COLONIZING NORTHERN LANDSCAPES: POPULATION GENETICS AND PHYLOGEOGRAPHY OF WOOD FROGS (*LITHOBATES SYLVATICUS*) IN THE JAMES BAY AREA

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ABSTRACT

The genetic structuring of populations can be influenced by present processes and past events. One of the largest historical events to affect the distribution and genetic characteristics of present-day North American biota is the Pleistocene glaciation. Thus, the study of post-glacial colonization patterns of species in northern landscapes can relay important ecological information, as species had to expand their range extensively following the retreat of the glaciers and are often at the terminal end of their expansion. These species consequently exhibit the genetic fingerprints of sequential founder events, in turn decreasing the genetic variation available for adaptation. Using amphibians to investigate post-glacial range expansion is advantageous, as they have limited dispersal abilities revealing fine-scale patterns and they are thought to be one of the first vertebrates to colonize post-glacial habitat. Therefore, to model the phylogeography of a primary colonizer and the population structure of anurans in northern landscapes, population genetics analyses of wood frogs (*Lithobates sylvaticus*) were performed in the James Bay area.

Wood frogs were sampled from 17 localities around James Bay and genetic analyses were conducted with seven microsatellite loci and mitochondrial DNA sequences of the ND2/tRNA^{TRP} genes. Results show that the post-glacial recolonization of the James Bay area by wood frogs originated from the putative refugium in western Wisconsin, an area known as the Driftless Area. Two routes were taken by founders to colonize the James Bay area: one north-west of Lake Superior, colonizing western Ontario, and one through the Upper Peninsula of Michigan, colonizing southern and eastern Ontario and western Québec. Interestingly, the meeting of the two lineages south-west of James Bay led to the establishment of a zone of higher genetic variation than expected under the founder effect hypothesis. Additionally, population structure analyses revealed the segregation of three genetic populations east, north-west, and south-west of the bay, the latter showing the highest genetic variation and likely representing a zone of secondary contact. This study shows that past events such as post-glacial range expansions can explain present patterns of genetic variation and population structure, and that studies in northern landscapes may be very useful in understanding genetic patterns throughout the range of a species.

RÉSUMÉ

La variabilité génétique des populations d'une espèce peut être influencée par des évènements actuels, qu'ils soient d'origine anthropique ou naturelle, ainsi que par des évènements historiques. Un des évènements historiques majeur qui a influencé la distribution de la faune et de la flore nord américaine aujourd'hui est sans nul doute la dernière ère glaciaire du Pléistocène. À la retraite des glaciers, les espèces survivantes ont pu sortir de leur refuge glaciaire et peupler les aires nouvellement disponibles vers le nord. Les paysages nordiques offrent une venue intéressante pour ces études de recolonisation car toutes espèces y vivant présentement ont dû agrandir leur distribution extensivement, en suivant de près la retraite des glaciers. Ces espèces sont généralement reconnues comme des colonisateurs primaires. Suite à la colonisation de nouveaux habitats et la création de populations par un petit nombre d'individus, l'espèce perd progressivement de sa variabilité génétique originale, un processus nommé effet fondateur. Les amphibiens sont d'excellents modèles pour étudier la colonisation post-glaciaire car ils ont une capacité de dispersion limitée, ce qui permet l'étude de l'expansion de leurs populations avec une plus grande résolution, et ils sont considérés comme colonisateurs primaires, étant les premiers vertébrés à peupler le nord à la suite de la retraite des glaciers. Donc, pour représenter la répartition des caractères génétiques d'un colonisateur primaire dans un paysage nordiques, la phylogéographie et la génétique des populations de grenouilles des bois (*Lithobates sylvaticus*) ont été étudiées dans la région de la Baie James.

Des grenouilles des bois de 17 localités de la région de la Baie James ont été échantillonnées pour déterminer le polymorphisme présent au niveau de sept marqueurs microsatellites et d'une séquence mitochondriale. Par ces analyses, il a été découvert que deux lignées provenant d'un refuge glaciaire de l'ouest du Wisconsin ont peuplé la région de la Baie James. Ces deux lignées, une entrant au Canada par une route à l'ouest du Lac Supérieur et une traversant la péninsule supérieure du Michigan, ont chacune colonisé une côte de la Baie James et se sont rencontrées dans la région de Moosonee, au sud-ouest de la baie. Cette zone de contact secondaire présente une plus grande variabilité génétique que le reste de la région et, d'une certaine manière, influence la structure des populations de la grenouille des bois autour de la baie. Trois populations génétiques ont été révélées, une à l'est, une au nord-ouest, et une au sud-ouest, cette dernière représentant la zone avec la plus grande variabilité génétique (zone de contact). Avec un faible flux génétique entre les trois populations génétiques, il se peut qu'il y ait une introgression limitée entre les lignées comme cela a déjà été suggéré pour des différents clades de la grenouille des bois. En conclusion, cette étude démontre l'importance d'inclure les évènements historiques dans une étude de génétique des populations et que les études dans des paysages nordiques contribuent à une bonne compréhension de la répartition actuelle des caractères génétiques d'une espèce.

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Andrée

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GLOSSARY OF SCIENTIFIC TERMS

Although other variants exist to these definitions, the terms described here will be used in this thesis according to the following definitions.

Admixture a composite genepool in which at least some individuals can trace ancestry to more than one population.

Bayesian analysis a method of statistical analysis beginning with prior probability distributions for the model parameters and updates based on observed data to arrive at a posterior probability distribution.

Bottleneck a large reduction in population size, often followed by a recovery.

Clade All lines of descendents from one refugium; separate refugia = separate clades.

Drift random change of allele frequencies due to stochastic events, particularly occurring in small populations.

Genetic distance extent to which groups of individuals differ from one another with respect to allele frequencies or DNA sequences at particular loci.

Haplotype DNA sequence that describes variants identified in mitochondrial DNA.

Lineage one line of descendants originating from a particular glacial refugium; all lineages sourcing from one refugium = a clade.

Locality an arbitrary grouping of individuals of a single species inhabiting a general area, not assuming equal chances of mating or close proximity between individuals.

Microsatellite interspersed repetitive DNA represented by short tandem repeats (in this case tri- and tetra-nucleotide) flanked by unique sequences and distributed throughout the genome; they generally show high levels of genetic polymorphism due to the addition or deletion of repeat subunits.

Mitochondrial DNA (mtDNA) the circular chromosome present in mitochondria; haploid, generally maternal in heritance with no recombination.

Null allele an allele that consistently fails to amplify to detectable levels via the polymerase chain reaction.

Phylogenetics study of the evolutionary relationship between lineages.

Phylogeography the study of principles and processes governing the geographic distribution in space and time of genealogical lineages; often used to interpret current geographic patterns of genetic variation in light of historical events, thus providing a temporal perspective to studies that invoke the influence of recent ecological changes as explanations for the current distribution of organisms.

Population a statistically-supported grouping of individuals into clusters with genetic similarities, or a supported grouping of individuals defined in other published articles. Please note that the use of the term *population* in scientific expressions such as *population genetics* or *population structure* has its own inherent meaning as a scientific term in the domain of genetic analyses.

[Glacial] refugium (plural = refugia) the location where a species survived the ice ages, which implies an area of suitable unglaciated habitat, allowing the survival of the refugees.

Single nucleotide polymorphism (SNP) a nucleotide position within a sequence that exhibits at least two distinct nucleotides among sampled individuals; an index of variability between sequences.

INTRODUCTION

Population genetics studies, including phylogeography, aim to describe the variations of allele frequencies between and within populations in space and time (Silva & Russo 2000; Arbogast *et al.* 2002; Zhang & Hewitt 2003; Evanno *et al.* 2005). The patterns discovered can be influenced by both present processes and past events (Hewitt 1999; Gaggiotti 2004). Molecular analyses, with selected DNA markers, provide tools to estimate the impact of processes ranging from gene flow, dispersal, divergence, genetic drift, and natural selection, to estimates of population size, reproductive success, mate choice, and the relative fitness of different genotypes (Bohonak 1999; Garnier *et al.* 2001; DeYoung & Honeycutt 2005; Islam *et al.* 2008; Lazrek *et al.* 2011). Past events can have significant impacts on the distribution of genetic diversity throughout a species' range this emphasizes the importance of studying the effects of historical events on population genetics (Hewitt 1999). For example, present genetic subdivisions between populations could be explained by past range expansions from different source populations (e.g. Nesbo *et al.* 1999). At the Last Glacial Maximum (LGM, 23 000-18 000 years ago; Hewitt 2004), North America was covered by ice as far south as 40°N including the Great Lakes (Hewitt 1996), dramatically affecting the spatial and temporal structure of biota (Webb & Bartlein 1992; Avise 2000; Shafer *et al.* 2010). North American biota were then restricted to a few ice-free locations that held some degree of suitable habitat, also known as glacial refugia (Holderegger & Thiel-Egenter 2009). Various locations were used as refugia by North American biota, depending on the species' previous distribution and habitat requirements (see inset in Figure 1 in Lee-Yaw *et al.* (2008) for a summary of the locations of putative amphibian refugia identified in the literature).

The Pleistocene Epoch is renowned for its rapid, severe, and relatively short climatic oscillations (Cox & Moore 2000; Hewitt 1996). With these oscillations, populations regularly went through local advances and retreats, where smaller population expansions and contractions were nested within larger cycles, driven by changes in climate (Hewitt 1996). During reversals, when temperatures dropped after a warmer period, expanding populations were eliminated so that only a few established populations survived in locally suitable locations. Successions of such events created multiple genetic structuring events, which altered genetic variability in the glacial refugia (Hewitt 1996). In addition, surviving refugial populations were restricted and often geographically isolated, resulting in genetic differentiation over time (Hewitt 1996; Shafer *et al.* 2010). Refugial genomes were thus created, allowing for divergent intraspecific clades. When a warmer phase led to another expansion, the surviving genomes persisted and spread, creating a shift in genomic occurrence.

Post-glacial range expansion

One of the largest climatic changes happened in the late Wisconsin to mid-Holocene (18 000 to 6000 BP), where conditions went from full ice age to full interglacial conditions (Hewitt 1996). As the mean temperature rose and the ice front made its final retreat, previously inhospitable habitat became open once more to colonizing species. As such, major range changes ensued as species either tracked suitable environment or were forced to adapt to or expand into new habitat (Coope 1994; Hewitt 1996, 2000). Abrupt decreases in population size (population bottleneck) occur when small numbers of colonizing individuals move away from their glacial refugium and found new populations (Amsellem *et al.* 2000). Due to the extreme reduction of population size and genetic drift, allelic frequencies in the newly founded populations are usually different from their ancestral refugial population and less common alleles have a higher probability of becoming extinct (Ibrahim *et al.* 1996; DeYoung & Honeycutt 2005). Thus, a tendency for loss of alleles is observed, reducing genetic diversity in the newly founded population (Maruyama & Fuerst 1984; Amsellem *et al.* 2000), a concept termed the founder effect

(Conner & Hartl 2004). Successive bottlenecks, through the creation of new populations further and further away from the refugium, generate a path of reduced genetic variation. Populations that remain in the refugial vicinity most often present a larger genetic pool than populations that went through extensive colonization, making refugial populations and putative colonization routes somewhat traceable through population genetics studies (Bernatchez & Dodson 1991; Avise 2000; Hewitt 2004; Petit 2011).

This genetic pattern can be observed in many organisms from widely different taxa, where the populations inhabiting post-glacial regions exhibit reduced genetic diversity as compared to populations of the same species occurring in an area where they have been established for longer periods of time (Merilä *et al.* 1997; Hewitt 2001; Haase *et al.* 2003; Garnier *et al.* 2004; Canestrelli *et al.* 2006; Bryja *et al.* 2010). For instance, most previously glaciated regions of North America have been colonized by species expanding their range northward since the Pleistocene (Conroy & Cook 2000; Hewitt 1999, 2000). Genetic variation was thus gradually reduced through a series of founder events, creating a cline of decreasing genetic diversity from low to high latitudes (Sage & Wolff 1986; Cwynar & MacDonald 1987; Beatty & Provan 2011). Therefore, northern populations are expected to have reduced levels of genetic variation when compared with southerly conspecific populations (Sage & Wolff 1986; Merilä *et al.* 1997; Soltis *et al.* 1997; Conroy & Cook 2000).

Historical events at the population level, such as range expansions and colonization events, have important impacts on gene flow and can create diagnostic geographic associations (Templeton 1998). For example, the colonizing strategy of the species will affect the anticipated pattern of gene flow (Petit 2011); rapidly expanding species will differ in genetic pattern when compared to slowly expanding species (Hewitt 1996, 2000). For example, if a species is slowly extending its range via a gradual moving front, then a higher proportion of ancestor alleles would be retained with less genome divergence among populations (Ibrahim *et al.* 1996; Hewitt 2000, 2001; Poljak *et al.* 2010). On the other hand, rapidly expanding species establishing populations abruptly in a new geographic region through long-distance colonization (leptokurtic dispersal) would have a decreased probability of retaining a high proportion of the alleles found in the source population, with derived genomes spread over large areas of the colonized range (Hewitt 1996, 2000; Ibrahim *et al.* 1996). These different strategies can affect a species' genetic composition over its area of distribution. Further studies concerning the dispersal capabilities and the population dynamics of the expanding species will provide additional insights.

Secondary contact zone

Increases in genetic diversity within a population can happen through mutation, recombination during sexual reproduction, or through gene flow (Hansson *et al.* 2000). Intraspecific introgression at a secondary contact zone between colonizing clades coming from different refugia during post-glacial recolonization provides evidence of the latter. This type of admixture may contribute to the adaptive potential of colonizing populations by creating novel genotypes with new combinations of traits, masking deleterious mutations, and even counteracting inbreeding depression that is often observed in isolated or small populations (Keller & Waller 2002; Petit *et al.* 2003; Verhoeven *et al.* 2010). Thus, secondary contact zones may help colonizing species by increasing their adaptability to new environments (Petit *et al.* 2004; Cosacov *et al.* 2010). An example of this is seen in lake trout (*Salvelinus namaycush*), where the increase in genetic variation created by the secondary contact of separate clades coming from multiple refugia enabled the colonizing front to adapt to new environments and to evolve into its present day distribution (Wilson & Mandrak 2004). However, there may be negative consequences of admixture. Depending on the specific dynamics between the species and its environment, admixture could hinder

locally adapted traits present in the established population, altering the fitness consequences of the introgressed individuals (Crémieux *et al.* 2010; Verhoeven *et al.* 2010). Crémieux *et al.* (2010) reported decreased fitness of a local perennial herb in cases where maladapted individuals from distant or ecologically distinct environments hybridized with local populations. These two opposing outcomes of admixture demonstrate the importance of historical and evolutionary studies as they might eventually help explain contemporary genetic patterns and guide conservation and reintroduction programs.

Ecology and genetics in northern landscapes

With their harsh climate, short aestival seasons, vast areas, and remoteness, logistical and financial challenges make the high latitudes of the northern hemisphere quite hazardous and complex to explore. These challenges make the acquisition of baseline genetic data for certain species or populations difficult. However, genetic studies in northern landscapes, although limited in numbers, can reveal interesting ecological insights about species and their histories (Gulve 1994; Conroy & Cook 2000; Moore *et al.* 2011). They could produce crucial information about a species' ecology such as its adaptive potential (e.g. Janhunen *et al.* 2010; Rossetto *et al.* 2011), its population dynamics (e.g. Dalén *et al.* 2006; Bush *et al.* 2011), and often its post-glacial history (e.g. McLeod & MacDonald 1997). Additionally, northern landscapes offer the opportunity to study species in relatively pristine habitat. Attributes of populations in undisturbed settings can be documented and subsequently compared with studies in other areas concerning the effects of fragmentation and anthropogenic activities on species distribution and population structure (e.g. Pechmann & Wilbur 1994; Seppä & Laurila 1999; Moore *et al.* 2011). Most importantly, research in northern landscapes is essential because of their global significance with regards to the storage of soil carbon as peat and in view of their potential response to increased temperatures, making them one of the most sensitive ecozones to environmental disturbances (Bridgham *et al.* 1995). With climate and land-use change, increased human presence, and increased resource exploitation, northern ecosystems may be at risk. Increasing our knowledge of the interactions between species' genetics and northern landscapes could help formulate land-use protocols and support climate change initiatives.

In theory, northern taxa are primary colonizers, assumed to be the earliest species to reinvade previously glaciated terrain, closely following the retreating edge of the glaciers (Hewitt 1999; Lee-Yaw *et al.* 2008). Under this assumption, northern populations should present markedly different phylogeographic patterns than more southern populations and potentially different dispersal strategies. For instance, under the assumption that primary colonizers are fast expanding species with long-distance dispersal capabilities (Holman 1992; Placyk *et al.* 2007; Lee-Yaw *et al.* 2008), northern taxa would have expanded their range following glacial retreat through a succession of founder events coupled with exponential population growth (Nichols & Hewitt 1994), which eventually would have created increasingly high levels of genetic homogeneity in the North (Hewitt 1996). Furthermore, colonizing lineages are often at the terminal point of their post-glacial expansion in northern landscapes. Therefore, many northern species are at the periphery of their distribution. Since they are furthest away from their source due to range expansions, these populations usually have limited potential to adapt to novel environments because of reduced genetic variation availability (Verhoeven *et al.* 2010). Peripheral populations are also more prone to extirpation due to stochastic or catastrophic demographic events (Pechmann & Wilbur 1994; Lesica & Allendorf 1995). Additionally, they are predicted to be at higher risk than central and continuous populations because of the quality and isolated character of their habitat (Lesica & Allendorf 1995). Peripheral populations habitually live in sub-optimal habitat conditions, requiring a high percentage of the individual's metabolic energy, which can reduce growth and limit reproduction to a critical limit (Hewitt 1996). Consequently, these induced stresses can structure

populations by limiting dispersal capabilities, which subsequently increases geographic isolation, reduces effective population size, and promotes genetic drift. As a result, genetic diversity decreases and populations may exhibit greater differentiation.

History and ecology of the James Bay area

The Ontario coast of James Bay is considered to be located near the centre of glaciation for the Pleistocene ice-sheets (Martini 1981b) and, since the retreat of the glaciers 8-7000 years BP, James Bay's coasts have been rebounding relatively fast in response to the glacio-isostatic adjustment (73 cm per century, suggested by 25 years of records, Barnett 1966; Webber *et al.* 1970). In fact, James Bay may represent the site of maximal recorded glacial depression in North America (Barnett 1966). With the retreat and subsequent melting of the glaciers, this depression filled with melt water and the Tyrrell Sea was created 7600 years BP. Hence, the James Bay area was covered from approximately 7600 years BP to 3500 BP by the Tyrrell Sea (Martini *et al.* 1979; Martini 1981a; Dyke 2004). Thus, with post-glacial succession, colonization of the James Bay area by plant and animal species is relatively recent.

The climatic conditions in the James Bay area are quite unique relative to its latitude. The presence of the world's largest northern inland sea (made of Hudson Bay and James Bay) permits the descent of polar air masses without obstruction and conveys to the area a subarctic to boreal climate, weather conditions usually seen at higher latitudes (Badzinski *et al.* 2001; Desroches *et al.* 2010; Macdonald & Kuzyk 2011). As a consequence, the continuous permafrost zone dips to the lowest latitudes in the world north-west of James Bay (Martini 1986). In fact, a cline from southern forested permafrost-free areas to a northern tundra area with continuous permafrost is observed around the bay, inferring varying geomorphological conditions to habitat in north-eastern Ontario and north-western Québec (Martini 1981b).

Most of the area covered by this research project falls within the Hudson Bay Lowlands, one of the largest wetlands of the world (Martini 1981b). These lowlands, as their name implies, are comprised of low-lying plains that are poorly drained, forming patterns of intricately linked wetlands throughout north-eastern Manitoba, Ontario's Far North, and north-western Québec (Hutton & Black 1975). Most of the surface is covered by marine clay deposited in the time of the Tyrrell Sea and thus tends to have poor drainage (Hutton & Black 1975). Extensive peat bogs and muskegs cover the clay, while string and quaking bogs are gradually filling lakes and ponds all over the area (Hutton & Black 1975). Alternatively, the north-east coast of James Bay is categorized under the Taiga Shield ecozone (Wiken *et al.* 1996). The landscape displays ample evidence of glacial history with eskers, drumlins, and copious numbers of lakes and wetlands filling in the millions of depressions left by the advancing and retreating glaciers (Wiken *et al.* 1996). Within 20 – 100 km from the bay, peatlands are numerous, with tamarack (*Larix laricina*) and black spruce (*Picea mariana*) dominating the tree layer. Jack pines (*Pinus banksiana*) are also very abundant around eskers and forest fires are quite common in the area (Wiken *et al.* 1996). Majors rivers segregate the coast and are opportunistically dammed for hydroelectric purposes (Hydro-Québec 2011). With the construction of the James Bay Road from Matagami to Radisson in the 1970s, human influences on the landscape are slightly more present on the Québec coast than on the Ontario coast (Muma 2009). Socio-economically, the James Bay region is increasingly receiving attention because of mining exploration, hydroelectric projects, tourism, and community development. This increase in anthropogenic activities underlines the importance of ecological research such as environmental impact assessments and the acquisition of ecological baseline data, where population genetics would help in describing the genetic health and evolutionary potential of species.

An amphibian species to study primary colonization in northern landscapes: the wood frog (Lithobates sylvaticus)

Amphibians in northern landscapes present an interesting opportunity for study; they are considered one of the earliest vertebrates to colonize post-glacial land, making them primary colonizers of northern landscapes (Holman 1992). Amphibians also possess certain characteristics that make them ideal for population genetics and phylogeographical research projects. First, they are relatively easy to sample and they have a complex life cycle that exploits land and water habitats that often includes ontogenetic trophic level shifts (Diamond 1996). This means that they may serve as early warning signs to the degradation of their terrestrial and aquatic environments (Blaustein *et al.* 1994; Diamond 1996), which may affect populations genetically through the addition of ecological barriers. Second, compared to other vertebrates, amphibians are relatively poor dispersers and highly philopatric, which allows for high population structuring (Beebee 2005). They may provide important details with regard to the ecology of the landscape at both small and large scales (Austin *et al.* 2002; Beebee 2005). However, their poor dispersal capabilities also increase their vulnerability to drastic changes in population size (Blaustein *et al.* 1994; Seppä & Laurila 1999), emphasizing the importance of genetic research for conservation and genetic rescue programs (Beauclerc *et al.* 2010). Finally, amphibians are undergoing major global declines, with 1910 of the planet's 6312 known amphibians in danger of extinction (IUCN 2011). With 41% of amphibian species threatened and few conservation efforts (Hoffmann *et al.* 2010), population genetic research opportunities are numerous and in high demand.

Fig. 1 Wood frog caught on Akimiski Island, NU, June 15th, 2009.

The James Bay area houses one of the most widespread amphibian species in North America: the wood frog (*Lithobates sylvaticus*). Wood frogs are one of the best ecologically-described species of anuran (e.g. Storey & Storey 1984; Berven 1990; van Buskirk & Relyea 1998; Donald *et al.* 2011; Gahl *et al.* 2011). Additionally, in light of their freeze tolerance (McNally *et al.* 2002) and their use of a wide array of habitats from forested patches to vernal ponds (Berven 1982; MacCulloch 2002; Rubbo & Kiesecker 2005), they are abundant and widespread in the James Bay area. This facilitated sampling and permitted a comprehensive study of the genetic makeup of the species' populations throughout the study area. Thus, to model the phylogeography of a primary colonizer and the structure of amphibian populations in northern landscapes, genetic analyses of wood frog populations in the James Bay area were performed. This study required the use of a focal species whose post-glacial range expansions closely followed the retreat of the glaciers. The wood frog is ideal for this purpose, as it is thought to be among the first herpetological species to colonize northern landscapes following glacial retreat (Holman 1992). The wood frog is a small ranid (3.5 - 7 cm in length for adults), easily recognizable by its dark mask extending

backward from the eye (Figure 1), and it is the most widespread frog species in Canada, its range extending farther north (past the Arctic Circle) than any other North American amphibian or reptile (Figure 2; Conant & Collins 1998). The average radius of the genetic neighbourhood of wood frogs has previously been documented at 1266 ± 170 m (Berven & Grudzien 1990). Through dispersal, wood frogs could thus potentially sustain enough gene flow between populations within a 1000 m radius to inhibit local differentiation (Berven & Grudzien 1990). However, adult wood frogs are known to be highly philopatric, the juvenile stage being mostly responsible for among population migrations and gene flow (Berven & Grudzien 1990; Meier 2007). This permits and facilitates genetic analyses with nuclear and mitochondrial markers, as the lack of sex-biased dispersal in wood frogs (Berven & Grudzien 1990) would not skew mitochondrial results towards a matrilineal model and patterns discerned by both types of markers can be compared. Furthermore, due to its dispersal capabilities, the wood frog exhibits leptokurtic dispersal during range expansions (Lee-Yaw *et al.* 2008), behaviour that is likely to lead to a loss of genetic variation during colonizing events towards northern landscapes (Ibrahim *et al.* 1996; Latch *et al.* 2009).

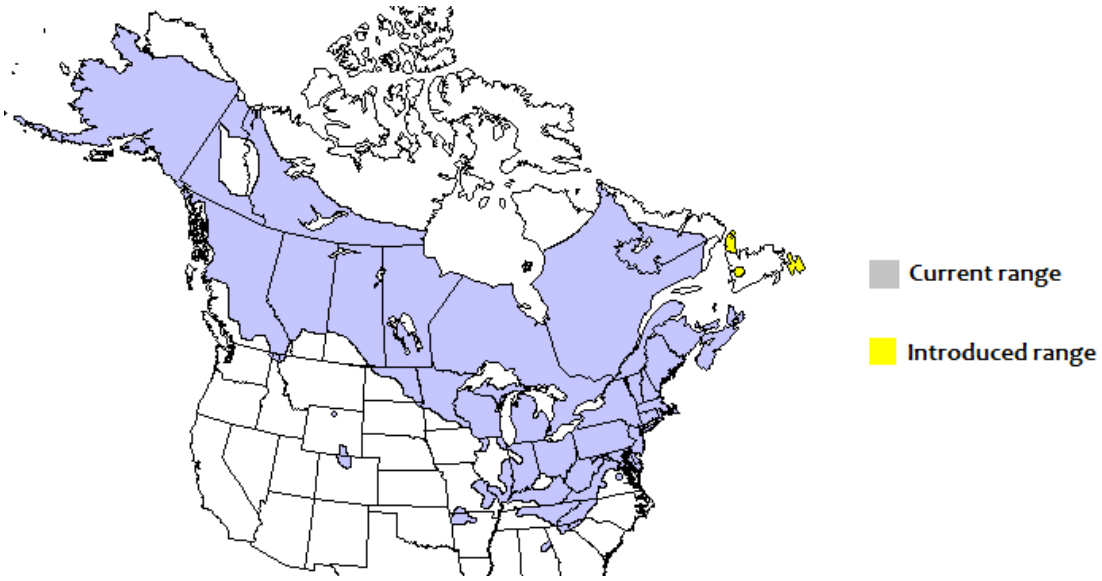


Fig. 2 Current estimated range of the wood frog (*Lithobates sylvaticus*) delineated by blue shading (USGS 2006).

In the provinces of Ontario and Québec, the coasts of James Bay and Hudson Bay act as the northern and north-western barrier, respectively, to species dispersal. Thus, wood frogs living in this vicinity are at the periphery of their distribution. Post-glacial range expansion in the wood frog is known to have occurred from multiple glacial refugia (Lee-Yaw *et al.* 2008). Ontario was populated by a wood frog clade originating from a refugium south of Lake Superior and west of Lake Michigan (the Western Clade from the western Wisconsin refugium; Lee-Yaw *et al.* 2008), whereas most of Québec was populated by a clade that expanded from a refugium located near the west border of the state of Maryland (the Eastern Clade from the northern Appalachian refugium; Lee-Yaw *et al.* 2008). Interestingly, Lee-Yaw *et al.* (2008) suggested the presence of a secondary contact zone between these two clades in western Québec, but no samples were taken from this area and the geographic location of such a contact zone was not confirmed. If a contact zone is discovered in the James Bay area, increased genetic variation would be expected in the region through admixture. However, if James Bay wood frog populations originate from one refugium only, phylogeographic analyses would place the most recent common ancestor of both Ontario and Québec James Bay coasts just south of the bay.

With the extent and scale of their colonization range, a gradual decline of genetic diversity is expected from the southern coast to the northern coast of James Bay by founder effect. Since James Bay acts as a barrier to the longitudinal dispersal of Ontario and Québec's Far North wood frogs, a certain degree of structure is expected between the two coasts, especially if they were populated by clades from different refugia. Considering the scale of the study area (close to 600 000 km^2) relative to the dispersing capabilities of the study species, gene flow patterns are also expected to follow an isolation-by-distance model. Reduced gene flow around the bay, and other genetic or physical barriers, have the potential to increase population structure and promote local differentiation. As considerable morphological variation is observed throughout the wood frog's geographic range (Berven 1982), northern populations exhibiting a higher prevalence of a light mid-dorsal stripe (Schueler & Cook 1980; Desroches *et al.* 2010), proportionally shorter hind limbs (Conant & Collins 1998), and differential tadpole labial tooth morphology (Altig 1970), this could potentially reveal signs of divergence. However, environmental influences may override underlying genetic differences between latitudes as the species could be expressing phenotypic plasticity, such that individuals bear traits that allow them to complete their life cycles in a wide array of biotic and abiotic conditions (Marshall & Jain 1968; Laugen *et al.* 2003).

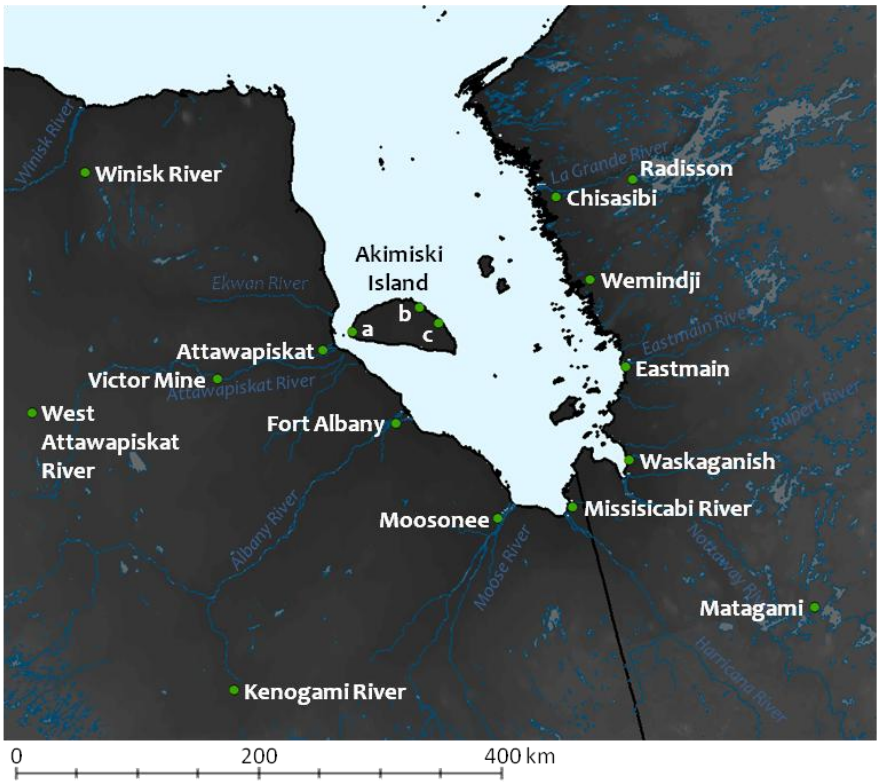
The aim of this study was to combine information on polymorphisms in nuclear and mitochondrial DNA to reveal how post-glacial range expansion has shaped the genetic diversity and structure of wood frog populations in the James Bay area. In particular, the role of the founder effect and admixture in shaping genetic patterns following a northward range expansion were evaluated. First, the amount of genetic variation in wood frog populations around James Bay and the degree of structuring between them was estimated using microsatellite markers in order to study the patterns of gene flow throughout the study area, including Akimiski Island. Second, the post-glacial history of the James Bay populations was investigated by reconstructing the colonization routes out of refugia using mitochondrial DNA.

Therefore, the tested hypotheses for this study were:

1. through founder effects, a reduction in genetic diversity will be correlated with a latitudinal increase;
2. due to the dispersal ability of wood frogs and the relative homogeneity of the landscape, the genetic structure of populations will follow a pattern of isolation-by-distance;
3. a contact zone or a divergence point will be observed south of the bay, depending on the refugial origin of the colonizing populations.

Field capture methods

Individuals were caught either using a small dipnet or by hand. To minimize the chance of spreading amphibian diseases from one individual to another, the frogs were manipulated with non-powdered gloves which were flipped inside-out or changed between each individual. All equipment (boots, nets, and other gear used) was disinfected with a solution of 15% bleach between sampling sites. When adults or metamorphs could not be caught, tadpoles and eggs were collected. In such cases, one fertilized egg per mass and tadpoles from different parts of the pond were sampled to minimize the probability of collecting siblings. For adults and metamorphs, a toe clip was taken for DNA analyses (Laurentian Animal Care protocol #2009-03-04), while the whole body was kept for tadpoles. Tissue samples were preserved in 95% ethanol and then stored at -20°C until laboratory analysis.



Akimiski Island localities:
a – Akimiski Island West Point
b – Akimiski Island Research Area
c – Akimiski Island East

Fig. 3 Study area and sampled localities around James Bay. Map drawn with ArcGIS 10.

Sampling and DNA extractions

The DNA material used in this study was extracted from 463 wood frog tissue samples collected from 17 localities around James Bay (Figure 3). All 463 samples were genotyped for nuclear microsatellites while only 76 were used for sequencing. DNA was extracted from toe clips, tadpole tail pieces, and egg nucleus using the DNeasy Blood and Tissue kit from Qiagen according to the manufacturer's instructions. To ensure that an adequate amount of DNA was extracted, the resulting extractions were quantified for nucleic acids using Thermo Scientific's NanoDrop 8000 spectrophotometer. Extractions were then diluted to 5 ng μL^{-1}, with the exception of samples comprising less than 10 ng μL^{-1}.

Nuclear DNA microsatellites, PCR conditions, and genotyping

Nine previously developed specie specific microsatellite loci were chosen for genetic variation and population structure analyses: RsAAT182, RsyD88, RsyD70, RsyD55, RsyD40, RsyC63, RsyC41, RsyC23, and RsyC11. These loci were selected from Newman & Squire (2001; RsAAT182) and Julian & King (2003; all others) after the initial screening for allele polymorphism and adequate amplification. These nine loci were amplified using a polymerase chain reaction (PCR) protocol modified from Crosby *et al.* (2009). Amplifications started with initial denaturation at 94°C (45 s), followed by 35 cycles of denaturation at 94°C (45 s), annealing at 52°C (RsyC70, RsAAT182, and RsyD40), 54°C (RsyD55, RsyC23, and RsyC63), or 55°C (RsyD88, RsyC41, and RsyC11) (45 s), extension at 72°C (30 s) and a final elongation step at 72°C (5 min). Amplification products were pooled together according to annealing temperature, forming a post-PCR triad (3.75 µL of PCR product from each microsatellite amplification, completing the volume to 15 µL with PCR-grade water), enabled by the use of forward primers labelled with the distinct fluorescent dyes FAM, NED, VIC, and PET (Applied Biosystems) within each triad (see Table 1). Pooled products were sent to Génome Québec Innovation Centre at McGill University in Montréal for the microsatellite genotypes to be resolved.

Table 1 Microsatellite characteristics and multiplexing arrangements.

Annealing temperature (°C)	Microsatellite locus	Published allelic lengths	Fluorescent dye
52	RsyD70	140-360	FAM
	RsAAT182	81-136	VIC
	RsyD40	145-360	NED
54	RsyD55	150-250	FAM
	RsyC23	185-245	NED
	RsyC63	125-255	PET
55	RsyD88	110-240	FAM
	RsyC41	80-135	VIC
	RsyC11	105-185	NED

Amplification and sequencing of the mitochondrial DNA control region

A 650-bp region of the NADH dehydrogenase subunit two and transfer RNA TRP mitochondrial genes (ND2/$tRNA^{TRP}$) was amplified in 76 selected individuals using the primers L4882b (Macey *et al.* 2000) and H5532 (Macey *et al.* 2001). This mitochondrial region has previously been shown to be appropriate for phylogeographic analyses in the wood frog (Lee-Yaw *et al.* 2008). Individuals chosen for sequencing were selected according to their geographic location and to the type of tissue that was processed (*i.e.* toe clips, tadpole tail pieces, or egg nucleus), to cover the entirety of the studied area with on average four samples by locality (see below). Amplification reactions were run with a total volume of 25 µL (2-10 ng of template DNA, 1x PCR buffer, 1.5 nM of $MgCl_2$, 0.6 µM of each primer, 0.2 mM of dNTP mix, and 0.5U of Taq DNA polymerase) for 35 cycles of 45 s at 94°C, 30 s at 56°C, and 60 s at 72°C, with an initial denaturing step of 2 min at 94°C and a final elongation step of 10 min at 72°C. Samples were sequenced at Génome Québec Innovation Centre at McGill University with Applied Biosystem's 3730xl DNA Analyzer technology.

Data analysis

Inference of population genetics characteristics using microsatellite markers

To compare the genetic characteristics present in the different localities around James Bay, each of the 463 samples were assigned to one of the 17 geographic regions. Hereafter, the term “locality” is

used instead of the term "population" (see glossary) as such groupings do not necessarily represent true genetic or demographic populations in the James Bay area (see below); some of the individuals within the same locality are separated by extensive geographic distances and might not represent a single breeding population.

MICRO-CHECKER 2.2.3 (van Oosterhout *et al.* 2004) tested for the presence of null alleles and scoring errors, while ARLEQUIN 3.5.1.2 (Excoffier & Lischer 2010) was used to test for linkage disequilibrium (LD) between pairs of loci within each locality and deviations of loci from Hardy-Weinberg equilibrium (HWE). At this stage, two microsatellite loci (RsyD70 and RsyC23) deviated strongly from HWE at all localities and were thus removed from further analysis (see below). Genetic diversity throughout the 17 localities was quantified by the number of alleles (Na) present in each locality and locus, the observed (H_o) and expected (H_e) heterozygosities, as well as by the unbiased expected heterozygosity (UH_e) for each locality using GENALEX 6.3 (Peakall & Smouse 2006). The inbreeding coefficient (F) and its significance were calculated with FSTAT 2.9.3.2 (Goudet 2002). Given the variability in the numbers of individuals collected per locality, genetic diversity was also assessed using allelic richness and private allelic richness through rarefaction calculated in HP-RARE (Kalinowski 2005) to correct for sample sizes. The geographic partitioning of microsatellite genetic variation at different hierarchical levels was tested with an AMOVA in ARLEQUIN. While only six loci met the accepted level of five percent of missing data per locus, an AMOVA on all loci recovered similar results and thus all seven loci were used for calculations.

To infer dispersal patterns exhibited by James Bay wood frogs, the genetic structure of each locality was examined through estimates of the fixation index (F_{ST}), through isolation-by-distance analysis, and finally with Bayesian clustering algorithms. Pairwise genetic distances (F_{ST}) between all 17 localities were calculated with FSTAT. Isolation-by-distance was assessed throughout the study area by testing the correlation between pairwise genetic and geographic distance using the regression of F_{ST} $(1-F_{ST})^{-1}$ versus the natural logarithm of geographic distances between localities, as suggested by Rousset (1997). For geographic distances, the shortest distance that crossed the least volume of salt water (continental distances) between each locality around the bay was measured using the distance tool in MAPSOURCE 6.13.7 (Garmin Ltd. 1995-2008). Isolation-by-distance was also tested within each cluster of localities identified according to the results of the clustering analysis (see below) with the same procedure. All isolation-by-distance tests and calculations were performed with GENALEX.

Defining a population's geographic boundaries can be problematic and requires extensive ecological studies *in situ* (Berryman 2002). Population geneticists mitigate this problem by defining populations according to the genetic relatedness of their individuals (genetic population). Clustering methods currently provide the best option available to tackle this challenge (Manel *et al.* 2005). Thus, to infer spatial genetic structure of the species in the study area, clustering was realized with TESS 2.3.1 (Chen *et al.* 2007). TESS uses a Bayesian clustering algorithm based on spatial networks between individuals to assign them to a maximal number of population clusters (K). The program also allows the defining of spatial priors for individuals within a network, permitting the modification of their neighbourhood system (Chen *et al.* 2007). The model without admixture with the neighbour-joining algorithm was run *a priori*, to provide an upper bound on the number of clusters in the data, with 20 replicates of 50 000 periods with 20 000 burn-in points, for K=2 to 20 (since it was not certain if each of the 17 localities could be considered as a genetic entity or if they were subdivided or grouped) and with a spatial interaction parameter of 0.6. Pritchard *et al.* (2009) recommended running the model without admixture before the model with admixture because it discerns if individuals are discretely from one population or another and it is often more powerful than the admixture model at detecting subtle

structure. Alternatively, individuals may have mixed ancestry. Therefore, the admixture model was subsequently run to determine the presence of clines (Durand *et al.* 2009). The Convolution Gaussian prior for spatial admixture (BYM) model was run with a linear degree of trend, up to the value of (K+1) initially determined by the model without admixture. Starting from the Neighbour-Joining tree, 20 repetitions of 100 000 sweeps and 30 000 burn-in points were run. In addition, a ACSII-Raster map was input to inform the program which areas were of suitable habitat. Chen *et al.* (2007) suggested that combining analyses using TESS and STRUCTURE was advantageous to infer spatial genetic structure of populations and locating discontinuities in allele frequencies. TESS ranks higher for correctly estimating the number of genetic populations and for identifying recent migrants but STRUCTURE is more efficient at assigning individuals in high geographical admixture and detecting clinal variations (Chen *et al.* 2007). Thus, the program STRUCTURE 2.3.1 (Pritchard *et al.* 2000) was used as well to obtain additional insights into patterns of gene flow around the study area and to confirm the number of genetic populations (clusters – K). The individuals were assigned to different clusters with five iterations of 500 000 sweeps with 100 000 burn-in points, testing for multiple K values, to insure that no subgroupings were missed. The resulting log-likelihoods (Ln P(D)) of each run were then compared for each K.

Calculations of HWE-sensitive indices

Two of the nine microsatellite loci were excluded from the analyses because of large and significant departures from HWE, but all seven remaining loci deviated slightly from HWE. Without the assumptions of HWE being met, some measures of population structure could be erroneous. Chapuis & Estoup (2007) observed that the presence of null alleles can significantly increase the apparent structure between populations. Analyses such as F_{ST}, AMOVA, and Bayesian clustering are often sensitive to deviations from HWE and thus extra efforts are needed to confirm the effect of this disequilibrium on the subsequent analyses (Schoville *et al.* 2011). In the case of F_{ST} measures, FSTAT software enables tests for genetic structure that does not assume HWE within samples (log-likelihood G; Goudet *et al.* 1996). Therefore, these values were used to assess the connectivity between each pair of localities and, subsequently, to assess isolation-by-distance. As for the other HWE-sensitive analyses, jackknifing across loci was used to verify the weight of the disequilibrium (Morin *et al.* 2009). Identical structuring patterns were recovered throughout the analyses. Consequently, the analyses proceeded with the seven chosen loci.

Phylogeographic analysis using nucleotide polymorphisms in a mitochondrial DNA sequence

To assess the history of the wood frog's range expansion in the study area in more detail, their phylogeographic relationships were investigated using single nucleotide polymorphisms (SNPs) in the ND2/tRNA^{TRP} mitochondrial genes. Sequences were aligned and manually edited using CODONCODE 3.7.1 (CodonCode Corporation 2011), and subsequently, the number of unique haplotypes was determined by COLLAPSE 1.2 (Posada 2006). Since populations closer to glacial refugia are expected to exhibit elevated levels of genetic diversity relative to other populations (Hewitt 1996, 2000; Provan & Bennett 2008), gene diversity ($\hat{H}$, the estimated probability that two haplotypes randomly chosen from a site are different) and nucleotide diversity (π, average proportion of nucleotide differences between all possible pairs of two randomly chosen sequences within each locality) were calculated for each locality. In addition to these values, the number of polymorphic sites (S), and the mean number of pairwise differences between haplotypes (d) were calculated using DNASP 5.10 (Librado & Rozas 2009). A haplotype network was estimated with a statistical parsimony algorithm in TCS (Clement *et al.* 2000). To determine the number and source of colonizing lineages present in the study area, several phylogenetic trees were built using the Maximum Likelihood (ML) and the Neighbour-Joining (NJ) inference methods implemented in MEGA5 (Tamura *et al.* 2011). These different methods were used to infer genealogical

relationships using different algorithm, giving greater confidence in results. The NJ algorithmic method groups haplotypes according to a distance matrix of pairwise differences between the sequences while ML uses a character-based model to compare characters at each site in the alignment (Hall 2011). Technically, ML methods tend to be slightly more efficient at recovering a tree closer to the true topology, but when the assumptions associated with the ML method are not met, the NJ method performs slightly better (Tateno *et al.* 1994), hence the use of both tree-building methods in this study. ML and NJ trees using the haplotypes from James Bay allowed the definition of phylogroups within the study area. *Lithobates catesbeiana* and *L. septentrionalis* (Genbank accession numbers EF122833 and AY206487, respectively) were used as outgroups.

James Bay haplotypes were also compared to haplotypes from two identified clades (the Western and Eastern Clades, GenBank accession numbers listed in Appendix A; Lee-Yaw *et al.* 2008) using the NJ algorithm with the p-distance method (Nei & Kumar 2000). These clades represent lineages that probably originated from at least two putative glacial refugia: the western Wisconsin and the northern Appalachian refugia (Lee-Yaw *et al.* 2008). Putative source areas were identified by estimating the closeness of the phylogenetic relationship between James Bay haplotypes and haplotypes from known locations south of the study area through a NJ tree with 1000 bootstrap replicates. The relationship between genetic similarity and geographic position was further tested through a spatial analysis of molecular variance (SAMOVA 1.0; Dupanloup *et al.* 2002), defining groupings (K) of geographically adjacent populations that maximized the proportion of genetic variance due to differences between groups. The analysis was conducted for K values ranging from 2 to 5, with 100 initial conditions, and tested for genetic structure within geographically neighbouring clusters of localities.

RESULTS

Microsatellite variability in the James Bay localities

Overall, 463 individuals were genotyped with 96.1% success, ranging from 89.6% (RsyD55) to 98.5% (RsyD40) success by locus. When considering all individuals, higher homozygosity than expected under the HWE was observed at all seven microsatellite loci. However, stuttering might explain these scoring errors in two of the loci (RsyD88, and RsyC41) and when analysing each region separately, only two loci exhibited an excess of homozygotes in more than three regions (RsAAT182, and RsyD88). This excess is also reflected in each region's inbreeding coefficient (F; Table 2), implying that three out of 17 localities exhibit significant inbreeding. These results are also observed after Bonferroni (Dunn-Sidak) adjustments of 95% confidence interval. Overall, 8.1% of all locus pairs (357) within localities demonstrated significant evidence of LD ($p < 0.05$), with LD not limited to any particular pair, indicating low multilocus interactions (Banks *et al.* 2010; Andras *et al.* 2011).

Table 2 Metrics of genetic variation observed in 17 localities surrounding James Bay (463 individuals).

#	Locality	Coordinates (UTM)	N	Na	P	Ne	Ar	Pa	H_o	H_e	UH_e	F	
1	Winisk River	16 U 612161 6066779	12	7.86	0	5.38	2.95	0.17	0.725	0.714	0.746	0.029	
2	West Attawapiskat River	16 U 534035 5854126	14	9.00	1	6.44	3.13	0.26	0.850	0.781	0.810	-0.051	
3	Victor Mine	17 U 302685 5861030	5	5.14	0	3.92	2.92	0.15	0.814	0.681	0.758	-0.084	
4	Attawapiskat	17 U 402733 5865479	28	10.29	1	5.53	2.99	0.16	0.746	0.764	0.778	0.042	
5	Akimiski Island West Point	17 U 433378 5875453	12	6.00	0	4.10	2.91	0.10	0.770	0.726	0.776	0.013	
6	Akimiski Island Research Area	17 U 498032 5883686	161	11.71	4	5.49	2.89	0.16	0.718	0.742	0.745	0.036	
7	Akimiski Island East	17 U 512418 5866014	8	5.14	0	3.64	2.88	0.22	0.826	0.690	0.765	-0.100	
8	Fort Albany	17 U 453175 5785282	30	13.29	0	8.68	3.36	0.35	0.808	0.863	0.879	0.082	
9	Kenogami River	16 U 681653 5578084	19	12.71	4	8.01	3.34	0.48	0.815	0.849	0.872	0.067	
10	Moosonee	17 U 524482 5679815	39	15.14	2	9.17	3.34	0.46	0.817	0.862	0.874	0.066	
11	Missisicabi River	17 U 593694 5674268	6	6.43	0	4.98	3.02	0.44	0.690	0.698	0.762	0.102	
12	Matagami	18 U 356894 5527981	5	5.14	0	4.02	2.90	0.20	0.714	0.689	0.765	0.074	
13	Waskaganish	17 U 654246 5704224	14	8.00	1	5.25	2.95	0.22	0.687	0.731	0.759	0.098	
14	Eastmain	17 U 670353 5787741	32	10.29	1	5.85	3.00	0.33	0.693	0.772	0.785	0.119	*
15	Wemindji	17 U 656324 5872782	36	9.57	1	5.18	2.85	0.20	0.660	0.729	0.740	0.108	
16	Chisasibi	17 U 643771 5953897	23	8.71	0	5.55	3.01	0.24	0.684	0.779	0.797	0.144	*
17	Radisson	18 U 319671 5951829	19	8.86	0	5.24	2.94	0.21	0.636	0.747	0.768	0.175	*

Na = number of different alleles; P = number of private alleles; Ne = number of effective alleles; Ar = allelic richness corrected for sample size; Pa = private allelic richness corrected for sample size; H_o = observed heterozygosity; H_e = expected heterozygosity; UH_e = unbiased expected heterozygosity; F = inbreeding coefficient; asterisk show significant value for $p < 0.001$

The seven loci were polymorphic in all sampling localities, the total number of alleles per locus varying from 11 to 39 (mean 21.4 ± 9.1). The mean allelic richness in the localities over all loci was 3.02 ± 0.17, ranging from 2.85 (Wemindji, QC) to 3.36 (Fort Albany, ON). Levels of genetic diversity (considering Na, Ne, Ar, Pa, and H_o per locality) were higher south-west of the bay, near Fort Albany, Kenogami River, and Moosonee, in Ontario. On the other hand, lowest diversity was seen on the east coast of Akimiski Island (locality 7) in Nunavut (Ar = 2.88) and near the Victor Mine (locality 3) in Ontario (Ar = 2.92; Table 2).

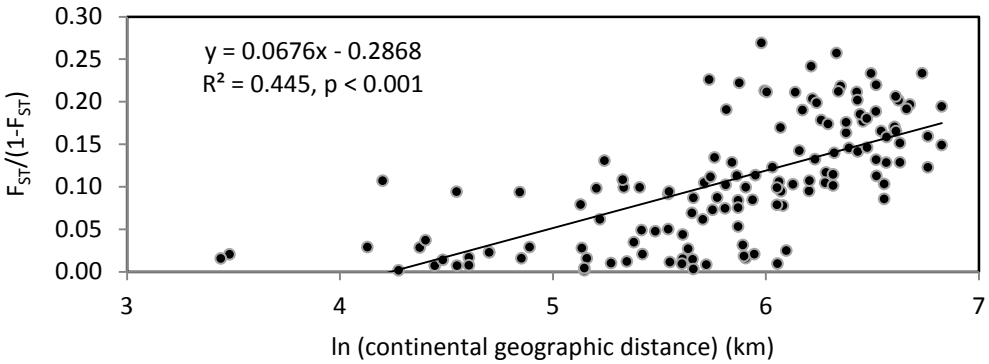


Fig. 4 Isolation-by-distance pattern in the James Bay area. Regression of corrected genetic differentiation [estimated by $F_{ST}/(1 - F_{ST})$] against natural logarithm of continental geographic distances (km) for all pairs of sampled regions.

Genetic structuring around James Bay

Comparing pairwise genetic distances (F_{ST}) to continental distances, a significant relationship was detected ($R^2 = 0.445$, $p < 0.001$; Figure 4). The analysis of microsatellite variation using the TESS model without admixture, showed a marked plateau in the deviance information criterion (DIC) of the data at K = 3 (Figure 5a). The model with admixture and the ACSII-Raster map outlined three distinct clusters as well, with individuals from localities 1 to 7 (NORTH-WEST; which represents the northernmost localities in Ontario and on Akimiski Island, NU) assigned to cluster 1; individuals from localities 8 to 10 (SOUTH-WEST; represented by Fort Albany, Kenogami River, and Moosonee in Ontario) to cluster 2; and individuals from localities 11 to 17 (EAST; all the Québec localities plus Missisicabi River in Ontario) to cluster 3 (Figure 6). The admixture analysis did recover lower DIC values when the maximal number of clusters equalled four, but at this configuration, the program isolated one individual into a separate fourth cluster, all other clusters remaining identical. Thus, this interpretation was excluded. Moreover, the Bayesian analysis using STRUCTURE did not reveal variations in the cluster assignment and the log likelihood also reached a plateau for three clusters (Figure 5b). The hierarchical AMOVA revealed that most of the genetic variance comes from within localities, with very low variation coming from among the localities within a same genetic population (Table 3). The division between all three genetic populations was still supported, with 13% of the variation resulting from such grouping.

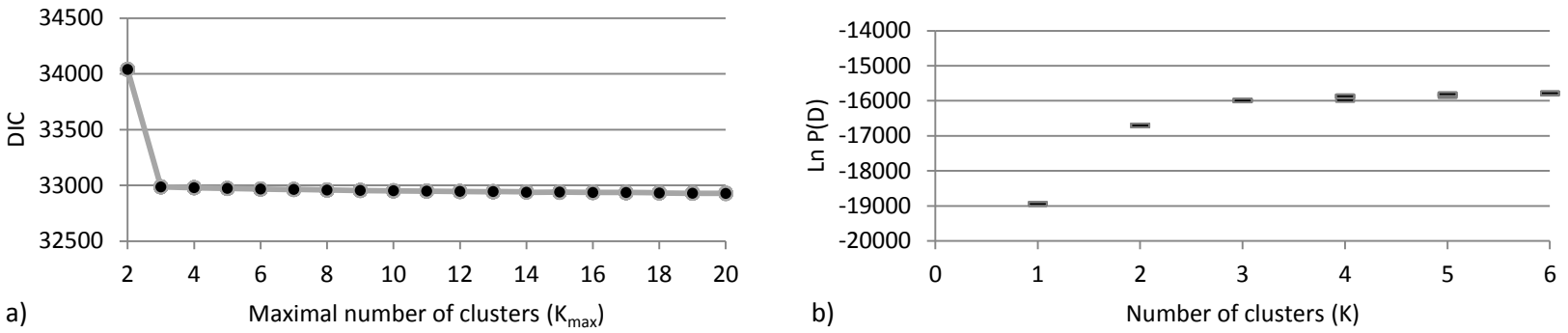


Fig. 5 Maximal number of clusters discovered by Bayesian clustering analyses. a) The Deviance Information Criterion (DIC) as a function of K_{max} as implemented in TESS v. 2.3, model without admixture (Chen *et al.* 2007). b) Plot of the log probability of the data [LnP(D)] as a function of the numbers of clusters (K) calculated by STRUCTURE v. 2.3.1 (Pritchard *et al.* 2000; Falush *et al.* 2003; Hubisz *et al.* 2009), supporting TESS results.

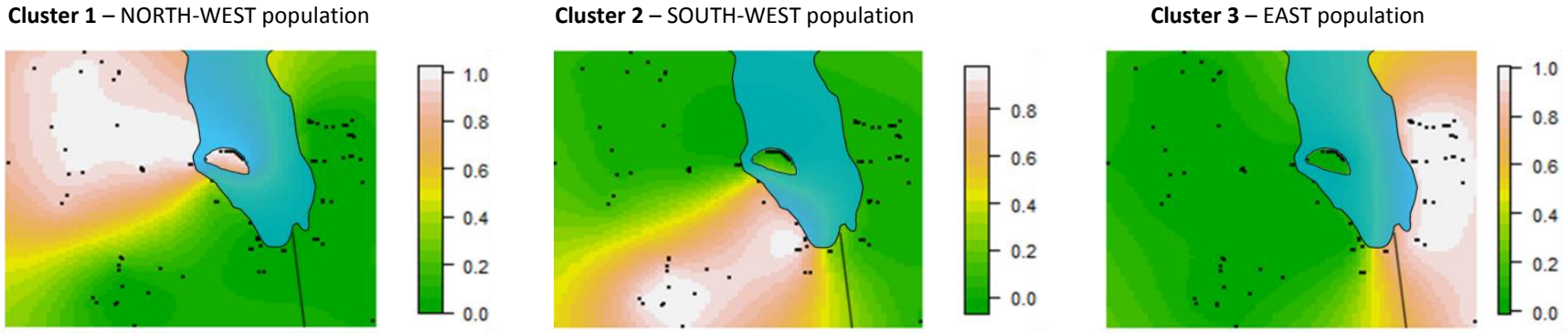


Fig. 6 Spatial interpolation of admixture proportions for the data set built with 463 individual genotypes from wood frogs in the James Bay area, analysed with TESS when the maximal number of clusters equaled three. Colour bands represent the probability of membership of individuals in the area to proper cluster. Vertical line delineates the geographic border of Ontario and Québec. The interpolation was drawn with the R Software, the R script taken from the TESS directory (Durand *et al.* 2009).

Table 3 Hierarchical AMOVA with groupings of localities by genetic populations; all fixation indices are significant with $p < 0.001$.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*Fixation indices*	
Among populations	2	200.08	0.332	13.00	F_{CT}	0.130
Among localities within populations	14	55.45	0.043	1.68	F_{SC}	0.019
Within localities	909	1977.39	2.175	85.32	F_{ST}	0.147
Total	925	2232.92	2.550			

Mitochondrial DNA ND2/tRNA^{TRP} diversity

Table 4 Measures of genetic diversity according to the ND2/tRNA^{TRP} mitochondrial sequences from 76 individuals.

#	Phylogroup	Locality	N	Haplotypes represented - #(inds.)	d	π	S	$\hat{H}$
1	1, 2b	Winisk River	5	10(4); 19(1)	1.6	0.003	4	0.4
2	2b	West Attawapiskat River	6	10(4); 12(1); 16(1)	1.0	0.002	3	0.6
3	2b	Victor Mine	3	10(3)	0	0	0	0
4	2b	Attawapiskat	4	10(4)	0	0	0	0
5	1 and 2b	Akimiski Island West Point	4	10(3); 19(1)	2.0	0.003	4	0.5
6	2b	Akimiski Island Research Area	5	5(2); 10(3)	0.6	0.001	1	0.6
7	2b	Akimiski Island East	2	10(2)	0	0	0	0
8	2b	Fort Albany	5	10(3); 16(1); 20(1)	0.8	0.001	2	0.7
9	1, 2a, and 2b	Kenogami River	7	4(3); 10(1); 16(1); 25(1); 27(1)	4.0	0.007	10	0.9
10	1 and 2a	Moosonee	9	2(1); 3(1); 4(2); 11(1); 13(1); 15(1); 17(1); 22(1)	5.2	0.009	16	1.0
11	1	Missisicabi River	2	1(1); 6(1)	1.0	0.002	2	1.0
12	1	Matagami	3	4(1); 14(1); 23(1)	2.0	0.003	3	1.0
13	1	Waskaganish	3	1(1); 26(1); 28(1)	3.3	0.005	5	1.0
14	1	Eastmain	5	4(1); 6(3); 15(1)	1.0	0.002	2	0.7
15	1	Wemindji	6	1(2); 6(1); 7(1); 9(1); 18(1)	1.7	0.003	4	0.9
16	1	Chisasibi	3	1(1); 8(1); 24(1)	2.0	0.003	3	1.0
17	1	Radisson	4	6(2); 9(1); 21(1)	2.3	0.004	4	0.8

d = mean number of pairwise differences between haplotypes; π = nucleotide diversity; S = number of polymorphic sites; $\hat{H}$ = gene diversity.

A fragment of 609 bp was successfully amplified and sequenced in 76 individuals representing all 17 localities. It included 28 polymorphic sites defining 28 unique haplotypes and there were no gaps among the sequences. Low sequence divergence was observed among haplotypes, the highest value being 1.8%, while mean divergence was 0.8 ± 0.3%. Overall, gene diversity ($\hat{H}$) was 0.856 ± 0.001, while nucleotide diversity (π) was 0.005 ± 0.0000002. The geographic distribution of genetic diversity was quite variable, with seven out of 28 haplotypes (25%) recorded in more than one region, and the mean number of haplotypes per locality being 2.9 ± 1.8, with Moosonee having eight haplotypes (Table 4; Figure 7). The number of polymorphic sites (S) within localities ranged from 0 to 16, with the highest values reported for the Kenogami River and Moosonee localities (S = 10 and 16, respectively). These localities also exhibited high values of nucleotide diversity (π; Table 4). Gene diversity ($\hat{H}$), which takes into account sample size (Nei & Chesser 1983), exhibited higher variation in localities 8 to 17 than in localities 1 to 7 (Table 4). This dichotomy is also seen in the geographic distribution of the mtDNA haplotypes, as no haplotypes present in localities 1 to 8 are present in localities 10 to 17, and *vice versa*.

Phylogeography and geographic structuring of haplotype diversity

Phylogenetic comparisons between James Bay haplotypes and Western and Eastern Clade haplotypes clearly revealed that wood frogs in the James Bay area are descendents from one clade only; the Western Clade (mean genetic distance between James Bay and Western Clade haplotypes = 0.8%, mean genetic distance between James Bay and Eastern Clade haplotypes = 6.8%). Although the Maximum Likelihood (ML) tree had low bootstrap support and could not easily distinguish any significant relationships (Figure 8), the Neighbour-Joining (NJ) tree supported the separation of each coast of James Bay into two lineages. Two phylogroups were defined with a high bootstrap value (99%), thus, remaining comparisons were conducted with the NJ tree (Figure 9). Haplotypes from the Québec side and south of James Bay clustered together (phylogroup 1 – localities 9 to 17, with haplotype 19 being found in localities 1 and 5), as did haplotypes found south-west and north-west of the bay (phylogroup 2 – localities 1 to 10). Two subgroups are also delineated in phylogroup 2 (2a and 2b, although not with significant bootstrap support), representing the phylogeographic division between haplotypes from the area north-west of James Bay and the haplotypes found more centrally in the Kenogami River and Moosonee regions (Figure 9). When haplotypes from the Western Clade (as identified in Lee-Yaw *et al.* 2008) are included in the NJ analysis, phylogroup 1 is identified as more closely related to haplotypes from southern, central, and eastern Ontario and as far east as Montréal, QC, whereas phylogroup 2a is more closely related to haplotypes found in Thessalon and Surluga, ON, and phylogroup 2b is more closely related to haplotypes found in Wild Goose Park and Mills Block, ON (Figure 10, Appendix B). The haplotype network (Figure 11) revealed low resolution of relationships between the haplotypes with no clear root, but when compared with the three genetic populations discovered by microsatellite analyses, the three groupings become more evident.

SAMOVA

Spatial analysis with SAMOVA was repeated for values of K from 2 to 8 to uncover where the variance among groups relative to the total variance (F_{CT}) reached a plateau. The highest value of F_{CT} was recovered at K = 6, while subsequent analyses had F_{CT} values that plateaued or even decreased (Table 5). However, at K = 6 some groups constituted of only one locality, which suggested an absence of geographic structure (Heuertz *et al.* 2004; Godbout *et al.* 2005). Indeed, all groupings over K = 3 split adjacent localities or isolated single localities, implying no significant geographic structure for K values greater than three (Dupanloup *et al.* 2002; Godbout *et al.* 2010). Thus, the grouping pattern recovered with SAMOVA at K = 3 was considered the optimal configuration. The three groups identified clustered localities 1 to 8, 9 and 10, and 11 to 17 together, representing the areas north-west, south-west, and east of the bay, which supports the three phylogroups (1, 2a, and 2b) identified in the NJ tree (Figure 9). Significant variation among groups (50.4%) was as important as the variation within localities (51%), which can be expected as values of gene diversity are quite elevated in at least half the localities (Table 4). Variation among localities within the same group was not significant (Table 5). Additionally, unlike what was observed in the phylogenetic trees, when K = 2 the south-west haplotypes from localities 9 and 10 were grouped with the east coast localities, suggesting a closer relationship between them than is proposed by the NJ tree. In the case of the north-west coast of James Bay, throughout all K-groupings, localities 1 to 8 always clustered together, likely revealing the strength of their association (Table 5).

Table 5 Fixation indices and percentage of variation for K = 2 to K = 8 groups of localities identified by SAMOVA (all results are significant at $p < 0.001$ unless otherwise indicated).

Number of groups (K)	Group composition	Fixation indices			Percentage of variation		
		F_{SC}	F_{ST}	F_{CT}	Among groups	Among localities within groups	Within localities
2	1-8; 9-17	0.099	0.529	0.477	47.73	5.2 *	47.07
3	1-8; 9-10; 11-17	-0.029	0.489	0.504	50.44	-1.47 NS	51.03
4	1-8; 9-10; 11, 15-17; 12-14	-0.085	0.472	0.514	51.37	-4.13 NS	52.76
5	1-8; 9-10; 11, 15-17; 12, 14; 13	-0.117	0.472	0.527	52.7	-5.55 NS	52.84
6	1-8; 9; 10; 11, 15-17; 12, 14; 13	-0.170	0.466	0.544	54.40	-7.76 **	53.37
7	1-8; 9; 10; 11, 15, 17; 12, 14; 13; 16	-0.178	0.462	0.543	54.32	-8.13 NS	53.82
8	1-8; 9; 10; 11, 15; 12, 14; 13; 16; 17	-0.182	0.458	0.541	54.10	-8.33 NS	54.24

NS = Non-Significant

* $p < 0.05$

** $p < 0.01$

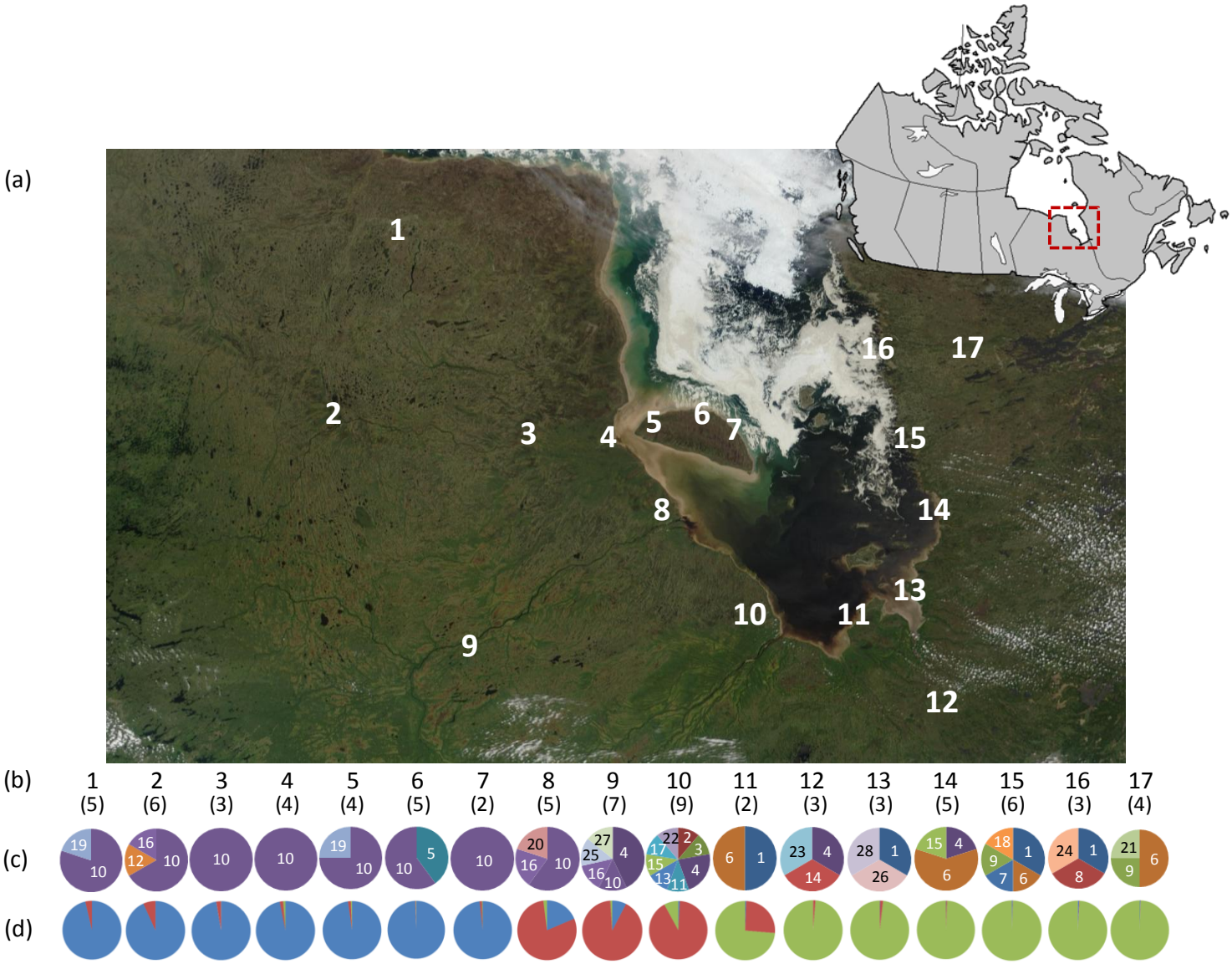


Fig. 7 Distribution of haplotypes throughout the study area. (a) Map of the James Bay area with sampling localities numbered as shown in Table 4. (b) Sampling localities, with the number of samples sequenced in brackets. (c) Pie charts of haplotype frequencies for each locality, with haplotype number written in each section. (d) Proportion of membership of each of the three genetic populations detected with microsatellite data and inferred with TESS. Satellite image from NASA (2002).

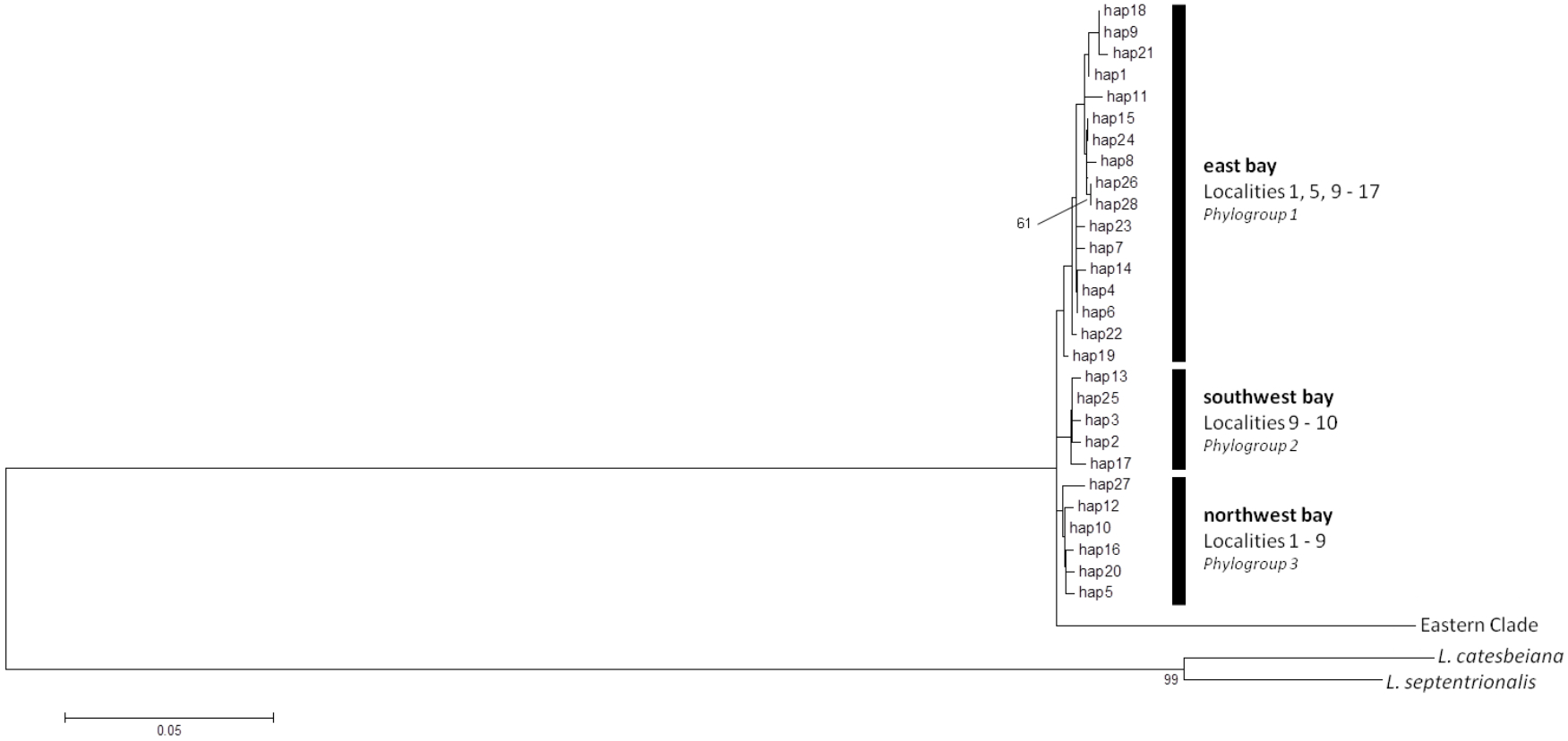


Fig. 8 Molecular phylogenetic analysis by Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993). A discrete gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 1.5158)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved all 28 nucleotide sequences from James Bay as well as haplotypes representing the Eastern Clade (as identified by Lee-Yaw *et al.* 2008) to show divergence. *Lithobates catesbeiana* and *L. septentrionalis* were aligned as outgroups. The bootstrap consensus tree was inferred from 1000 replicates (Felsenstein 1985) and only values >50% are shown. Analyses were conducted in MEGA5 (Tamura *et al.* 2011).

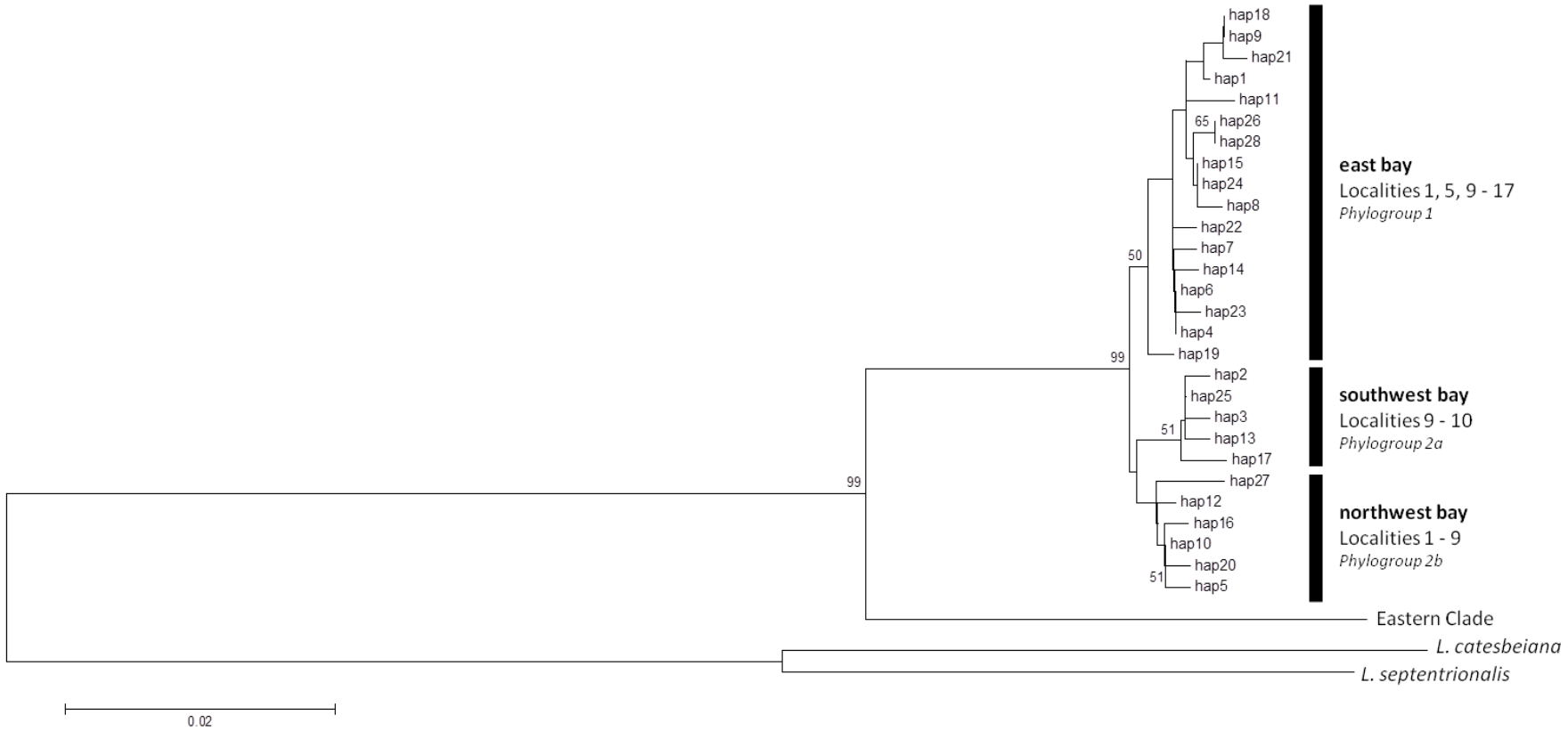


Fig. 9 Evolutionary relationships of the 28 James Bay haplotypes inferred using the Neighbour-Joining method (Saitou & Nei 1987). The bootstrap consensus tree was inferred from 1000 replicates (Felsenstein 1985) and only values >50% are shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei & Kumar 2000) and are in the units of the number of base differences per site. Eastern Clade represents the clade identified in Lee-Yaw *et al.* (2008). Analyses were conducted in MEGA5 (Tamura *et al.* 2011).

Fig. 10 Map of the phylogeographic relationships between haplotypes from southern Ontario and western Québec and from the James Bay area. Grey arrows represent the general direction and source of the colonizing waves into the study area. W1 and W2 represent two routes that colonized the west coast of James Bay, while E1 represent the route that colonized the east coast of James Bay. Map drawn with ArcGIS 10.

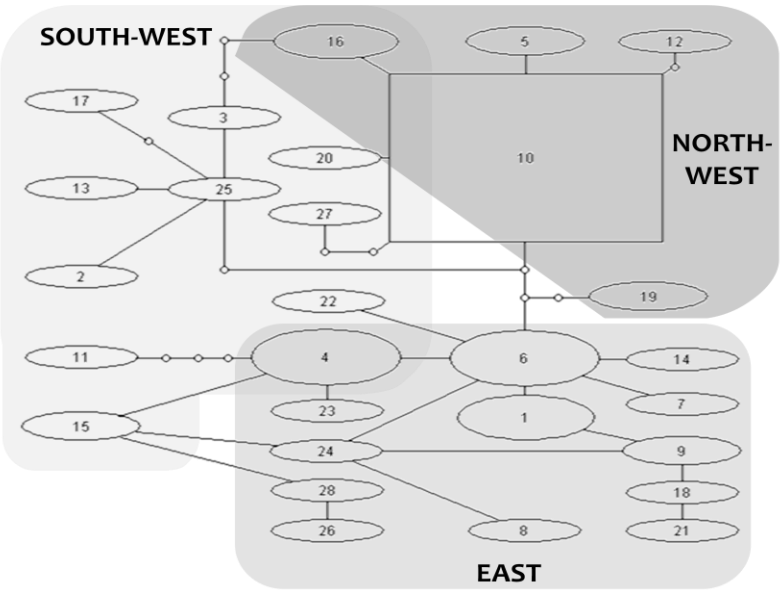


Fig.11 Haplotype network. Shaded areas represent the three genetic populations delineated by microsatellite analyses. A line indicates 1 bp difference. Numbers represent haplotype identity.

DISCUSSION

Population genetics analyses allow inferences about demographic, evolutionary, and ecological processes, revealing patterns not discernible through traditional ecological research (Gaggiotti 2004). Through the quantification of genetic polymorphisms in discrete DNA markers, population genetics has become an essential tool to describe the availability of genetic diversity and to assess the genetic health of diverse populations, information that is of high value to conservation biologists and wildlife managers (Hartl 2000). Due to variable rates of mutations (substitutions, slippage replications, etc.), different genetic markers can help elucidate population dynamics across different spatial or temporal resolutions (Manel *et al.* 2003; Gaggiotti 2004). For example, microsatellites, which are highly polymorphic on average and rapidly evolving, can provide estimates of contemporary genetic variation, connectivity, and migration between localities, giving insight into fine-scale ecological patterns (Selkoe & Toonen 2006). By contrast, mitochondrial DNA sequences, whose regions generally evolve more slowly than microsatellites, can retrieve patterns at larger spatial and deeper temporal scales, thus providing the necessary resolution to recover phylogenetic relationships and population history (Avise 1994; Sunnucks 2000). Due to those differences, the analyses presented here convey information on both present and past patterns of wood frog population genetics.

Present patterns of population genetics

Genetic variation in northern landscapes

Present patterns of wood frog population genetics can be observed through estimates of contemporary genetic variability and gene flow between localities, providing insights on survivorship and connectivity of James Bay wood frog populations. These estimates can be compared over the years to characterize the evolutionary potential of the species in the region. For instance, higher genetic variation was observed in the James Bay area than would be predicted for this northern latitude. Benefits of high genetic diversity include increased variation upon which natural selection can act (Verhoeven *et al.* 2010), increased colonization potential (Petit *et al.* 2004), and improved resistance to disease (Spielman *et al.* 2004). In theory, successive episodes of founder effects far from the source population (in this case, the glacial refugium of western Wisconsin; Lee-Yaw *et al.* 2008) are expected to decrease gradually the genetic diversity in populations invading recently deglaciated areas (Hewitt 1996, 2001; Merilä *et al.* 1997). When comparing the microsatellite data in this study with other published results (Figure 12; e.g. Crosby *et al.* 2009), James Bay localities do not exhibit significantly lower genetic variation than populations in southern Ontario, with the exception of allelic richness. It has to be noted that allelic richness was calculated by standardization to the smallest sample size (Kalinowski 2005), and because the minimum number of alleles per locality per locus was four (due to the high prevalence of non-amplified genotypes in one locus in one of the localities), the resulting allelic richness was much lower than if all alleles from every individual would have amplified adequately.

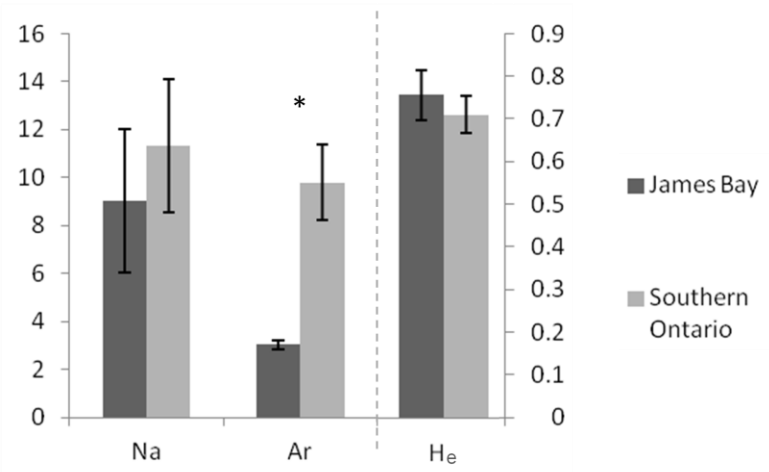


Fig. 12 Comparative genetic variation indices from microsatellite markers for James Bay and southern Ontario wood frog populations averaged over loci and localities (James Bay = 17 localities, 7 microsatellite loci; southern Ontario = 9 localities, 8 microsatellite loci). Number of alleles (Na), allelic richness (Ar) and expected heterozygosity (H_e) values are compared. Right scale corresponds to observed heterozygosity values while number of alleles and allelic richness are represented on left scale. Asterisk indicates that the difference between values is significant. Error bars represent standard error. Southern Ontario estimates were extracted from Crosby *et al.* (2009).

Although differences in levels of genetic variation were expected between northern and southern Ontario, two scenarios may explain the similar levels observed: 1) admixture of multiple lineages increased the genetic variation in an area further away from the refugium than was expected; 2) the microsatellite genetic variability reported in the southern Ontario populations reflect similar post-glacial colonization distances away from the refugium. In the case of genetic admixture, the level of genetic diversity observed in the James Bay area parallels other studies showing higher genetic variability north of the refugial area because of admixture between colonizing lineages (Petit *et al.* 2003; Walter & Epperson 2005). In a study of woody angiosperm taxa by Petit *et al.* (2003), populations with the highest genetic diversity were discovered at intermediate latitudes, north of the putative refugial grounds, due to the mixing of colonization routes and subsequent admixture. For the second scenario, southern Ontario was colonized by a lineage that entered Canada's Great Lakes region via the Upper Peninsula of Michigan and subsequently went south. This route represents considerable distance from the refugium and consequently, a loss of genetic variation by sequential founder effects and genetic drift would likely be observed (Hewitt 2001). With similar series of founder events, similar levels of genetic variation would thus be expected between the James Bay area and southern Ontario populations. However, when comparing genetic variation indices calculated by the quantification of polymorphisms in the ND2/$tRNA^{TRP}$ genes, three wood frog populations closer to the putative refugium (Lake Ann, Esmond, and Livingston Road in Michigan; Lee-Yaw *et al.* 2008) showed no significant differences in gene diversity ($\hat{H}$), mean number of pairwise differences (d), and nucleotide diversity (π) from the James Bay localities. This comparison further supports the first scenario of relatively high levels of genetic variability following admixture (see below – *Secondary contact zone*).

With admixture increasing genetic variation north of refugial grounds, similar patterns would be anticipated in other North American species exhibiting post-glacial range expansion towards the North. Indeed, many population expansions originated from multiple glacial refugia and secondary contact zones are common during their recolonization of post-glacial land towards the North (Walter & Epperson 2001, 2005; Zamudio & Savage 2003; Latch *et al.* 2009). For instance, the spring peeper (*Pseudacris*

crucifer) exhibits high levels of genotypic diversity north of its putative southern refugia due to the admixture of clades that have come into secondary contact in south-western Ontario through post-glacial recolonization (Austin *et al.* 2002). Although *P. crucifer* has not been reported to have taken shelter in the western Wisconsin refugium, it is present throughout the study area (with the exception of Akimiski Island, where no calls, egg masses, or individuals were noticed in the summers of 2008 and 2009; *personal observation*, see Desroches *et al.* 2010 for confirmation in other years). A phylogeographic study of this species in the James Bay area would allow a comparison of the levels of genetic variation remaining after their range expansion to northern landscapes.

The special case of Akimiski Island

According to the founder effect hypothesis, island populations that were originally colonized by individuals derived from mainland sources would exhibit lower levels of heterozygosity and carry fewer alleles per locus relative to the mainland source population (Lande 1995; Keller & Waller 2002; DeYoung & Honeycutt 2005; Franks 2010). Under this scenario, Akimiski Island wood frog populations should be genetically depleted compared to James Bay coast populations. Thus, it is interesting to observe similar heterozygosity levels on the island than on the Ontario mainland. Anuran presence on this island is puzzling, because it lies 20 km away from the mainland circled by James Bay's saline waters. The salinity level of the surface waters is generally low (10 – 24‰, summer measures; Martini 1981b), but is elevated enough to restrict survival of anuran species to no more than a few hours (Davenport & Huat 1997). Also, this island was never in direct contact with the mainland, considering the time since the LGM. After the retreat of the glaciers, the rise in water level, and the continental isostatic rebound, Akimiski Island emerged from the Tyrrell Sea without having any contact with the surrounding land, because at that time the sea covered the mainland about 100 km south of the present shoreline (Martini & Glooschenko 1984). However, the presence of anurans on this island (American toad, *Anaxyrus americanus*; boreal chorus frog, *Pseudacris maculata*; and wood frog, *Lithobates sylvaticus*; *personal observations*) and the relatively high genetic diversity suggest repeated immigration events from the mainland (Kolbe *et al.* 2004; Fernández-Mazuecos & Vargas 2011). Other recent studies also observed higher levels of genetic diversity on islands (Algar & Losos 2011; Désamoré *et al.* 2011), reporting that with frequent dispersal events between mainland and island areas, due to geographic proximity or high mobility of the organisms, typical bottleneck signs in island populations seem to be rapidly erased. In the case of Akimiski Island, possible scenarios for wood frog immigration may be: 1) the inflow of hibernating individuals from vegetative life rafts ripped from the banks by heavy spring flow from the Attawapiskat and Ekwan Rivers, guided by the same currents creating sediment plumes[1], which then settle against the coasts of Akimiski Island (Figure 13), and 2) the random drift of individuals from the mainland to the island by river currents with spring melt creating a freshwater layer that allows the anurans to survive the migration[2]. Either way, wood frogs seem to travel in a unidirectional manner towards the island and slowly disperse from West to East. As the area is still under isostatic rebound, the future may bring interesting modifications with regards to the rate of immigration and emigration of species if a land

[1] Martini & Glooschenko (1984) confirm that the Attawapiskat River plumes have significant effects on the AIS zone (named by the authors to designate the south-west shore of Akimiski Island; see Figure 1 in Martini & Glooschenko 1984), which could propose an entry point for anurans on the island.

[2] As a side note, currents moving through the Akimiski Straight were measured at 0.8 m/sec in the summer of 1978 (Martini 1981b). Considering that the shortest distance between the mainland and Akimiski Island is 17 km at low tide (Martini & Grinham 1984; although this route would be almost impossible for anurans to take because of the direction of the currents), frogs floating and following this route would take a minimum of six hours to reach the coast of Akimiski Island.

bridge forms between the island and the mainland (average depth of James Bay is 28 m (El-Sabh & Koutitonsky 1977) and the Akimiski Straight may be shallower; Martini & Grinham 1984).

Fig. 13 Satellite image of Akimiski Straight and the sediment plumes from the Ekwan and Attawapiskat River (Martini 1981b).

Genetic structure of wood frog populations in the James Bay area

One of the biggest challenges in the field of ecology is the definition of natural populations (Berryman 2002). In this study, the label "population" was carefully used, as the 17 localities subjectively grouped samples which could not be demographically defined as a population. The term "population" was thus first used with James Bay related data to represent groupings in the study area after Bayesian clustering of microsatellite data, where three distinct genetic populations were defined. Analysis of the structure within and among these three populations (AMOVA) revealed that 13% of the microsatellite variation was observed among the populations, whereas most of the variation (85%) came from within the localities. Mitochondrial data (SAMOVA) supported a similar structure of three distinct clusters, with the exception of locality 8 grouping with the first seven localities instead of localities 9 and 10. Such clustering maximized the proportion of genetic variance among groups (Dupanloup *et al.* 2002). Highest variance was reported within localities, which may confirm the arbitrariness of the 17 divided regions. The population structure analyses support the grouping of the 17 localities into three genetic populations, which, in turn, cover an extremely large area and cannot possibly represent a collection of interbreeding individuals. Therefore, this suggests that gene flow and connectivity exist between localities within the three defined populations. Patterns of gene flow estimated through nuclear and mitochondrial DNA analyses outline the significant variance between the three populations (13% and 50%, respectively) and may also suggest the presence of constraints to dispersal between these genetic populations. Similarly, connectivity influences the persistence of populations (Hess 1996; Herfindal *et al.* 2010), which is a fundamental concept in ecological studies and in conservation biology. Gene flow, which gives insight on the movements of individuals between localities, was non-significant between 46.3% of the F_{ST} -pairwise comparisons. Highest differentiation between adjacent localities occurred between localities 10 – 11, and between localities 2/3/4 and 9, mapping the geographic limits between the three genetic populations (Appendix C). Further detailed analyses of these splits would be needed to

detect if any geographic barriers or other genetic phenomena are segregating wood frog populations in these specific areas (see below).

James Bay is without doubt the largest physical barrier to gene flow in the area. With its size and the salinity of its waters, James Bay affects population genetic structure mostly through isolation-by-distance, since the shortest distance between eastern and western localities is around the bay. However, when isolation-by-distance was tested within each genetic populations identified in the microsatellite section, no isolation-by-distance was observed within the three groups of localities (although such relationship was nearly significant for the EAST population; $R^2 = 0.167$; $p = 0.059$).

Furthermore, two localities possessed most of the private alleles (Akimiski Island Research Area and Kenogami River). Greater numbers of private alleles are expected in larger sample sizes, such as in the case of the Akimiski Island Research Area locality, but the abundance of private alleles at the Kenogami River locality suggests differentiation (Beauclerc *et al.* 2010). Additionally, the Kenogami River locality was the only one with haplotypes from both sides of the bay (both phylogroups 1 and 2; Figures 7 and 9), also suggesting that modern gene flow from the NORTH-WEST population to the EAST population is uncommon (Conroy & Cook 2000), or that there is limited introgression between lineages upon secondary contact (Lee-Yaw *et al.* 2008). With selection and population disequilibrium, Walter & Epperson (2005) also observed little gene flow across a zone of admixture, characterized by high genetic diversity and sharp boundaries forming a steep cline with surrounding low diversity areas. Furthermore, limited introgression between wood frog lineages was already proposed by Lee-Yaw *et al.* (2008), with reproductive isolation possibly limiting hybridization between the Western and Eastern Clades. The observed reduction in gene flow at the edges of the three genetic populations suggests the presence of such phenomenon, but further research into the reproductive isolation and contemporary selective pressures in the area would be needed to propose adequate hypotheses.

Evolutionary and ecological importance of colonizing northern landscapes

Being at the northern edge of the species distribution presents adaptive challenges, as peripheral populations often encounter climatic conditions at their extreme physiological limit, less suitable habitats, increased isolation, segmented distributions, small effective sizes, increased stress, and higher susceptibility to extinction (Mayr 1963; Templeton *et al.* 1990; Lesica & Allendorf 1995; Brooks 2000). All these characteristics have genetic consequences, from high levels of genetic subdivision and population structure to low emigration rates due to reduced genetic diversity and local adaptation (Johnson & Watts 1994; Holliday *et al.* 2011; Row 2011). In fact, peripheral populations may play a key role in speciation events because of their unique genetic characteristics (Bush 1975; Brussard 1984; Brooks 2000; Friesen 2007). Genetic analyses suggest that wood frog populations in the James Bay area do not exhibit typical peripheral population characteristics as there is relatively high gene flow between localities clustered in the same genetic population, they present higher levels of genetic variation than what is expected for northern landscapes, and their genetic populations cover large areas suggesting low levels of genetic subdivision. These characteristics are known to benefit a species' survival as well as enhance their capacity to establish new populations and persist in new environments, characteristics that are of value in northern landscapes (Sgrò *et al.* 2011; Weeks *et al.* 2011).

Inbreeding, excess of homozygosity, and genotyping errors

Heterozygote deficiency is relatively common in natural populations, as inbreeding, assortative mating, the Wahlund effect (Wahlund 1928), or natural selection may occur and can cause an excess in homozygosity (Gaffney *et al.* 1990; Castric *et al.* 2002; van Oosterhout *et al.* 2004; Karlsson & Mork

2005). In this study, three hypotheses could explain the observed excess of homozygotes. First, null alleles in all loci may be responsible for the observed trend in homozygosity (Foltz 1986; Lemer *et al.* 2011). However, it is relatively easy to confound the presence of null alleles with genotyping errors, which have been reported to affect parameters such as homozygosity and overestimates of inbreeding coefficient (Morin *et al.* 2009). Whereas DNA quality could affect the number of genotyping errors, high-quality tissue samples such as skin and bone tissue generally exhibit less genotyping errors than non-invasive DNA collection samples such as hair and faeces samples (Morin *et al.* 2009). Second, non-random sampling across life stages may also have played a role in the observed deficiency of heterozygotes. Even though efforts were made to prevent sampling of related individuals, the collection of eggs, tadpoles, and metamorphs may have increased the probability of sampling two related individuals. Third, there might be undiagnosed subdivisions within localities, with the admixture of two or more sets of closely-related individuals in the locality (Wahlund effect; Wahlund 1928), leading to an excess in homozygotes. With the extent of the sampled area (close to 600 000 km^2), and individuals being grouped into general geographic clusters (localities), it is not surprising to observe Hardy-Weinberg disequilibrium in these groupings that most likely do not follow the main assumptions of the Hardy-Weinberg principle (in particular, random mating, no migration, and no selection; Hartl 2000).

Past patterns inferred by phylogeographic analyses

Historical events shape contemporary patterns in the distribution of genetic diversity (Rey & Turgeon 2007; Girard & Angers 2011). In fact, some studies report that historical processes have a more important role in shaping current genetic variation than recent demographic processes (Lougheed *et al.* 1999; Jordan *et al.* 2009). Thus, phylogeographic and population genetic analyses can help infer past events that may help explain contemporary population structure. Wood frogs are considered primary colonizers, closely following the margins of the retreating Laurentide ice sheet and colonizing newly available habitat (Placyk *et al.* 2007). However, the pattern of the wood frog's post-glacial range expansion deviates slightly from the expected leading-edge model because wood frogs in North America originate from multiple refugia and secondary contact zones are present between colonizing clades and lineages (Lee-Yaw *et al.* 2008). Thus, the observed patterns of gene flow in wood frog populations in the James Bay area result from past specific colonization behaviours that, in turn, were influenced by past geological events that occurred in the studied area.

Results from this thesis showed that the James Bay coasts have been colonized by wood frogs from the Western Clade, originating from the putative western Wisconsin refugium. This refugium is hypothesized to have been located in south-western Wisconsin (Lee-Yaw *et al.* 2008), more precisely in the region known as the Driftless Area (Braun 1950). This region has never been covered by continental ice sheets and was never surrounded simultaneously by ice on both sides during the Last Glacial Period, despite being situated north of the glacial boundary (Braun 1950; Hobbs 1999). The Driftless Area is also known to act as refugial grounds for multiple species, plant and animal (Braun 1950; Jackson *et al.* 2000; Rowe *et al.* 2004; Godbout *et al.* 2005; Heilveil & Berlocher 2006; Howes *et al.* 2006; Feldhamer 2007; Beatty & Provan 2011). Thus, when the ice front finally retreated (six advances and retreats are believed to have occurred during the Pleistocene before the final retreat after the LGM; e.g. Hewitt 1996, 1999; Avise 2000; Cox & Moore 2000), species predisposed to primary colonization from this refugium were able to begin dispersing northward early on.

Colonization routes

Phylogenetic analyses support the presence of two populating lineages, named LS (Lake Superior) and UPM (Upper Peninsula of Michigan). The NJ tree separates phylogroups 1 and 2,

representing these distinct lineages out of the western Wisconsin refugium. According to Lee-Yaw *et al.* (2008), one lineage entered Canada west of Lake Superior (LS) and another through the Upper Peninsula of Michigan (UPM), following the retreat of the glaciers. The pattern of haplotype diversity in the James Bay area supports the presence of these two lineages, as gene diversity ($\hat{H}$) values from localities 1 to 8 consistently exhibit lower values than localities 9-17, suggesting a geographic split (Xavier *et al.* 2010). However, larger sample sizes would be needed to infer significance to this comparison (Godbout *et al.* 2005). Nevertheless, another clear pattern is revealed by the haplotype distribution throughout the studied area. While Kenogami River (locality 9) is the only locality containing haplotypes from both east and west of the bay, no haplotypes observed in localities 1 through 8 were present in localities 10 to 17, and *vice versa*. Such dichotomy strongly supports the hypothesis of two separate lineages colonizing each coast of James Bay separately.

Since the retreat of the glaciers in Ontario and in the James Bay area is relatively recent, timing of divergence between these two lineages (UPM and LS, represented by phylogroups 1 and 2, respectively) may be problematical. The shallow DNA divergence observed between the haplotypes from both phylogroups (Smith & Green 2004; Gidis *et al.* 2011), and the low bootstrap support within phylogroup branches (Conroy & Cook 2000) imply a recent radiation. However, by comparing the outline of deglaciation in North America and associating it with the post-glacial range expansion of a primary colonizer such as the wood frog, a rough estimate of the chain of events leading to the colonization of the James Bay area by wood frogs may be inferred. According to Dyke (2004), both the Lake Superior and the Upper Peninsula of Michigan routes into Canada were free of ice by approximately 10 500 years BP. However, it took until 9000 years BP before wood frogs were able to move north of the Great Lakes region. By 7600 years BP, all of Ontario was free of glacial ice, although most of Ontario's Far North was covered by the Tyrrell Sea (Dyke 2004). The James Bay area is actually considered to be near the centre of glaciation of the Pleistocene ice-sheets (Martini 1981b), and thus, when deglaciation took place, the retreating ice front started splitting into two masses just south of the bay around 7800 year BP. James Bay's south-east coast was the first to be deglaciated (although it was still covered by water; Dyke 2004). By 7600 years BP, the only large obstacle in Ontario to wood frog colonization towards the North was the Tyrrell Sea, which covered the area presently known as the Hudson Bay Lowlands. With the shape of the sea and Ontario's Far North free of ice, wood frogs would have been able to colonize north-western Ontario early on. Genetic analyses show a general decreasing trend of genetic variation from west of the studied area (locality 2) toward the west coast of the bay (locality 4). Following the founder effect model, this would suggest a colonization route coming from the west, which supports the W1 lineage colonizing north-western Ontario then moving across towards the west coast of James Bay as the Tyrrell Sea receded with continental rebound (Figure 10). The Québec shores of James Bay were still in close proximity to the glaciers at 7600 years BP, making climatic conditions quite different to those on the Ontario side (Dyke 2004). It took until 7200 years BP for the glaciers to recede out of the James Bay area and until after 5000 years BP for the glaciers to disappear from Québec altogether (Dyke 2004). At this time, the James Bay area covered in this study was completely submerged under the Tyrrell Sea. It took several thousand years for its waters to recede and the present shoreline of James Bay to emerge, making the coasts of James Bay only very recently colonisable (after 3500 years BP; Martini *et al.* 1979; Martini 1981a). The Québec side of James Bay, as is seen through patterns of decreasing genetic variability, was colonized by wood frogs in a south to north direction.

According to phylogenetic analyses, wood frogs apparently colonized the coasts of James Bay in three distinct waves. One of the waves (E1) originated from the UPM lineage, crossing the Great Lakes via the Upper Peninsula of Michigan and colonizing southern Ontario and western Québec (Lee-Yaw *et al.* 2008). In fact, phylogroup 1 is more closely related to haplotypes from Highway 15, Van Dusen Farm,

Lagoon Park, Grundy Lake in Ontario, and Morgan Arboretum in Québec, than to haplotypes from western Ontario. The other two waves (W1 and W2) originated from the LS lineage following a colonization path around Lake Superior north-west from Wisconsin into Ontario (see Figure 6 in Lee-Yaw *et al.* 2008). The W1 wave originated from the Thunder Bay area colonizing the area north-west of James Bay (phylogroup 2b from the NJ tree), while the W2 wave moved further along a line from the Thessalon and Surluga region (Figure 10). Hence, with the genetics patterns highlighted above, it is likely that W1 colonized the north-west coast of James Bay from a westerly direction, that E1 colonized the east coast of the bay in a northward ascension, with some individuals also heading towards the west coast, and that W2 added alleles to the contact zone south-west of the bay where W1 and E1 individuals met (Figure 10).

The case of haplotype 19

Although the pattern of colonization is reasonably clear based on these phylogeographic analyses, haplotype 19 is enigmatic. Haplotype 19 is only observed in localities 1 and 5, north-west of James Bay. However, in both phylogenetic trees (ML and NJ), haplotype 19 separates from phylogroup 1, which encompasses all haplotypes east of the bay. Geographically, this clustering pattern is difficult to explain and further sampling would be needed. Nonetheless, the position of haplotype 19 in both the NJ and ML trees and in the haplotype network suggests a possible ancestral haplotype pattern (Weiss *et al.* 2002; Miraldo *et al.* 2011). This haplotype appears to be the closest common relative of phylogroup 1 and 2b (Figure 11) and its position in the NJ tree suggests that it may be an ancestral haplotype to phylogroup 1 (Figure 9). However, the relative abundance of haplotype 19 is low (2 individuals out of 76), and according to Fernández-Mazuecos & Vargas (2011), ancestral haplotypes should be widely distributed throughout the area. In the future, larger numbers of sequences could possibly uncover missing haplotypes, in turn explaining the relationship between haplotype 19 and the phylogroups recovered here.

Secondary contact zone

The SOUTH-WEST genetic population (Figure 6) was identified as the area of secondary contact between the LS and UPM wood frog lineages. According to microsatellite and mitochondrial analyses, this population contains the highest genetic variation, a phenomenon associated with genetic admixture (Durand *et al.* 2009; Sakaguchi *et al.* 2011). Multiple routes of colonization and zones of high genetic diversity where expanding populations met were also observed in a study by Austin *et al.* (2002) on spring peepers (*Pseudacris crucifer*). They identified a zone of secondary contact by the discovery of haplotypes from divergent clades in the same vicinity. This phenomenon is also observed here, where divergent haplotypes from phylogroups 1 and 2 (2a and 2b) are sympatrically distributed at locality 9; hence indicating the location of the zone of admixture (Austin *et al.* 2002). A secondary contact zone between the LS and UPM lineages was already proposed to be present in northern Ontario (Lee-Yaw *et al.* 2008), and although the entire zone of contact has not been mapped out, it is now clear that part of this zone does exist south-west of James Bay.

Phylogenetic method comparison

Two methods of phylogenetic inference were used to uncover the colonization pattern of James Bay wood frog populations: Maximum Likelihood (ML) and Neighbour-Joining (NJ). Interestingly, key differences were observed in the resulting phylogenetic trees between these two methods. First, although the ML and NJ trees both recovered phylogroups with matching haplotype composition, the arrangement of phylogroups 2 and 3 are not the same. In the ML tree, three phylogroups are defined:

one regrouping localities from east of the bay (phylogroup 1 – localities 9-17), one representing localities from south-west of the bay (phylogroup 2 – localities 9 and 10), and the third comprising the haplotypes found north-west of the bay (phylogroup 3 – localities 1-9). Localities encompassing haplotypes from more than one phylogroup are Moosonee and Kenogami River (if haplotype 19 is not considered – see above). The relationships between all three phylogroups are of equal hierarchy, which may suggest three waves of colonization towards the North as well as demonstrate the close relatedness of the diverging branches. In the case of the NJ tree, phylogroup 2 encompasses all the haplotypes observed west of the bay, but is subdivided (2a and 2b) similarly to phylogroups 2 and 3 in the ML tree. This subdivision may imply that phylogroups 2a and 2b are more closely related to each other than to phylogroup 1. Second, since bootstrap values were very low in the ML tree, no relationships between branches were supported significantly. In both ML and NJ trees, bootstrap values within phylogroups were lower than 65%. The weakness of relationships between phylogroups is also reflected in the haplotype network (Figure 11), whereby the low resolution of the network (presence of loops) emphasizes both the shallow divergence between the sequences and the shallow phylogeographic relationships between them (Panchal & Beaumont 2007; Bisconti *et al.* 2011). Recent colonization events and modern lineage divergence resulted in non-significant differentiation between haplotypes. It is worth mentioning that comparing lineages originating from the same clade decreases the chances of recovering significant relationships because of the shallow hierarchy.

Conclusion and future research opportunities

Population genetics characteristics of wood frogs in the James Bay area can be largely explained by the species' post-glacial range expansion out of the western Wisconsin refugium and through the subsequent admixture between colonization routes at a secondary contact zone south-west of the bay. The presence of this secondary contact zone increased the observed genetic variation in the area and is likely responsible for the population structure. The north-western and south-western coasts of James Bay were populated primarily by two colonization waves from the LS lineage, while the eastern coast was colonized by one wave from the UPM lineage. The Ontario and Québec coasts of James Bay thus exhibit genetic characteristics reflecting their divergent histories, which are evident through phylogeographic analyses.

One application that would greatly aid in understanding the causes of the distribution of genetic differences observed in wood frog populations and possibly other species in the James Bay area is the study of landscape genetics. The topography of coastal areas from the mouth of the Winisk River to the Eastmain River is flat and uniform, with the exceptions of wide valleys carved by rivers through the soft Quaternary sediments (Prest 1970). Nine major rivers (Winisk, Ekwan, Attawapiskat, Albany, Moose, Harricana, Nottaway, Broadback, and Rupert River) and countless smaller tributaries run through the Hudson Bay Lowlands (Prinsenberg 1980; Desroches *et al.* 2010; Déry *et al.* 2011). The north-eastern coast of James Bay (Taiga Shield ecozone) has higher relief than the Hudson Bay Lowlands, but is still entrenched by large river systems, many exploited for their hydroelectric potential (Hydro-Québec 2011). The Moose and Harricana Rivers south of the bay and the Albany River west of the bay outline the geographic limits between all three genetic populations, perhaps comprising barriers to the dispersal of wood frogs. However, with the presence of other major rivers ostensibly having no effect on the genetic structure of wood frogs, future research should look into the effects of the landscape's hydrology on anuran population connectivity in the James Bay area. For example, small waterways may act as colonizing corridors (Adams *et al.* 2005; Spear *et al.* 2005), but larger rivers with increased width and flow may more likely act as barriers to anuran movement and dispersal (Lee-Yaw *et al.* 2009; Moore *et al.* 2011). The roles of the James Bay rivers on gene flow would thus need to be better described for a

more comprehensive view of their relationship with each other, especially with impending climate change and hydroelectric developments altering streamflow into the bay (Déry *et al.* 2011).

Alternatively, other landscape features may better predict the genetic structure observed in the James Bay area. Several studies suggest that older landscape features, such as ridges and other topographical features no longer evident in the landscape, have had a greater impact on contemporary genetic structure than present physical barriers (Lougheed *et al.* 1999; Gascon *et al.* 2000; Jordan *et al.* 2009). This impact may emphasize the importance of landscape history and of phylogeographic studies of range expansions in post-glacial landscapes. Landscape genetic approaches would thus greatly help in understanding the roles of past geological features (such as late intercontinental seas or glacial ridges) and of the present landscape's heterogeneity (including hydrology) on the connectivity of habitats from the wood frog's perspective (Holderegger & Wagner 2006).

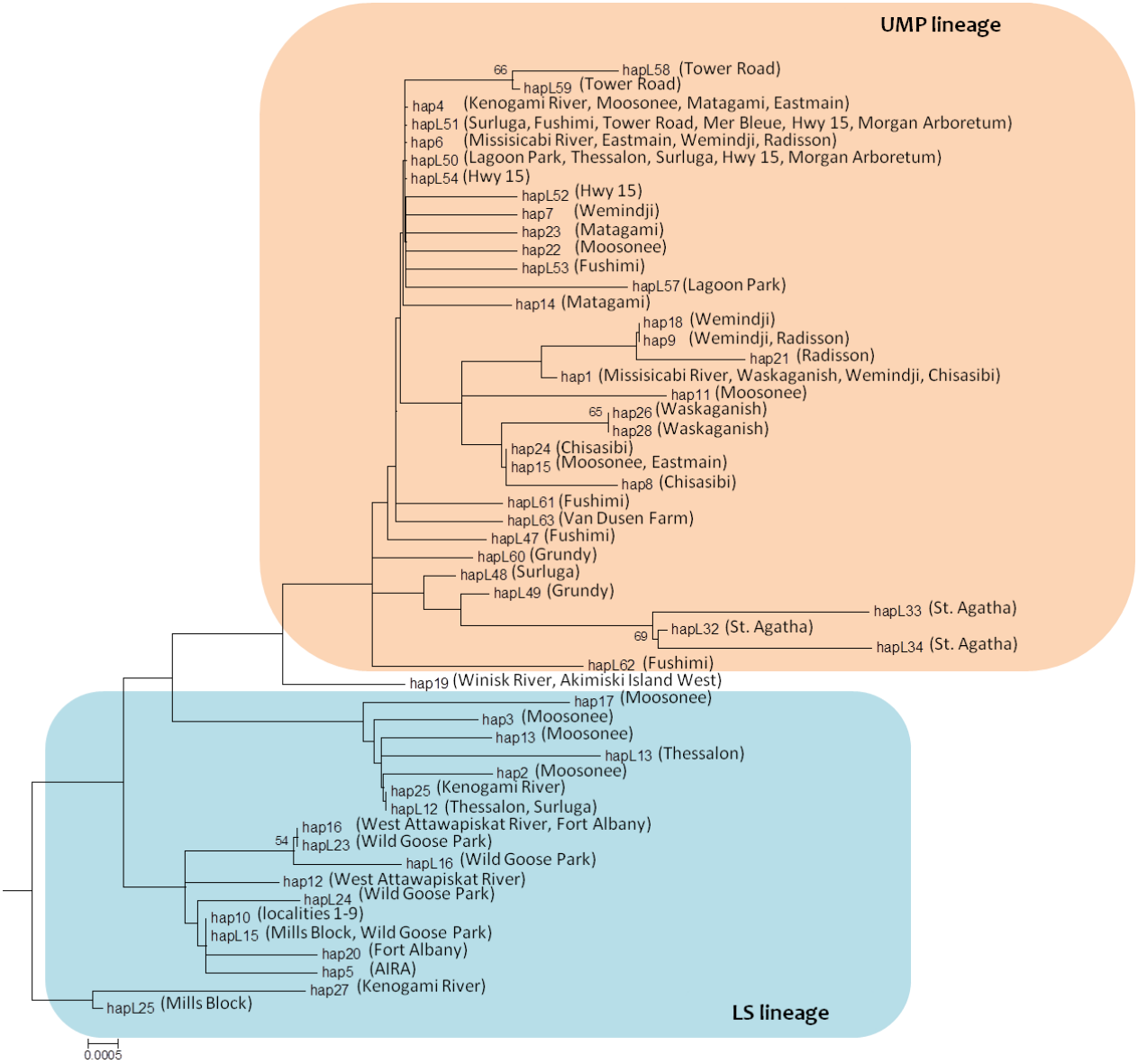
This research project constitutes a first step towards understanding the distribution of genetic diversity in wood frog populations in the James Bay area. It will provide access to more extensive research aiming to discern the causes of such genetic patterns and represents baseline data of high importance when considering the changing climate and its effects on northern landscapes. In fact, the results presented here have implications for conservation and environmental impact assessment studies. While morphological studies are not always effective at discerning patterns of phylogeography (Conroy & Cook 2000), disentangling the genetic makeup of the wood frog in the James Bay area allowed for a better understanding of its post-glacial history. Such temporal investigation may provide information on the evolutionary trends followed by northern species and may also unveil other barriers and influences on their population structure. This information is in turn critical for conservationists and local community members, as understanding how species responded to previous climatic changes is becoming more and more important to predict future behaviours and distributions with regards to the current period of climate change.

APPENDIX A – Genbank accession numbers of haplotypes from Lee-Yaw *et al.* (2008) used to infer phylogeographic relationships.

Western Clade haplotypes	
hapL12	EU203480
hapL13	EU203481
hapL15	EU203482
hapL16	EU203485
hapL23	EU203486
hapL24	EU203487
hapL25	EU203488
hapL32	EU203494
hapL33	EU203495
hapL34	EU203496
hapL47	EU203511
hapL48	EU203519
hapL49	EU203509
hapL50	EU203505
hapL51	EU203507
hapL52	EU203510
hapL53	EU203512
hapL54	EU203506
hapL57	EU203513
hapL58	EU203520
hapL59	EU203521
hapL60	EU203522
hapL61	EU203525
hapL62	EU203523
hapL63	EU203524

Eastern Clade haplotypes	
hapL66	EU203337
hapL71	EU203345
hapL74	EU203340
hapL75	EU203391
hapL95	EU203367
hapL97	EU203369
hapL98	EU203375
hapL101	EU203373
hapL102	EU203395
hapL105	EU203399

APPENDIX B – Evolutionary relationships of the Western Clade in Ontario and Québec inferred using the NJ method (Saitou & Nei 1987). The bootstrap consensus tree was built from 1000 replicates (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei & Kumar 2000) and are in the units of the number of base differences per site. There were a total of 542 bp positions compared in the final dataset. Analyses were conducted with MEGA5 (Tamura *et al.* 2011).



APPENDIX C – Pairwise F_{ST} (below the diagonal) and probability that the genotypes are identical between sampling sites when all loci are combined (above the diagonal) for wood frog localities across the studied area. Significant values are indicated by bold type. Please note that the Akimiski Island East locality did not amplify genotypes for one locus and thus probability values are lacking.

Locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 Winisk River	-	0.1474	0.1382	0.0125	0.0055	0.0004	NA	0.0004	0.0004	0.0004	0.0007	0.0007	0.0004	0.0004	0.0004	0.0004	0.0004
2 West Attawapiskat River	0.0203	-	0.7614	0.0471	0.5125	0.0004	NA	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
3 Victor Mine	0.0467	0.0017	-	0.5349	0.3540	0.3890	NA	0.0007	0.0011	0.0004	0.0029	0.0085	0.0022	0.0004	0.0004	0.0007	0.0015
4 Attawapiskat	0.0150	0.0093	0.0165	-	0.6232	0.0004	NA	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
5 Akimiski Island West Point	0.0580	0.0083	0.0282	0.0201	-	0.0967	NA	0.0015	0.0018	0.0004	0.0136	0.0180	0.0022	0.0007	0.0004	0.0007	0.0015
6 Akimiski Island Research Area	**0.0305**	**0.0158**	0.0101	**0.0071**	0.0282	-	NA	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
7 Akimiski Island East	0.0780	0.0203	0.0119	0.0226	0.0277	0.0154	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8 Fort Albany	**0.0904**	**0.0694**	0.0733	**0.0861**	0.0857	**0.1156**	0.0902	-	0.0081	0.0018	0.0007	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
9 Kenogami River	**0.0868**	**0.0679**	0.0647	**0.0803**	0.0775	**0.1095**	0.0865	0.0144	-	0.2529	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
10 Moosonee	**0.0968**	**0.0722**	**0.0800**	**0.0903**	**0.0838**	**0.1185**	0.0928	0.0156	0.0032	-	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
11 Missisicabi River	0.2046	**0.1691**	0.1818	**0.1844**	0.1602	**0.2121**	0.1755	0.0977	**0.1016**	**0.0967**	-	0.1960	0.0342	0.0004	0.0004	0.0004	0.0004
12 Matagami	0.1644	**0.1456**	0.1405	**0.1514**	0.1227	**0.1745**	0.1503	**0.0958**	**0.1046**	**0.0949**	0.0455	-	0.0423	0.0044	0.0555	0.0239	0.1338
13 Waskaganish	**0.1802**	**0.1680**	0.1744	**0.1746**	0.1450	**0.1947**	0.1658	**0.1004**	**0.0933**	**0.0894**	0.0223	0.0336	-	0.0412	0.0015	0.0004	0.0827
14 Eastmain	**0.1678**	**0.1420**	**0.1481**	**0.1598**	0.1169	**0.1792**	0.1495	**0.1020**	**0.0947**	**0.0862**	**0.0581**	0.0263	0.0071	-	0.0007	0.0004	0.0074
15 Wemindji	**0.1893**	**0.1608**	**0.1564**	**0.1750**	**0.1271**	**0.1892**	0.1587	**0.1247**	**0.1239**	**0.1140**	**0.0420**	0.0181	0.0155	0.0138	-	0.0004	0.0243
16 Chisasibi	**0.1297**	**0.1094**	0.1138	**0.1275**	0.1013	**0.1419**	0.1140	**0.0921**	**0.0789**	**0.0730**	**0.0701**	0.0243	**0.0477**	**0.0271**	**0.0358**	-	0.3070
17 Radisson	**0.1628**	**0.1374**	0.1368	**0.1528**	0.1165	**0.1710**	0.1315	**0.1028**	**0.0936**	**0.0900**	**0.0505**	0.0095	0.0113	0.0043	0.0076	0.0017	-

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