# The Phylogeographic History of the Wood Frog (*Rana sylvatica*)

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## Abstract

Although the range dynamics of North American amphibians during the last glacial cycle are increasingly better understood, the recolonization history of the most northern regions and the impact of southern refugia on patterns of genetic diversity in these regions are not well reconstructed. In this study I present the phylogeographic history of a widespread and primarily northern frog, *Rana sylvatica*. For this study, 45 individuals from 34 localities were surveyed for a 700 b.p. fragment of cytochrome b and 551 individuals from 116 localities were surveyed for 650 b.p. of the NADH dehydrogenase subunit 2 and $tRNA^{TRP}$ mitochondrial genes. Phylogenetic analyses revealed two distinct clades corresponding to eastern and western populations. Phylogeographic patterns within each of these clades revealed similarities as well as differences from patterns found in other species. Specifically, the results corroborate eastern refugia located in the southern Appalachians near present-day North and South Carolina and in the interior plains in the lower Ohio River Valley. Current Maritime populations form a subclade amongst eastern populations and appear to have been colonized from the southern refugium. However, a more northern refugium located in the Appalachian highlands seems to have been source for most other northeastern wood frog populations. *Rana sylvatica* populations in the Great Lakes region appear to have been colonized from a western refugium located in present-day Wisconsin. This refugium was also a likely source for populations in the species' expansive northwestern range since there is no evidence to support additional, more western refugia.

## Résumé

Malgré le fait que la compréhension de l'expansion de la distribution des amphibiens d'Amérique du Nord depuis la dernière glaciation est en continuelle évolution, l'histoire de la recolonisation des régions les plus nordiques et l'impact des refuges de plus faible latitudes sur les patrons de diversité génétique ne sont pas bien reconstitués. Dans cette étude, je présente l'histoire phylogéographique d'une grenouille répandue et originellement nordique, *Rana sylvatica*. Pour ce faire, 45 individus provenant de 34 sites ont été étudiés pour un fragment de 700 p.b. du gène mitochondrial cytochrome b et 551 individus provenant de 116 sites ont été analysé pour un fragment de 650 b.p. de la sous-unité 2 de la région NADH deshydrogénase ainsi que pour le gène mitochondrial ARNtTRP. L'analyse phylogénétique de ces gènes a révélé deux clades distincts correspondants aux populations de l'est et de l'ouest du continent. Les divers patrons phylogéographiques retrouvés dans chaque clade montrent à la fois des similitudes et des différences avec les patrons retrouvés chez d'autres espèces. Plus spécifiquement, les résultats corroborent la présence d'un refuge situé au sud des Appalaches près des actuelles Carolines (du Nord et du Sud) et des plaines du sud de la Vallée de la Rivière Ohio. Les populations modernes des maritimes forment un sous-clade parmi les populations de l'Est, et semblent avoir été colonisées depuis les refuges plus au sud. Toutefois, un refuge plus nordique, situé sur les plateaux appalachiens, semble être la source de la majorité des populations du nord-est de l'aire de distribution des grenouilles des bois. Les populations de *Rana sylvatica* de la région des Grand Lacs semblent quant à elles être issues d'un refuge plus à l'ouest situé de nos jours au Wisconsin. Ce refuge serait aussi la source probable des populations dont la distribution est la plus nordique dans l'ouest du continent américain, puisque aucune donnée ne montre l'existence d'un autre refuge.

For Squirt, one of many.

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## Preface and Contributions of Authors

This thesis was prepared and completed in accordance with the "Thesis Submission Guidelines" of the Faculty of Graduate and Postdoctoral Studies of McGill University. Supervision was provided by Dr. David Green. Sample collection was organized and conducted by the author and by J.T. Irwin. Molecular work was conducted in the laboratories of Dr. Ehab Abouheif and Dr. Thomas Bureau by J.A.Lee-Yaw (ND2/tRNA$^{TRP}$ gene) and J.T. Irwin (cytochrome b gene). Both J.A.Lee-Yaw and J.T. Irwin were responsible for sequence editing and alignment. J.A.Lee-Yaw conducted all analyses and wrote the manuscript included in the thesis with input from D.M. Green and J.T. Irwin.

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# General Introduction and Literature Review

## 1.1 Introduction

The Quaternary Period, comprised of the Pleistocene Epoch (1.8 mya to 11.5 kya) and the current Holocene Epoch (11.5 kya to present), has been characterized by repeated episodes of glacial advance and retreat. In response to these climatic events, temperate species have made several, large-scale (primarily north-south) migrations (Huntley and Webb, 1989; Hewitt, 1996). These migrations have continually altered the configuration of populations within species (Hewitt, 1996) and the composition of entire biological communities (Williams *et al.*, 2004) and have therefore influenced levels of population connectivity, the distribution of genetic diversity and the selective environments encountered by species. Understanding evolutionary processes in northern regions is thus augmented by an examination of the specific biogeographic responses of species to Quaternary events.

Increasingly, studies are using genetic markers and phylogeographic methodology to verify coarse-scale patterns of Quaternary range adjustments inferred from the fossil and pollen records and to further resolve the histories of specific populations. In the following discussion, I review the phylogeographic information available for North American amphibians with the goal of outlining general patterns in their responses to Quaternary climatic events. Most studies to date have utilized sequence (haplotype) data from mitochondrial genes because base substitutions in the mitochondrial genome occur at a rate suitable for tracking recent events and because the mitochondria is uniparentally inherited without recombination to confound genealogical history (Avise, 2004). Thus reference is made to this type of data unless otherwise stated. The last glacial cycle is specifically discussed, because events from this cycle are the best preserved in the paleoenvironmental record and because these events have had the most recent impact on the present-day distribution of biological diversity. The implications of Quaternary range adjustments for the evolutionary history of northern amphibians and for the conservation of this taxonomic group in North America are discussed.

## 1.2 Amphibians as a Focal Group

Amphibians have several attributes that make them an appropriate taxonomic group with which to delineate the biogeographic effects of Quaternary events. Their limited vagility (compared to larger mammals and birds), high site fidelity (Vosconceles and Clahoun, 2004; Blihovde, 2006) and sensitivity to geographic barriers (Rittenhouse and Semlitsch, 2006) make them Category I species in the classification scheme of Avise (2000). That is, they generally exhibit high levels of genetic structure among populations, particularly in relation to the physical landscape (Lougheed *et al.*, 1999; Spear *et al*, 2005; Funk *et al.*, 2005; Kraaijeveld-Smit *et al.*, 2005; Stevens *et al.*, 2006). Thus amphibians are natural candidates for tracing population history with genetic markers. Furthermore, many North American amphibians have wide ranges, encompassing both previously glaciated and unglaciated terrain, as well as many putative colonization barriers. The signature of Quaternary events captured in the genetic structure of amphibians over large areas can thus inform broader hypotheses about the effects of specific landscape features on patterns of range adjustment made by a multitude of species.

In addition to being informative with respect to the general effects of Quaternary events on species' distributions, specific information about temperate amphibian populations is increasingly required for conservation purposes. Currently in the United States and Canada, 48 amphibian species are listed as endangered, threatened or of special concern under federal programs. Multiple, complex factors threaten the survival of existing amphibian populations (see Blaustein and Kiesecker, 2002; Beebee and Griffiths, 2005). Of these, habitat loss and fragmentation are major factors contributing to population declines (Cushman, 2006). Predictions regarding the ultimate consequences of these modern-day threats can be informed by determining the responses of amphibian populations to habitat loss associated with historical climate change. Furthermore, delineating the historical affinities between populations with genetic markers is useful for guiding conservation initiatives such as reserve design and population reintroductions.

Holman (2000, 2001) outlined the various stresses imposed by the Pleistocene glaciations on northern amphibians. Included in his list were: 1) community obliteration by advancing ice 2) topographic changes 3) climatic changes and 4) vegetation change. Although these factors broadly affected all northern taxa, several aspects of amphibian biology need to be considered when making predictions regarding the responses of amphibians to environmental change. First, as ectotherms, amphibians are dependent on environmental temperatures for metabolic function. Overwinter survival and summer growth rate are limited by temperature regimes: most amphibians cannot withstand freezing temperatures or very cold climates. Second, amphibians require moist conditions for efficient respiration, hydration and breeding and thus the distribution of species is highly influenced by patterns of precipitation. Third, most temperate species are closely associated with forest habitat (taiga or boreal, cool mixed and deciduous forests) and thus the distribution of these biomes governs the potential distribution of most species. Finally, as mentioned previously, amphibians have limited mobility compared to other taxa and are sensitive to dispersal barriers. Thus understanding range adjustments made by temperate amphibians in response to Quaternary events requires specific attention to the distribution of equable climates and forested environments, as well as consideration of the role of geographic features in shaping migration pathways.

## 1.3 Amphibian Responses to Glacial Advance

### *1.3.1 General Inferences Drawn from the Paleoenvironmental Record*

The height of the last glacial advance occurred 21,000 - 18,000 years ago during what is referred to as the Wisconsinan glacial episode in North America. At its maximum, glacial ice reached latitudes as far south as 40 degrees N (Figure 1.1) and achieved thicknesses of up to 3 km (Sutcliffe, 1986). Paleoclimatic simulations and the plant macro-fossil and pollen records suggest that the distribution of climatic zones was dramatically different during this period. Temperatures throughout the northern hemisphere were, on average, 5°C lower

than present (Broccoli and Manabe, 1987; Webb, 1992) with regions near the ice more than 20°C colder (Kutzbach and Guetter, 1986). These cool conditions would have restricted the ranges of all but the most cold-adapted amphibians to more southern regions of the continent where temperatures were warmer. Precipitation patterns were also markedly different. Drier conditions existed inland (Pielou, 1991) and in the Pacific Northwest (Thompson and Anderson, 2000) while the southwestern United States (currently under desert conditions) likely received abundant precipitation due to a southern diversion of the jet stream (Webb and Bartlein, 1992; Bartlein *et al.*, 1998). Thus apart from the southwest, coastal areas likely offered the most suitable conditions for amphibian species in terms of moisture levels. Coastal areas were also more exposed and provided large tracts of territory for terrestrial species during the height of the last glaciation due to reductions in sea level that accompanied the formation of the glaciers (Sutcliff, 1986; Pielou, 1991).

Vegetation patterns recorded in the palynological record provide insight into the distributions of northern forests and, by extension, the potential distribution of their inhabitants during the last glaciation. Although some tree species were found directly adjacent to the ice edge in many regions of North America, trees made up a low percentage of the vegetation in these otherwise, parkland- or tundra-like communities (Thompson and Anderson, 2000; Williams *et al.*, 2004; Yansa, 2006). Apart from pockets of boreal forest in the northeast (Williams *et al.,* 2004) and of cool conifer forest in the Pacific Northwest (Thompson and Anderson, 2000) most regions near the ice would have been unsuitable for amphibians. Instead, forest biomes were largely compressed to the south. For example, the temperate-deciduous forest and warm, mixed forest that occupies most of the Eastern United States today was restricted to the Florida Peninsula during the last glacial maximum while most of eastern North America south of the ice was covered by the cool, mixed forest that is found at higher latitudes today (Williams *et al.*, 2004).

Although climatic and vegetational shifts during periods of glacial advance are generally thought to have led to the expansion of species' southern

distributions (Hewitt, 1996), concordance between the amphibian fossil record and species' current geographic distributions suggests that the ranges of most North American amphibians remained largely unchanged in unglaciated regions of the continent (Fay, 1988; Holman, 1995, 2000). In some cases, existing (or rapid evolution of) physiological tolerances may have permitted species to maintain their latitudinal positions south of the ice despite environmental change (Fay, 1988; Holman, 2000). In other cases, persistence at the same latitude during glacial advance and retreat may have involved tracking suitable climate altitudinally (Hewitt, 1996). Southern range expansion by northern species on the other hand, may have potentially been limited by competition with existing southern species (Crespi *et al.*, 2003), which is thought to limit many species' ranges today (Case and Taper, 2000; Gross and Price, 2000).

Range contraction from the north along with topographic influences would have fragmented the ranges of many amphibians during periods of glacial advance. Thus, based on palynological and paleontological evidence, amphibian range dynamics during the Pleistocene glaciations are expected to fit a model of contraction into multiple, southern refugia. The fossil record places many eastern species in the coastal plains (e.g. present-day Florida and Texas) as well as in the southern Appalachian region (e.g. present-day Tennessee and West Virginia) during the height of the last glaciation (Holman, 1995). In the west, amphibian fossils dating to this time period are documented from the Pacific Coastal region (e.g. in present-day California) as well as the Great Plains (e.g. present-day Nevada and Kansas). Accordingly, phylogeographic surveys are expected to reveal ancestral haplotypes and evidence of genetic differentiation owing to historical isolation (Hewitt, 1993) in these regions. These regions should also harbour higher levels of genetic diversity than more recently colonized regions that were subject to founder effects during the colonization processes (Hewitt, 1996; Petit *et al.*, 2003).

### *1.3.2 Genetic Patterns East of the Mississippi River*

Many widespread eastern amphibians demonstrate phylogeographic patterns consistent with range contraction into multiple, glacial refugia (Figure

1.1, Table 1). For instance, Austin *et al.* (2004) found evidence for allopatric divergence between eastern and western *Rana catesbeiana* populations separated by the Mississippi River. The distribution of presumed ancestral haplotypes in this species is consistent with the hypothesis that these frogs persisted in glacial refugia in the Gulf and Atlantic coastal plains (Austin *et al.*, 2004). This finding is in agreement with the habitat and climatic conditions described above, as well as with the fossil records for north-central Florida and the coastal areas of Texas (Holman, 1995). The genetic patterns observed in several other eastern taxa also point to persistence in coastal areas during the Pleistocene (Table 1). For example, Zamudio and Savage (2003) found evidence for the expansion of *Ambystoma maculatum* populations from Atlantic coastal plain refugia near the Carolinas. Similarly, Church *et al.* (2003) found the highest levels of within-population genetic variation in this region for eastern *Ambystoma tigrinium tigrinium* populations, consistent with a refugium in the area.

Apart from coastal refugia, eastern species appear to have also persisted in more northerly refugia in the Appalachian Mountains and interior lowlands (Figure 1.1, Table 1). For instance, Austin *et al.* (2002) found higher levels of nucleotide diversity in populations of *Pseudacris cruficer* in Tennessee relative to other populations suggesting that populations in this region are older than populations elsewhere. Furthermore, populations of this species in the southern Appalachian Mountains and Ozark highlands are genetically distinct, which is consistent with allopatric divergence within multiple refugia in these regions (Austin *et al.*, 2004). Although *Pseudacris* species are absent from the fossil record of the Ozark Region (Holman, 1995), specimens of *Pseudacris crucifer* dating to the late Wisconsinan are found in a variety of Appalachian locations (Holman, 1995; 2003). Even further north, *Ambystoma tigrinium tigrinium* individuals in the Blue Ridge Mountains of Virginia are highly divergent from other northeastern populations and are thought to have existed as an isolated population during much of the Pleistocene (Church *et al.*, 2003). Thus the Appalachian Mountains played an important role in the glacial range dynamics of

eastern amphibians, separating interior and coastal plain refugia as well as harbouring distinct refugia where pockets of suitable habitat persisted.

### *1.3.3 Genetic Patterns West of the Mississippi River*

In the west, genetic evidence also supports the existence of multiple coastal and inland refugia (Figure 1.1, Table 1), many of which are associated with various mountain ranges. For example, coastal populations of *Ascaphus truei* in the Olympic, North Cascades, Coastal Range and Siskiyou Mountains form discrete mitochondrial groups that likely began to diverge during the late Pliocene or early Pleistocene and continued to diverge in isolated refugia during the Pleistocene glaciations (Nielson *et al.*, 2001). Even deeper divergence between coastal and Rocky Mountain populations of this species, along with high levels of haplotype divergence among some Rocky Mountain populations, suggests that this species also persisted in multiple, inland, mountain refugia (Nielson *et al.*, 2001). Results from other studies further support these findings. A distinct coastal refugium in the Klamath-Siskiyou Mountains was one of two refugia identified for *Dicamptodon tenebrosus* in a study by Steele and Storfer (2006). Similarly, Carstens *et al.* (2004) identified a Rocky Mountain refugium for *Plethodon idahoensis* based on estimates of population growth inferred from demographic analysis and patterns of range expansion inferred from Nested Clade Analysis. The Columbia River Valley also appears to have supported amphibian populations during glacial advance based on patterns observed for both *Dicamptodon tenebrosus* (Steele and Storfer, 2006) and *Plethodon larselli* (Wagner *et al.*, 2005). In contrast to steppe-like conditions elsewhere in the Pacific Northwest, this region appears to have harboured pockets of cool, coniferous forests during the height of the Wisconsinan (Thompson and Anderson., 2000), which would have been suitable habitat for these species.

There is limited information available about the refugial locations of western species currently distributed east of the Rocky Mountains. However, in line with amphibian fossil deposits found in Clark County, Nebraska (Holman, 1995), Hoffman and Blouin (2004) found ancestral haplotypes for *Rana pipiens* in the northern plains, indicating that the species persisted somewhere near present-

day Nebraska during the Pleistocene. Jaeger *et al.* (2005) provide evidence to support *Bufo punctatus* refugia further south in the Mojave (Eastern California/Nevada) and Chihuahuan (Texas) deserts. This species is adapted to desert habitats and has a current distribution lying almost entirely below the historic extent of glacial ice. Thus the phylogeographic patterns observed for *Bufo punctatus* speak to the types of range adjustments made by those southern inhabitants of the Great Plains adapted to more arid conditions.

### *1.3.4 The Distribution of Endemic Species*

In general, the amphibian refugial locations identified in the literature based on genetic patterns (see Table 1) align well with the geological, palynological, and paleontological evidence. Specifically, northern species appear to have persisted in multiple, southern refugia. Further support for the location of putative refugia outlined in the phylogeographic literature comes from the distributions of narrowly distributed amphibians. Many of these endemics have populations within or adjacent to presumed refugia (Table 2). Phylogenetic reconstruction and molecular dating suggest that at least some of these species arose prior to the Quaternary (e.g. Carstens *et al.*, 2004; Jones *et al.*, 2006). The overlap of these species' distributions with the locations of proposed amphibian refugia therefore supports the notion that these areas have consistently harboured amphibian populations throughout periods of glacial advance.

## 1.4 Amphibian Responses to Glacial Retreat

### *1.4.1 General Inferences Drawn from the Paleoenvironmental Record*

It has been estimated from the fossil record that, in response to shifting climates and vegetation, amphibians were able to begin expanding their ranges into previously glaciated areas as early as 13,000 ybp (Holman, 1995; 2000). Tree species largely tracked temperature and precipitation changes (Williams *et al.*, 2004), driving shifts in the distribution of various biomes and thus influencing the availability of suitable habitat for amphibians. The taiga biome captured the largest portion of previously glaciated North America, expanding rapidly from

northern pockets into the ice-free corridor that opened up between the Laurentian and Cordilleran ice sheets ~12,000 ybp (Strong and Hills, 2005) and eventually encompassing most of Canada (Williams *et al.*, 2004). Species of amphibians associated with this biome thus gained the most habitat following glacial retreat and, where barriers did not impede migration, were potentially able to make large, rapid range adjustments.

In the east, as the Laurentian ice sheet retreated inland away from the coasts, cool, mixed forest expanded north and moved into the Maritime region (Williams *et al.*, 2004), opening up a coastal corridor for forest species into the northeast (Pielou, 1991). At more southern latitudes in the east, temperate, deciduous and warm, mixed forests expanded from the southeast to encompass regions south of the Great Lakes (Williams *et al.*, 2004). Meanwhile, steppe conditions in the Pacific Northwest were replaced by cool, conifer and boreal forests (Thompson and Anderson, 2000). The Great Plains made a brief transition from spruce parkland to deciduous parkland and finally to prairie forbs and grasses in response to increasing aridity and soil drying during the Pleistocene-Holocene transition (Williams *et al.*, 2004; Yansa, 2006). This dry region, including a narrow extension of prairie that extended as far east as Lake Erie (the Prairie Peninsula: Transeau, 1935), would have been inhospitable to many amphibians, preventing the east-west migration of individuals south of the former glacial maximum and delaying the northward expansion of populations from the Interior and Great Plains.

Although forest habitat became rapidly available across much of the continent, northern range expansion required contending with numerous topographic changes associated with glacial retreat. Melting ice created many pro-glacial lakes during the period between ~15,000 and 8,000 years ago (Pielou, 1991). The largest of these included Glacial Lakes Lake Missoula and Lake Columbia in the west, Glacial Lakes Lake Agassiz and Lake McConnell in the Prairies, and the many precursors to the present-day Great Lakes in the east (especially Glacial Lake Algonquin). Drainage patterns associated with these lakes underwent many changes in response to isostatic rebound and the formation

and breaking of ice dams before leaving us with the present-day Columbia, Mississippi and Saint Lawrence river basins among others (Pielou, 1991). Sea levels also rose, submerging much of the coastal plains and, in combination with isostatic depression, gave rise to the Champlain Sea—an inlet of the Atlantic ocean that covered parts of Ontario, Quebec, New York and Vermont from 12,000 ybp to about 10,000 ybp (Pielou, 1991).

Unlike very mobile taxa or species able to disperse by wind or air, topographic features would have had a large impact on the timing and routing of amphibian range expansion. In combination with expansion from multiple glacial refugia, topographic features would have served to promote the presence of multiple genetic lineages within the post-glacial ranges of many widespread North American amphibians. Accordingly, recently colonized (generally northern) regions are expected to harbour populations with divergent mitochondrial DNA haplotypes. The location of specific genetic breaks should correspond with existing or historical landscape features, depending on the region in question and the timing of species' individual range expansions.

### *1.4.2 Genetic Patterns East of the Mississippi River*

The expansive glacial Lake Algonquin system and the partially saline Champlain Sea would have served as formidable barriers to the earliest colonizing amphibians in the east, preventing expansion from the Appalachian region. However, some species would have been able to follow the western retreat of the Laurentian ice sheet into areas north of the Great Lakes. For instance, *Rana pipiens* haplotypes north of Lake Superior belong to a distinct western clade of this species, suggesting that one colonization route taken by the species was an eastward path of expansion from a western refugium (Route 4, Figure 1.2) (Hoffman and Blouin, 2004). Subsequent draining of Lake Algonquin and the Champlain Sea ~10,000 ybp would have allowed additional populations of this and other species to move north from eastern refugia (Route 7 in Figure 1.2, Table 1). Hence, *Pseudacris crucifer* appears to have entered the north primarily via a corridor east of the Great Lakes (Austin *et al.*, 2002). Still other populations appear to have penetrated the Great Lakes area via Michigan and southwestern

Ontario (Holman,1992; 1995; Austin *et al.*, 2002) following the westward retreat of the Prairie Peninsula (Route 6 in Figure 1.2, Table 1). Apart from the Great Lakes and their predecessors, northern genetic breaks within and among eastern taxa line up with the Mississippi River (Hoffman and Blouin, 2004; Moriarty and Cannatella, 2004), suggesting that this feature also impacted colonization patterns. Surprisingly, the Appalachian Mountains, although playing a role in the genetic structuring of populations during glacial advance, do not seem to have been much of a barrier to expanding amphibians populations (e.g. see expansion patterns outlined for *Ambystoma maculatum* in Zamudio and Savage, 2003). Nevertheless, the topographic relief associated with the Appalachian highlands likely promoted a primary route of expansion along the Atlantic coastal plain (e.g. *Ambystoma maculatum*: Zamudio and Savage, 2003; *Ambystoma t. tigrinium*: Church *et al.*, 2003).

### *1.4.3 Genetic Patterns West of the Mississippi River*

In the west, Lake Missoula would have been a formidable barrier to expanding amphibian populations. In addition, the repeated, catastrophic draining of this lake as ice dams burst, reformed and burst again, left vast regions of the present-day Columbia Plateau periodically flooded, delaying the permanent establishment of northern amphibian populations until ~12,000 ybp. The present-day distribution of species suggests that following the final draining of Lake Missoula, species moved north along the Coast and Rocky Mountains (Figure 1.2). However, migration into the north would have been impeded by the Columbia River, and appears to have largely occurred following the "capture" of southern populations during episodic changes in the river's course (Wagner *et al.*, 2005). The subsequent recapture of northern populations accounts for the presence of "northern" haplotypes of *Plethodon larsellii* (Wagner *et al.*, 2005) and *Dicamptodon tenebrosus* (Steele and Storfer, 2006) south of the present-day Columbia River. Other than these few, anomalous haplotypes, the river has generated clear genetic demarcations between northern and southern populations of species (Monsen and Blouin, 2003; Wagner *et al.*, 2005; Steele and Storfer, 2006).

East of the Rocky Mountains, low levels of pairwise sequence divergence in the western clade of *Rana pipiens* fit a model of rapid range expansion (Hoffman and Blouin, 2004). The species, a primary colonizer according to Holman (1992), may have been able to rapidly follow the boreal forest into the north. However, the general lack of genetic structure in its post-glacial range indicates that its northward expansion was largely unimpeded by physical barriers and thus may have occurred after the retreat of Lake Agassiz. Studies of additional taxa are needed to determine which, if any, landscape features in the northern Great Plains influenced patterns of amphibian range expansion.

### *1.4.4 Southern Range Contraction*

While expanding their northern ranges into previously glaciated regions, some species may have also undergone range contraction along their southern margins in response to changing environmental conditions there (Hewitt, 1996; Green *et al.*, 1996). Range contraction occurs when the rate of population extinction exceeds the rate of population establishment or rescue (Green *et al.*, 1996). Populations become increasingly isolated and, as a result of genetic drift, begin to demonstrate high levels of population differentiation and low levels of allelic or haplotypic diversity (Hewitt, 1993; Hampe and Petit, 2005). Few phylogeographic studies have looked for the genetic signature of southern range contraction. However, Green *et al.* (1996) used 20 polymorphic allozyme loci to address these predictions and, consistent with patterns expected under range contraction, found evidence of allelic loss and high levels of differentiation among southern populations of *Rana pretiosa* (now *R. lutreiventris*).

## 1.5 Implications of Quaternary Range Adjustments

### *1.5.1 The Evolutionary History of North American Amphibians*

*Speciation and Lineage Diversification.* It has been proposed that relative to other events the Pleistocene glaciations have had a disproportionate influence on recent speciation in temperate regions (see Austin *et al.*, 2004 for review). For instance,

based on a survey of 47 herpetofauna species from around the world, Avise (2000) contends that 57% of phylogroup separations date to the Pleistocene (15% if a slower molecular clock is enforced). However, debate over the importance of the Pleistocene to speciation continues (Klicka and Zink, 1997; Arbogast and Kenagy, 2001; Weir and Schuluter, 2004; Knowles and Richards, 2005). Although most speciation events reported for North American amphibians predate the Quaternary (Table 3), the isolation of populations in separate refugia during periods of glacial advance did promote lineage diversification within many amphibian species (Table 3) and likely helped to preserve and further promote diversification initiated during earlier periods (Austin *et al.*, 2002). For instance, Jaeger *et al.* (2005) propose that range contraction during periods of glacial advance may have led to the extinction of intermixed populations in historical contact zones between continental clades of *Bufo punctatus*, thereby preventing erosion of a deep phylogenetic split initiated during the late Miocene/early Pliocene. Thus Pleistocene events have played an important role in population differentiation and the generation and maintenance of incipient species.

*The Evolution of Species' Niches.* Quaternary events have undoubtedly influenced the history of adaptation within species. On one hand, environmental change during the Quaternary may be viewed as a destructive force since population extinction along the receding edges of species' ranges during both glacial advance and retreat and would have led to a loss of genetic variation. Some of this variation may have been adaptive if species exhibited local adaptation across their historical ranges, as observed across some species' ranges today (Olsson and Uller, 2003). At the same time, changing climates and community assemblages would have subjected species to new environmental pressures. Given that the evolutionary response of species to selection can be rapid (on the order of decades: Stockwell *et al.*, 2003; Phillips and Shine, 2006), these events were likely a major force in the evolution of species' niches.

*The Evolutionary Potential of Northern Populations.* Patterns of expansion since the last glacial retreat have had a disproportionate influence on the amount and

distribution of genetic variation within species in previously glaciated regions and thus the evolutionary potential of populations in these regions. Generally, populations in northern areas are characterized by low levels of genetic diversity owing to the effects of successive founder events (Hewitt, 1996) (but see sections below). However, as outlined by Hewitt (1996), differences in the rate in which northern genetic diversity declines likely exist between those species that undertook northern range expansion very early (primary colonizers) and those that migrated later (secondary colonizers). Primary colonizers would have met unoccupied territory and, free from competition, would have undergone exponential population growth causing them to demonstrate severe founder effects. Secondary colonizers would have met occupied habitat, restricting them to logistic population growth and causing them to lose genetic diversity more slowly along expansion fronts (Hewitt, 1996). In line with this model, several of the primary colonizers listed under the classification scheme of Holman (1992) demonstrate rapid loss of genetic diversity (at least at neutral markers) and low levels of population structure in the north (*Pseudacris crucifer*: Austin *et al.*, 2002; *Ambystoma maculatum*: Zamudio and Savage, 2003; *Rana pipiens*: Hoffman and Blouin, 2004). Fewer studies have examined patterns within secondary colonizers. However, Smith and Green (2004) examined northern populations of *Bufo fowerli*, a secondary colonizer of the Great Lakes region, and found high levels of haplotype diversity, a large proportion of within population genetic variance and a small proportion of among population variance, all of which are consistent with the greater retention of genetic diversity expected for secondary colonizers (Hewitt, 1996). Thus the evolutionary potential of northern amphibian populations is likely impacted by the generally low levels of genetic diversity resulting from recent colonization, with some differences existing between primary and secondary colonizers.

*Northern Contact Zones*. Species and populations colonizing the north from multiple, genetically distinct refugia, should come into secondary contact roughly at the geographic midpoint between refugial locations (Anderson, 1949; Remington, 1968; Swenson and Howard, 2005). Thus some northern regions

should represent "melting pots" of genetic diversity, potentially harboring even more diversity than refugial "source" populations (Petit *et al.*, 2003). For example, southeastern Ontario represents a region of secondary contact between divergent populations of many eastern species (*P. crucifer*: Austin *et al.*, 2002; *A. maculatum*, Zamudio and Savage, 2003; *R. catesbeiana*: Austin *et al.*, 2004; *B. fowerli*: Smith and Green, 2004). The outcome of secondary contact between different mitochondrial groups will vary depending on the degree to which the groups are reproductively isolated.

### *1.5.2 The Conservation of North American Amphibians*

*Unique Biotic Regions.* Identifying discontinuities in patterns of biological diversity is a necessary step for developing conservation strategies to preserve the maximum complement of diversity. Shared glacial refugia and congruent patterns of post-glacial range expansion among North American amphibians have produced several general discontinuities in genetic diversity across the continent. These genetic breaks, along with regional patterns in the distributions of species, serve to delineate major regions of amphibian diversity (Figure 1.3, Table 4). These regions closely correspond with the distribution of biomes in North America, further indicating that the amphibian populations and species that they harbour are part of distinct biological communities and thus warrant independent consideration in the conservation assessment and planning process.

*Populations of Conservation Priority.* Populations that harbour high levels of genetic diversity are considered by some to be of high conservation priority. For temperate species, these populations are expected to be located in refugial areas (e.g. Figure 1.1) and in regions of secondary contact (Petit *et al.*, 2003). Southern refugial areas may be particularly important from a conservation perspective. Not only do they harbour high levels of genetic diversity, but they may also harbour unique alleles not represented in the gene pool of the limited number of founders that colonized other areas (Hampe and Petit, 2005). Furthermore, these populations may be at a greater risk of extinction than populations elsewhere. As

a result of southern range contraction, many are small and isolated from other populations (Green *et al.*, 1996) and are thus not only more likely to go extinct but are also unlikely to be rescued from neighbouring regions should they go extinct (Hampe and Petit, 2005).

## 1.6 Outstanding Questions and Motivation for Present Study

Despite several studies describing amphibian responses to events associated with Pleistocene, the recolonization history of the most northern regions and the impact of southern refugia on patterns of genetic diversity in these regions is not well understood. For instance, studies of eastern taxa have generally included very few samples north or east of the Great Lakes. The relationship between northeastern populations in the Maritime Provinces and more southern populations and the impact of the St. Lawrence River on population structure thus remains unexplored. Similarly, in the west, most studies have focused on populations in the Pacific Northwestern USA and southern British Columbia. Thus while we have a general picture of range dynamics in refugial areas and areas near the southern extent of the last glacial maximum, the ultimate consequences of these dynamics for patterns of diversity in the previously glaciated north remain largely speculative.

This deficit in our knowledge is unfortunate given that both our understanding of evolutionary dynamics in the north and our ability to design appropriate conservation strategies for northern species hinge upon an understanding of patterns of genetic diversity and population connectivity. From a conservation perspective particularly, information about the history of northern populations is increasingly required. Northern populations may harbour unique adaptations to extreme climatic conditions. Yet these populations are expected to be among the most highly impacted by contemporary climate change.

The present research was conducted with the goal of characterizing patterns of genetic diversity and population connectivity in a widely distributed, primarily northern amphibian, *Rana sylvatica* (the wood frog). The species' complete colonization of the north coupled with its freeze tolerance abilities and

relatively high dispersal capabilities (compared to other amphibians) suggests that it was the first amphibian to reinvade northern areas following glacial retreat. Thus its phylogeographic history should offer new insights as to the effects of the Pleistocene on primary colonizing, highly vagile amphibians. Using genealogical relationships among mitochondrial DNA haplotypes and patterns of genetic diversity across the range of the species, I describe the post-glacial history of this primary colonizer. I specifically ask: 1) Does *R. sylvatica* exhibit the marked degree of genetic structure found in other amphibians? 2) Did *R. sylvatica* persist in refugial areas similar to those inferred for other primary colonizing amphibians? 3) What was the ultimate impact of various refugia on northern colonization?

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Table 1.1. Summary of the phylogeographic histories of North American amphibians studied to date. Letters for putative refugia and numbers for putative colonization routes refer to those in Figures 1.1 and 1.2 respectively.

| **Species** | **Refugia** | **Colonization Routes** | **Physiogeoraphic Influences** | **Source** |
|---|---|---|---|---|
| Northern Leopard Frog (*Rana pipiens*) | H, K, L, M | 4, 6, 7, 8, 9 | Mississippi River; Great Lakes | (Hoffman and Blouin, 2004) |
| Spring Peeper (*Pseudacris crucifer*) | I, M, L | 5, 6, 7. 8, 9 | Prairie Pennisula; Appalachian Mountains; Great lakes; Apalachicola River | (Austin *et al.*, 2002 and Austin *et al.*, 2004) |
| American Bullfrog (*Rana catesbeiana*) | J, N | 5, 8 | Mississippi River; Appalachian Mountains | (Austin *et al.*, 2004) |
| Spotted Salamander (*Ambystoma maculatum*) | L, M, N | 8, 9 | Fall line | (Zamudio and Savage, 2003) |
| Fowler's Toad (*Bufo fowleri*) | - | 6, 7 | Great Lakes | (Smith and Green, 2004) |
| Easter Tiger Salamander (*Ambystoma tigrinum tigrinum*) | N, O | 7 | Apalachicola River; Altamaha River | (Church *et al.*, 2003) |
| Larch mountain salamander (*Plethodon larselli*) | B | 1 | Columbia River | (Wagner *et al.*, 2005) |
| Pacific giant salamander (*Dicamptodon tenebrosus*) | B, C | 1, 3 | Columbia River; Willamette river | (Steele and Storfer, 2006) |
| Western spotted frog (*Rana pretiosa*) | - | 2 | Cascade and Rocky Mountains | (Green *et al.*, 1996) |
| Red spotted toad (*Bufo punctatus*) | E, G | - | Rio Grande; Colorado River | (Jaeger *et al.*, 2005) |
| Woodhouse's toad (*Bufo woodhousii*) | E, F, H | - | - | (Masta *et al.*, 2003) |
| Cascades frog (*Rana cascadae*) | Potentially A, I | - | Columbia river | (Monsen and Blouin 2003) |
| Tailed frog (*Ascaphus truei*) | D (Potentially A, C) | 1, 2, 3 | - | (Nielson *et al.*, 2001) |
| Coeur d'Alene Salamander (*Plethodon idahoensis*) | D | 2 | - | (Carstens *et al.*,2004) |

Table 1.2. List of narrowly distributed salamanders and frogs with ranges that overlap with or lie adjacent to putative glacial refugia identified in the phylogeographic literature for North American amphibians.

| Refugium | Caudata | Anura |
|---|---|---|
| Olympic Peninsula (A) | *Ambystoma gracile*<br>*Ambystoma macrodactylum macrodactylum*<br>*Plethodon vehiculum*<br>*Taricha granulosa* | *Rana aurora* |
| Columbia River Drainage (B) | *Ambystoma gracile*<br>*Ambystoma macrodactylum macrodactylum*<br>*Plethodon vehiculum*<br>*Taricha granulosa* | *Rana aurora* |
| Klamath-Siskiyou Mountains (C) | *Ambystoma macrodactylum sigillatum*<br>*Aneides ferreus*<br>*Taricha granulose*<br>*Plethodon elongatus*<br>*Plethodon stormi*<br>*Plethodon asupak* | *Rana aurora* |
| Northern Rocky Mountains (D) | - | *Ambystoma macrodactylum krausei* |
| Mojave Desert (E) | - | *Spea intermontana* |
| Wyoming (F) | - | *Bufo baxteri* |
| Northern Chluahuan Desert (G) | - | *Scaphiopus couchii*<br>*Bufo debilis*<br>*Hyla arenicolor* |
| Great Plains (H) | - | *Bufo hemiophrys* |
| Ozark-Central Highlands (I) | *Eurycea lucifuga*<br>*Plethodon albagula* | - |
| Gulf Coastal Plain (J) | *Plethodon mississippi* | *Rana grylio*<br>*Scaphiopus holbrookii*<br>*Scaphiopus hurterii*<br>*Bufo valliceps*<br>*Gastrophryne carolinensis*<br>*Hyla avivoca*<br>*Hyla cinerea*<br>*Hyla gratiosa*<br>*Hyla squirella* |
| Appalachians 1 (K) | *Ambystoma barbouri*<br>*Eurycea lucifuga*<br>*Plethodon richmondi* | - |

| Refugium | Caudata | Anura |
|---|---|---|
| Appalachians 2 (L) | *Aneides aeneus*<br>*Desmognathus marmoratus*<br>*Desmognathus monticola*<br>*Desmognathus quadramaculatus*<br>*Desmognathus welteri*<br>*Eurycea lucifuga*<br>*Eurycea wilderae*<br>*Plethodon richmondi*<br>*Plethodon welleri*<br>*Pseudotriton ruber nitidus* | - |
| Southern Appalachians (M) | *Desmognathus marmoratus*<br>*Desmognathus monticola*<br>*Desmognathus quadramaculatus,*<br>*Eurycea lucifuga*<br>*Eurycea wilderae*<br>*Plethodon chattahoochee*<br>*Pseudotriton ruber schencki* | - |
| Coastal Plains (N) | *Plethodon grobmani* | *Rana grylio*<br>*Rana virgatipes*<br>*Scaphiopus holbrookii*<br>*Bufo quercicus*<br>*Bufo terrestris*<br>*Gastrophryne carolinensis*<br>*Hyla cinerea*<br>*Hyla gratiosa*<br>*Hyla squirella* |
| Northern Applachians (O) | *Desmognathus monticola* | - |

Table 1.3. Levels of sequence divergence and estimated timing of lineage diversification for major lineages within and among North American amphibians.

| **Taxa** | **Order** | **Mitochondrial Gene(s) Sequenced** | **Mean Levels of Genetic Divergence**[Ψ] | **Estimated Timing of Divergence**[¥] | **Source** |
|---|---|---|---|---|---|
| ***Among Major Lineages Within Species*** | | | | | |
| *Pseudacris crucifer* | Anura | Cyt b | 2.34 – 6.62% (HKY) | Pliocene [Miocene at earliest] (4.35-8.7 mya)** and Pliocene (2.47 – 4.94 mya)** | Austin *et al.*, 2004 |
| *Rana catesbeiana* | Anura | Cyt b | 1.24 – 2.76% (HKY) | Early Pleistocene [Late Pliocene at earliest] (~1.14 – 2.28 mya)** | Austin *et al.*, 2004 |
| *Rana muscosa* | Anura | ND1, $tRNA^{ILE}$, $tRNA^{GLN}$, $tRNA^{MET}$, ND2, $tRNA^{TRP}$, $tRNA^{ALA}$, $tRNA^{ASN}$, $tRNA^{CYS}$, $tRNA^{TYR}$, COI, $O_L$ | 2.9% and 1.8-2%* | Pliocene (2.2 mya) and Pleistocene (1.4 and 1.5 mya) [§] | Macey *et al.*, 2001 |

| Taxa | Order | Mitochondrial Gene(s) Sequenced | Mean Levels of Genetic Divergence[ψ] | Estimated Timing of Divergence[¥] | Source |
|---|---|---|---|---|---|
| *Bufo punctatus* | Anura | Cyt b | 6.69 – 6.8% | Early Pliocene (4.8 – 4.9 mya)**[§] | Jaeger *et al.*, 2005 |
| *Pseudacris Regilla* | Anura | Cyt b | 1.71 – 6.5% | Pliocene (3.2 mya)** and Pleistocene (0.9 – 1 mya) | Recuero *et al.*, 2006 |
| *Rana cascadae* | Anura | ND1, $tRNA^{LEU}$, $tRNA^{ILE}$, $tRNA^{GLN}$, $tRNA^{MET}$ | 3.2 – 4.0 % | Pliocene or late Miocene (2.3 – 6.1 mya)[§] | Monsen and Blouin, 2003 |
| *Ascaphus truei* | Anura | Cyt b, ND2 | 1.3 – 12.1 % (GTR+I) | Late Miocene/ Early Pliocene (rate and dates not specified | Nielson *et al.*, 2001 |
| *Bufo woodhoussii* | Anura | 16S, $tRNA^{LEU}$, ND1 | 0.002*-0.54% | Pleistocene (0.461 mya and 0.177 mya) | Masta *et al.*, 2003 |
| *Dicamptodon tenebrosus* | Caudata | Cyt b, CR | 1.95 % (3.2% HKY+I+G corrected) | Mid-Pleistocene (~0.8 mya) | Steele and Storfer, 2006 |

| Taxa | Order | Mitochondrial Gene(s) Sequenced | Mean Levels of Genetic Divergence[Ψ] | Estimated Timing of Divergence[¥] | Source |
|---|---|---|---|---|---|
| *Ambystoma tigrinium tigrinium* | Caudata | D-loop and adjacent intron | 0.5 – 2.1% | Late Pliocene, early Pleistocene (1.4 – 2.1 mya) and Pleistocene (0.3 -0.5 mya, 0.6 – 1 mya) | Church *et al.*, 2003 |
| *Ambystoma californiense* | Caudata | CR | Deepest divergence = 2.25% | Early Pleistocene (0.74-0.92 mya) | Shaffer *et al.*, 2004 |
| *Desmognathus marmoratus* | Caudata | ND2 | not specified | Tertiary (8.9 – 10.8 mya) | Jones *et al.*, 2006 |
| *Desmognathus orestes* | Caudata | Cyt b | 4.9% | Pliocene (3-5 mya)[§] | Mead *et al.*, 2001 |
| *Batrachoseps wrighti* | Caudata | Cyt b | 3.6% | Pleistocene (1 mya) [§] | Miller *et al.*, 2005 |
| *Taricha t. sierrae* | Caudata | Cyt b | not specified | Pliocene (2.6-3 mya) [§] | Kutchta and Tan, 2006 |
| *Taricha t. torosa* | Caudata | Cyt b | not specified | Pleistocene (1.4-1.7 mya) and Pliocene (1.9-2.3 mya) [§] | Kutchta and Tan, 2006 |

| Taxa | Order | Mitochondrial Gene(s) Sequenced | Mean Levels of Genetic Divergence[ψ] | Estimated Timing of Divergence[¥] | Source |
|---|---|---|---|---|---|
| ***Among Closely Related Species/Subspecies*** | | | | | |
| *Rana catesbeiana* species group | Anura | Cyt b and ND2 | not specified | Late Miocene or Pliocene (~9 – 11 mya); Pleistocene divergence of *R. okaloosae*/ *R. clamitans* | Austin *et al.*, 2003 |
| *Rana boylii* species group | Anura | ND1, $tRNA^{ILE}$, $tRNA^{GLN}$, $tRNA^{MET}$, ND2, $tRNA^{TRP}$, $tRNA^{ALA}$, $tRNA^{ASN}$, $tRNA^{CYS}$, $tRNA^{TYR}$, COI, $O_L$ | 7.2 – 12.1%* | Miocene (5.5 – 9.3 mya)** § | Macey *et al.*, 2001 |
| *Plethodon vandykei* species group | Caudata | Cyt b | mean not specified but ranges = 26.4-27%; 8.5-10.6% (HKY + Γ) | likely Pliocene (~3.75 mya) | Carstens *et al.*, 2004 |

| Taxa | Order | Mitochondrial Gene(s) Sequenced | Mean Levels of Genetic Divergence[Ψ] | Estimated Timing of Divergence[¥] | Source |
|---|---|---|---|---|---|
| *Eurycea multiplicata* species complex | Caudata | Cyt b, ND4 | Mean not specified but ranges = 13.37-15.68%; 11.39-13.26%; 14.52-15.13%; 13.37-15.68% | Miocene (6.7-31.4 mya, 5.7.26.5 mya, 7.3-30.3 mya, 6.7-31.4 mya)[§] | Bonett and Chippindale, 2004 |
| *Desmognathus ochrophaeus* species complex | Caudata | Cyt b | 6.52-15.58% (K2P) | Miocene/Pliocene (4.3-15.58 mya)** [§] | Mead *et al.*, 2001 |
| *Taricha torosa* | Caudata | Cyt b | not specified | Pliocene (7-13 Mya) [§] | Kutchta and Tan, 2006 |

[Ψ] Refers to uncorrected pairwise sequence differences unless a model of evolution is specified in parentheses
[¥] Numbers given in parentheses are approximate dates
* Averages were calculated from data provided
**Specific timing was not given in article but was calculated based on molecular clock specified in article
[§] Study applied a molecular clock without testing for rate heterogeneity

Table 1.4. A list of representative taxa within each of the biotic regions defined by distributions of amphibians and the location of phylogeographic breaks within and among species (underlined). See Figure 1.3 for map of regions.

| Region | Representative Caudata | Representative Anura |
|---|---|---|
| North Pacific Coast (1) | *Ambystoma gracile* | *Ascaphus truei* |
| | *Ambystoma m. macrodactylum* | *Rona aurora* |
| | *Aneides vagrans* | *Rana cascadae* |
| | *Dicamptodon tenebrosus* | |
| | *Ensatina e. oregonensis* | |
| | *Plethodon vehiculum* | |
| | *Plethodon larsellii* | |
| | *Taricha granulosa* | |
| South Pacific Coast (2) | *Aneides lugubris* | *Bufo b. halophilus* |
| | *Ensatina e. eschscholtzii* | *Rana a. draytonii* |
| | *Taricha torosa* | |
| Intermountain (3) | *Ambystoma m. columbiamum* | *Bufo b. boreas* |
| | | *Spea intermontana* |
| Rocky Mountains (4) | *Ambystoma m. krausei* | *Bufo b. boreas* |
| | *Plethodon idahoensis* | *Rana luteiventris* |
| | | *Ascaphus montanus* |
| Great Plains (5) | *Ambystoma t. mavortium* | *Bufo hemiophrys* |
| | *Ambystoma t. melanostictum* | *Bufo cognatus* |
| | | *Bufo w. woodhousii* |
| | | *Spea bombifrons* |
| | | *Acris c. crepitans* |
| | | *Pseudacris maculata* |
| | | *Rana sylvatica* |
| South Central (6) | - | *Bufo cognatus* |
| | | *Bufo debilis* |
| | | *Bufo punctatus* |
| | | *Bufo valliceps* |
| | | *Bufo speciosus* |
| | | *Bufo w. australis* |
| | | *Hyla arenicolor* |
| | | *Rana berlandieri* |
| | | *Scaphiopus couchii* |
| Canadian Shield (7) | *Ambystoma laterale* | *Bufo americanus* |
| | *Eurycea bislineata* | *Pseudacris crucifer* |
| | | *Rana clamitans (melanota)* |
| | | *Rana septentrionalis* |
| | | *Rana pipiens* |
| | | *Rana sylvatica* |

| Region | Representative Caudata | Representative Anura |
|---|---|---|
| Great Lakes (8) | *Ambystoma jeffersonianum*<br>*Ambystoma maculatum*<br>*Notophthalmus viridescens* | *Hyla versicolor*<br>*Pseudacris triseriata*<br>*Rana septentrionalis*<br>*Pseudacris crucifer*<br>*Rana palustris*<br>*Rana catesbeiana* |
| Interior Lowlands (9) | *Ambystoma texanum*<br>*Notophthalmus v. louisainensis*<br>*Plethodon albagula* | *Bufo americanus*<br>*Bufo fowleri*<br>*Hyla versicolor*<br>*Pseudacris crucifer*<br>*Rana clamitans (melanota)*<br>*Rana palustris*<br>*Rana sphenocephala*<br>*Rana catesbeiana* |
| Midwestern (10) | *Ambystoma jeffersonianum*<br>*Ambystoma laterale*<br>*Amybstoma maculatum*<br>*Ambystoma t. tigrinium*<br>*Ambystoma texanum*<br>*Plethodon glutinosus* | *Bufo americanus*<br>*Bufo fowleri*<br>*Pseudacris crucifer*<br>*Acris crepitans*<br>*Pseudacris triseriata*<br>*Rana catesbeiana* |
| Interior Highlands (11) | *Ambystoma maculatum*<br>*Desmognathus conanti*<br>*Plethodon Mississippi* | *Bufo a. charlesmithi*<br>*Bufo fowerli*<br>*Pseudacris crucifer* |
| Appalachian (12) | *Ambystoma jeffersonianum*<br>*Ambystoma laterale*<br>*Ambystoma maculatum*<br>*Ambystoma t. tigrinium*<br>*Cryptobranchus allenganiensis*<br>*Desmognathus fuscus*<br>*Desmognathus monicola*<br>*Desmognathus ochrophaeus*<br>*Eurycea bislineata*<br>*Gyrinophilus porphyriticus*<br>*Pseudotriton r. ruber* | *Bufo americanus*<br>*Bufo fowerli*<br>*Hyla versicolor*<br>*Pseudacris crucifer*<br>*Rana palustris*<br>*Rana pipiens*<br>*Rana catesbeiana* |

| Region | Representative Caudata | Representative Anura |
|---|---|---|
| Coastal Plains (13) | *Amybstoma talpodiem*<br>*Amphiuma means*<br>*Amybstoma t. tigrinium*<br>*Notophthalmus v. louisainensis*<br>*Plethodon chlorobryonis*<br>*Plethodon grobmani* | *Bufo quercicus*<br>*Bufo terrestris*<br>*Gastrophryne carolinensis*<br>*Hyla cinerea*<br>*Hyla gratiosa*<br>*Hyla squirella*<br>*Rana c. clamitans*<br>*Rana grylio*<br>*Rana virgatipes*<br>*Scaphiopus holbrookii*<br>*Pseudacris nigrita*<br>*Pseudacris crucifer* |

Figure 1.1. The approximate extent of ice during the height of the last glaciation and the locations of amphibian refugia identified in the literature. A= Olympic Peninsula; B = Columbia River; C = Klamath-Siskiyou Mountains; D = Northern Rocky Mountains; E = Mojave Desert; F = Wyoming; G = Northern Chluahuan Desert; H = Great Plains; I = Ozark-Central Highlands; J = Gulf Coastal Plain; K = Appalachians 1; L = Appalachians 2; M = Southern Appalachians; N = Coastal Plains; O = Northern Appalachians

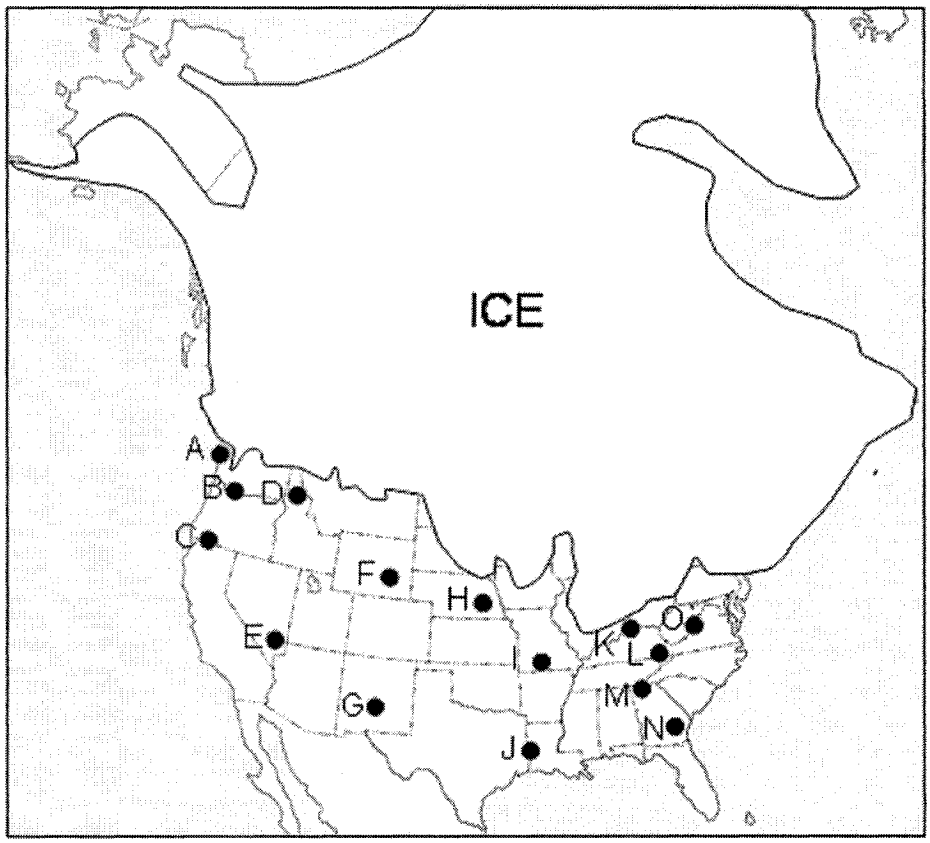


Figure 1.2. Post-glacial range adjustments made by amphibians in North America

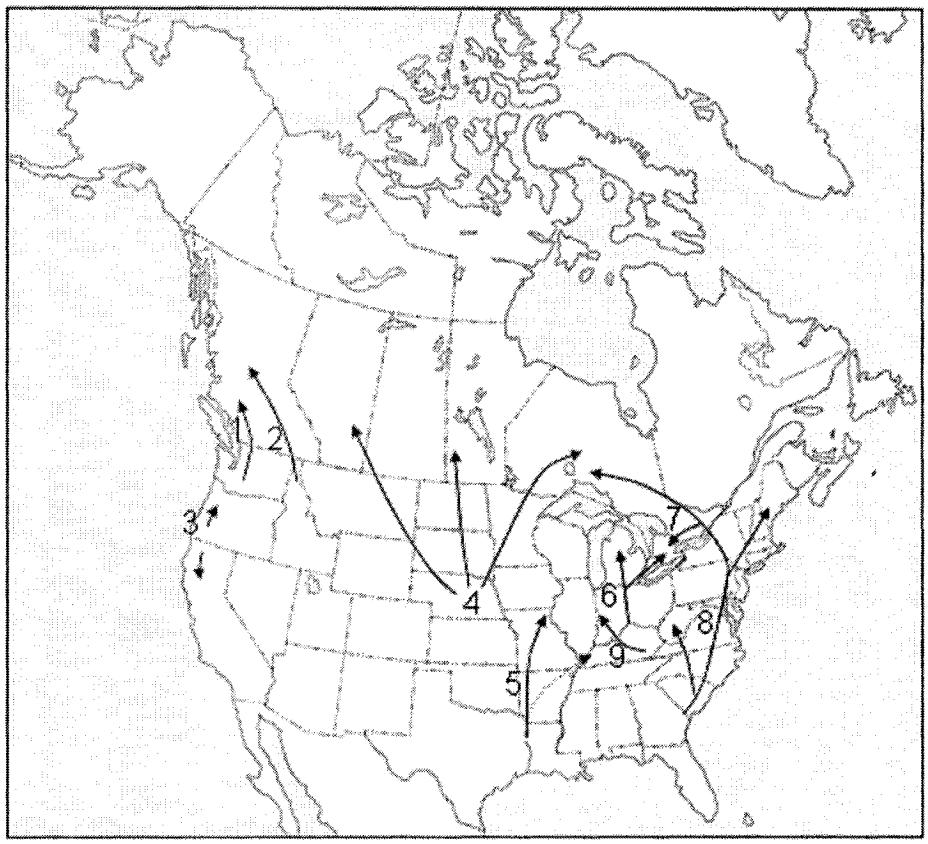
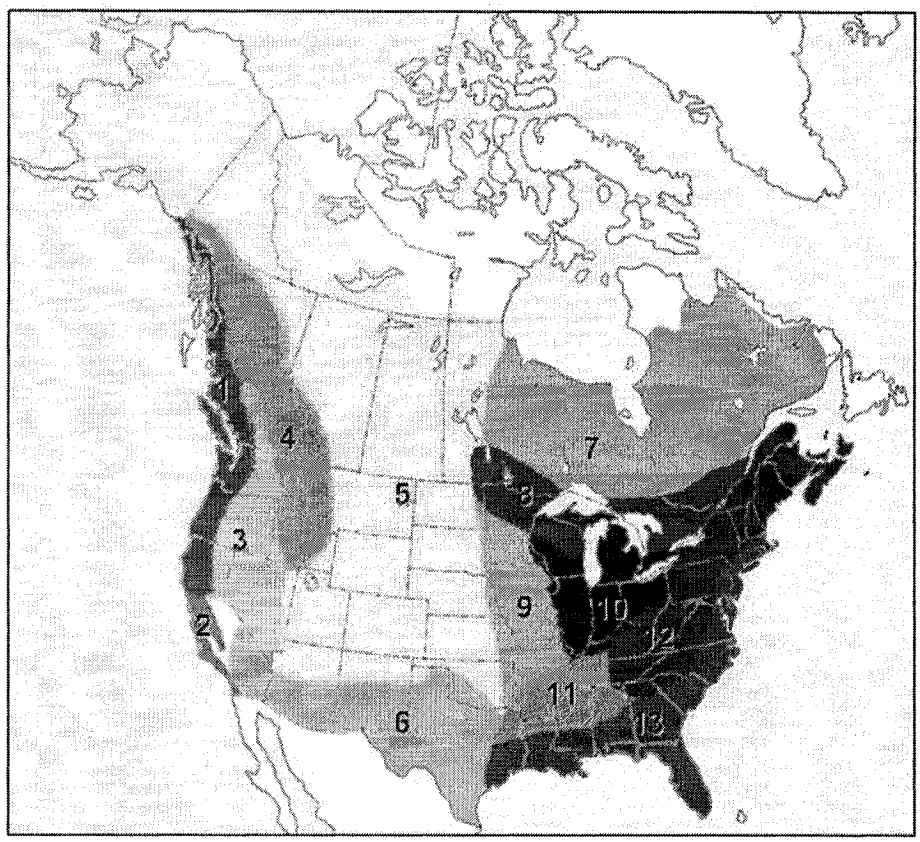


Figure 1.3. Biotic regions of North American amphibians defined by the distribution of common range limits and phylogeographic patterns observed within and among taxa. Regional boundaries are drawn fuzzy to emphasize the large amount of variation among taxa



# Manuscript

**Cryptic lineages and post-glacial range expansion from northern refugia by the wood frog, *Rana sylvatica*.**

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Running Title:

The post-glacial history of *Rana sylvatica*

## 2.1 Introduction

Climate change during the Pleistocene resulted in dramatic changes to species' distributions (Webb and Bartlien, 1992; Hewitt, 1996). Northern taxa were particularly affected as repeated episodes of glacial advance and retreat caused their ranges to alternatively shift north and south (Green et al., 1996; Hewitt, 1996). The most recent of these range adjustments have set the context for evolutionary processes in the north, influencing the distribution of genetic diversity, the amount of genetic variation available for local adaptation and the outcome of secondary contact between populations. Thus, interpretation of evolutionary phenomena in temperate regions requires an understanding of range adjustments associated with the last glacial cycle.

Amphibians are choice species for examining the consequences of the Pleistocene. Many exhibit limited dispersal (but see reviews by Marsh and Trenham, 2001, and Smith and Green, 2005), high site fidelity (Vosconceles and Clahoun, 2004; Blihovde, 2006) and sensitivity to geographic barriers (Rittenhouse and Semlitsch, 2006), all of which promote genetic structure in relation to the physical landscape (Lougheed *et al.*, 1999; Spear *et al*, 2005; Funk *et al.*, 2005; Kraaijeveld-Smit *et al.*, 2005; Stevens *et al.*, 2006). In addition, many amphibians have distributions encompassing previously glaciated and unglaciated terrain. The signature of Pleistocene events captured in the genetic structure of these species can thus inform hypotheses about general patterns of range adjustments during this period. Furthermore, conservation concern for amphibians is increasing (Green, 2003; Stuart *et al.*, 2004; Crushman, 2006; Rissler *et al.*, 2006) and many species face imminent threats to population persistence. Understanding the dynamics of range changes, such as those made during the Pleistocene, thus has particular relevance for this group.

Although species responded individually to the Pleistocene, many similarities exist amongst North American amphibians. Eastern species generally demonstrate multiple, divergent lineages in the north, reflecting expansion from disjunct glacial refugia (Austin *et al.*, 2002; Church *et al.*, 2003; Zamudio and

Savage 2003; Austin *et al.*, 2004; Smith and Green, 2004). The Appalachian mountains played an important role, harbouring refugia (Church *et al.*, 2003) and filtering populations into interior and coastal plain refugia (Zamudio and Savage, 2003; Austin *et al.*, 2004; Hoffman and Blouin, 2004). Phylogenetic breaks are generally arranged longitudinally (Austin *et al.*, 2004) and, in addition to the Appalachian mountains, have been influenced by the Great Lakes (Austin *et al.*, 2002; Smith and Green, 2004), the Mississippi River (Austin *et al.*, 2004; Moriarity and Cannatella, 2004; Hoffman and Blouin, 2004) and the Prairie Peninsula (Austin *et al.*, 2002).

In the west, smaller geographic distributions and a higher degree of endemism are consistent with the more varied topography. Some taxa persisted in a single refugium during the last glaciation (Wagner *et al.*, 2005), while others appear to have persisted in multiple refugia (Neilson *et al.*, 2001; Steele and Storfer, 2006). Putative refugial locations include coastal areas in the Klamath-Siskiyou Mountains (Steele and Storfer, 2006) and the Columbia River Valley (Steele and Storfer, 2006), as well as inland refugia in the Clearwater (Carstens *et al.*, 2004) and Salmon (Neilson *et al.*, 2001) river drainages of Idaho. In line with topographic features oriented both east to west (e.g. Columbia river) and north to south (e.g. the Cascades mountains), major phylogeographic divisions are oriented both longitudinally (Neilson *et al.*, 2001; Shaffer *et al.*, 2004) and latitudinally (Shaffer *et al.*, 2004; Wagner *et al.*, 2005; Steele and Storfer, 2006; Recuero *et al.*, 2006).

Although the biogeographic histories of North American amphibians are increasingly understood, the post-glacial history of the most northern regions and the impact of southern refugia on patterns of genetic diversity in these regions are not well reconstructed. Northern taxa—presumably the earliest species to invade previously glaciated terrain, may exhibit phylogeographic differences from other species. For instance, under a leading edge model of range expansion (Hewitt, 1996), primary colonizers invaded the north via long distance dispersal events coupled with exponential population growth (Nicoles and Hewitt, 1994; Hewitt, 1996). As a result of successive founder effects, these species should demonstrate

increasingly vast areas of genetic homogeneity in the north (Hewitt, 1996). This pattern differs from that expected for secondary colonizers, which are restricted to logistic population growth as a result of competitive effects and retain more genetic diversity owing to less severe founder effects and slower expansion (Hewitt, 1996).

Here we present a range-wide phylogeographic survey of the wood frog (*Rana sylvatica*), a predominantly northern amphibian. The geographic range of this species is extensive, ranging northwest into the Yukon and Alaska, east and west across Canada and south into the eastern United States along the Appalachian Mountains (Figure 2.1). In light of this remarkable northern distribution, as well as its freeze tolerance capabilities, high dispersal abilities (Berven and Grudzien, 1990; Newman and Squire, 2001; Crosby *et al.*, unpublished m.s.) and use of a variety of habitats, including tundra, *R. sylvatica* is thought to have been the earliest amphibian to extend its range following glacial retreat (Holman, 1992). Thus the wood frog is expected to demonstrate very low levels of genetic diversity in the north and, compared to other amphibians, may be characterized by less genetic structure.

The overlap of the southern portion of *R. sylvatica*'s range with the postulated glacial refugia of many eastern amphibians also represents an opportunity to determine the impact of previously identified refugia and colonization routes on the overall genetic structure of a widespread, northern species. For instance, the spring peeper (*Pseudacris crucifer*) another eastern woodland amphibian, appears to have entered the north and moved west along a pathway extending from an Appalachian refugium, east and then north over the Great Lakes (Austin *et al.*, 2002). If the wood frog shared a similar biogeographic history in this region, populations north and west of the Great Lakes may share haplotypes with populations in the Appalachians. Alternatively, western populations of the wood frog could have been founded by a western refugium, perhaps in Beringia as proposed for other, non-amphibian taxa (Elias, 1992; Soltis *et al.*, 1997; Eddingsaas *et al.*, 2004; Oliver *et al.*, 2006; Loehr *et al.*, 2006), or south of the glacial ice.

In the present study, we use the genealogical relationships among mitochondrial DNA haplotypes and patterns of genetic diversity across the range of the wood frog to elucidate the post-glacial history of this species. Specifically, we set out to determine whether this species exhibits the marked degree of genetic structure found in other amphibians in relation to the post-glacial landscape and to test whether the wood frog shared similar refugia and followed the same colonization pathways as these other amphibians.

## 2.2 Materials and Methods

### *2.2.1 Sampling*

An initial set of 45 samples was collected by J. Irwin from 35 widely spaced localities (Figure 2.1 and Appendix 1) during the summer of 2000 and spring of 2001. These samples were analyzed first using cytochrome b (see below). Further sampling was conducted by J. Lee-Yaw during the periods of March-August, 2005 and May 2006 and combined with additional samples obtained from private or institutional collections, yielded a second dataset of 551 individuals, representing 116 sites from across the range (Figure 2.1 and Appendix 1). This larger dataset was analyzed for the ND2/tRNA$^{TRP}$ mitochondrial region (see below). Sampling at all locations involved capturing adult frogs by hand or dip net, removing a toe from each front foot using a sterilized blade and releasing the individuals. When adult frogs could not be found, eggs or tadpoles were collected. Eggs were collected from different clutches and tadpoles were collected from different parts of the pond to avoid sampling closely related kin. Eggs were allowed to hatch and the tadpoles euthanized. A subset of tadpoles from all populations where eggs or tadpoles were collected was raised to metamorphosis in the lab to verify species' identity. All tissue was stored in 95% ethanol.

### *2.2.2 Molecular Methods*

*DNA Amplification and Sequencing*: DNA was extracted from toes using a Qiagen DNeasy tissue kit. DNA was extracted from tadpoles using the protocol outlined by Fetzner (1999). For the initial set of samples, a 700 b.p. region of the cytochrome b (cyt b) mitochondrial gene was amplified using primers *ralu1* (Bos and Sites, 2001) and modified H15502 (Babik *et al.*, 2004) following the methods outlined in Babik *et al.*, 2004). For the second dataset, a 650 b.p. region of the NADH dehydrogenase subunit two and transfer RNA TRP mitochondrial genes (ND2/tRNA$^{TRP}$) were amplified using the primers L4882 (Macey *et al.*, 2000) and H5532 (Macey *et al.*, 1998). The utility of this second region for inferring intraspecific phylogenetic relationships has been previously demonstrated (Neilson *et al.*, 2001; Austin *et al.*, 2003). Reaction volumes for polymerase chain reaction (PCR) amplification of this region were 25 µl and amplification involved a denaturation step at 94°C for 2 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, primer annealing at 62°C for 45 seconds, and elongation at 72°C for 1 minute and 10 seconds. A final elongation step at 72°C for five minutes was added followed by cooling to 4°C. A negative control was included for all PCR reactions.

All PCR products were sent to the Genome Quebec Innovation Centre at McGill University for direct sequencing. Briefly, PCR products were filtered to remove unused PCR reagents and purified from a gel. BigDye Terminator v3.1 (Applied Biosystems) and 2 µl (10 µM) of primer were used in the sequencing reaction. Sequencing reactions consisted of 25 cycles performed on a Gene Amp PCR System 9700 (Applied Biosystems) with the following conditions: 96°C for 10 seconds, 50°C for five seconds, 60°C for four minutes. Products were sequenced on an Applied Biosystems DNA Analyzer 3730XL automated sequencer. Sequences were verified by eye in CHROMAS LITE (Technelysium Pty Ltd, 1998-2005).

*Alignment*: Sequences were aligned in CLUSTAL X (Thompson *et al.*, 1997) and the alignments were imported into MACCLADE 4.0 (Maddison and Maddison,

2000) for further, manual editing. There were no gaps and the few characters with any ambiguity were excluded from analysis. The online program COLLAPSE 1.2 (Posoda 2006) was used to collapse redundant haplotypes. This step resulted in final datasets of 19 unique cyt b haplotypes and 195 unique ND2/tRNA$^{TRP}$ haplotypes.

### *2.2.3 Data Analyses*

*Phylogenetic Reconstruction:* Phylogenetic relationships among haplotypes were evaluated using two criteria: Neighbour-Joining as implemented in PAUP* 4.0 (Swafford, 2002) and maximum likelihood using the genetic algorithm implemented in GARLI 0.942 (Zwickl, 2006). For the analyses of the cyt b data, morphologically similar frogs of the brown frog group (*R. luteiventris* and *R. aurora*) were used as outgroups. With recent work placing the *R. catesbeiana* group as the sister group to *R. sylvatica* (Hillis and Wilcox, 2005) and *R. septentrionalis* and *R. virgatipes* as basal within that group (Austin *et al.*, 2003), *R. septentionalis* and *R. virgatipes* were used as outgroups in the analyses of ND2/ tRNA$^{TRP}$ (Gene bank accession numbers AY206487 and AY206491 respectively). The best model of nucleotide evolution for these analyses was determined using the Akaike Information Criterion (AIC) in MODELTEST 3.7 (Posada and Crandall, 1998). GARLI analyses were conducted using the default settings of the program. Three runs were conducted: two using random start trees and one using a neighbour-joining start tree. Support for the NJ tree was estimated with 500 non-parametric bootstrap replicates. Support for the maximum likelihood tree obtained by GARLI was estimated with 200 bootstrap replicates. Due to the limited number of sequences per locality, the cyt b data was only used in tree reconstruction and is not included in the methods below.

*SAMOVA:* In addition to phylogenetic methods for identifying major lineages in the ND2/tRNA$^{TRP}$ dataset, we used Spatial Analysis of Molecular Variance (Dupanloup *et al.*, 2002) as implemented in the program SAMOVA version 1 (Dupanloup *et al.*, 2002) to define groups of geographically adjacent populations

that are maximally differentiated from each other. The program uses a simulated annealing procedure to sample different partitionings of input populations into a user-defined number of groups ($k$) and chooses the grouping of populations that maximizes the proportion of genetic variance that is due to differences between groups (Dupanloup *et al.*, 2002). For the first set of analyses, all populations were included and the analysis was conducted for $k$=2 through to $k$=7 groups. To ensure that the final configuration of populations was not influenced by the initial configuration, each analysis was conducted with 100 start conditions. One thousand coalescent simulations were conducted to generate a null distribution against which the significance of the test statistic ($F_{CT}$) could be tested. SAMOVA was also used to explore structure within each of the major clades identified in the phylogenetic analyses.

*Nested Clade Analysis*: To explore the association between geography and haplotype frequency and to infer population processes that have influenced current genetic structure, we conducted nested clade analysis (NCA) (Templeton, 1995). The program TCS version 1.21 (Clement *et al.*, 2000) was used to generate an intraspecific haplotype network according to the algorithm of Templeton *et al.* (1992). This algorithm calculates the probability of parsimony for an increasing number of pairwise sequence differences until the probability falls below 0.95, at which point the limits of statistical parsimony have been met (Templeton *et al.*, 1992). Haplotypes are connected using the number of mutational differences associated with this limit as the maximum number of mutational connections allowed between pairs of sequences in the network. Network ambiguities were resolved according to rules based on coalescent theory provided in Pfenninger and Posada (2002).

The nesting rules outlined in Templeton *et al.* (1987) and Templeton and Sing (1993) were applied to generate a hierarchy of nested haplotypes within the network. Briefly, starting from the tips, haplotypes (zero-step clades) separated by one mutational step were nested into one-step clades. The process was repeated nesting clades into higher level clades until the entire network had been nested. The program GeoDis version 2.5 (Posada *et al.* 2000) was used to test the

significance of the geographical association of clades and nested clades. For each clade with geographic and genetic variation, the program first performs a permutational contingency analysis to test for an association between geographic and genetic variation. The program calculates 1) clade distance (Dc), a measure of the geographical spread of a clade and 2) nested clade distance (Dn), a measure of the spread of a clade from the geographic centre of the entire nesting clade. These statistics were then compared to distributions of Dc and Dn generated by 1000 permutations of clades against sampling locations to test the null hypothesis of a random distribution of clades across locations. Significantly small and large values of either of these statistics implicate different historical or population processes. An updated version of the inference key of Templeton *et al.* (1995) provided on the GeoDis website was used to distinguish between various scenarios underlying observed geographical patterns within clades.

*Patterns of Genetic Diversity*: Populations within refugial areas are expected to demonstrate elevated levels of genetic diversity relative to populations within other areas. To identify putative refugia, gene diversity ($\hat{H}$, the probability that two haplotypes randomly chosen from the population are different) and mean pairwise sequence differences ($\pi$) were calculated in ARLEQUIN version 3.01 (Exoffier *et al.*, 2005), using Tamura distances (Tamura, 1992). These measures of genetic diversity were calculated for all major clades identified in the phylogenetic analyses, for sampling locations where $n>3$ and for groups of sites located in putative refugial areas and along putative colonization pathways according to what has been observed for other species (Table 1). To test whether patterns of diversity within major clades identified in the phylogenetic analyses are consistent with the assumption of neutrality, we used DNASP (Rozas *et al.*, 2003) to compare the ratio of nonsynonymous and synonymous substitutions within and among lineages according to the MK test (McDonald and Kreitman, 1991).

## 2.3 Results

### *2.3.1 Summary of Sequences*

For cyt b, 700 b.p. were successfully sequenced. There were 186 variable sites (for the ingroup only), of which 138 were parsimony-informative. Pairwise sequence divergence among the 19 haplotypes ranged from 0.0014 to 0.074. For ND2/ $tRNA^{TRP}$, 574 b.p were successfully sequenced. For this region, there were 139 variable sites, of which 89 were parsimony-informative. Pairwise sequence divergences between 195 unique haplotypes ranged from 0.002 to 0.089.

### *2.3.2 Phylogenetic Results*

The optimal model of evolution for the cyt b dataset was K81uf+G (with rate matrix: a-c = 1, a-g = 6.3334, a-t = 0.4450, c-g = 0.4450, c-t = 6.3334, g-t = 1; gamma rate-heterogeneity shape = 0.2934; base frequencies: a = 0.2395, c = 0.3187, g = 0.1365, t = 0.3052) as determined by AIC in MODELTEST 3.7 (Posada and Crandall, 1998). Both neighbour-joining and the genetic algorithm recovered similar optimal topologies. The best maximum likelihood tree recovered applying the genetic algorithm had a –ln L = 2171.90 (Figure 2.2). An optimal model of evolution for the ND2/$tRNA^{TRP}$ dataset was TIM+I+G (with rate matrix: a-c = 1, a-g =35.0949, a-t = 0.3817, c-g = 0.3817, c-t = 9.3582, g-t = 1; base frequencies: a = 0.2915, c = 0.2783, g = 0.0915, t = 0.3387; gamma rate-heterogeneity shape = 1.2070 and proportion of invariant sites = 0.4774) Once again, both phylogenetic analyses recovered congruent topologies. The two main clades recovered were also consistent with those recovered for the cyt b dataset. The best maximum likelihood tree recovered applying the genetic algorithm had a –ln L = 2814.06 (Figure 2.2).

The most obvious feature across all topologies is the division of haplotypes into two main clades corresponding to eastern and western localities with levels of sequence divergence (uncorrected p-distances) between these groups ranging from 0.063 to 0.074 for cyt b and 0.057 to 0.089 for ND2/$tRNA^{TRP}$. The eastern clade contains individuals from the Appalachian

mountains region, the southern part of the American Midwest, Quebec and the Maritimes. The western clade contains individuals from the northern part of the American Midwest, the Great Lakes region, all of western Canada and isolated populations in the southwestern United States. Only three locations harbour a mixture of eastern and western haplotypes: two locations in western New York (sites 34 and 35 in Appendix 1) contain eastern and western ND2/tRNA$^{TRP}$ haplotypes and a site in Arkansas (site d in Appendix 1) contains both eastern and western cyt b haplotypes. All of these sites occur along the boundary between the two clades, west of the Appalachian Mountains. Within the eastern clade of the ND2/tRNA$^{TRP}$ dataset, haplotypes from the Maritime states and provinces fall into a distinct subclade with high support, although sister group relationships are not discernable from the phylogenetic analyses (Figure 2.2). Haplotypes from this subclade are unique to Maritime populations and these locations harbour no other eastern haplotypes. A second eastern subclade with more limited support contains haplotypes from the southern Appalachians and Kentucky.

### *2.3.4 SAMOVA*

The results of the phylogenetic analyses were confirmed by SAMOVA of the ND2/tRNA$^{TRP}$ dataset. When $k$ was set to two, SAMOVA grouped all populations containing eastern haplotypes in one group and all populations containing western haplotypes in the second group. Differences among the eastern and western regions (groups) accounted for 85.85% of the genetic variation, while differences among populations within regions and within populations accounted for 9.64% and 4.51% of the variation respectively. Setting $k = 3$ recovered the Maritime eastern subclade in addition to the division between eastern and western clades. In this case, 90.02% of the variation was explained by differences among regions, 5.03% by differences among populations within regions and 4.95% by differences within groups. The other eastern subclade (from the southern Appalachians and Kentucky) observed in the phylogenetic analyses was not readily pulled out by the SAMOVA until $k$ was set to six.

To expedite examination of groups within each of the main phylogenetic groups, we conducted SAMOVAs for the eastern clade (without the Maritime

populations) and western clade independently. For the eastern clade, SAMOVA was able to find groups of populations for which the proportion of variation due to differences between groups was >64% when $k$=2. This value went down as group number increased. In all cases, the groups chosen by SAMOVA contained at least one group composed of a single population (Table 2). The first populations to fall out as their own groups were from the American Midwest. The southern Appalachian populations also formed a group at higher values of $k$. For the western clade, the proportion of variance due to differences between groups increased as group number increased. Setting $k$=2 to $k$=3 pulled out groups consisting mainly of populations from Michigan, Ohio and Ontario. Setting higher values of $k$ tended to further subdivide these populations rather than resolve additional groups in the west.

### *2.3.5 Nested Clade Analysis*

In concordance with results from the phylogenetic analyses and SAMOVAs, cladogram estimation using statistical parsimony resulted in three distinct networks corresponding to eastern, western and maritime ND2/tRNA$^{TRP}$ haplotypes (Figure 2.3). These networks could not be connected within the limits of parsimony, in this case equal to 10 mutational steps. However, when the number of mutational steps permitted was increased by a minimum of 16 steps, the Maritime network was connected to haplotypes found in the Carolinas.

Significant associations with geography were found for 12 of the nested groups in the eastern network, two in the Maritime network and seven in the western network. Inferences for these clades are presented in Table 3 and Figure 2.4. In general, both the eastern and western clades are characterized by restricted gene flow with isolation by distance and contiguous range expansion. Allopatric fragmentation is also inferred for clade 2-21 in the western network, which encompasses the geographically isolated population in Colorado. Past fragmentation and/or long distance dispersal is invoked to explain patterns in clade 2-3 in the eastern network, which includes sampling locations from the southern Appalachians (sites 48, 52, 53). Patterns at the largest nesting level in the Maritime network suggest restricted gene flow with isolation by distance.

### *2.3.6 Patterns of Genetic Diversity*

At the clade level, levels of gene diversity were similarly high in the eastern clade (0.9226 ± 0.0001 95% CI) and western clade (0.9114 ± 0.0002 95% CI) and lower in the Maritime clade (0.7425 ± 0.005 95% CI). At the population level, gene diversity was high (>0.5) for most sites in the eastern clade (Appendix 2). Unexpectedly, sites in previously glaciated areas of the Appalachian region generally exhibited the highest values of gene diversity, whereas sites in the southern portion of the Appalachian mountains and American Midwest generally exhibited lower levels of gene diversity. For the western clade, gene diversity was high in most sites in Michigan and Ontario and low for many sites further west. In the Maritime subclade, only sites in New Brunswick exhibited consistently high levels of gene diversity. When populations were grouped according to putative refugial areas and along putative colonization pathways, levels of gene diversity in the southern Appalachians and Midwest (putative refugial areas) were generally as high but not uniformly higher than levels of gene diversity observed elsewhere (Figure 2.5). In contrast, regions within the western clade maintained the same general trend of decreasing levels of gene diversity from Michigan northwest to Alaska (Figure 2.5).

The mean number of pairwise sequence differences were low in all clades (eastern: $\pi = 3.32 \pm 0.02$ 95% CI; western: $\pi = 3.93 \pm 0.03$ 95% CI; Maritime: $\pi = 1.50 \pm 0.07$). As expected, several sites in the southern portion of the Appalachians and the American Midwest had higher levels of $\pi$ than those observed in more northern sites (Appendix 2). In the west, the highest levels of $\pi$ were found for sites in Michigan and northern Ontario and the lowest levels found in northwestern populations (Appendix 2). Similar trends in $\pi$ were observed when populations were grouped into putative refugial areas and areas along putative colonization pathways (Figure 2.5). The assumption of selective neutrality within each region was not rejected by the MK test ($p=0.77$, Fisher's exact test).

## 2.4 Discussion

Our initial analyses using cytochrome b revealed a distinct split between eastern and western wood frog populations. This result prompted us to sample the species' range more intensely to further resolve phylogenetic patterns. After testing three mitochondrial regions for levels of sequence divergence on a subset of samples, we also decided to analyze this larger dataset with ND2/tRNA$^{TRP}$ in attempt to gain more phylogenetic resolution than that afforded by cytochrome b. This second analysis confirmed the distinct split between eastern and western wood frog populations as well as revealed that Maritime populations form a distinct subclade amongst eastern populations. Although initial divergence may have predated the Pleistocene, the lack of mixing observed is consistent with a hypothesis of isolation in and expansion from multiple glacial refugia. Low levels of genetic diversity in the north and the limited phylogenetic structure within clades is consistent with rapid range expansion.

### *2.4.1 Phylogeographic history of the eastern lineage*

Patterns of genetic diversity in the eastern clade suggest that wood frog occupied a southern refugium in the Carolinas and an interior plain refugium in the American Midwest along with several other amphibians (*A. maculatum*: Zamudio and Savage, 2003; *A. t. tigrinium*: Church *et al.*, 2003; *R catesbeiana* and *P. crucifer*: Austin *et al.*, 2004; *R. pipiens*: Hoffman and Blouin, 2004). Pairwise sequence differences are high here relative to more northern populations, indicating that these regions harbour more divergent haplotypes, as expected for refugial areas. The fossil record also verifies the presence of *R. sylvatica* in these areas during the late Pleistocene (Holman, 1995). Although we would have also expected haplotype diversity within these regions to be higher than other regions, NCA points to restricted gene flow among many of these populations and so these populations may have experienced some haplotype loss as a result of population isolation and genetic drift.

The highest levels of both gene diversity and pairwise sequence differences for eastern wood frog populations were found in Virginia and

Pennsylvania, indicating that the species also likely persisted in a third, more northern glacial refugium (Figure 2.6). A refugium as far north as central Virginia has been proposed for the tiger salamander (Church *et al.*, 2003) and wood frog fossils dating to the late Wisconsinan have been found as far north as Pennsylvania (Holman, 1995). This putative northern refugium appears to have had the biggest impact on the colonization of the northeast since populations in this area share widely-distributed, ancestral haplotypes (66 and 119) with more northern populations (Appendix 1).

Several lines of evidence suggest that these putative refugia were geographically distinct. For instance, haplotypes from the southern highlands form a subclade in our phylogenetic analyses, sharing only one haplotype with populations in neighboring Virginia. Haplotypes from the interior plains are distantly related to southern highland haplotypes in the haplotype network and are not found elsewhere in the range. The NCA also points to past fragmentation between populations in the Carolinas and Pennsylvania, potentially indicating isolation in separate refugia. Finally the structure identified by SAMOVA indicates that populations from these areas are genetically distinct, consistent with a history of geographic separation.

### *2.4.2 The Maritime Subclade*

Although our phylogeny does not reveal the sister group to the distinct Maritime haplotypes and the Maritime network did not connect to the eastern network within the limits of statistical parsimony used in haplotype network estimation, increasing the number of mutational steps permitted in the network estimation procedure connected the Maritime network to haplotypes in the Carolinas. Thus the Maritime region was likely colonized by individuals moving north along the coast from this southern refugium. This colonization path, as opposed to colonization from adjacent, interior populations, has been previously proposed, in line with observations that more of the continental shelf was exposed during the last glaciation (Pielou, 1991). Colonization of the Maritimes along the coast may have proceeded early, preventing subsequent invasion from more interior regions.

### *2.4.3 Phylogeographic history the western lineage*

Patterns of genetic diversity (*e.g.* complete homogeneity in Alaska) do not support a western refugium for the wood frog. Instead, we observe the highest levels of genetic diversity for the west in Michigan, Ohio and Ontario. Although these areas were covered by the Laurentide Ice Sheet ~14,000 ybp, part of neighbouring Wisconsin was ice-free (Holliday *et al.*, 2002) and may have contained forest habitat (Jackson *et al.*, 2000). Furthermore, other forest-dwelling species are thought to have persisted in refugia in this region (Rowe *et al.*, 2004; Howes *et al.*, 2006). With its adaptation to cold climates, *R. sylvatica* is physiologically equipped to have maintained populations this far north.

Colonization out of a Wisconsin refugium likely proceeded via two routes (Figure 2.6). The ordering of haplotypes in the haplotype network suggests that one route entered the Great Lakes region via the upper peninsula of Michigan, consistent with one of the routes proposed by Holman (1992). Specifically, haplotypes from Wisconsin and upper Michigan lie interior in the network to haplotypes from Michigan and Ontario, which in turn lie interior to haplotypes from Ohio. This pattern indicates that populations entered Michigan and Ontario from the Upper Peninsula, as opposed to coming from the south. This pattern is surprising because ice and glacial Lake Algonquin would have blocked this route up to 9, 900 ybp, making wood frogs a relatively new arrival to the Great Lakes area. The lack of wood frog fossils in Michigan deposits dating 13,000 to 11,700 years ago (Holman, 2000), indicates that the species may have still been absent from the state during a period when other species were reinvading the region from the south, providing further support for delayed colonization via this northern route.

A second colonization path likely went northwest from Wisconsin. Although we only had one sample from Wisconsin, we found widespread, northern haplotypes (1 and 15) in the Minnesotan populations adjacent to the putative refugium, indicating that range expansion took place from that vicinity. This arm of colonization covers a territory stretching approximately 5,000 km and thus range expansion from a Wisconsin refugium would have occurred at a

minimum rate of about 0.5 km/year, which is substantial for an amphibian. Finally, we also found haplotype 15 in northwestern Ontario, indicating that this western arm of colonization also moved east over Lake Superior. Northern Ontario thus appears to represent a zone of contact between western colonists moving east from Minnesota and west from southern Ontario (Figure 2.6).

### *2.4.4 Implications of the isolated western populations*

The isolated populations in Wyoming, Colorado and Arkansas likely represent relict populations from a previously wider distribution in the southwest. Holman (2003) documents *R. sylvatica* fossils from Nebraska and Kansas and other disjunct populations are found in Idaho and Missouri (Muths *et al.*, 2005) indicating that the western range of *R. sylvatica* was once more extensive. The Colorado population contains a unique haplotype. NCA points to allopatric fragmentation between this population and those in the putative refugial area, indicating that the Colorado population was likely isolated and the southern range already fragmented during the last glaciation. In contrast, the occurrence of widespread haplotype 1 in Wyoming indicates that this area had a more recent connection with the post-glacial range. Such a connection raises the possibility that an additional, now extinct, refugium in the northern Plains, such as that proposed for *R. pipiens* (Hoffman and Blouin, 2004), played a role in the colonization of northwestern populations.

### *2.4.5 Comparison with other amphibians*

The wood frog demonstrates comparably less phylogenetic structure than other recolonizing amphibians and although colonization patterns were influenced by the Great Lakes and their proglacial antecedents, we did not detect phylogeographic structure in relation to the Appalachian Mountains. A selective sweep could theoretically produce similar patterns (Bazin *et al.*, 2006). However, the MK test comparing the ratio of non-synonymous to synonymous substitution within and among clades failed to reject the hypothesis of selective neutrality, indicating that the structure observed within clades is not likely to be confounded by direct selection on the ND2/tRNA$^{TRP}$ gene. Furthermore, another amphibian

with a comparably extensive northern distribution, *R. pipiens*, also demonstrates limited phylogenetic structure at a mitochondrial gene (Hoffman and Blouin, 2004) pointing to a shared demographic history rather than selection as a cause of these patterns.

The colonization history of the wood frog is different from that of other amphibians. Most of the northeast appears to have been colonized from an Appalachian refugium located further north than most amphibian refugia. Furthermore, eastern haplotypes do not penetrate Southern Ontario or Michigan as they do in *P. crucifer* (Austin *et al.*, 2002), *A. maculatum* (Zamudio and Savage, 2003), *R. catesbeiana* (Austin *et al.*, 2004), *P. crucifer* (Austin *et al.*, 2004) and *B. fowleri* (Smith and Green, 2004). Instead, the eastern lineage is restricted to the Laurentian Mountains in Quebec (although additional sampling of populations further north is required to delineate exact clade boundaries) and frogs inhabiting the Great Lakes region are part of a larger, western clade. Potentially, wood frogs, arriving earlier than other species, encountered ice or the Champlain Sea and were denied access to the eastern route taken by later arrivals over the Great Lakes. Instead, the Great Lakes region was colonized by western populations, which appear to have persisted in a northern refugium in Wisconsin. Northwestern populations also appear to have originated from this refugium, although we cannot reject the potential existence of a now-extinct refugial population in the northern Plains.

### *2.4.6 Broader implications and future directions*

We have shown a distinct split between eastern and western populations of the wood frog with levels of sequence divergence comparable to levels reported for some amphibian species pairs (*R. aurora–R. muscosa* , *R. cascadae–R. muscosa*: Macey *et al.*, 2001; *R. catesbeiana–R. clamitans*, *R. catesbeiana –R. okaloosae*: Austin *et al.*, 2003; *R. aurora–R. cascadae*: Macey *et al.*, 2001, Shaffer *et al.*, 2004; *P. vandykei–P. idahoensis*: Carstens *et al.*, 2004). This split roughly lines up with some of the morphological variation historically used to subdivide the species into subspecies (Wright and Wright, 1949). Although Martof and Humphries (1959) made a compelling case against these subspecies

designations by providing environmental explanations for observed morphological variation, they observed several anomalies in morphological patterns that could potentially arise from genetic differences among the distinct clades observed presently. For instance, they note that Great Lake populations are more similar to northern populations than expected based on environmental conditions—an occurrence which could be in part due to the shared genetic history of Great Lake and northwestern populations. They also note that the sharpest gradient in body size occurs west of the Appalachian Mountains, across an area that encompasses populations from both the eastern and western mitochondrial clades. Thus, our results provide impetus for a re-examination of regional morphological patterns in light of the genetic boundary between eastern and western populations.

It is also intriguing that although we sampled extensively along the putative boundary between populations in the eastern and western clades, we found very few localities with haplotypes from both lineages. Likewise, we found a clear geographic demarcation between populations in the Maritime subclade and other eastern populations. This result suggests that there has been limited introgression between clades and raises questions as to the factors sustaining these boundaries. For instance, some degree of reproductive isolation among clades may exist and future studies will address this possibility.

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Table 2.1. List of populations included in each of the regions for which haplotype diversity and mean pairwise sequence differences were measured. Population numbers correspond to those in Appendix 1.

| Clade | Region | Populations Included | N |
|---|---|---|---|
| Eastern | Midwest | 62 - 64 | 14 |
| | Virginia | 42 | 5 |
| | Carolinas | 52 -56 | 25 |
| | PA | 43 - 51 | 68 |
| | NY | 29 – 33, 36 - 41 | 55 |
| | NewEngld | 18 - 28 | 65 |
| | StLawrence | 1 - 9 | 75 |
| | Maritime | 10, 11, 13 - 17 | 38 |
| Western | N.MI | 66 - 69 | 17 |
| | S.MI & N.OH | 57, 58, 60, 61, 65 | 9 |
| | MN & ND | 83 - 89 | 13 |
| | L. Superior W | 76, 77 | 14 |
| | L. Superior E | 74, 75, 78 | 19 |
| | S.E. ON | 71 - 73, 79 - 82 | 30 |
| | Prairies | 90 – 93, 97 - 102 | 23 |
| | BC & ALBT | 103, 104, 106 - 108 | 19 |
| | Yukon | 105, 109 - 114 | 10 |
| | Alaska | 115, 116 | 10 |

N = total number of individuals in region

Table 2.2. Fixation indices and percentage of variation explained by each source for groups of populations identified by SAMOVAs within each clade.

| Number of Populations | Group Compositions | Fixation Indices | | | Percentage of Variation (%) | | |
|---|---|---|---|---|---|---|---|
| | | $F_{SC}$ | $F_{ST}$ | $F_{CT}$ | Among Groups | Among Populations | Within Populations |
| **Eastern Clade** | | | | | | | |
| 2 | 56; all others | 0.34 | 0.76 | 0.64 | 64.18 | 12.04 | 23.78 |
| 3 | 56; 63; all others | 0.31 | 0.73 | 0.61 | 61.18 | 12.05 | 26.78 |
| 4 | 56; 63; 43; all others | 0.31 | 0.72 | 0.59 | 59.51 | 12.50 | 27.99 |
| 5 | 56; 63; 55; 59; all others | 0.29 | 0.70 | 0.57 | 57.48 | 12.26 | 30.25 |
| 6 | 42; 56; 63; 62, 64; 52-55; all others | 0.16 | 0.64 | 0.57 | 57.48 | 6.69 | 35.83 |
| 7 | 56; 63; 52-55; 42; 62; 64; all others | 0.15 | 0.64 | 0.58 | 57.67 | 6.57 | 35.76 |
| **Maritime Clade** | | | | | | | |
| 2 | 15; all others | 0.18 | 0.69 | 0.62 | 61.90 | 6.87 | 31.23 |
| 3 | 15; 16; all others | 0.10 | 0.58 | 0.54 | 53.99 | 4.41 | 41.60 |
| 4 | 15; 16; 13; all others | 0.01 | 0.52 | 0.51 | 51.55 | 0.60 | 47.85 |

| Number of Populations | Group Compositions | Fixation Indices | | | Percentage of Variation (%) | | |
|---|---|---|---|---|---|---|---|
| | | $F_{SC}$ | $F_{ST}$ | $F_{CT}$ | Among Groups | Among Populations | Within Populations |
| **Western Clade** | | | | | | | |
| 2 | 12, 57, 58, 60, 61, 65, 66-68, 71-75, 78-82; all others | 0.63 | 0.83 | 0.54 | 54.29 | 28.79 | 16.92 |
| 3 | 12, 66-68, 71-75, 78-81; 82, 57, 58, 60,61, 65; all others | 0.56 | 0.83 | 0.61 | 61.39 | 21.49 | 17.12 |
| 4 | 12, 66, 67, 71-73, 75, 78-81; 68; 57, 58, 60, 61, 65, 82; all others | 0.51 | 0.83 | 0.66 | 65.79 | 17.43 | 16.77 |
| 5 | 12, 66, 67, 71-75, 78-81; 57, 58, 60, 61, 65, 82; 94; 68; all others | 0.44 | 0.83 | 0.70 | 69.65 | 13.27 | 17.08 |
| 6 | 69, 70, 87, 74; 94; 57, 58, 60, 61, 65, 82; 68; 12, 66, 67, 71-73, 75, 78-81; all others | 0.39 | 0.82 | 0.71 | 71.27 | 11.13 | 17.59 |

| Number of Populations | Group Compositions | Fixation Indices | | | Percentage of Variation (%) | | |
|---|---|---|---|---|---|---|---|
| | | $F_{SC}$ | $F_{ST}$ | $F_{CT}$ | Among Groups | Among Populations | Within Populations |
| 7 | 76, 77, 83-85, 88, 89, 90, 91, 93; 12, 66, 67, 71-73, 75, 78-81; 68; 57, 58, 60, 61, 65, 82; 69, 70, 74, 87; 94; all others | 0.27 | 0.80 | 0.73 | 73.16 | 7.21 | 19.62 |

Table 2.3. Inferences for all clades in the nested clade analysis that had a significant association with geography. Inferences key provided at: http://darwin.uvigo.es/software/geodis.html (November 2005).

| Clade | Chain of Inference | Inference |
|---|---|---|
| ***Eastern Network*** | | |
| Clade 1-3 | 1-2-11-12:No | Contiguous range expansion |
| Clade 1-7 | 1-2-3-5-6-7: Yes | Restricted gene flow/dispersal but some long distance dispersal |
| Clade 2-3 | 1-19-20-2-3-5-15: No | Past fragmentation and/or long distance colonization |
| Clade 2-4 | 1-2-3-5-6-7: Yes | Restricted gene flow/dispersal but with some long distance dispersal |
| Clade 2-14 | 1-2-3-4: No | Restricted gene flow with isolation by distance |
| Clade 3-1 | 1-2-3-4: No | Restricted gene flow with isolation by distance |
| Clade 3-2 | 1-2-3-4: No | Restricted gene flow with isolation by distance |
| Clade 3-4 | 1-2-11-12: No | Contiguous range expansion |
| Clade 3-5 | 1-2-3-4: No | Restricted gene flow with isolation by distance |
| Clade 4-1 | 1-2-3-5-6 | Insufficient genetic resolution to discriminate between range expansion/colonization and restricted dispersal/gene flow |
| Clade 4-2 | 1-2-11-12: No | Contiguous range expansion |
| Clade 5-1 | 1-2-11-12: No | Contiguous range expansion |
| ***Maritime Network*** | | |
| Clade 1-50 | 1-2-3-5-15-16-18: No | Geographical sampling inadequate to discriminate between fragmentation, range expansion and isolation by distance |
| Clade 3-6 | 1-2-3-4: No | Restricted gene flow with isolation by distance |
| ***Western Network*** | | |
| Clade 2-21 | 1-19: No | Allopatric fragmentation |
| Clade 3-7 | 1-2-3-5-6 | Insufficient genetic resolution to discriminate between range expansion/colonization and restricted dispersal/gene flow |
| Clade 3-8 | 1-2-3-4: No | Restricted gene flow with isolation by distance |
| Clade 3-9 | 1-19-20-2-11-17-4: No | Restricted gene flow with isolation by distance |
| Clade 3-10 | 1-19-20: No | Inadequate geographical sampling |
| Clade 3-11 | 1-19-20: No | Inadequate geographical sampling |
| Clade 4-4 | 1-2-11-12: No | Contiguous range expansion |

Figure 2.1. Sampling locations for our mtDNA surveys of *Rana sylvatica*. The grey shading represents the approximate geographic range of *R. sylvatica* (after Stebbins, 2003). Open squares represent localities sequenced only for cytochrome b. Black circles represent sampling localities included only in the ND2/tRNA$^{TRP}$ dataset. Black triangles represent localities included in both datasets. Full site details are provided in Appendix 1

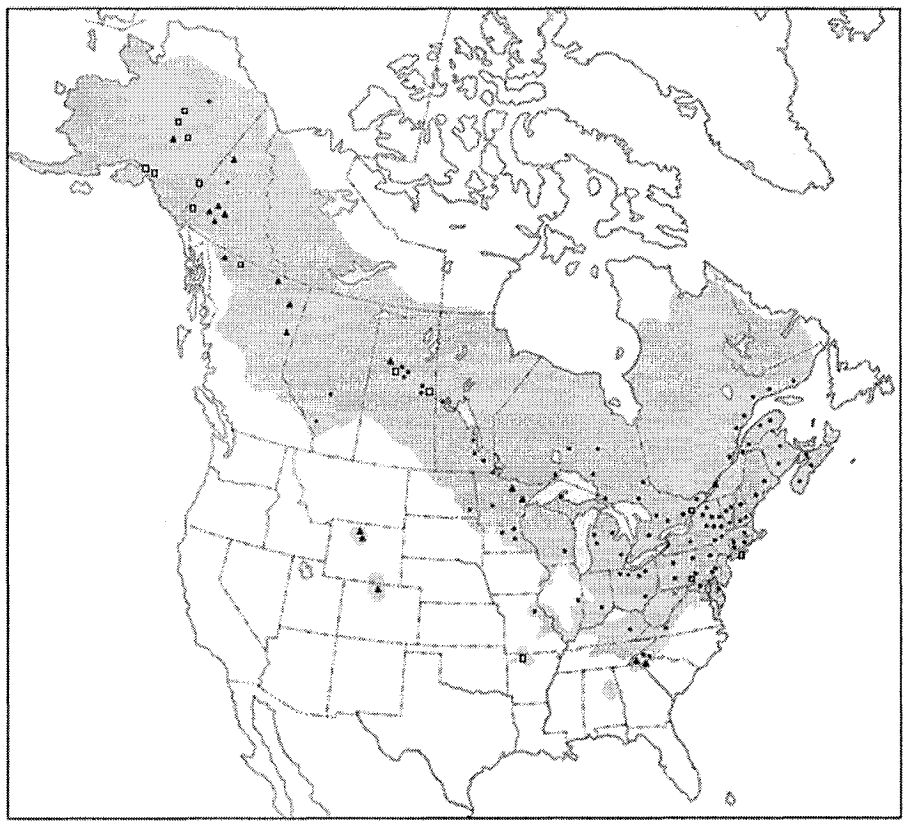


Figure 2.2. Maximum likelihood trees for *Rana sylvatica* based on (a) 700 b.p. of cytochrome b from 45 individuals representing 34 sites and (b) 574 b.p. of ND2/tRNA$^{TRP}$ from 551 individuals representing 116 sites across the species' range. Numbers above nodes indicate bootstrap support. An asterisk indicates that haplotypes form a polytomy. The relative geographic locations of major clades are noted

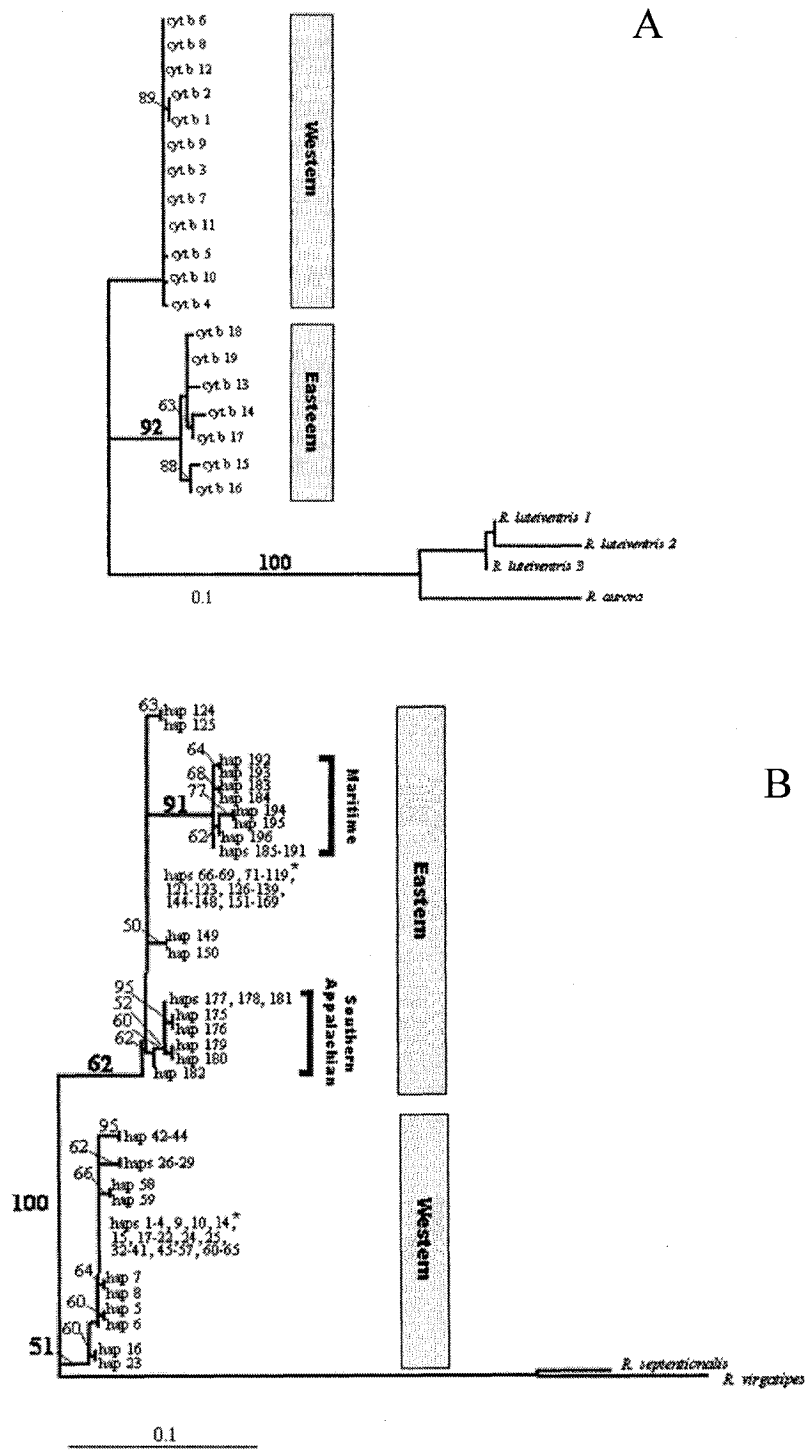
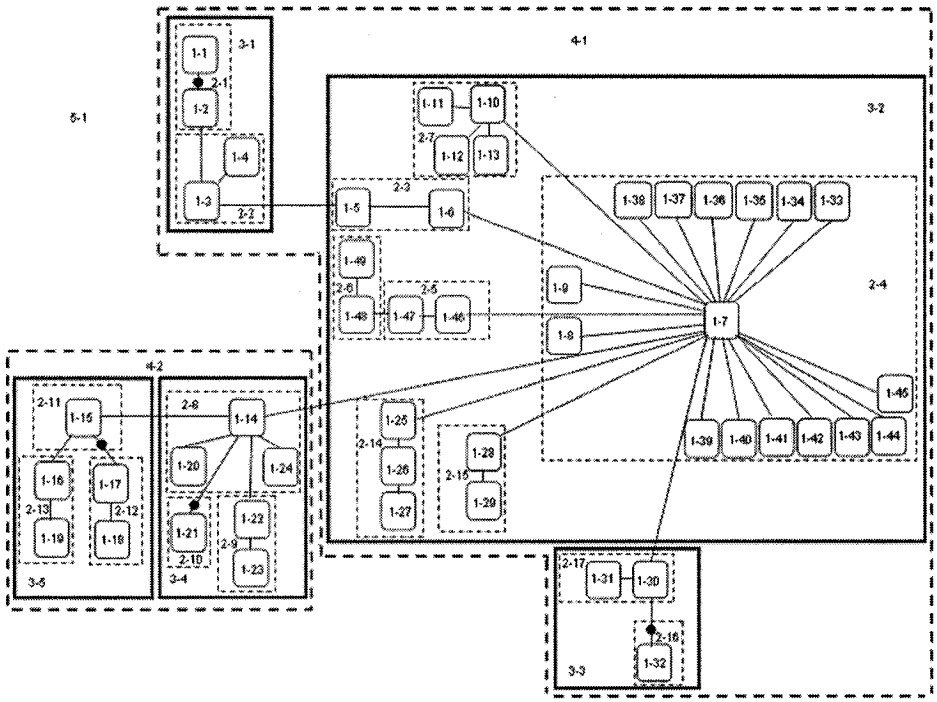
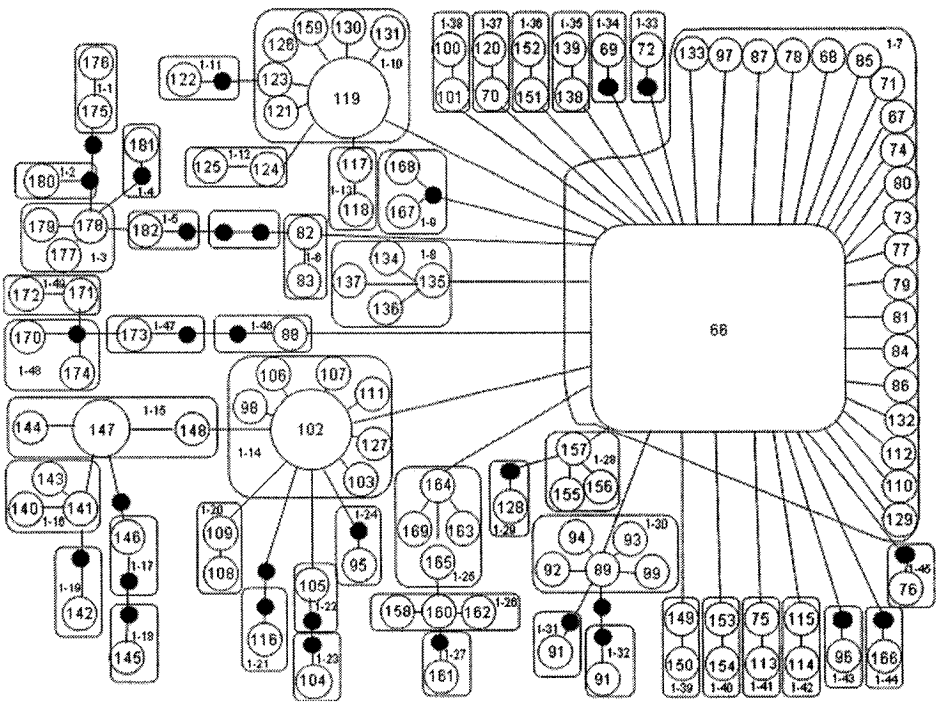


Figure 2.3. Haplotype networks identified at the 95% limit of statistical parsimony for 195 haplotypes of *Rana sylvatica* from 551 individuals sequenced for 574 bp of ND2/tRNA$^{TRP}$. Lines indicate a connection within 10 mutation steps. Missing haplotypes are shown as black circles. (a) One-step clades of the Eastern Network (b) Higher step clades of the Eastern Network (c) The Maritime network (d) One- and two- step clades of the Western Network (e) Higher step clades of the Western Network

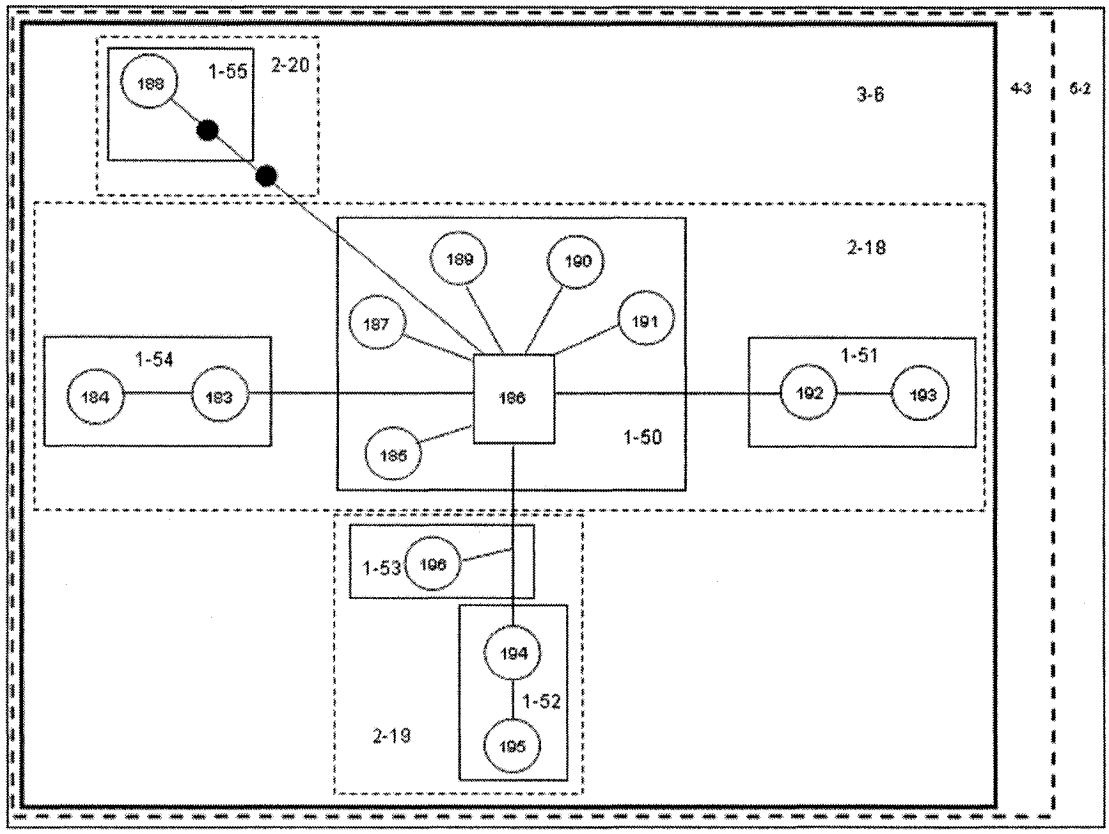


A



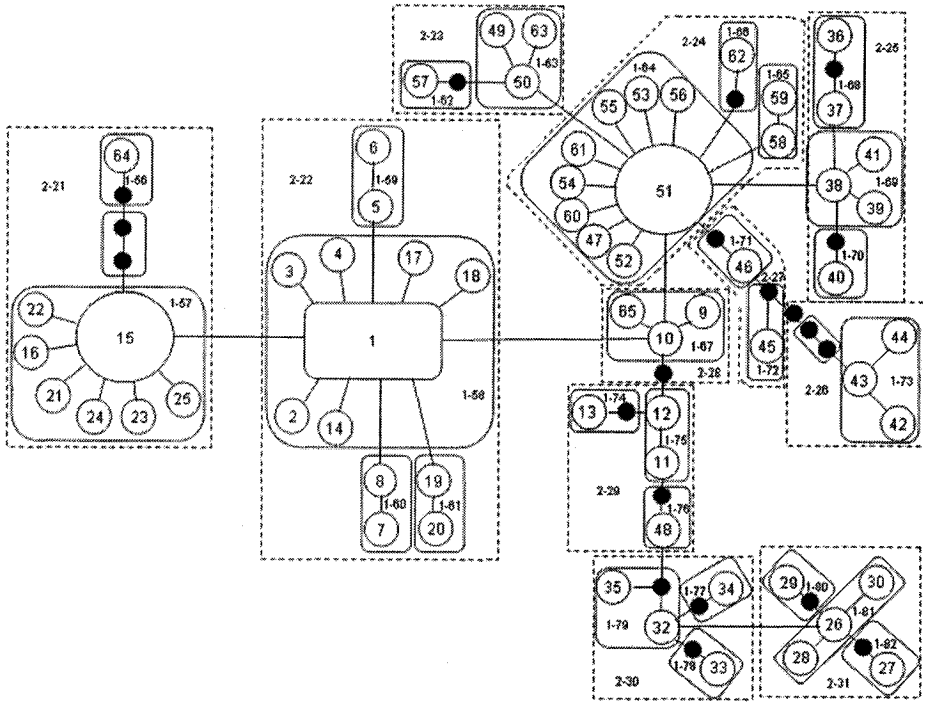
B

Figure 2.3 continued.

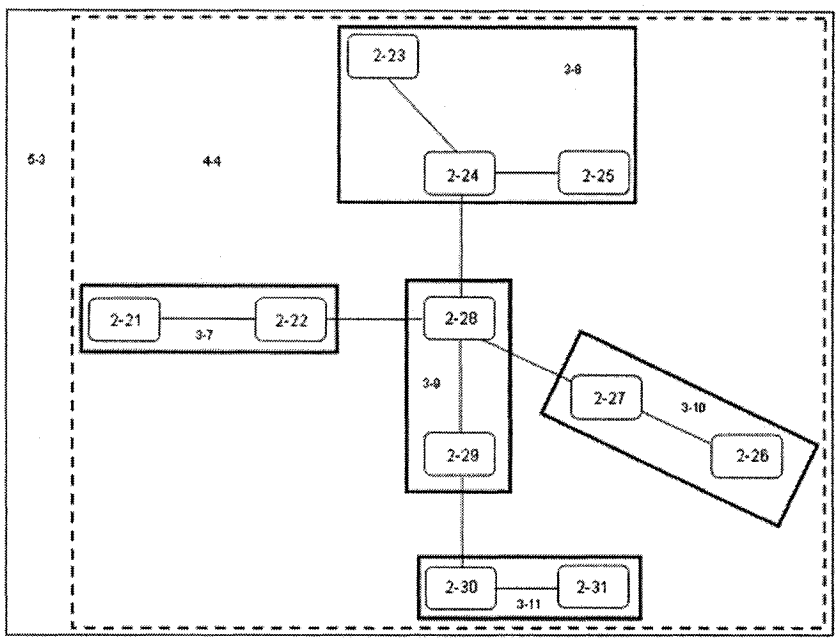



C

Figure 2.3 continued.

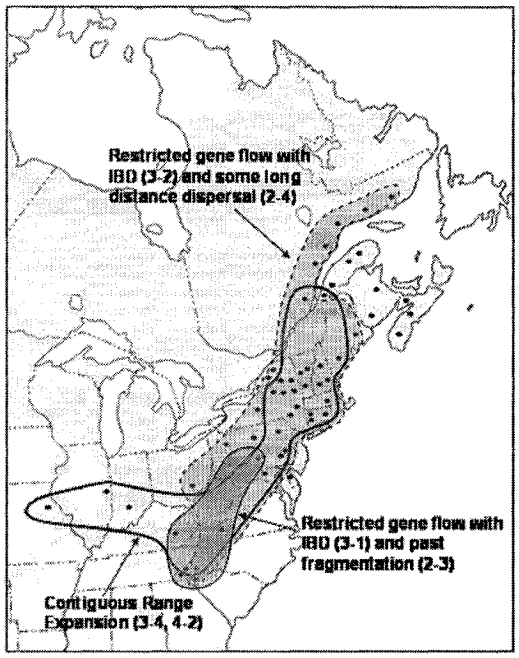



D

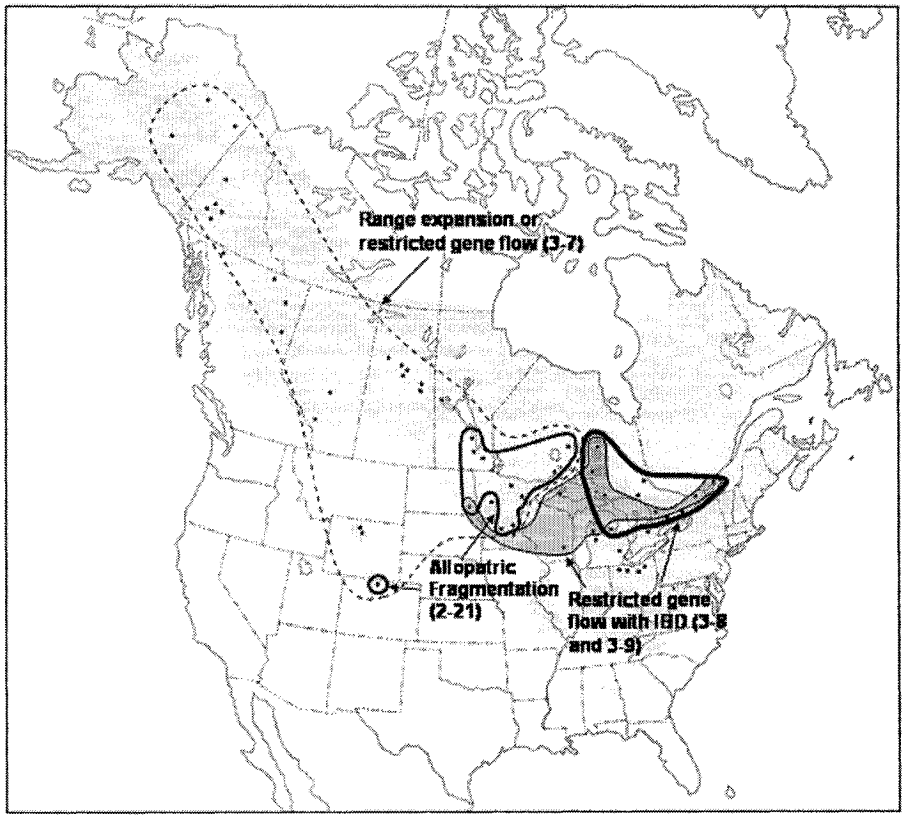



E

Figure 2.4. Selected nested clade analysis inferences for 195 *Rana sylvatica* mitochondrial haplotypes. (a) Inferences for the Eastern Network. (b) Inferences for the Western Network. Additional inferences are given in Table 3

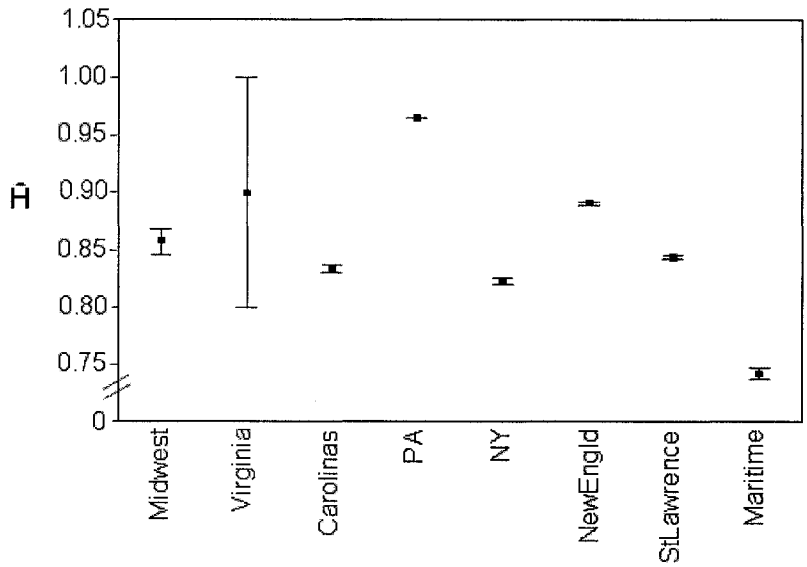



A

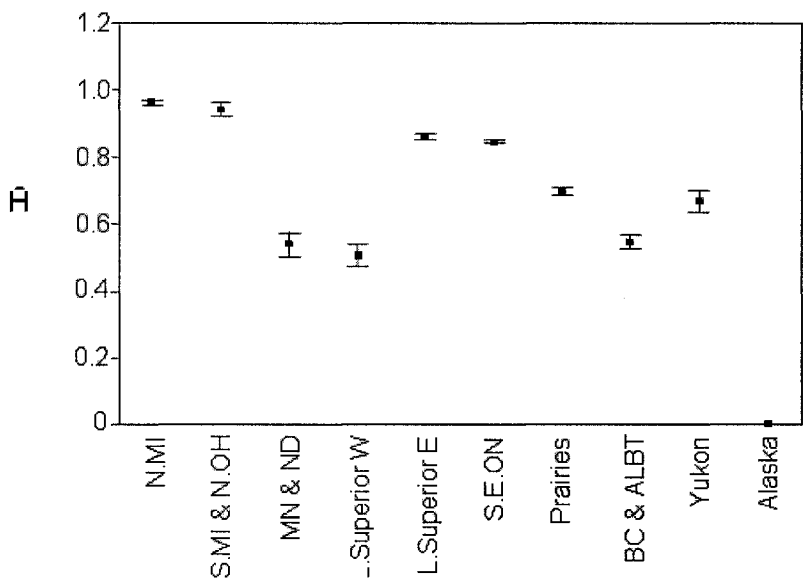



B

Figure 2.5. Indices of genetic diversity for regional groupings of 551 ND2/tRNA$^{TRP}$ sequences of *Rana sylvatica*. Haplotype diversity for regions within (a) the eastern clade and (b) the western clade. Mean number of pairwise sequence differences ($\pi$) for regions within (c) the eastern clade and (d) the western clade. Populations included in each region are given in Table 1

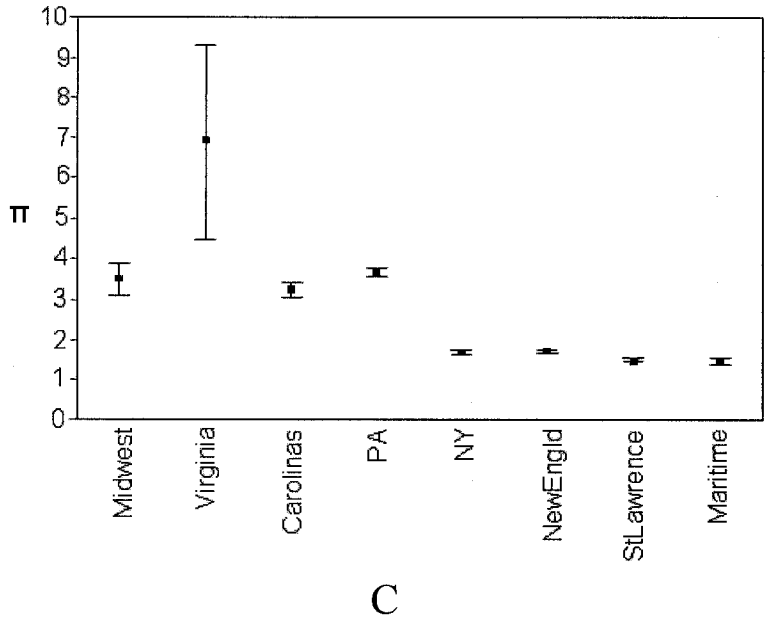



A

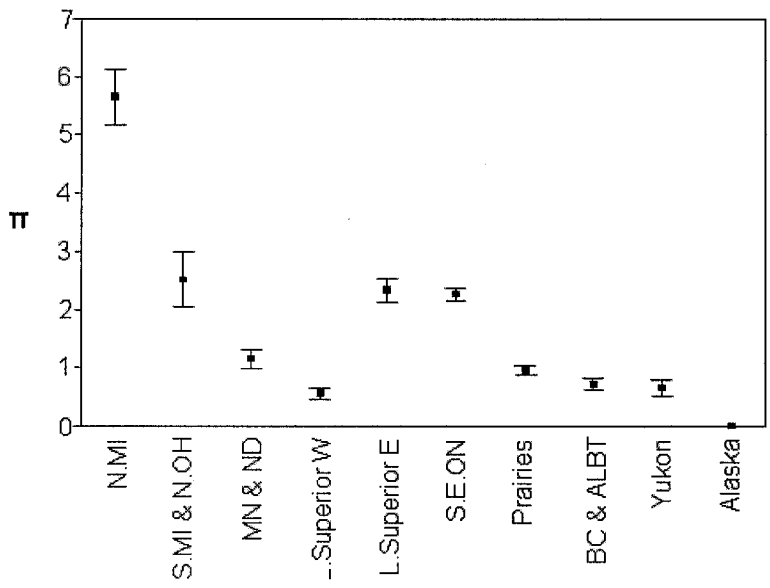



B

Figure 2.5 continued.

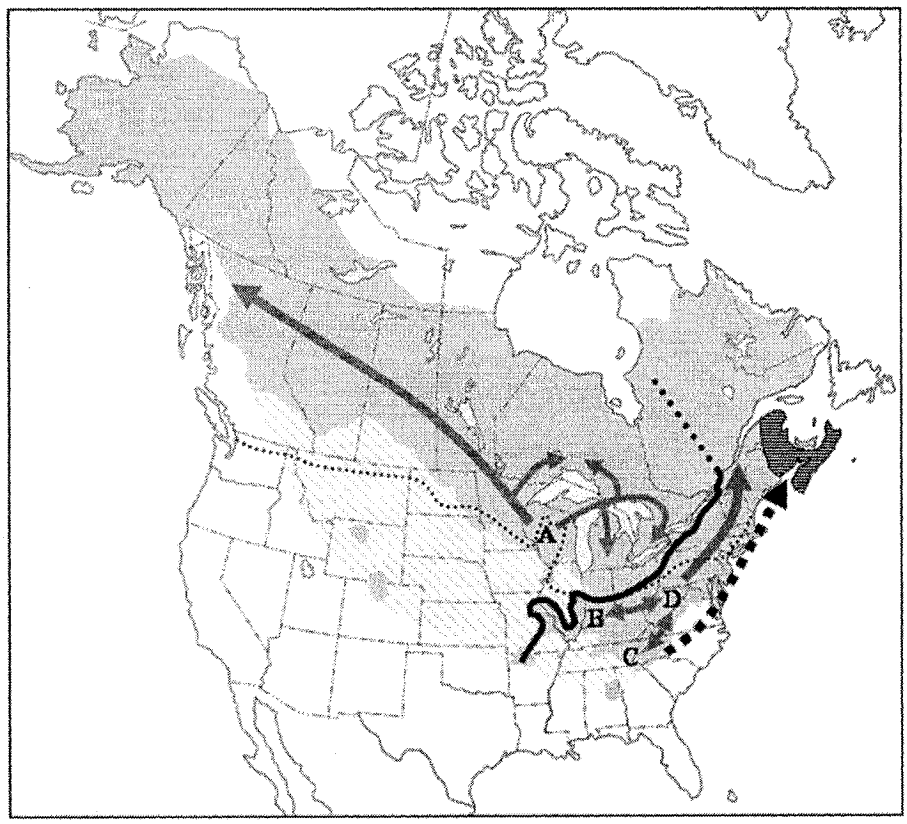



C




D

Figure 2.6. Summary of the post-glacial history of *Rana sylvatica*. The species' current range is shaded gray. Left slanting stripes indicating the potential historic extent of the range. The thin dotted line running east-west approximates the maximal southern extent of ice during the last glaciation. The solid black line indicates the boundary between eastern and western clades, with the thick dotted line indicating that the exact northern boundary between the clades is unknown. The Maritime clade encompasses the region indicated by black, horizontal stripes. Putative refugial areas are identified: A=western Wisconsin refugium, B=interior plains refugium, C=Southern highlands refugium, D=Northern Appalachian refugium. Grey arrows represent putative colonization pathways. The black, dashed arrow represents the putative connection between populations in the Carolinas and Maritimes



# Final Conclusion and Summary

## 3.1 General Conclusion

In the introduction I outlined what is known about the biogeographic responses of amphibians to environmental changes of the last glacial cycle. I hypothesized that disparities would exist between the phylogeographic patterns of northern, primary-colonizing species and southern, more recent arrivals to the north, owing to differences in the landscape and conditions encountered by species moving north at different times. The genetic patterns revealed in my study of the wood frog (*Rana sylvatica*) suggest that this northern species expanded its geographic range primarily from high-latitude refugia adjacent to the maximum extent of the glacial ice (although it also appears to have shared several, more southern refugia in the east with other species). With the exception of Maritime populations, which appear to have been derived from ancestral populations in the southeastern USA, eastern wood frog populations are derived from ancestral populations that were located somewhere near present-day Pennsylvania. Populations in and to the west of Ontario form a separate group and appear to be derived from a refugial population in present-day Wisconsin.

Apart from detailing the specific history of the wood frog, this study provides new insight into the biogeographic history of northern amphibians more generally. Refugia identified for the wood frog are further north than any previously identified for amphibians, indicating that the most northerly amphibians may have persisted at higher latitudes and thus been part of different biotic assemblages than more southern species during the last glaciation. Widespread mitochondrial DNA haplotypes and low levels of phylogenetic structure in wood frog populations in the north indicate that post-glacial range expansion by the wood frog was rapid. For instance, the western range of the wood frog, an area of over 5,000 km, was colonized in less than 10,000 years. Thus the region was colonized at a minimum rate of about 0.5 km a year. This rate is substantial for an amphibian and sheds new light on the rate at which amphibians are capable of making range adjustments. Nevertheless, despite evidence of its vagility, the wood frog expanded its range contiguously rather than via the series of long distance dispersal events that would be predicted under the

Leading Edge model (Hewitt, 1996) for range expansion. Indeed, a model of contiguous range expansion may be more appropriate for amphibians than a model of expansion by rare long distance migration events. Unlike larger vertebrates, wind-blown plant propagules or winged insects and birds, most amphibians are unlikely to make the long distance movements demanded by the model. Furthermore, unlike some organisms where a single pregnant female can potentially found a new population, the occasional, long-distance dispersing amphibian still requires a mate for successful population establishment. Thus it is more likely that amphibian range expansion involved a number of dispersers, moving relatively similar distances away from a range front.

This study also has implications for the conservation of temperate amphibians in North America. In terms of the wood frog specifically, I demonstrated that the species is comprised of three distinct geographic groups (eastern and western clades and a Maritime subclade). Although this species is not of particular conservation concern throughout most of North America, populations in Missouri are officially listed as endangered. These populations are part of a larger eastern lineage. However, they do harbour unique haplotypes and thus current efforts to preserve these populations should help maintain the species' pool of genetic variation.

The general patterns observed in the wood frog also provide new insight as to the location of regional genetic discontinuities in North America. Although, the genetic break between eastern and western wood frog populations roughly supports the Appalachian and Atlantic Coastal biotic region (outlined in the Introduction, Figure 1.3), the patterns observed for the wood frog suggest that the regional breaks deduced from more southern species may oversimplify levels of diversity in the north. For instance, the Maritimes may be a unique region, independent from the Appalachian region. Likewise, shield populations in Ontario and Quebec may not represent a single genetic region, as might be predicted from patterns observed in other eastern species. Differences between the wood frog and other species highlight the importance of obtaining information from many species when drawing conclusions about the biogeographic history of a particular

group of organisms. At this point, additional studies of northern amphibian species and populations are required to determine whether the phylogeographic patterns observed for the wood frog apply more generally to North American amphibians found at higher latitudes.

# Appendices

Appendix 1. Collection sites and summary of samples and haplotypes from each site for the cytochrome b dataset and the ND2/tRNA$^{TRP}$ dataset.

| Site I.D. | Site Name | Approximate Coordinates (DD) | | N | Haplotype (count) |
|---|---|---|---|---|---|
| | | Latitude | Longitude | | |
| **Samples from the cyt b dataset** | | | | | |
| a | NYRS1 | 40.867 | -72.763 | 1 | cyt 17 (1) |
| b | PARS1 | 39.931 | -77.441 | 1 | cyt 19 (1) |
| c | WVRS1 | 37.921 | -80.237 | 1 | cyt 16 (1) |
| d | ARRS1 | 35.958 | -92.218 | 2 | cyt 14 (2) |
| e | ONRS1 | 44.874 | -75.656 | 1 | cyt 10 (1) |
| f | QCRS1 | 45.434 | -73.945 | 1 | cyt 1 (1) |
| g | MNRS2 | 47.198 | -93.537 | 1 | cyt 9 (1) |
| h | SCRS1 | 34.500 | -82.660 | 1 | cyt 15 (1) |
| i | NCRS1 | 35.092 | -83.630 | 1 | cyt 13 (1) |
| j | MNRS1 | 46.592 | -92.677 | 1 | cyt 12 (1) |
| k | CORS1 | 40.580 | -105.070 | 1 | cyt 8 (1) |
| l | WYRS1 | 44.525 | -107.217 | 1 | cyt 12 (1) |
| m | WYRS2 | 44.621 | -107.292 | 1 | cyt 12 (1) |
| n | SKRS1 | 55.111 | -107.727 | 1 | cyt 11 (1) |
| o | SKRS10 | 54.549 | -103.669 | 1 | cyt 3 (1) |
| p | SKRS2 | 55.478 | -108.047 | 1 | cyt 12 (1) |
| q | BCRS1 | 59.948 | -131.996 | 1 | cyt 6 (1) |
| r | BCRS2 | 59.917 | -131.869 | 1 | cyt 12 (1) |
| s | BCRS3 | 59.747 | -127.350 | 1 | cyt 12 (1) |
| t | BCRS4 | 58.883 | -123.054 | 1 | cyt 12 (1) |
| u | BCRS5 | 56.392 | -121.132 | 1 | cyt 7 (1) |
| v | YKRS1 | 61.844 | -140.128 | 1 | cyt 9 (1) |
| w | YKRS2 | 61.137 | -135.359 | 1 | cyt 12 (1) |
| x | YKRS3 | 61.290 | -135.528 | 1 | cyt 12 (1) |
| y | YKRS4 | 62.996 | -136.497 | 1 | cyt 12 (1) |
| z | YKRS5 | 64.056 | -138.937 | 2 | cyt 12 (2) |
| aa | YKRS7 | 60.785 | -136.296 | 1 | cyt 12 (1) |
| bb | AKRS1 | 64.858 | -147.807 | 2 | cyt 12 (2) |
| cc | AKRS4 | 65.881 | -149.717 | 1 | cyt 3 (1) |
| dd | AKRS6 | 67.409 | -150.084 | 1 | cyt 12 (1) |
| ee | AKRS9 | 61.481 | -149.131 | 1 | cyt 12 (1) |
| ff | AKRS10 | 62.284 | -145.358 | 1 | cyt 4 (1) |
| gg | AKRS11 | 63.143 | -142.072 | 1 | cyt 12 (1) |
| hh | AKRS8 | 63.906 | -149.070 | 1 | cyt 2 (1) |
| **Samples from the ND2/tRNA$^{TRP}$ dataset** | | | | | |
| 1 | Des Grands Jardins, Quebec | 47.702 | -70.703 | 10 | 102(7), 107(1), 108(1), 148(1) |
| 2 | Port Cartier Correctional Facility, Quebec | 50.035 | -66.873 | 9 | 66(1), 71(2), 134(1), 135(2), 136(1), 137(2) |
| 3 | Natashquan, Quebec | 50.188 | -61.824 | 11 | 66(11) |
| 4 | Matanec, Quebec | 50.297 | -65.894 | 5 | 66(2), 101(1), 135(2) |
| 5 | Hwy 389, Quebec | 49.237 | -68.233 | 8 | 66(6), 74(1), 112(1) |
| 6 | Hwy 385, Quebec | 48.874 | -69.086 | 10 | 66(6), 97(1), 129(2), 149(1) |
| 7 | Parc JC, Quebec | 47.249 | -71.386 | 8 | 75(7), 113(1) |
| 8 | St. Francois, Quebec | 48.081 | -69.039 | 10 | 95(1), 98(1), 102(4), 106(1), 109(1), 111(1), 127(1) |
| 9 | Duchenier, Quebec | 48.184 | -68.698 | 4 | 102(2), 105(1), 109(1) |

| Site I.D. | Site Name | Approximate Coordinates (DD) | | N | Haplotype (count) |
|---|---|---|---|---|---|
| | | Latitude | Longitude | | |
| 10 | Matane, Quebec | 48.660 | -67.334 | 10 | 186(9), 193(1) |
| 11 | Gaspesie, Quebec | 48.981 | -66.204 | 3 | 185(1), 186(2) |
| 12 | Morgan Arboretum, Quebec | 45.434 | -73.945 | 9 | 48(1), 50(1), 51(5), 55(1), 56(1) |
| 13 | Kouchibouguac, New Brunswick | 46.769 | -65.012 | 5 | 186(1), 187(2), 188(1), 191(1) |
| 14 | Mahoney Corner, Nova Scotia | 45.683 | -63.587 | 5 | 186(5) |
| 15 | Kejimkujik, Nova Scotia | 44.438 | -65.197 | 5 | 194(4), 195(1) |
| 16 | Hwy 7, Nova Scotia | 44.780 | -62.902 | 5 | 189(1), 190(3), 192(1) |
| 17 | St. George, New Brunswick | 45.133 | -66.855 | 5 | 183(1), 184(1), 186(2), 196(1) |
| 18 | Hwy 11, Maine | 44.304 | -69.964 | 5 | 66(1), 119(1), 127(2), 160(1), |
| 19 | Osborn, Maine | 44.845 | -68.332 | 4 | 102(2), 119(1), 159(1) |
| 20 | Brewster Forest, New Hampshire | 42.916 | -72.099 | 7 | 66(1), 67(2), 68(1), 91(1), 94(1), 163(1) |
| 21 | Pawtuckaway, New Hampshire | 43.078 | -71.174 | 2 | 138(2) |
| 22 | White Brook, New Hampshire | 43.996 | -71.351 | 11 | 66(5), 119(2), 138(1), 157(3) |
| 23 | White Lake, New Hampshire | 43.838 | -71.215 | 7 | 66(2), 88(1), 150(1), 160(2), 162(1) |
| 24 | White Mountains West, New Hampshire | 44.109 | -71.900 | 8 | 66(3), 72(1), 78(1), 112(1), 133(1), 138(1) |
| 25 | Little Pond, Vermont | 42.879 | -73.097 | 7 | 66(2), 86(1), 101(1), 119(1), 158(1), 160(1) |
| 26 | Groton, Vermont | 44.276 | -72.281 | 5 | 66(2), 84(1), 119(2) |
| 27 | Granville, Vermont | 43.949 | -72.825 | 4 | 66(2), 103(1), 160(1) |
| 28 | Hampden, Massachusetts | 42.116 | -71.845 | 5 | 66(3), 70(1), 166(1) |
| 29 | New Hartford, Connecticut | 41.800 | -73.043 | 5 | 66(4), 155(1) |
| 30 | Stratford, Connecticut | 41.199 | -73.132 | 2 | 81(1), 89(1) |
| 31 | Voluntown, Connecticut | 41.574 | -71.865 | 3 | 66(1), 77(1), 98(1) |
| 32 | Catskills, New York | 42.184 | -74.186 | 5 | 66(1), 80(1), 139(1), 154(1), 156(1) |
| 33 | Frick Parking, New York | 41.975 | -74.859 | 10 | 66(2), 69(1), 76(1), 85(1), 102(3), 119(1), 161(1) |
| 34 | Blueberry Patch, New York | 42.493 | -76.821 | 10 | 32(2), 66(5), 99(1), 117(1), 160(1) |
| 35 | Herkimer/Hamilton, New York | 43.393 | -74.835 | 10 | 66(4), 73(1), 92(1), 110(1), 120(2), 164(1) |
| 36 | Road of Hope, New York | 43.304 | -74.232 | 9 | 66(2), 96(3), 102(1), 151(1), 153(1), 169(1) |
| 37 | County Rd 24, New York | 43.197 | -73.759 | 5 | 66(4), 79(1) |
| 38 | Keene, New York | 44.228 | -73.760 | 5 | 66(2), 80(1), 90(1), 102(1) |
| 39 | Second Pond, New York | 44.243 | -74.292 | 6 | 66(4), 81(1), 165(1) |
| 40 | Degrasse, New York | 44.332 | -75.015 | 1 | 66(1) |
| 41 | Everton, New York | 44.680 | -74.442 | 4 | 66(4) |
| 42 | Montgomery, Virginia | 37.21544 | -80.136 | 5 | 146(2), 176(1), 178(1), 181(1) |
| 43 | Tufton, Maryland | 39.493 | -76.769 | 1 | 118(1) |

| Site I.D. | Site Name | Approximate Coordinates (DD) | | N | Haplotype (count) |
|---|---|---|---|---|---|
| | | Latitude | Longitude | | |
| 44 | Ashland, Delaware | 39.786 | -75.686 | 5 | 66(1), 68(2), 138(2) |
| 45 | Buckingham Mt., Pennsylvania | 40.317 | -75.041 | 10 | 66(2), 121(1), 122(2), 138(1), 160(4) |
| 46 | Paul's Revere, Pennsylvania | 40.531 | -75.153 | 10 | 66(2), 112(1), 123(1), 138(3), 160(3) |
| 47 | Pine Grove Furnace, Pennsylvania | 40.034 | -77.298 | 10 | 93(1), 114(1), 115(1), 116(1), 128(1), 130(2), 170(2), 179(1) |
| 48 | Tunnel Road, Pennsylvania | 40.067 | -79.253 | 10 | 82(2), 83(1), 117(1), 173(2), 174(1), 179(2), 180(1) |
| 49 | Minister Creek, Pennsylvania | 41.572 | -79.229 | 10 | 119(3), 124(1), 125(1), 126(1), 149(1), 167(1), 171(1), 172(1) |
| 50 | Spooky Swamp, Pennsylvania | 41.452 | -76.575 | 5 | 66(1), 100(1), 119(1), 131(1), 153(1) |
| 51 | Delaware, Pennsylvania | 41.295 | -75.094 | 7 | 66(2), 87(1), 132(1), 152(1), 159(1), 164(1) |
| 52 | SCRS1, South Carolina | 34.500 | -82.660 | 5 | 178(1), 182(4) |
| 53 | BC, North Carolina | 35.092 | -83.630 | 5 | 168(1), 177(2), 178(1), 182(1) |
| 54 | CMGL, North Carolina | 35.404 | -81.065 | 5 | 147(1), 178(4) |
| 55 | FLTTP, North Carolina | 36.049 | -82.409 | 5 | 104(1), 177(4) |
| 56 | Bighill, Kentucky | 37.524 | -84.244 | 5 | 175(5) |
| 57 | Killdeer Plains, Ohio | 40.735 | -83.288 | 1 | 35(1) |
| 58 | Ranger, Ohio | 41.529 | -83.942 | 1 | 28(1) |
| 59 | Zaleski, Ohio | 39.278 | -82.392 | 1 | 167(1) |
| 60 | Swamp Cottonwood, Ohio | 41.005 | -82.154 | 1 | 32(1) |
| 61 | Crall Woods, Ohio | 41.056 | -82.434 | 1 | 29(1) |
| 62 | Jennings, Indiana | 38.580 | -85.374 | 5 | 143(1), 144(4) |
| 63 | Daniel Boone, Missouri | 38.779 | -91.393 | 5 | 142(2), 145(3) |
| 64 | Vermilion, Illinois | 40.243 | -87.780 | 4 | 140(1), 141(3) |
| 65 | Livingston Rd, Mighigan | 42.640 | -83.577 | 5 | 26(2), 27(1), 30(2) |
| 66 | Esmond, Mighigan | 44.378 | -83.760 | 6 | 36(1), 37(1), 38(1), 39(1), 40(1), 41(1) |
| 67 | Bitely, Michigan | 43.755 | -85.860 | 3 | 45(1), 46(2) |
| 68 | Lake Ann, Michigan | 44.704 | -85.833 | 5 | 11(1), 42(1), 43(2), 44(1) |
| 69 | Keweenaw, Michigan | 46.855 | -88.480 | 3 | 9(3) |
| 70 | Wisconsin | 43.368 | -88.231 | 1 | 65(1) |
| 71 | Van Dusen Farm, Ontario | 45.192 | -77.771 | 1 | 63(1) |
| 72 | Lagoon Park, Ontario | 45.379 | -79.217 | 3 | 50(2), 57(1) |
| 73 | Grundy, Ontario | 45.913 | -80.545 | 2 | 49(1), 60(1) |
| 74 | Thessalon, Ontario | 46.339 | -83.489 | 4 | 12(2), 13(1), 50(1) |
| 75 | Surluga, Ontario | 47.992 | -84.743 | 5 | 12(1), 48(1), 50(2), 51(1) |
| 76 | Mills Block, Ontario | 48.437 | -89.334 | 9 | 15(8), 25(1) |
| 77 | Wild Goose Park, Ontario | 49.695 | -87.271 | 5 | 15(2), 16(1), 23(1), 24(1) |
| 78 | Fushimi, Ontario | 49.840 | -83.917 | 10 | 12(1), 47(1), 51(5), 53(1), 61(1), 62(1) |
| 79 | Tower Road, Ontario | 47.055 | -79.782 | 4 | 51(2), 58(1), 59(1) |
| 80 | Mer Bleue, Ontario | 45.403 | -75.558 | 5 | 51(5) |

| Site I.D. | Site Name | Approximate Coordinates (DD) | | N | Haplotype (count) |
|---|---|---|---|---|---|
| | | Latitude | Longitude | | |
| 81 | Hwy 15, Ontario | 44.541 | -76.198 | 5 | 50(1), 51(2), 52(1), 54(1) |
| 82 | St. Agatha, Ontario | 43.423 | -80.615 | 10 | 32(8), 33(1), 34(1) |
| 83 | Coon Lake, Minnesota | 45.327 | -93.127 | 2 | 15(1), 21(1) |
| 84 | MNRS2, Minnesota | 47.198 | -93.537 | 3 | 15(3) |
| 85 | MNRS4, Minnesota | 46.592 | -92.677 | 1 | 15(1) |
| 86 | Ottertail, Minnesota | 46.426 | -95.557 | 1 | 1(1) |
| 87 | Washington, Minnesota | 45.222 | -92.759 | 1 | 65(1) |
| 88 | Wright, Minnesota | 45.317 | -93.934 | 1 | 15(1) |
| 89 | ND, North Dakota | 46.442 | -97.681 | 4 | 10(1), 15(3) |
| 90 | Balmoral, Manitoba | 50.237 | -97.301 | 5 | 15(3), 1(1), 22(1) |
| 91 | Chatfield, Manitoba | 50.776 | -97.547 | 5 | 15(4), 22(1) |
| 92 | MB, Manitoba | 53.606 | -101.455 | 2 | 1(2) |
| 93 | Tyndall, Manitoba | 50.186 | -96.636 | 5 | 1(1), 15(4) |
| 94 | CORS1/2, Colorado | 40.580 | -105.070 | 5 | 64(5) |
| 95 | WYRS1, Wyoming | 44.525 | -107.217 | 2 | 1(2) |
| 96 | WYRS2, Wyoming | 44.621 | -107.292 | 1 | 20(1) |
| 97 | SKRS2, Saskatchewan | 55.478 | -108.000 | 1 | 2(1) |
| 98 | SKRS3, Saskatchewan | 55.240 | -106.852 | 1 | 1(1) |
| 99 | SKRS4, Saskatchewan | 55.001 | -106.000 | 1 | 17(1) |
| 100 | SKRS5, Saskatchewan | 54.888 | -105.750 | 1 | 18(1) |
| 101 | SKRS6, Saskatchewan | 54.681 | -104.742 | 1 | 1(1) |
| 102 | SKRS8, Saskatchewan | 54.278 | -104.573 | 1 | 1(1) |
| 103 | Ministik, Alberta | 53.403 | -112.997 | 5 | 1(2), 7(1), 8(1), 14(1) |
| 104 | Foothills, Alberta | 51.045 | -114.063 | 3 | 5(1), 6(1),1(1) |
| 105 | BCRS2, British Columbia | 59.917 | -131.869 | 2 | 1(2) |
| 106 | BCRS3, British Columbia | 59.747 | -127.350 | 1 | 1(1) |
| 107 | BCRS4, British Columbia | 58.883 | -123.054 | 5 | 1(4), 19(1) |
| 108 | BCRS5, British Columbia | 56.392 | -121.132 | 5 | 1(5) |
| 109 | YKRS2, Yukon | 61.137 | -135.359 | 1 | 1(1) |
| 110 | YKRS3, Yukon | 61.290 | -135.528 | 2 | 1(1), 19(1) |
| 111 | YKRS4, Yukon | 62.996 | -136.497 | 1 | 18(1) |
| 112 | YKRS6, Yukon | 64.056 | -138.937 | 1 | 1(1) |
| 113 | YKRS7, Yukon | 60.785 | -136.296 | 4 | 1(1), 3(2), 4(1) |
| 114 | YKRS10, Yukon | 63.260 | -135.060 | 1 | 1(1) |
| 115 | AKRS7, Alaska | 65.881 | -149.717 | 5 | 1(5) |
| 116 | AKRS8, Alaska | 63.906 | -149.070 | 5 | 1(5) |

Appendix 2. Measures of genetic diversity among *Rana sylvatica* ND2/tRNA$^{TRP}$ sequences for sampling locations where n>3. Populations number correspond to those listed in Appendix 1.

| Population No. | $\hat{H}$ | S.D. | 95% C.I. | $\pi$ | S.D. | 95% C.I. |
|---|---|---|---|---|---|---|
| **Eastern Clade** | | | | | | |
| 1 | 0.53 | 0.18 | 0.05 | 0.80 | 0.63 | 0.18 |
| 2 | 0.92 | 0.07 | 0.02 | 1.73 | 1.11 | 0.36 |
| 3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 4 | 0.80 | 0.16 | 0.10 | 1.00 | 0.80 | 0.49 |
| 5 | 0.46 | 0.20 | 0.07 | 0.50 | 0.47 | 0.17 |
| 6 | 0.64 | 0.15 | 0.04 | 0.76 | 0.61 | 0.18 |
| 7 | 0.25 | 0.18 | 0.07 | 0.25 | 0.31 | 0.12 |
| 8 | 0.87 | 0.11 | 0.03 | 1.40 | 0.94 | 0.27 |
| 9 | 0.83 | 0.22 | 0.18 | 1.00 | 0.83 | 0.66 |
| 18 | 0.90 | 0.16 | 0.10 | 1.61 | 1.13 | 0.70 |
| 19 | 0.83 | 0.22 | 0.18 | 1.84 | 1.32 | 1.05 |
| 20 | 0.95 | 0.10 | 0.04 | 2.97 | 1.76 | 0.75 |
| 22 | 0.75 | 0.10 | 0.03 | 0.95 | 0.70 | 0.19 |
| 23 | 0.90 | 0.10 | 0.04 | 1.63 | 1.09 | 0.46 |
| 24 | 0.89 | 0.11 | 0.04 | 1.51 | 1.01 | 0.37 |
| 25 | 0.95 | 0.10 | 0.04 | 1.62 | 1.09 | 0.46 |
| 26 | 0.80 | 0.16 | 0.10 | 1.00 | 0.80 | 0.49 |
| 27 | 0.83 | 0.22 | 0.18 | 1.51 | 1.12 | 0.90 |
| 28 | 0.70 | 0.22 | 0.14 | 1.20 | 0.91 | 0.56 |
| 29 | 0.40 | 0.24 | 0.15 | 0.80 | 0.68 | 0.42 |
| 32 | 1.00 | 0.13 | 0.08 | 2.41 | 1.56 | 0.97 |
| 33 | 0.91 | 0.08 | 0.02 | 2.28 | 1.36 | 0.40 |
| 34 | 0.76 | 0.13 | 0.04 | 16.15 | 7.87 | 2.30 |
| 35 | 0.84 | 0.10 | 0.03 | 2.08 | 1.27 | 0.37 |
| 36 | 0.89 | 0.09 | 0.03 | 2.35 | 1.41 | 0.46 |
| 37 | 0.40 | 0.24 | 0.15 | 0.40 | 0.44 | 0.27 |
| 38 | 0.90 | 0.16 | 0.10 | 1.20 | 0.91 | 0.56 |
| 39 | 0.60 | 0.22 | 0.11 | 1.00 | 0.78 | 0.39 |
| 41 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 42 | 0.90 | 0.16 | 0.10 | 6.91 | 3.92 | 2.43 |
| 44 | 0.80 | 0.16 | 0.10 | 1.20 | 0.91 | 0.56 |
| 45 | 0.82 | 0.10 | 0.03 | 2.48 | 1.46 | 0.43 |
| 46 | 0.84 | 0.08 | 0.02 | 1.54 | 1.00 | 0.29 |
| 47 | 0.96 | 0.06 | 0.02 | 5.64 | 2.96 | 0.86 |
| 48 | 0.93 | 0.06 | 0.02 | 5.28 | 2.79 | 0.81 |
| 49 | 0.93 | 0.08 | 0.02 | 3.75 | 2.06 | 0.60 |
| 50 | 1.00 | 0.13 | 0.08 | 2.21 | 1.46 | 0.90 |
| 51 | 0.95 | 0.10 | 0.04 | 2.30 | 1.43 | 0.61 |
| 52 | 0.40 | 0.24 | 0.15 | 0.40 | 0.44 | 0.27 |
| 53 | 0.90 | 0.16 | 0.10 | 3.64 | 2.21 | 1.37 |
| 54 | 0.40 | 0.24 | 0.15 | 2.43 | 1.57 | 0.97 |
| 55 | 0.40 | 0.24 | 0.15 | 3.66 | 2.22 | 1.38 |
| 56 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 62 | 0.40 | 0.24 | 0.15 | 1.20 | 0.91 | 0.56 |
| 63 | 0.60 | 0.18 | 0.11 | 3.64 | 2.21 | 1.37 |

| Population No. | $\hat{H}$ | S.D. | 95% C.I. | $\pi$ | S.D. | 95% C.I. |
|---|---|---|---|---|---|---|
| 64 | 0.50 | 0.27 | 0.21 | 0.50 | 0.52 | 0.42 |
| **Maritime Clade** | | | | | | |
| 10 | 0.20 | 0.15 | 0.05 | 0.20 | 0.27 | 0.08 |
| 13 | 0.90 | 0.16 | 0.10 | 2.21 | 1.46 | 0.90 |
| 14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 15 | 0.40 | 0.24 | 0.15 | 0.40 | 0.44 | 0.27 |
| 16 | 0.70 | 0.22 | 0.14 | 1.81 | 1.24 | 0.77 |
| 17 | 0.90 | 0.16 | 0.10 | 1.81 | 1.24 | 0.77 |
| **Western Clade** | | | | | | |
| 12 | 0.72 | 0.16 | 0.05 | 0.67 | 0.56 | 0.18 |
| 65 | 0.80 | 0.16 | 0.10 | 1.61 | 1.13 | 0.70 |
| 66 | 1.00 | 0.10 | 0.05 | 1.67 | 1.13 | 0.57 |
| 68 | 0.90 | 0.16 | 0.10 | 4.05 | 2.43 | 1.50 |
| 72 | 0.67 | 0.31 | 0.36 | 1.34 | 1.10 | 1.25 |
| 74 | 0.83 | 0.22 | 0.18 | 2.52 | 1.70 | 1.36 |
| 75 | 0.90 | 0.16 | 0.10 | 1.81 | 1.24 | 0.77 |
| 76 | 0.22 | 0.17 | 0.05 | 0.22 | 0.29 | 0.09 |
| 77 | 0.90 | 0.16 | 0.10 | 1.00 | 0.80 | 0.49 |
| 78 | 0.78 | 0.14 | 0.04 | 1.81 | 1.14 | 0.33 |
| 79 | 0.83 | 0.22 | 0.18 | 1.17 | 0.93 | 0.74 |
| 80 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 81 | 0.90 | 0.16 | 0.10 | 0.80 | 0.68 | 0.42 |
| 82 | 0.38 | 0.18 | 0.05 | 0.80 | 0.63 | 0.18 |
| 89 | 0.50 | 0.27 | 0.21 | 1.00 | 0.83 | 0.67 |
| 90 | 0.70 | 0.22 | 0.14 | 0.80 | 0.68 | 0.42 |
| 91 | 0.40 | 0.24 | 0.15 | 0.40 | 0.44 | 0.27 |
| 93 | 0.40 | 0.24 | 0.15 | 0.40 | 0.44 | 0.27 |
| 94 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 103 | 0.90 | 0.16 | 0.10 | 1.40 | 1.02 | 0.63 |
| 107 | 0.40 | 0.24 | 0.15 | 0.00 | 0.00 | 0.00 |
| 108 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 113 | 0.83 | 0.22 | 0.18 | 1.17 | 0.93 | 0.74 |
| 115 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 116 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

$\hat{H}$ = Gene (haplotype) Diversity; $\pi$ = mean number of pairwise sequence differences; S.D. = standard deviation; C.I.= confidence interval