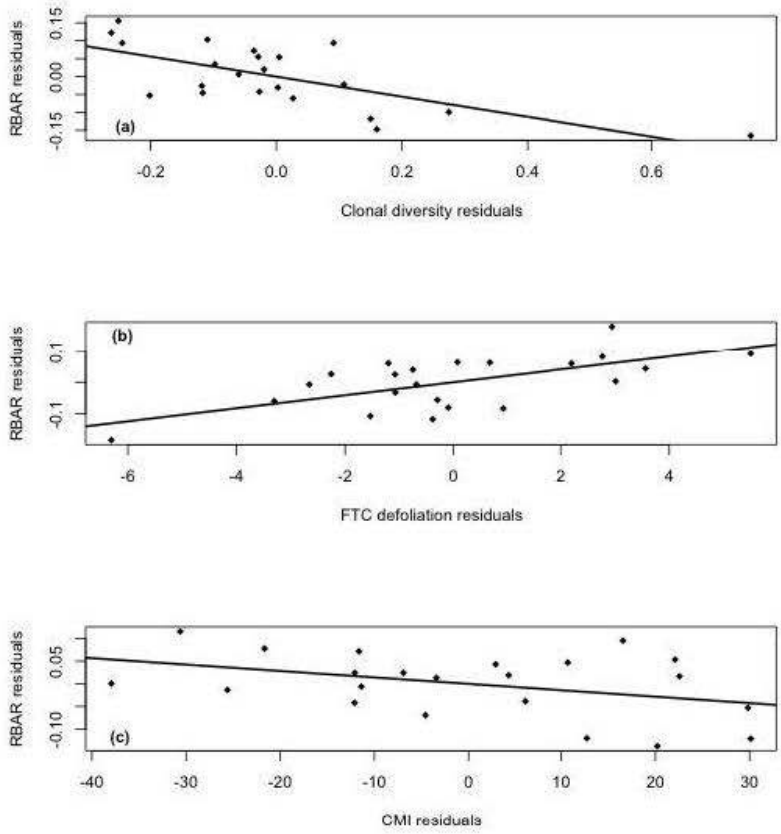

Figure 4.4 Partial regression plots showing the relationship between (a) RBAR residuals vs clonal diversity residuals, (b) RBAR residuals vs forest tent caterpillar extreme defoliation residuals and (c) RBAR residuals vs CMI residuals for 22 populations of Populus tremuloides that were located across boreal Canada.

4.6.2. Modelling growth responses to genetics and environmental data

4.6.2.1. Annual growth and inter-tree growth variability

Tree basal area increment (TBAI) was best explained by regional differences in CMI. Genetic diversity (H_o, A and D) could not explain the differences in TBAI that have been observed in the Canadian boreal forest. Mitton and Grant (1980) and Jelinski and Cheliak (1992) have suggested that heterozygotes may have greater physiological versatility than homozygotes, which would permit more flexible responses to environmental variation. In general, heterozygotes had no better growth than homozygotes in terms of TBAI (Figure 4.2) and mean H_o was not significant. Clonal richness had no effect on the standard deviation of TBAI (data not shown), meaning that mean growth of all genotypes varied with similar amplitude in response to external environmental conditions. Our results supported a relationship between CMI and TBAI, as has been previously reported by Hogg et al. (2005) in the western boreal forest. In a recent study, Anyomi et al. (2014) also showed a decline in aspen productivity with high moisture deficits at a continental scale. In their study, King et al. (2013) reported that tree ring-width variation in European larch (*Larix decidua*) and Norway spruce (*Picea abies*) that were growing in the Alps were more strongly driven by climate than by genetics at regional and larger scales.

A high MS value is characteristic of high inter-annual variation in growth (Cook and Kariukstis 1990; Biondi and Qeadan 2008; Schreiber et al. 2013) and is an approximation of the time series standard deviation (Bunn et al. 2013). Hogg et al. (2002a) demonstrated that defoliation by FTC was the most important factor causing inter-annual variation in annual growth before pointing out the combined influence of inter-annual variation in the CMI and FTC defoliation on those variations in growth. During years of severe early season defoliation by insects, anomalously pale-coloured, low-density tree rings are formed (Hogg et al. 2005), and reduced growth or no growth is observed. Our results (Figure 4.3) supported the idea that the frequency

of these outbreaks (N_{FTC}) increased inter-annual variation, given that year-to-year growth variability was observed. Climatic conditions are the other important component that would explain this variation. A more favourable climate (high CMI) reduced inter-annual differences in growth, thereby lowering MS. Regions with moister climate are less subject to extreme drought events and, thus, variation in inter-annual growth responses are expected to be more homogeneous in eastern than in western Canada. The fact that genetic variables (H_o, D and A) did not significantly influence variation in MS demonstrated that even in extreme climatic conditions, there were no differences in growth response among genotypes within sites. It is possible that higher levels of diversity could allow the trees to have a more flexible response in the case of poorer than normal environmental conditions (Nicotra et al. 2010). Contrary to our hypothesis, clonal (R or D) and genetic diversity (H_o, A) of the population did not explain variation in MS and higher levels of diversity could not buffer growth reductions and variation during extreme events.

4.6.2.2. Mean inter-tree correlation (RBAR)

Our results partly validated our hypothesis for a relationship between genetic characteristics and growth responses in aspen. RBAR was mainly influenced by clonal diversity and N_{FTC}. Clonal diversity was the most important factor. It increased the differences between individual chronologies and the average growth response per site. In contrast, N_{FTC} had a homogenising effect on RBAR (Figure 4.4), suggesting that insects similarly and severely affect all of the trees and genotypes. Osier and Lindroth (2001) showed that the primary source of variation in gypsy moth (*Lymantria dispar*) performance was aspen genotype (different ranges of resistance against this insect), which we did not observe here, most probably because we focused only on severe defoliation. Severe defoliation is known to reduce tree growth (Moulinier et al. 2014), which could affect all genotypes. Consequently, the growth response was more homogeneous within ramets (high RBAR, with low R) of the

same genotype than between ramets of different genotypes. In general, a complex assemblage of different genotypes (high R) would likely increase differences in genotype growth patterns (phenotypic responses for this trait) to the same environmental factors of low intensity, whereas severe events would affect them strongly and similarly. The effect of other environmental factors, such as moisture, could not be isolated because of the predominant effects of N_{FTC} and clonal diversity, together with the noise that was introduced by these factors. Differences in genetic diversity could not explain variation in RBAR and no signal was detected. This response can probably be explained by the high level of genetic diversity that was measured by H_o and by the fact that H_o is quite homogenous, even when RBAR varies among sites. Phenotypic differences that were observed among clones (genotypes), rather than overall genetic diversity, likely created most of the variability and are the source of the differences among chronologies.

4.6.3. Conclusions and perspectives

The current study is the first continental-scale study to examine trembling aspen radial growth in relation to population genetic data in the Canadian boreal forest. In general, the assumption that has been made regarding growth studies is that inter-annual variation in growth is driven primarily by physiological responses to climatic variables (Parmesan 2006; King et al. 2013). This work confirmed previous findings (Hogg et al. 2005), showing that environmental disturbances are the main drivers of average and inter-annual growth variation and that annual growth variation is more strongly driven by climate than by genetics at regional and broader scales.

Higher aspen TBAI was not correlated with higher genetic diversity. We can not exclude the possibility that the absence of correlation between "multilocus heterozygosity" and the fitness of our trees (evaluated by different radial growth measures) could be due to an insufficient number of markers considered to represent the genome-wide diversity (Hansson and Weserberg, 2002). However the correlation

between genome-wide heterozygosity and multi-locus estimates is relatively high with a median value across studies of 0.83 for microsatellites (Mittel et al. 2015). Mittel et al. (2015) argued that a relatively small number of microsatellite loci and number of individuals genotyped are sufficient to give an accurate estimate of genome-wide diversity. However they also shown that the ability of current molecular genetic diversity estimates to predict average heritability has an upper bound of 0.26 and that additional genotyping will probably not improve the ability of these markers to predict adaptive potential.

Genetic factors should subsequently be taken into account in future studies, since our results have shown their importance in growth synchronicity. Multi-clonal aspen stands would likely be, on average, more resilient to year-to-year events of small intensity (e.g. climate or insect outbreaks) and less sensitive to rapid declines because their growth responses were less homogeneous due to the complex assemblage of many different genotypes. Our results emphasize the importance of trying to maintain highly diverse (genetic and clonal diversity) aspen stands. It is especially crucial in the context of global change where an alteration in the genetic composition could lead to a reduction in the ability of a stand to resist and recover from environmental disturbances (Jump and Peñuelas 2005), thereby causing extensive stand decline, especially in the western part of the species' range (e.g. Hogg et al. 2008; Worrall et al. 2008; Worrall et al. 2013).

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4.8. References

Anyomi KA, Lorenzetti F, Bergeron Y, Leduc A (2014) Stand dynamics, humus type and water balance explain aspen long term productivity across Canada. Forests 62:416-432

Applequist MB (1958) A simple pith locator for use with off-center increment cores. Journal Forest 562:141

Arnaud-Haond S, Duarte CM, Alberto F, Serrão EA (2007) Standardizing methods to address clonality in population studies. Mol Ecol 1624:5115-5139

Beaulieu J, Bousquet J (2010) Facteurs génétiques affectant la variabilité des cernes annuels chez les espéces arborescentes nordiques. In: Payette S., Filion L. (eds) La dendroécologie: principes, méthodes et applications. Presses de l'Université Laval, Québec City (QC), Canada, pp. 137-163

Becker M (1989) The role of climate on present and past vitality of silver fir forests in the Vosges mountains of northeastern France. Can J For Res 199:1110-1117

Biondi F, Qeadan F (2008) Inequality in paleorecords. Ecology 894:1056-1067

Briffa KR, Jones PD (1990) Basic chronology statistics and assessment. In: Cook ER,

Kairiukstis LAE (eds) Methods of dendrochronology: Applications in the environmental sciences. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 137-152

Briffa KR, Schweingruber FH, Jones PD, Osborn TJ, Harris IC, Shiyatov SG,

Vaganov EA, Grudd H (1998) Trees tell of past climates: but are they speaking less clearly today? Philos Trans R Soc Lond B Biol Sci 353:65-73

Bunn AG (2008) A dendrochronology program library in R (dplR). Dendrochronologia 262:115-124

Bunn AG, Jansma E, Korpela M, Westfall RD, Baldwin J (2013) Using simulations and data to evaluate mean sensitivity (zeta) as a useful statistic in dendrochronology. Dendrochronologia 313:250-254

Burnham KP, Anderson DR (2004) Multimodel inference: Understanding AIC and BIC in model selection. Sociol Method Res 332:261-304

Callahan CM, Rowe CA, Ryel RJ, Shaw JD, Madritch MD, Mock KE (2013) Continentalscale assessment of genetic diversity and population structure in quaking aspen (*Populus tremuloides*). J Biogeogr 409:1780-1791

Cole CT, Anderson JE, Lindroth RL, Waller DM (2010) Rising concentrations of atmospheric CO2 have increased growth in natural stands of quaking aspen (*Populus tremuloides*). Glob Change Biol, 16: 2186-2197

Cook EK, Kariukstis AL (1990) Methods of dendrochronology - applications in the environmental sciences. Kluwer Academic Publishers and International Institute for Applied Systems Analysis, Dordrecht, The Netherlands

Cook ER (1985) A time series analysis approach to tree ring standardization. PhD thesis, University of Arizona, Tucson, AZ.

Cook ER, Pederson N (2010) Uncertainty, emergence, and statistics in dendrochronology. In: Hughes MK, Swetnam TW, Diaz HF (eds) Dendroclimatology: Progress and prospects. Springer Science and Bussiness Media, New York, USA, pp. 77-112

Cook ER, Peters K (1981) The smoothing spline: a new approach to standardizing forest interior tree-ring width series for dendroclimatic studies. Tree-Ring Bl 41:45-53

Cornelius J (1994) Heritabilities and additive genetic coefficients of variation in forest trees. Can J For Res 242:372-379

Dayanandan S, Rajora OP, Bawa KS (1998) Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*). Theor Appl Genet 966-7:950-956

DeWoody J, Rowe CA, Hipkins VD, Mock KE (2008) "Pando" lives: molecular genetic evidence of a giant aspen clone in central Utah. West N Am Naturalist 684:493-497

Dorken ME, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodonverticillatus* (Lythraceae). Journal of Ecology 893:339-350

Drobyshev I, Gewehr S, Berninger F, Bergeron Y (2013) Species-specific growth responses of black spruce and trembling aspen may enhance resilience of boreal forest to climate change. J Ecol 1011:231-242

Environment Canada (2014) Calculation of the 1971 to 2000 Climate Normals for Canada. http://climate.weather.gc.ca/climate_normals/normals_documentation_e.html?docID=1971 (Accessed May 3 2014)

Fox J, Weisberg S (2011) An *R* Companion to Applied Regression, Second Edition. Thousand Oaks, California. Available at: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion. (Accessed: February 20 2015)

Frey BR, Lieffers VJ, Hogg EH, Landhäusser SM (2004) Predicting landscape patterns of aspen dieback: mechanisms and knowledge gaps. Can J For Res 347:1379-1390

Fritts HC (1976) Tree rings and climate. Academic Press, New York, USA

Grant MC, Mitton JB, Linhart YB (1992) Even larger organisms. Nature 3606401:216-216

Gray LK, Gylander T, Mbogga MS, Chen P-Y, Hamann A (2010) Assisted migration to address climate change: recommendations for aspen reforestation in western Canada. Ecol Appl 215:1591-1603

Gylander T, Hamann A, Brouard JS, Thomas BR (2012) The potential of aspen clonal forestry in Alberta: Breeding regions and estimates of genetic gain from selection. PLoS ONE 78:e44303. doi: 10.1371/journal.pone.0044303

Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness in natural populations. Mol Ecol 11 2467-2474.

Hanson EJ, Azuma DL, Hiserote BA (2003) Site index equations and mean annual increment equations for Pacific Northwest Research Station forest inventory and analysis inventories, 1985-2001. US Department of Agriculture, Forest Service Pacific Northwest Research Satation, Portland, OR. Research Note PNW-RN-533 26p

Hogg EH (1997) Temporal scaling of moisture and the forest-grassland boundary in western Canada. Agr Forest Meteorol 841–2:115-122

Hogg EH (2001) Modeling aspen responses to climatic warming and insect defoliation in western Canada. In: Proceedings of the Sustaining Aspen in Western Landscapes Symposium, Grand Junction, CO (ed). USDA Forest Service Proceedings RMRS-P-18. Rocky Mountain Research Station, Ft. Collins, CO, pp. 325-337

Hogg EH, Brandt JP, Kochtubajda B (2002a) Growth and dieback of aspen forests in

northwestern Alberta, Canada, in relation to climate and insects. Can J For Res 325:823-832

Hogg EH, Brandt JP, Kochtubajda B (2005) Factors affecting interannual variation in growth of western Canadian aspen forests during 1951-2000. Can J For Res 353:610-622

Hogg EH, Brandt JP, Michaelian M (2008) Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Can J For Res 386:1373-1384

Hogg EH, Hart M, Lieffers VJ (2002b) White tree rings formed in trembling aspen saplings following experimental defoliation. Can J For Res 3211:1929-1934

Hogg EHT, Bernier PY (2005) Climate change impacts on drought-prone forests in western Canada. Forest Chron 815:675-682

Huang J-G, Stadt KJ, Dawson A, Comeau PG (2013) Modelling growth-competition

relationships in trembling aspen and white spruce mixed boreal forests of western Canada. PLoS ONE 810:e77607. doi: 10.1371/journal.pone.0077607

Jelinski DE, Cheliak WM (1992) Genetic diversity and spatial subdivision of *Populus tremuloides* (Salicaceae) in a heterogeneous landscape. Am J Bot 797:728-736

Jump AS, Peñuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. Ecol Lett 89:1010-1020

King GM, Gugerli F, Fonti P, Frank DC (2013) Tree growth response along an elevational gradient: climate or genetics? Oecologia 1734:1587-1600

Kullback S, Leibler RA (1951) On information and sufficiency. Ann Math Stat 22:79-86

LeBlanc DC (1990) Relationships between breast-height and whole-stem growth indices for red spruce on Whiteface Mountain, New York. Can J For Res 209:1399-

1407

Lebourgeois F, Gomez N, Pinto P, Mérian P (2013) Mixed stands reduce Abies alba tree-ring sensitivity to summer drought in the Vosges mountains, western Europe. Forest Ecol Manag 303:61-71

Lemprière T, Bernier PY, Carroll AL, Flannigan MD, Gilsenan RP, McKenney DW, Hogg EH, Pedlar JH, Blain D (2008) The importance of forest sector adaptation to climate change. Information Report NOR-X-416E. Natural Resouces Canada, Canadian Forest Service, Northern Forestry Centre, Edmonton, AB. 78p

Leonelli G, Denneler B, Bergeron Y (2008) Climate sensitivity of trembling aspen radial growth along a productivity gradient in northeastern British Columbia, Canada. Can J For Res 385:1211-1222

Li H, Wang X, Hamann A (2010) Genetic adaptation of aspen (Populus tremuloides) populations to spring risk environments: a novel remote sensing approach. Can J For Res 4011:2082-2090

Little EL (1971) Atlas of United States Trees, Volume 1, Conifers and Important Hardwoods. U.S. Department of Agriculture, Forest Service, Washington, DC. Miscellaneous Publication 1146, 200 maps

Long JN, Mock K (2012) Changing perspectives on regeneration ecology and genetic diversity in western quaking aspen: implications for silviculture. Can J For Res 4212:2011-2021

Mazerolle MJ (2013) AICcmodavg: model selection and multimodel inference based on (Q)AIC(c). R package version 1.35. http://cran.rproject.org/web/packages/AICcmodavg/index.html. (Accessed February 20 2015)

Meirmans PG, Van Tienderen PH (2004) genotype and genodive: two programs for the analysis of genetic diversity of asexual organisms. Mol Ecol Notes 44:792-794

Mérian P (2012) POINTER and DENDRO - Two applications under R software for analyzing tree response to climate using dendroecological approach. Revue Forestière Française 646:789-798

Mérian P, Lebourgeois F (2011) Size-mediated climate–growth relationships in temperate forests: A multi-species analysis. Forest Ecol Manag 2618:1382-1391

Michaelian M, Hogg EH, Hall RJ, Arsenault E (2011) Massive mortality of aspen following severe drought along the southern edge of the Canadian boreal forest. Global Change Biol 176:2084-2094

Mittell EA, Nakagawa S, Hadfield JD (2015) Are molecular markers useful predictors of adaptive potential? Ecol Lett 18 772-778.

Mitton JB, Grant MC (1980) Observations on the ecology and evolution of quaking aspen, *Populus tremuloides*, in the Colorado Front Range. Am J Bot 202-209

Mitton JB, Grant MC (1984) Associations among protein heterozygosity, growth rate, and developmental homeostasis. Annu Rev Ecol Syst 15:479-499

Mitton JB, Grant MC (1996) Genetic variation and the natural history of quaking aspen. Bioscience 461:25-31

Mock KE, Rowe CA, Hooten MB, Dewoody J, Hipkins VD (2008) Clonal dynamics in western North American aspen (*Populus tremuloides*). Mol Ecol 1722:4827-4844

Moulinier J, Lorenzetti F, Bergeron Y (2014) Growth and mortality of trembling aspen (*Populus tremuloides*) in response to artificial defoliation. Acta Oecol 550:104-112

Namroud MC, Tremblay F, Bergeron Y (2005) Temporal variation in quaking aspen (*Populus tremuloides*) genetic and clonal structures in the mixedwood boreal forest of eastern Canada. Ecoscience 121:82-91

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P,

Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 1512:684-692

Osier TL, Lindroth RL (2001) Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. J Chem Ecol 277:1289-1313

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Ann Rev Ecol Evol S 371:637-669

Peterson EB, Peterson NM (1992) Ecology, management, and use of aspen and balsam poplar in the prairie provinces, Forestry Canada Northwest Region, Northern Forestry Center, Canada. Edmonton, Canada Special Report 1. 252p

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2013) nlme: Linear and nonlinear mixed effects models, R package version 3.1-113. http://cran.rproject.org/web/packages/nlme/index.html. (Accessed: February 20 2015)

R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.R-project.org/. (Accessed: February 20 2015)

Rahman MH, Dayanandan S, Rajora OP (2000) Microsatellite DNA markers in *Populus tremuloides*. Genome 432:293-297

Régnière J, St-Amant R, Béchard A (2013) BioSIM 10 User's manual, Information Repport LAU-X-137E. Natural Resources Canada, Laurentian Forest Centre, Québec City, QC, Canada. ftp://ftp.cfl.scf.rncan.gc.ca/regniere/software/BioSIM/Doc/LAU-X-137E.zip (Accessed September 10 2013)

Rinn F (1996) TSAP – Time Series Analysis and Precipitation. Version 3 Reference Manual. Heidelberg,

Schreiber SG, Ding C, Hamann A, Hacke UG, Thomas BR, Brouard JS (2013) Frost hardiness vs. growth performance in trembling aspen: an experimental test of assisted

migration. J Appl Ecol 504:939-949

Smulders MJM, Van Der Schoot J, Arens P, Vosman B (2001) Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L.). Mol Ecol Notes 13:188-190

Valentine HT (1985) Tree-growth models: Derivations employing the pipe-model theory. J Theor Biol 1174:579-585

Verbeke G, Molenberghs G (2009) Linear mixed models for longitudinal data. Springer Science and Bussiness Media, New York, USA

Wigley TML, Briffa KR, Jones PD (1984) On the average value of correlated time series, with applications in dendroclimatology and hydrometeorology. J Clim Appl Meteorol 232:201-213

Worrall JJ, Egeland L, Eager T, Mask RA, Johnson EW, Kemp PA, Shepperd WD (2008) Rapid mortality of *Populus tremuloides* in southwestern Colorado, USA. Forest Ecol Manag 2553:686-696

Worrall JJ, Rehfeldt GE, Hamann A, Hogg EH, Marchetti SB, Michaelian M, Gray LK (2013) Recent declines of *Populus tremuloides* in North America linked to climate. Forest Ecol Manag 299: 35-51

Wyman J, Bruneau A, Tremblay MF (2003) Microsatellite analysis of genetic diversity in four populations of *Populus tremuloides* in Quebec. Can J Bot 814:360-367

Yang Y, Huang S, Meng SX, Trincado G, VanderSchaaf CL (2009) A multilevel individual tree basal area increment model for aspen in boreal mixedwood stands. Can J For Res 3911:2203-2214

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer Science and Bussiness Media, New York, USA

4.9. Appendices

Appendix 4.1 Calculation method to obtain the DBH without the bark.

The BAIs were calculated backwards with the radius of the last year of growth (2010) deducted from the DBH (R=DBH/2). Bark width can be of considerable thickness in aspen (i.e. 7 to 12 % of DBH; Applequist 1958; Fowler 1991, 1993). To avoid overestimation of the radius, the inside diameter DBH_i (DBH without bark) was obtained by the following equation: $DBH_i = B_f * DBH$, where B_f is the bark factor and DBH, the measured diameter including the bark. B_f was calculated according to Fowler 1993 as follows:

$$B_f = 0.9214 - 0.000842 \times H_t + 0.007588 \times \ln(H_t)$$

where H_t is total tree height (in inches). On 50 trees with two complete cores (pith included) and known DBH, the BAI series calculated from the DBH_i did not differ significantly from those obtained with the "classical" method (i.e. from the distance to the pith).

Appendix 4.2 Calculation of the years with severe defoliation for the 1959–2009 period.

For each plot and year during the 1959–2009 period, years with severe defoliation were calculated as follows:

(1) Years with no missing rings: a severe defoliation corresponded to at least 33 % of the white rings, and where 95 % of trees displayed a ring-width in a given year that was two-times narrower than that observed in the previous year.

(2) Years with missing rings: the proportion of missing rings during a period of two to four successive years of defoliation varied between 5 and 97.5 %, with greater proportions for the second and third years of outbreak (data not shown). Severe defoliations were defined as those years following a year of severe defoliation that had been defined according to (1), for which (a) at least one missing-ring was detected and (b) a maximum of 5 % of the trees displayed a ring-width in a given year that was twice as large as that observed in the previous one.

CHAPITRE 5 CONCLUSION GÉNÉRALE

Bien que les études génétiques sur le peuplier (*Populus tremuloïdes* Michx.) soient récemment en augmentation (e.g. Namroud *et al.* 2005a; Namroud *et al.* 2005b; Ally *et al.* 2008; Mock *et al.* 2008; Mock *et al.* 2012; Callahan *et al.* 2013), très peu d'entre elles se sont intéressées aux dynamiques de la diversité génétique et de la structure clonale de cette espèce au niveau transcontinental au Canada (Mock *et al.* 2012; Callahan *et al.* 2013). L'objectif principal de cette thèse était d'évaluer les différences régionales de diversité génétique et de structure clonale, de comprendre leurs origines (facteurs historiques, environnement, perturbations) et de mesurer l'effet de la structure génétique des populations sur les variations de la réponse de croissance du peuplier. Pour cela les travaux se sont appuyés sur un cadre multidisciplinaire alliant plusieurs domaines de recherche différents dont la dendrochronologie, la génétique et la biogéographie. Chaque chapitre de la thèse repose sur l'utilisation de marqueurs microsatellites pour l'identification des clones et l'évaluation de la diversité génétique des populations étudiées. Différentes questions ont été abordées, en se basant sur l'utilisation des données moléculaires. En effet, le chapitre 2 visait à identifier les zones de refuges glaciaires et les voies de recolonisation du peuplier faux tremble dans la partie ouest de l'Amérique du Nord grâce à un échantillonnage intensif. Dans ce cas, les marqueurs microsatellites ont été utilisés pour reconstruire des processus démographiques passés. Le chapitre 3 avait pour but d'évaluer la diversité génétique et la structure clonale du peuplier dans la forêt boréale canadienne et la tremblaie-parc et l'effet des perturbations naturelles (climat, feux, fragmentation) sur la composition génétique des peuplements. Ici les microsatellites nous ont permis de caractériser la diversité génétique et la structure clonale avant de quantifier l'influence des perturbations naturelles sur celles-ci. Finalement, le chapitre 4 nous a permis de mesurer l'effet de la structure génétique des populations sur les variations de la réponse de croissance du peuplier. Dans ce cas, la diversité génétique et clonale ont été évaluées à l'aide des marqueurs microsatellites et mises en relation avec les différences de croissance observées. L'analyse moléculaire offre la possibilité de répondre à un grand nombre de questions

de recherche différentes concernant la dynamique des écosystèmes de la forêt boréale.

5.1. Discussion générale

Nous n'avons pas mis en évidence la présence de refuges glaciaires que ce soit en Béringie ou en Alberta au pied des Montagnes rocheuses dans la zone du *Ice-free corridor* (Chapitre 2). Nos résultats confirment ceux de Callahan *et al.* (2013) qui ont observé une faible structure génétique dans les populations de tremble du nord de leur aire de répartition (Canada et Alaska). Dans notre étude, nous avons détecté peu ou pas de structure en raison des faibles niveaux de variation des estimés de distances génétiques (Nei et F_{ST}). Nous n'avons pas observé d'évidence d'isolation avec la distance entre les sites, même à de très grandes distances les unes des autres (100 - 3000 km). Ceci indique donc que dans cette zone, le tremble semble être issu des mêmes lignées d'origine.

L'étude transcontinentale de la structure clonale et de la diversité génétique des remblaies démontre qu'il existe un patron homogène indépendamment des conditions climatiques rencontrées et un rôle faible des perturbations naturelles (Chapitre 3). Le pourcentage des clones composés d'une seule tige et la diversité clonale observée étaient élevés. Ceci confirme à l'échelle continentale, les résultats de Namroud *et al.* (2005b) qui, au Québec, avaient montré que plus de 75% des clones étaient composés d'une seule tige et que la diversité clonale était élevée. Ceci suggère: 1) que des évènements de reproduction sexuée se sont produits sur l'ensemble de sites après des perturbations importantes (c.à.d. coupe totale, feux de forêt et dépérissement) associé au drageonnement dans le cas où le peuplier était déjà présent sur le site ; 2) la présence d'auto-éclaircie des ramets intra-clones (genets) avec une longue durée depuis le dernier feu. L'établissement par graines se fait après suppression complète de la canopée. De plus, au niveau de la croissance, les drageons surpassent généralement les semis quand des peupliers sont déjà présents sur le site.

L'établissement par graines est certainement moins fréquent après des perturbations secondaires (c.à.d., défoliations, chablis, sècheresse) car les conditions écologiques (c.à.d., température du sol, lumière) ne sont généralement pas optimales pour l'établissement et la survie des semis (MacKenzie 2010). Le grand nombre d'interconnexions racinaires entre les arbres (entre clones et ramets) pourrait influencer la régénération de génotypes qui étaient morts depuis de nombreuses années et conservés dans le sol (Jelinkova *et al.* 2009). Quand les tiges d'un peuplement de peupliers sont supprimées à travers un processus d'auto-éclaircie, leurs racines peuvent survivre comme faisant partie d'un système racinaire commun plus vaste. Ceci pourrait donc être une des raisons pour laquelle des clones à ramet unique ont été observés sur la plupart des sites mixés avec des clones multi-ramets. Ceci est similaire à ce que Mock *et al.* (2008) ont observé dans le clone Pando où des génotypes distincts sont trouvés au milieu de très grands clones.

La diversité génétique varie entre les sites (H_o, N_a, Trip) et est, en partie, expliquée par les conditions environnantes (Chapitre 3). L'hétérozygotie observée (H_o) augmente faiblement avec des taux de brulage faibles et des conditions humides (faible CMI). Cela confirme, en partie, les travaux de Turner *et al.* (2003), qui ont suggéré que des perturbations épisodiques de grande taille peuvent jouer un rôle clé sur la structure des populations, la génétique et l'évolution du tremble. Contrairement à Balloux *et al.* (2003), aucune relation entre la présence de grands clones et H_o ou N_a n'a été trouvée. Le nombre moyen d'allèles par locus (N_a) et la proportion de la triploïdie (Trip) étaient inférieurs et supérieurs, respectivement, avec l'augmentation de la fragmentation du paysage. Le nombre moyen d'allèles par locus (N_a) doit être interprété avec prudence, car cette mesure est dépendante de la taille de l'échantillon (Leberg, 2002). La réduction du N_a en raison d'un paysage fragmenté pourrait être la conséquence de flux de gènes plus faible entre les populations. Cependant, les traits reproductifs propres au tremble (pollinisation par le vent, exogamie) devraient avoir un effet tampon contre la perte de diversité résultant de la fragmentation.

Nous avons montré qu'en incluant des données génétiques, les conditions environnementales demeurent les facteurs principaux expliquant les variations de croissance (Chapitre 4). Ceci confirme les résultats de King *et al.* (2013) qui ont montré que la variation de la largeur des cernes de croissance du Mélèze européen (*Larix decidua*) et de l'épinette de Norvège (*Picea abies*) dans les Alpes, était principalement influencée par le climat plutôt que par la génétique. Mitton & Grant (1980) et Jelinski & Cheliak (1992) ont suggéré que les hétérozygotes pourraient avoir une plus grande flexibilité de réponse aux variations environnementales comparativement aux homozygotes. Dans notre étude, les hétérozygotes n'ont pas montré une croissance supérieure aux homozygotes en ce qui concerne la surface terrière. Aucun effet de la génétique n'a permis d'expliquer ces variations de croissance (TBAI et MS). En effet, le climat et le nombre d'années avec une sévère défoliation par la livrée des forêts (N_{FTC}) influencent principalement la variabilité annuelle de croissance (MS) chez le peuplier. Par ailleurs, seules, les conditions climatiques influencent la croissance (TBAI) confirmant les résultats obtenus par Hogg *et al.* (2002a, 2005) qui ont montré que l'effet combiné des variations interannuelles de l'indice d'humidité (*Climate Moisture Index*; CMI) et des défoliations par la livrée des forêts expliquent principalement les variations de sensibilité moyenne chez le tremble. Une étude récente de Anyomi *et al.* (2014) montre également une baisse de la productivité du tremble avec des déficits en eau élevés à l'échelle continentale. En effet, au cours des années où les épisodes de défoliation sévère se produisent tôt en saison, des cernes de couleur pâle et de faible densité se forment (Hogg *et al.* 2002b; Hogg *et al.* 2005), et une croissance très réduite, voire nulle, est observée. Inversement, des conditions climatiques favorables tendent à réduire les variations interannuelles de croissance conduisant à des valeurs plus faibles de sensibilité moyenne. Ainsi, dans les régions avec un climat plutôt humide comme dans l'est du Canada, moins sujettes à des périodes de fortes sècheresses, on a tendance à observer des variations de croissances interannuelles moins fortes (homogénéité de la variation) que dans l'ouest du Canada.

Enfin, la diversité clonale réduite alors que le nombre cumulé d'années avec une sévère défoliation par la livrée des forêts augmente les niveaux de synchronicité de la réponse de croissance. Osierand Lindroth (2001) ont montré que la principale source de variation des performances de croissance (défoliation plus importante) par les chenilles du Bombyx disparate (*Lymantria dispar ;* un autre défoliateur du tremble) était le génotype de chaque tremble ayant des différents seuils de résistance à l'insecte (teneurs en composés phénoliques et métaboliques secondaires). Nous n'avons pas observé ce phénomène dans notre étude, probablement dû au fait que nous nous sommes concentrés juste sur les défoliations sévères (connues pour réduire la croissance des arbres (Moulinier *et al.* 2014)) qui pourraient affecter tous les individus d'un peuplement indépendamment de leurs génotypes respectifs. Par conséquent, les réponses de croissance étaient plus homogènes entre les tiges d'un même génotype qu'entre les tiges de génotypes différents. Un assemblage complexe de différents génotypes aura tendance à augmenter les différences de croissance entre les génotypes (réponse phénotypique pour ce trait) en réponse à une contrainte environnementale de faible intensité alors que les évènements sévères affecteront fortement et de manière uniforme tous les génotypes. Le maintien de la diversité des peuplements de peuplier permettrait de maintenir une grande variabilité de patrons et de réponse de croissance aux variations de l'environnement et pourrait favoriser la résilience des peuplements diversifiés sous les climats futurs.

Le tremble est une espèce qui livre petit à petit ses secrets. Longtemps laissé de côté et peu étudié en raison de sa facilité à se régénérer et son faible intérêt économique comparativement aux conifères, son écologie et sa dynamique n'est pas si simple. A travers cette thèse, les bases de la compréhension de l'organisation et des dynamiques génétiques commencent à être appréhendées. Ainsi, cette étude apporte des informations sur l'histoire démographique de cette espèce depuis la dernière glaciation mais aussi sur les différences génétiques observées à travers la forêt boréale et les liens avec la croissance.

5.2. Perspectives

Cette thèse constitue une étape importante pour une meilleure compréhension d'une espèce clé en forêt boréale. La possibilité de comparer différentes régions aux caractéristiques bioclimatiques et environnementales diverses grâce à un échantillonnage homogène est un atout. Nous avons ainsi pu caractériser précisément les variations de la structure génétique et clonale et répondre à des questions plus précises comme le rôle du climat et des perturbations sur cette dernière ou bien l'effet de la génétique en plus des variables environnementales sur la croissance radiale du tremble. Dans le futur, il serait bon de développer et d'utiliser une autre approche moléculaire grâce à la génomique et plus particulièrement les SNPs (polymorphisme nucléotidique) afin de valoriser un jeu de données riche de diversité et représentatif de l'ensemble de la forêt boréale nord-américaine. Cela pourrait permettre de répondre à des questions telles que : i) y a-t-il des SNPs sous sélection dans certaines zones de l'étude où les changements environnementaux et le dépérissement sont déjà forts (zone sud de la tremblaie-parc) ? ii) existe-t-il des gènes d'adaptation propres à des conditions environnementales définies (conditions climatiques, résistance aux insectes) ? Toutes ces questions pourraient être accompagnées d'un échantillonnage complémentaire dans la partie nord de l'aire de répartition où le peuplier faux-tremble sera amené à s'étendre en réponse aux changements climatiques. Cet échantillonnage complémentaire pourrait permettre de comprendre et de suivre précisément les dynamiques de colonisation du tremble et d'évaluer sur quels gènes la pression de sélection s'effectue. D'un point de vue plus pratique, il pourrait être important de comprendre plus précisément : i) quels facteurs gouvernent l'apparition et la persistance d'individus triploïdes dans le paysage ? ii) qu'apporte la triploïdie comme avantage pour le tremble (physiologie, compétition, croissance) ? iii) quel est le rôle de la triploïdie dans l'adaptation aux changements climatiques ? Ainsi de nombreux axes de recherche qui mériteraient d'être développés et exploités dans le futur. Il y a encore bien de nombreuses choses à découvrir concernant cette espèce.

BIBLIOGRAPHIE

Abbot RJ, Smith LC, Milne RI, *et al.* (2000) Molecular analysis of plant migration and refugia in the arctic. *Science* **289**, 1343-1346.

Ally D, Ritland K, Otto SP (2008) Can clone size serve as a proxy for clone age? An exploration using microsatellite divergence in *Populus tremuloides*. *Molecular Ecology* **17**, 4897-4911.

Anderson LL, Hu FS, Nelson DM, Petit RJ, Paige KN (2006) Ice-age endurance: DNA evidence of a white spruce refugium in Alaska. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 12447-12450.

Anderson LL, Hu FS, Paige KN (2011) Phylogeographic history of white spruce during the last glacial maximum: uncovering cryptic refugia. *Journal of Heredity* **102**, 207-216.

Anyomi KA, Lorenzetti F, Bergeron Y, Leduc A (2014) Stand dynamics, humus type and water balance explain aspen long term productivity across Canada. *Forests* **6**, 416-432.

Arnold TG (2002) Radiocarbon dates from the ice-free corridor. *Radiocarbon* **44**, 437-454.

Balloux F, Lehmann L, de Meeûs T (2003) The population genetics of clonal and partially clonal diploids. *Genetics* **164**, 1635-1644.

Beatty GE, Provan JIM (2010) Refugial persistence and postglacial recolonization of North America by the cold-tolerant herbaceous plant Orthilia secunda. *Molecular Ecology* **19**, 5009-5021.

Beaulieu J, Bousquet J (2010) Facteurs génétiques affectant la variabilité des cernes annuels chez les espéces arborescentes nordiques. In: *La dendroécologie: principes, méthodes et applications* (eds. Payette S, Filion L), pp. 137-163. Presses de l'Université Laval, Québec City, Québec, Canada.

Bengtsson BO (2003) Genetic variation in organisms with sexual and asexual reproduction. *Journal of Evolutionary Biology* **16**, 189-199.

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

Bergeron Y, Chen HYH, Kenkel NC, Leduc AL, Macdonald SE (2014) Boreal mixedwood stand dynamics: Ecological processes underlying multiple pathways. *The Forestry Chronicle* **90**, 202-213.

Bergeron Y, Leduc A, Harvey BD, Gauthier S (2002) Natural fire regime: a guide for sustainable management of the Canadian boreal forest. *Silva fennica* **36**, 81-95.

Brais S, Harvey BD, Bergeron Y, *et al.* (2004) Testing forest ecosystem management in boreal mixedwoods of northwestern Quebec: initial response of aspen stands to different levels of harvesting. *Canadian Journal of Forest Research* **34**, 431-446.

Brubaker LB, Anderson PM, Edwards ME, Lozhkin AV (2005) Beringia as a glacial refugium for boreal trees and shrubs: new perspectives from mapped pollen data. *Journal of Biogeography* **32**, 833-848.

Callahan CM (2012) Continental-scale characterization of molecular variation in quaking aspen. Master's Thesis, Utah State University, Logan, Utah, USA.

Callahan CM, Rowe CA, Ryel RJ, *et al.* (2013) Continental-scale assessment of genetic diversity and population structure in quaking aspen (*Populus tremuloides*). *Journal of Biogeography* **40**, 1780-1791.

Cheliak WM, Dancik BP (1982) Genetic diversity of natural populations of a clone-forming tree *Populus tremuloides*. *Canadian Journal of Genetics and Cytology* **24**, 611-616.

Cole CT, Anderson JE, Lindroth RL, Waller DM (2010) Rising concentrations of atmospheric CO2 have increased growth in natural stands of quaking aspen (*Populus tremuloides*). *Global Change Biology* **16**, 2186-2197.

Cooke BJ, Lorenzetti F (2006) The dynamics of forest tent caterpillar outbreaks in Québec, Canada. *Forest Ecology and Management* **226**, 110-121.

Cooke BJ, Lorenzetti F, Roland J (2009) On the duration and distribution of forest tent caterpillar outbreaks in east-central canada. *Journal of the Entomological Society of Ontario* **140**, 3-18.

Cornelius J (1994) Heritabilities and additive genetic coefficients of variation in forest trees. *Canadian Journal of Forest Research* **24**, 372-379.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001-2014.

Cumming SG, Schmiegelow FKA, Burton PJ (2000) Gap dynamics in boreal aspen stands: Is the forest older than we think? *Ecological Applications* **10**, 744-759.

de Lafontaine G, Turgeon J, Payette S (2010) Phylogeography of white spruce (*Picea glauca*) in eastern North America reveals contrasting ecological trajectories. *Journal of Biogeography* **37**, 741-751.

DeWoody J, Rowe CA, Hipkins VD, Mock KE (2008) "Pando" lives: molecular genetic evidence of a giant aspen clone in central Utah. *Western North American Naturalist* **68**, 493-497.

Drobyshev I, Gewehr S, Berninger F, Bergeron Y (2013) Species-specific growth responses of black spruce and trembling aspen may enhance resilience of boreal forest to climate change. *Journal of Ecology* **101**, 231-242.

Dyke AS, Moore A, Robertson L (2003) Deglaciation of North America. Geological Survey of Canada Ottawa, Ontario, Canada.

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology* **10**, 305-318.

Gendreau-Berthiaume B, Kneeshaw DD, Harvey BD (2012) Effects of partial cutting and partial disturbance by wind and insects on stand composition, structure and growth in boreal mixedwoods. *Forestry* **85**, 551-565.

Grant MC, Mitton JB, Linhart YB (1992) Even larger organisms. *Nature* **360**, 216-216.

Gray LK, Gylander T, Mbogga MS, Chen P-Y, Hamann A (2010) Assisted migration to address climate change: recommendations for aspen reforestation in western Canada. *Ecological Applications* **21**, 1591-1603.

Gylander T, Hamann A, Brouard JS, Thomas BR (2012) The potential of aspen clonal forestry in Alberta: Breeding regions and estimates of genetic gain from selection. *Plos One* 7, e44303. doi: 44310.41371/journal.pone.0044303.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907-913.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**, 247-276.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **359**, 183-195.

Hogg EH (1997) Temporal scaling of moisture and the forest-grassland boundary in western Canada. *Agricultural and Forest Meteorology* **84**, 115-122.

Hogg EH (2001) Modeling aspen responses to climatic warming and insect defoliation in western Canada, Proceedings of the Sustaining Aspen in Western Landscapes Symposium, 13-15 June 2000, Grand Junction, CO, USA, 325-337.

Hogg EH, Bernier PY (2005) Climate change impacts on drought-prone forests in western Canada. *Forestry Chronicle* **81**, 675-682.

Hogg EH, Brandt JP, Kochtubajda B (2002a) Growth and dieback of aspen forests in northwestern Alberta, Canada, in relation to climate and insects. *Canadian Journal of Forest Research* **32**, 823-832.

Hogg EH, Brandt JP, Kochtubajda B (2005) Factors affecting interannual variation in growth of western Canadian aspen forests during 1951-2000. *Canadian Journal of Forest Research* **35**, 610-622.

Hogg EH, Brandt JP, Michaelian M (2008) Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. *Canadian Journal of Forest Research* **38**, 1373-1384.

Hogg EH, Hart M, Lieffers VJ (2002b) White tree rings formed in trembling aspen saplings following experimental defoliation. *Canadian Journal of Forest Research* **32**, 1929-1934.

Hu FS, Brubaker LB, Anderson PM (1996) Boreal ecosystem development in the northwestern Alaska range since 11,000 yr BP. *Quaternary Research* **45**, 188-201.

Huang J-G, Stadt KJ, Dawson A, Comeau PG (2013) Modelling growth-competition relationships in trembling aspen and white spruce mixed boreal forests of western Canada. *Plos One* **8**, e77607. doi: 77610.71371/journal.pone.0077607.

Huang J-G, Tardif J, Denneler B, Bergeron Y, Berninger F (2008) Tree-ring evidence extends the historic northern range limit of severe defoliation by insects in the aspen stands of western Quebec, Canada. *Canadian Journal of Forest Research* **38**, 2535-2544.

Hyun JO, Rajora OP, Zsuffa L (1987) Genetic variation in trembling aspen in Ontario based on isozyme studies. *Canadian Journal of Forest Research* **17**, 1134-1138.

Jackson LE, Wilson MC (2004) The ice-free corridor revisited. *Geotimes* **49**, 16-19.

Jelinkova H, Tremblay F, DesRochers A (2009) Molecular and dendrochronological analysis of natural root grafting in *Populus tremuloides* (Salicaceae). *American Journal of Botany* **96**, 1500-1505.

Jelinski DE, Cheliak WM (1992) Genetic diversity and spatial subdivision of *Populus tremuloides* (Salicaceae) in a heterogeneous landscape. *American Journal of Botany* **79**, 728-736.

Kanaga MK, Ryel RJ, Mock KE, Pfrender ME (2008) Quantitative-genetic variation in morphological and physiological traits within a quaking aspen (Populus tremuloides) population. *Canadian Journal of Forest Research* **38**, 1690-1694.

Keller SR, Olson MS, Silim S, Schroeder W, Tiffin P (2010) Genomic diversity, population structure, and migration following rapid range expansion in the Balsam Poplar, *Populus balsamifera*. *Molecular Ecology* **19**, 1212-1226.

King GM, Gugerli F, Fonti P, Frank DC (2013) Tree growth response along an elevational gradient: climate or genetics? *Oecologia* **173**, 1587-1600.

Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology* **11**, 2445-2449.

Leonelli G, Denneler B, Bergeron Y (2008) Climate sensitivity of trembling aspen radial growth along a productivity gradient in northeastern British Columbia, Canada. *Canadian Journal of Forest Research* **38**, 1211-1222.

Li H, Wang X, Hamann A (2010) Genetic adaptation of aspen (*Populus tremuloides*) populations to spring risk environments: a novel remote sensing approach. *Canadian Journal of Forest Research* **40**, 2082-2090.

Little EL (1971) Atlas of United States Trees, Volume 1, Conifers and Important Hardwoods, pp. 9 p, 200 maps. U.S. Department of Agriculture, Forest Service, Washington, DC. Miscellaneous publication 1146.

Loehr J, Worley K, Grapputo A, *et al.* (2006) Evidence for cryptic glacial refugia from North American mountain sheep mitochondrial DNA. *Journal of Evolutionary Biology* **19**, 419-430.

Lund ST, Furnier GR, Mohn CA (1992) Isozyme variation in quaking aspen in Minnesota. *Canadian Journal of Forest Research* **22**, 521-524.

MacKenzie N (2010) Ecology, Conservation and Management of Aspen: A Literature Review. Scottish Native Woods (Aberfeldy). 41 pp.

Madritch M, Greene S, Lindroth R (2009) Genetic mosaics of ecosystem functioning across aspen-dominated landscapes. *Oecologia* **160**, 119-127.

Man R, Kayahara GJ, Rice JA, MacDonald GB (2008) Response of trembling aspen to partial cutting and subsequent forest tent caterpillar defoliation in a boreal mixedwood stand in northeastern Ontario, Canada. *Canadian Journal of Forest Research* **38**, 1349-1356.

Mandryk CAS, Josenhans H, Fedje DW, Mathewes RW (2001) Late Quaternary paleoenvironments of Northwestern North America: implications for inland versus coastal migration routes. *Quaternary Science Reviews* **20**, 301-314.

Mitton JB, Grant MC (1980) Observations on the ecology and evolution of quaking aspen, *Populus tremuloides*, in the Colorado Front Range. *American Journal of Botany*, 202-209.

Mitton JB, Grant MC (1984) Associations among protein heterozygosity, growth rate, and developmental homeostasis. *Annual Review of Ecology and Systematics* **15**, 479-499.

Mitton JB, Grant MC (1996) Genetic variation and the natural history of quaking aspen. *Bioscience* **46**, 25-31.

Mock KE, Callahan CM, Islam-Faridi MN, *et al.* (2012) Widespread triploidy in western North American aspen (*Populus tremuloides*). *Plos One* 7, e48406. doi:48410.41371/journal.pone.0048406.

Mock KE, Rowe CA, Hooten MB, Dewoody J, Hipkins VD (2008) Clonal dynamics in western North American aspen (*Populus tremuloides*). *Molecular Ecology* **17**, 4827-4844.

Moulinier J, Lorenzetti F, Bergeron Y (2014) Growth and mortality of trembling aspen (*Populus tremuloides*) in response to artificial defoliation. *Acta Oecologica* **55**, 104-112.

Namroud MC, Leduc A, Tremblay F, Bergeron Y (2006) Simulations of clonal species genotypic diversity - trembling aspen (*Populus tremuloides*) as a case study. *Conservation Genetics* **7**, 415-426.

Namroud MC, Park A, Tremblay F, Bergeron Y (2005a) Clonal and spatial genetic structures of aspen (*Populus tremuloides* Michx.). *Molecular Ecology* **14**, 2969-2980.

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

Osier TL, Lindroth RL (2001) Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. *Journal of Chemical Ecology* **27**, 1289-1313.

Peros MC, Gajewski K, Viau AE (2008) Continental-scale tree population response to rapid climate change, competition and disturbance. *Global Ecology and Biogeography* **17**, 658-669.

Petit RJ, Aguinagalde I, de Beaulieu J-L, *et al.* (2003) Glacial Refugia: Hotspots But Not Melting Pots of Genetic Diversity. *Science* **300**, 1563-1565.

Power MJ, Marlon J, Ortiz N, *et al.* (2008) Changes in fire regimes since the Last Glacial Maximum: an assessment based on a global synthesis and analysis of charcoal data. *Climate Dynamics* **30**, 887-907.

Régnière J, St-Amant R, Béchard A (2013) BioSIM 10 User's manual. Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec, QC, Canada. Information Report LAU-X-137E. ftp://ftp.cfl.scf.rncan.gc.ca/regniere/ software/BioSIM/

Romme WH, Turner MG, Gardner RH, *et al.* (1997) A rare episode of sexual reproduction in aspen (*Populus tremuloides* Michx.) following the 1988 Yellowstone fires. *Natural Areas Journal* **17**, 17.

Romme WH, Turner MG, Tuskan GA, Reed RA (2005) Establishment, persistence, and growth of aspen (*Populus tremuloides*) seedlings in Yellowstone National Park. *Ecology* **86**, 404-418.

Schreiber SG, Ding C, Hamann A, *et al.* (2013) Frost hardiness vs. growth performance in trembling aspen: an experimental test of assisted migration. *Journal of Applied Ecology* **50**, 939-949.

Shafer A, Cullingham CI, Cote SD, Coltman DW (2010) Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology* **19**, 4589-4621.

Stevens MT, Turner MG, Tuskan GA, *et al.* (1999) Genetic variation in postfire aspen seedlings in Yellowstone National Park. *Molecular Ecology* **8**, 1769-1780.

Turner MG, Romme WH, Tinker DB (2003) Surprises and lessons from the 1988 Yellowstone fires. *Frontiers in Ecology and the Environment* **1**, 351-358.

Whitfield PH, Cannon AJ (2000) Recent Variations in Climate and Hydrology in Canada. *Canadian Water Resources Journal* **25**, 19-65.

Williams JW (2003) Variations in tree cover in North America since the last glacial maximum. *Global and Planetary Change* **35**, 1-23.

Wyman J, Bruneau A, Tremblay MF (2003) Microsatellite analysis of genetic diversity in four populations of *Populus tremuloides* in Quebec. *Canadian Journal of Botany* **81**, 360-367.