BIOGEOGRAPHY OF HIGHER DIPTERA IN GLACIAL AND POSTGLACIAL GRASSLANDS

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ABSTRACT

The presence of xeric grasslands on south-facing slopes in the Yukon Territory, Canada, has not been definitively explained. There are two competing hypotheses to explain their presence beyond the usual southern range of grasslands: (1) they are relicts of the Pleistocene mammoth steppe, widespread during the last glacial in Beringia, (2) they are relicts of the expansion of southern grasslands during the Hypsithermal, the warmest period of this last interglacial.

This thesis examines the biogeographical patterns of selected acalyptrate Diptera in Canadian glacial and postglacial grasslands, with emphasis on the persistence of Yukon grasslands in an otherwise boreal zone. Flies were studied from three regions: the southern Prairies (AB and MB), the Peace River region (AB) and the southern Yukon. The Peace River grasslands were used as a baseline of a community influenced by a Hypsithermal expansion. Community structure, at the species-level, of the family Chloropidae was compared across the three grassland regions. Additionally, phylogeographic patterns at the population level were studied in three species: *Incertella incerta* (Becker), *Meromyza columbi* Fedoseeva (Chloropidae) and *Trixoscelis fumipennis* Melander (Heleomyzidae).

Over 11,000 Chloropidae were used in the species-level analysis, representing 96 species. The community structure of the Chloropidae from Yukon grasslands was distinct from the community structure of southern grassland Chloropidae, including those from the Peace River region.

At the population level, two genes were analysed: cytochrome *c* oxidase subunit I and cytochrome *b*. For all three species, almost all haplotypes from the Yukon were unique, the Yukon individuals formed separate genetic lineages from the two southern regions and divergence times between the Yukon and southern populations suggested that populations diverged during the Pleistocene, prior to the end of the Wisconsinan glaciation.

Biogeographical analyses at both levels suggest that modern Diptera communities in Yukon xeric grasslands survived Pleistocene glaciations in Beringia. This implies that these Yukon boreal grasslands are not recent expansions of southern grasslands but are rather relicts of the Pleistocene mammoth steppe which was widespread in Beringia during the last ice age.

RÉSUMÉ

La présence de prairies dans le Yukon, Canada, sur les pentes aux versants arides et orientés vers le sud, demeure sans explication définitive. Il existe présentement deux hypothèses qui pourraient expliquer leur présence en dehors de leur habitat habituel : (1) ce sont des reliques de la steppe qui était répandue lors des cycles glaciaires du Pléistocène en Béringie; (2) ce sont des reliques de prairies qui se retrouvent présentement dans les Grandes Plaines, mais qui pendant la période hypsithermique, l'intervalle le plus chaud du présent cycle interglaciaire, auraient été davantage répandues.

Cette thèse considère les patrons biogéographiques de certains diptères acalyptères provenant de prairies canadiennes glaciaires et postglaciaires. Une importance particulière est accordée aux prairies du Yukon qui persistent dans une zone autrement dominée par la forêt boréale. Dans le cadre de cette thèse, des diptères ont été recueillis dans trois régions: les Grandes Plaines (AB et MB), la région de la rivière de la Paix (AB), et le sud du Yukon. Les prairies de la région de la rivière de la Paix représentent une communauté qui a été créée par l'expansion des prairies des Grandes Plaines lors de la période hypsithermique. Cette région a donc été utilisée comme référence des changements subis par une communauté suite à cet intervalle de réchauffement climatique. En outre, les patrons phylogéographiques de populations de trois espèces sont également étudiés : *Incertella incerta* (Becker), *Meromyza columbi* Fedoseeva (Chloropidae) et *Trixoscelis fumipennis* Melander (Heleomyzidae).

Plus de 11 000 spécimens de diptères de la famille Chloropidae ont été recueillis, comprenant 96 espèces. La structure de la communauté des Chloropidae provenant du Yukon était distincte comparativement à la communauté des Chloropidae provenant des régions des Grandes Plaines et de la rivière de la Paix.

À l'échelle des populations, nous avons analysé deux gènes : la sous-unité I de la cytochrome oxydase *c* et le cytochrome *b.* Pour les trois espèces, presque tous les haplotypes des individus provenant du Yukon étaient uniques ; les individus du Yukon formaient leur propre lignée généalogique par rapport aux individus des deux régions méridionales ; et le temps de divergence entre les populations du Yukon et celles des régions méridionales suggérait que ces populations ont divergé pendant le Pléistocène, avant la fin de la glaciation Wisconsinienne.

Les analyses biogéographiques à l'échelle des populations et des espèces suggèrent que les communautés modernes de diptères présentes dans les prairies arides du Yukon ont survécu

les glaciations du Pléistocène en Béringie. Il est donc probable que ces prairies du Yukon présentes dans une zone boréale sont des reliques de la steppe répandue en Béringie durant la dernière période glaciaire, plutôt qu'une extension récente des prairies méridionales.

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PREFACE

This thesis is manuscript-based and contains two chapters which will be submitted to peer-reviewed journals.

The first chapter includes an introduction to the thesis, along with a literature review which encompasses a historical summary of the changes in climate during the last ice age and this most recent interglacial. It also examines the literature pertaining to biotic changes during the Pleistocene and Hypsithermal, Diptera diversity, and holistic biogeographical approaches.

The two subsequent chapters are research manuscripts: Chapter 2 examines the community composition of Chloropidae (Diptera) in xeric grasslands in western Canada, while Chapter 3 is a phylogeographical study of selected higher Diptera on these same grasslands.

CONTRIBUTIONS OF AUTHORS

Chapter 2: Distribution and community structure of chloropid flies (Diptera: Chloropidae) in Nearctic glacial and postglacial grasslands

A.M. Solecki and T.A. Wheeler conducted the fieldwork for this project in 2012, and T.A. Wheeler was part of the collecting activities for most previous years. A.M. Solecki performed all laboratory work including specimen preparation and identification of newly collected specimens, and identification of older collection specimens, as well as preparing the initial drafts of this manuscript and completing the final revisions. T.A. Wheeler verified all Chloropidae identifications and provided research facilities. Both T.A. Wheeler and C.M. Buddle are principal investigators with the NBP and provided funding for this research. All authors contributed to the conceptualization and editing of this manuscript.

Chapter 3: Phylogeography of higher Diptera in glacial and postglacial grasslands in western North America

A.M. Solecki and T.A. Wheeler conducted the fieldwork required for collection of Diptera specimens. A.M. Solecki performed all laboratory work in specimen preparation, identification and the molecular analyses, as well as preparing the initial draft of this manuscript and completing final revisions. T.A. Wheeler provided research facilities and equipment for the taxonomic work. Both T.A. Wheeler and C.M. Buddle are principal investigators with the NBP and provided funding for this research and contributed to the conceptualization of this project. J.H. Skevington provided the laboratory facilities for the molecular work, as well as financial support and advice for the molecular portion of this project. All authors contributed to the editing of this manuscript.

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INTRODUCTION

Large scale abiotic events can have a major impact on biotic patterns at multiple scales from community structure, to species distributions, to population genetic structure within species. In North America, the Pleistocene glaciations are one of the most significant abiotic drivers of current distributions of species and populations. Because most of Canada was covered by ice for much of the Pleistocene, current species distributions are the result of dispersal from glacial refugia beyond the margins of the ice.

Despite the importance of glaciation in structuring the current biota, there have been fewer studies of glacial and postglacial impacts on distribution in North America compared to Europe. Most North American studies have focused on plants and vertebrates; there have been very few studies on terrestrial arthropods, even though they are the most species-rich taxa in previously glaciated regions.

Diptera are one of the most species-rich and ecologically diverse group of animals in Canada, especially in open habitats dominated by grasses and other herbaceous plants, but the impacts of glaciation on their distribution have been little studied.

In this study, we examine the origins of the higher Diptera in boreal grasslands in the Yukon Territory, Canada. These xeric grasslands are generally present only on south-facing slopes in what was once Beringia, an unglaciated land bridge which linked Asia and North America during the last glacial, and are disjunct from their widespread southern counterpart: the Prairies. It is unknown whether these grasslands and associated fauna survived the last glaciation in the Beringian refugium or migrated north from the Prairies during the Hypsithermal, the warmest period during this interglacial. The Peace River grasslands which are between the Yukon grasslands and the Prairies originated during the Hypsithermal; southern Prairie vegetation migrated north and, when the climate cooled, retreated south, leaving behind relict grassland vegetation in the valleys and tributaries of the Peace River. It is thought the Yukon grasslands may have originated in the same way. As such the Peace River grasslands provide a baseline of the expected community if the Yukon grasslands originated in this way.

CHAPTER 1. LITERATURE REVIEW

Historical Review

Pleistocene: The Wisconsinan glaciation

The effects of the ice sheets which covered most of the North American continent as recently as 20,000 years ago can still be seen in current landscapes. The Rocky Mountains' U-shaped valleys and the Great Lakes are only a few of the many remnants that the ice sheets have left.

Even though the continent bears obvious scars of glaciations, reconstructing glacial history remains elusive. The evidence for earlier glaciations was often erased by later ones, and even for the latter, some of the evidence is buried at sea or is difficult to interpret. Nonetheless, geomorphologists have been able to paint a fairly detailed picture of the last ice age, and particularly of the last glaciation, the Wisconsinan.

Over the past 1.8 million years, there have been at least 17 major glacial stages lasting 60,000–90,000 years, separated by interglacials 10,000–40,000 years in duration (Menzies, 2002). These oscillations are linked to Milankovitch cycles (Pielou, 1991), which shaped glacial movements, often creating asynchronous oscillations in regional ice margins (Menzies, 2002).

The late Wisconsinan glacial maximum is thought to have occurred asynchronously between 22,000–14,000 BP. At that time, the three continental ice sheets (Laurentide, Cordilleran and Innuitian) that made up the Wisconsinan complex were at their maximum extent and global sea levels were 120 m below current position (Menzies, 2002).

The Cordilleran Ice Sheet was a complex system of ice streams, intermontane icefields and piedmont glaciers (Hopkins, 1982). This ice sheet covered the western mountain belt south of the unglaciated parts of Yukon and Alaska (Dyke, 2004) and west, along the coasts of British Columbia and Washington. Because of diminished precipitation north and east of the central mountains, the interior of Alaska and the central and northern Yukon Territory were ice-free (Menzies, 2002).

The Laurentide Ice Sheet was centred over the Canadian Shield but extended west and south, through the Interior Plains. The eastern limits of the Laurentide Ice Sheet are unclear although the sheet likely reached the edge of the continental shelf off Labrador. The southern margin extended from Long Island, across southern New York, northern Pennsylvania, central

Ohio, Indiana, Illinois, Iowa, Wisconsin and Minnesota to the Dakotas into the foothills of the Rockies in Montana and west to Washington (Menzies, 2002). Unlike the maritime margins of the Cordilleran and Laurentide Ice Sheets, the southern margins are clearly delineated (Dyke *et al.*, 2002).

During their maximum extent, the Laurentide and Cordilleran Ice Sheets were joined for about 700 km from southeast Yukon to northeast British Columbia into Alberta (Menzies, 2002).

The existence of the Innuitian Ice Sheet was controversial for a long time though its existence is now accepted (Dyke *et al.*, 2002). It covered most of the Canadian Arctic Archipelago (The Queen Elizabeth Islands) north of 75 degrees latitude (Dyke, 2004). The margins of the ice sheet are uncertain as they most likely were offshore. The Innuitian Ice Sheet is known to have been extensive in the eastern and central archipelago, but the western islands may have contained some ice-free regions (Dyke *et al*., 2002).

Glacial Refugia

Most species that survived the last glacial maximum in North America, as well as previous glaciations, found refuge south of the ice sheets (Scudder, 1979; Beatty & Provan, 2010). However, a large landmass was left unglaciated in the northwest. The accumulation of biogeographic evidence from that region, such as rare endemic fauna and flora, as well as conspecific species between Siberia and Alaska led to the idea of a northern refugium: Beringia.

Beringia is by far the largest (Scudder, 1997a) and best documented refugium of the last glaciation (DeChaine, 2008). It encompasses far eastern Russia and northwestern North America, which were linked by a bridge of exposed land due to lower sea level during past glaciations (Harington, 2005). The region is thought to have remained unglaciated during the Quaternary because it was far from the nuclei of the major ice sheets and the lack of precipitation in the area prevented the growth of new glaciers (DeChaine, 2008). The Swedish botanist, Eric Hultén, was the first to coin the term "Beringia." He noticed that the current ranges of Alaskan plants indicated that a land bridge permitting the interchange of biota between Asia and Alaska had to have existed (Harington, 2005; Elias & Crocker, 2008). The body of evidence which supports the Beringia refugium hypothesis is wide and spans many fields such as geology, palynology, biogeography, phylogeography and paleontology (DeChaine, 2008).

Numerous reconstructions of the last glacial maximum show additional unglaciated areas in the northwest in the Canadian Arctic Archipelago, particularly on Banks and Melville Islands, suggesting these areas were refugia. This view was reinforced by the discovery of woolly mammoth fragments on both islands (England *et al*., 2009). While most glacial reconstructions show Beringia as separate from Banks Island, the few phylogeographic studies which have sampled in the island consider it to be part of a larger Beringian refugium (Tremblay & Schoen, 1999; Weider & Hobæk, 2003).

The development of phylogeography has allowed the discovery of previously unknown cryptic refugia across the continent. Paleoecological and geological data (historically used to infer refugia) is often insufficient for smaller areas such as islands and nunataks (unglaciated areas on a mountain ridges or peaks). Using molecular signals to infer refugia bypasses these limitations. The Alexander Archipelago, Haida Gwaii, the Canadian Arctic, as well as nunataks and ice free corridors within the ice sheets are North American ice age cryptic refugia for which there is mounting evidence (Shafer *et al*., 2010).

Holocene deglaciation

Following the last glacial maximum, the ice sheets began to recede. This did not occur at a steady pace, with some temporary, local readvances of the ice sheets (Pielou, 1991). At 18,000 BP, the ice sheet cover in North America was still at or near its glacial maximum, except along the southern Cordilleran Ice Sheet. The southwest Cordilleran ice margins were still growing to their maximum extent until approximately 14,000 BP (Dyke *et al.*, 2002). The Laurentide Ice Sheet maintained its near maximum size until 15,000 BP (Scuderi, 2002). Between 13,500 and 11,000 BP, there was a progressive net reduction of glaciated areas in North America, although the ice sheets still remained large and the Arctic ice margins were not strongly affected. Around this time, the Cordilleran Ice Sheet became detached from the Laurentide Ice Sheet. The timing of the opening is not clear, although it is unlikely that both ice sheets remained connected beyond 11,500 BP (Dyke, 2004).

By 10,000 BP, the Laurentide Ice Sheet was reduced to 35% of its last glacial maximum size and by 7,000 BP the ice sheet had almost disappeared (Scuderi, 2002). Throughout the Arctic islands, ice recession had been limited until 10,000 BP, after which time the ice started

receding more quickly. By 9,000 BP, the Innuitian and Laurentide Ice Sheets were separated and by 8,000 BP, in most places, the ice had receded to its modern margins (Dyke, 2004).

Climatic conditions during deglaciation were strongly influenced by solar energy and variation in the size and location of the Laurentide Ice Sheet. At around 9,000 BP, summer insolation was at its peak and a shift in air masses resulting from the reduction of that ice sheet had caused warm and dry conditions in North America. By 6,000 BP, summer insolation had decreased and the ice sheets disappeared, which caused air masses to shift again causing cooling until modern climatic conditions were reached by 3,000 BP. These climatic conditions have seen several interludes of renewed glaciations, such as the Little Ice Age between the 16th and mid-19th century (Scuderi, 2002).

The time frame between 9,000 and 6,000 BP corresponds to the Hypsithermal, the warmest time interval of the current interglacial (Pielou, 1991). This solar radiation maximum is thought to have occurred earlier in the northern Yukon: between 11,000 to 9,000 BP (Matthews & Telka, 1997). These warmer and drier conditions caused a northward displacement of plant species (and by extension, their associated fauna) which then would have receded back to their current distribution patterns following cooling (Strong & Hills, 2003).

Biotic responses to changing climate

Glaciations during the Pleistocene have left their mark on the land, affecting topography and river systems. However, fauna and flora were also affected. Changes in climate altered community composition, forcing cold-intolerant species south and others into refugia (Hewitt, 2000). Despite the ice having receded approximately 10,000 years ago, the continent is still rebounding (Menzies, 2002), many species are left in microclimatic remnants of their former expanse (Kuzmina *et al*., 2008) and populations, once disconnected by ice, still bear the genetic signature of their former separation (Soltis *et al*., 2006; Shafer *et al*., 2010). Both distributional shifts and molecular data have been used to infer the history of the continent.

Reconstructing the environment in Beringia during glaciation

The presence of Beringia as a refugium has been well established. Animals and plants were able to survive the harsh conditions of the glaciations in this area devoid of ice sheets.

Despite this knowledge, reconstructing the composition of the community that survived in Beringia has not been easy.

The biggest challenge in reconstructing the environment in ice-age Beringia has been consolidating seemingly conflicting lines of evidence. Schweger (1997) reviewed the paleocological reconstructions and surrounding controversy of ice-age Beringia, particularly the Yukon. In broad terms, the debate was centered on the type of vegetation present in Beringia throughout the last full-glacial maximum. It was known through fossil evidence that megafauna had survived in the North during glaciations; large grazing mammals such as saiga antelope, camels, bonnet-horned muskoxen and the emblematic woolly mammoth survived in a land that currently could not sustain them (Schweger, 1997; Guthrie, 2001). This suggested that past vegetation was dramatically different from current assemblages. Yet fossil pollen influx data suggested that the environment was discontinuous tundra, like that now found on dry rocky sites or above the treeline. Given such vegetation during glacial periods, large macrofauna would have had to be present only during the interstadials. This became the "production paradox" since grazing mammals clearly survived continually in Beringia during the last glacial. This conflicting evidence led to a change in methodology and prompted others to look at the data differently. Instead of fossil pollen influx, assemblages as a whole were examined, which showed a Beringia covered in arctic grassland or steppe that could have supported this well-documented grazing macrofauna (Schweger, 1997).

This particular debate, however, was centered on pollen analysis (Schweger, 1997). Notwithstanding the fact that issues with fossil pollen taxonomy could lead to misleading conclusions (Zazula *et a*l., 2003, 2006; Elias & Crocker, 2008), the inclusion of additional data over the years and other types of evidence, such as insect and plant fossils, has revealed a more complex vegetation landscape (Schweger, 1997).

Current data supports ecological mosaics due to local environmental factors such as altitude, soil moisture, or loess deposits (Zazula *et al*., 2003, 2006). In broad strokes (since there are exceptions within the described regions), much of the central land bridge was likely mesic, with poorly drained lowlands dominated by birch-graminoid tundra vegetation, although upland regions within the land bridge were xeric (Zazula *et al*., 2006; Elias & Crocker, 2008). Western Beringia and the interior of Eastern Beringia are thought to have been dominated by a steppe-tundra ecosystem, the Mammoth steppe. Biogeographic evidence suggests that the difference in

environmental conditions between the mesic land bridge and the xeric continental land masses became a barrier to some organisms; some western steppe weevils and megafauna, such as the woolly rhinoceros, failed to colonize Eastern Beringia (Elias & Crocker, 2008).

Due to the limitations of reconstructions (i.e. limited, partial fossil samples, taxonomic bias, methods, etc.), it is unlikely that this is a complete picture of Pleistocene Beringia. Recently, for instance, the use of DNA metabarcoding has revealed that the Pleistocene tundra-steppe was likely dominated by forbs, which would have provided a more nutrient-rich diet for large-mammals than graminoids. This observation would have gone unnoticed by traditional methods, since forbs are not high pollen producers like graminoids (Willerslev *et al*., 2014).

Perhaps the biggest challenge in reconstructing ice-age Beringia is the fact that the flora and fauna of Pleistocene Beringia has no modern analogue (Schweger, 1997; Zazula *et al*., 2003, 2006; Elias & Crocker, 2008). The pollen and macrofossil assemblages found in many sites in eastern Beringia, for instance, contain prairie-sage (*Artemisia frigida*), bunch-grasses and forbs. The discovery and identification of *A. frigida* is particularly important. In the absence of macrofossils, pollen can only be identified to the generic level as *Artemisia*. *Artemisia* species currently found in the north are *A. artica (norvegica)* and *A. globularia*, associated with tundra assemblages, and *A. tilesii*, associated with floodplains (Zazula *et al*., 2006). *Artemisia frigida*, on the other hand, is a good indicator of steppe vegetation, and along with the other plants, suggests steppe was extensive (Zazula *et al*., 2003, 2006). This plant, however, is no longer widespread in the arctic.

Although these steppe assemblages are generally considered to have no modern analogue, there are still areas which were formerly part of Beringia that contain pockets of steppe vegetation, including prairie-sage and grasses. These are present only on south-facing slopes where the soil is dry and net radiation is high. While the composition of current south-facing slope vegetation and fossil assemblages is not identical, the main plant genera and some species, like *A. frigida*, suggest that the flora on these south-facing slopes is at least a partial remnant of formerly widespread vegetation (Guthrie, 2001; Zazula *et al*., 2006).

Because one of the crucial components for the presence of steppe vegetation is aridity, in initial climate reconstructions, it was assumed that aridity in Beringia was only due to reduced precipitation; eastern Beringia had become more continental, and was under the influence of the nearby Laurentide Ice Sheet (Guthrie, 2001; Zazula *et al*., 2006). However, Guthrie (2001)

argued that clear skies played an additional role in shaping the vegetation. Increased continentality meant a reduced cloud cover which coupled with the high latitude of the Yukon would have resulted in high net radiation and increased absorption of solar energy by the soil surface. This would have caused accelerated evapotranspiration of soil and plants, deepening the active layer, despite colder overall temperatures, allowing plants like *Artemesia frigida* with deep roots to grow (Guthrie, 2001; Zazula *et al*., 2006). This would explain why steppe plants which were once widespread can still grow on south-facing slopes. Although the current climate with frequent low stratus clouds creates cool wet summers, the slope allows higher net radiation from the sun, creating an arid microclimate perfect for steppe vegetation (Guthrie, 2001).

The insects of Beringia: from the present to the past

Reconstructing insect assemblages of a past environment cannot be done without knowledge of the current assemblages (Matthews & Telka, 1997). Many of the species that survived the last glacial in Beringia are still extant. The current distribution of organisms, particularly with low mobility, is still influenced by this past event. Relatively little time has passed since the ice sheets retreated and the fauna and flora likely still have not reached equilibrium (Danks *et al*., 1997, Matthews & Telka, 1997).

Current distribution patterns of insects in Beringia

Although widespread species may have survived in Beringia if their current distribution includes the northwest, without additional evidence it is difficult to ascertain their postglacial history: whether they are new colonists from the south or have had multiple points of origin (e.g. the south and/or Beringia and/or the Palearctic) (Ball & Currie, 1997). Distributions of insects that have disjunct ranges and particularly those that are endemic to Beringia are easier to interpret. A relationship exists between high areas of endemism and refugia, and disjunct distributions allow speculations regarding barriers to continuous distributions. Many taxa whose distributions were continuous prior to glacial periods survived in disjunct refugia (Tribsch & Schönswetter, 2003).

Endemism

There are endemic species in many of the insect taxa in Beringia. Danks *et al*. (1997) estimate that about 7% of Yukon insects are endemic to East Beringia, based on the subset of insect families discussed in Danks & Downes (1997). Because of the incomplete taxonomic coverage of this publication, this figure may be inaccurate. Despite this gap, it represents, for the most part, the current state of knowledge.

Not surprisingly, taxa that are not diverse in the North, tend to be low in endemics. Odonates, for instance, are not diverse at high latitudes. Only one species surveyed in the Yukon has a Palearctic-East Beringian distribution: *Somatochlora sahlbergi* (Corduliidae) (Cannings & Cannings, 1997). Similarly, there is only one endemic Orthoptera: *Brunneria yukonensis* (Acrididae). It has been recorded in dry grassy areas of the southern Yukon and is thought to be restricted to this habitat (Boucher, 1998)

Heteroptera also decline with increasing latitudes; they are relatively rare in arctic ecozones and only 5.6% (216 species) of the North American fauna can be found in the Yukon. From this suborder, only one of the species surveyed in the Yukon, *Labopidea bermani* (Miridae), has an East-West Beringian distribution. That species is known to associate with *Saussuria angustifolia* (Asteraceae), a plant with an amphiberingian distribution associated with dry tundra. Several other Heteroptera species are associated with Beringia, but there is no pattern in their habits; one is semi-aquatic and the other terrestrial heteropterans are each associated with different habitats, from ground-dwelling to conifer hosts. If Beringia was a habitat mosaic, it is likely that each of these species could have found suitable habitat but no generalizations can be made (Scudder, 1997b).

Cicadellidae (Hemiptera) is, to a certain extent, an exception to this general pattern. While members of this family tend to dwindle in diversity (but not abundance) in the North, they are surprisingly diverse in the Yukon. Their diversity is higher in the northwest than the northeast and this pattern is attributed to the role of Beringia as a refuge. About 10% (14 species) of the Yukon leaf-hopper fauna is endemic, although 21 species are known from Beringia. Approximately a third of the eastern Beringian fauna is found along the Yukon River and main tributary valleys, some of which now sustain relict xeric grasslands on south-facing slopes. One species, *Chlorita nearctica* is known exclusively from these slopes. The other endemic Beringian

fauna is divided between species found on dry, grassy tundra and open forest, as well as coastal species (Hamilton, 1997).

Given that beetles have historically received more taxonomic treatment than other insect groups (Anderson, 1997) and that they are abundant in Pleistocene fossil assemblages, Coleoptera features prominently in Beringia literature (Danks & Downes, 1997). Currently, 913 species in 57 families have been recorded in the Yukon (Anderson, 1997). The proportion of diversity represented by each family is similar to general patterns across Canada. The most diverse families in the Yukon are Carabidae, Staphylinidae, Dytiscidae, Curculionidae and Chrysomelidae. Although the order of greatest diversity varies, these are also the most diverse families in Canada (except for Dytiscidae which is only the seventh most diverse in Canada, but third in the Yukon). There are 79 species in the Yukon known to be restricted to Beringia. The endemic Coleoptera fauna is in eight families: the aforementioned diverse families, and Elateridae, Scarabaeidae and Anthicidae (the latter three are represented by one species each) (Anderson, 1997).

Most of the endemic beetle species are presumed to be recently derived Beringian species (Anderson, 1997). Many are in families and genera that are widespread and species-rich in the North (e.g. Dytiscidae: *Agabus*; Curculionidae: *Dorytomus, Ceutorhynchus*). There are a few outliers, however, such as the genus *Pterostichus* (Carabidae). Sixteen closely related species from this genus, particularly the subgenus *Cryobius*, are restricted to Beringia. They have likely undergone radiation in Beringia over a long period of time. Two other endemic weevils have also likely been in Beringia for a long time. They are morphologically distinct, with no known close relative, and present in the fossil record. *Connatichela artemisiae* (Curculionidae) is the only member of its genus. It has been collected on *Artemisia* and is currently found only in East Beringia in close association with xeric south-facing slopes. *Lepidophous thulius* (Curculionidae) is another species that appears to be extremely differentiated. It is a species associated with dry steppe and tundra (Anderson, 1997).

The beetle endemic fauna is dominated by predators, which is a pattern present in all Coleoptera in the North (Anderson, 1997). Only 13 endemic plant-associated beetles are known from the Yukon. The majority of the endemics are found in tundra or dry open ground such as fell-field and steppe (Anderson, 1997).

Although each insect order and family has its own idiosyncracies, a few generalizations can be made. For terrestrial insects, a significant proportion of the endemic fauna is associated with tundra. In addition to Coleoptera which has many species associated with tundra or open ground, there is likewise a higher degree of endemism in species from tundra (particularly dry tundra) in Noctuidae (Lepidoptera) and Anthomyiidae (Diptera) (Griffiths, 1997).

The other habitat of note is xeric grasslands which are currently mostly restricted to south-facing slopes in the Yukon. Although the proportion of endemics present in this habitat is not as high as dry tundra, species found there are often restricted to these pockets of vegetation, such as *C. artemisiae*. As well, for the suborder Heteroptera, even though no species appear to be endemic, it is the only habitat which has a distinct fauna in the Yukon (Scudder, 1997b). This habitat is also distinctive in that it supports species that are disjunct from more southern distributions.

Disjunct distributions

Disjunct distributions are biogeographically informative. They can arise from the imposition of a barrier into a previously continuous distribution. In the case of the last glacial, the ice sheets forced fauna and flora out of their former ranges. Disjunctions have been observed between the insects of the Yukon and two other regions: eastern North America, and southern or west-central North America (Danks *et al*., 1997).

Disjunctions with eastern North America appear to be linked to habitat preference in certain cases. For instance, there is a pattern of disjunction in Sphaeroceridae (Diptera) between species from wet tundra in the northwest and peatlands in eastern North America (Marshall, 1997). Trichoptera species that share this pattern are lotic species (Wiggins & Parker, 1997). Other taxa for which this pattern has been noticed are *Megadelis piceae* (Curculionidae: Coleoptera) (Anderson, 1997) and *Eupithecia sharronata* (Geometridae: Lepidoptera) (Lafontaine & Wood, 1997). Although some of these disjunctions could be a result of undersampling, many appear to be real (Danks *et al*., 1997) but few explanations have been explored in depth.

Patterns of disjunction between the Yukon, and southern and west central North America are almost uniformly related to habitat. These species in the Yukon are often restricted to steppe habitats (or at least dry habitats) and are often widespread in grasslands in southern regions. Taxa

in which this pattern occurs include many species of Noctuidae, one Hesperidae, two Arctiidae (Lepidoptera) (Lafontaine & Wood, 1997), *Tychius tectus* (Coleoptera: Curculionidae) (Anderson, 1997), many species of Aculeata (Hymenoptera) (Finnamore, 1997), and many Diptera (Boucher, 1998). Authors tend to attribute this pattern to either species surviving in two refugia (South and Beringia) or species migrating north from the south once ice sheets started melting. Some authors mention the Hypsithermal as a period during which the latter phenomenon may have happened (e.g. Finnamore, 1997), although others reject this notion (e.g. Scudder, 1997b).

Examining patterns of change through the fossil record

The intense climatic changes during the last glacial forced species to either become extirpated or adapt by retreating into refugia and newly created niches. Past assemblages can be examined through the fossil record, and since many Pleistocene insect taxa are still extant today, they can be compared to modern assemblages (Danks *et al*., 1997).

Insect fossils have greatly contributed to extending the knowledge of the climate and environment during the last glacial/interglacial cycle as many insect fossils have been found in different types of sediment from the Quaternary period. By far, in any fossil assemblage, most identifiable fragments belong to Coleoptera. Their robust exoskeleton contributes to their preservation and because they are well documented, they are among the easier insect orders to identify (Coope, 1970; Matthews & Telka, 1997).

Insect fossil evidence shows large scale distributional shifts during the last glaciation, with two major refugia: Beringia and south of the ice-sheets (from Washington, through Montana, the Dakotas, into southeastern Wisconsin, Illinois, northeastern Pennsylvania and New York). While no insect fossils dating from the Pleistocene have been found on the east coast of North America, vertebrate fossils have been found on the eastern continental shelf, suggesting possible cryptic refugia (Elias, 2010).

By and large, like floristic patterns, fossil insect patterns show that there were many different habitats in Beringia. However, insect assemblages are based on niches or ecological habitat requirements that are difficult to define and not always equivalent to plant associations. The difficulty in comparing insect fossil assemblages (e.g. due to taphonomic biases, taxonomic diversity, etc.) and linking them to habitat has led to classification systems to make sense of the

insect fossil associations. For instance, Matthews (1983) suggested a system for North American insect fossil assemblages that is a mixture of ecological and taxonomic groupings based on abundance and niches (e.g. a *Cryobius* group representing members of this subgenus that are associated with mesic sites, but also other beetles; or a miscellaneous and phytophagous group which is just a "catch all" category) .While there are issues with this classification, he suggested grouping taxa into current ecological requirements and comparing percentages in assemblages (Matthews, 1983), a method still used today to describe fossil insect findings. Kuzmina *et al*. (2008) provides a list of the different classifications of eastern and western Beringian insect fauna. Her classification is simple and based in ecology and as such is the one adapted in this brief synthesis (Table 1.1).

Assemblages found in the fossil record from the late Pleistocene are often composed of groupings that are not found together today. One very common fossil assemblage consists of a grouping dominated by *Morychus* sp. and *Lepidophorus lineaticollis.* It is often described as an indicator of dry local conditions (Matthews & Telka, 1997; Elias *et al*., 2000), particularly when in association with other taxa: *Amara alpina, Pterostichus sublaevius, Coniocleonus zherichini* and *Lepidophorus thulius* (Matthews, 1983; Elias *et al*., 2000). *Connatichela artemisiae* is also part of this assemblage and is the only true indicator of a steppe community, even today (Elias *et al*., 2000). This is the insect fossil assemblage that represents the Pleistocene steppe-tundra (but steppe, and dry steppe and tundra in current associations (Table 1.1)). When comparing contemporary relict steppes on south-facing slopes and fossil assemblages, Berman *et al*. (2011) noted that the main difference between both is the absence of tundra species today from the relict steppes. Many of these tundra species, like the carabid *Pterostichus sublaevius* are now found north of the boreal zone (Berman *et al*., 2011).

This insect fossil Pleistocene steppe-tundra assemblage is not present uniformly throughout the Beringian record and it reinforces the notion of a habitat mosaic (Elias *et al*., 2000).

Exploring the past through molecular patterns: phylogeography

Using species distributions and assemblages, whether based on the fossil record or contemporary ranges, can lead to biased reconstructions; current ranges are not always known, fossil records are often incomplete (Danks *et al*., 1997) and it is difficult to draw conclusions

from the distributions of widespread species (Ball & Currie, 1997). Molecular characters, however, are universal and historical events, such as bottlenecks, leave a molecular signal.

Phylogeography is an analytical approach that uses phylogenetic methods in a geographic context to infer past historical processes in an effort to understand the processes influencing the distribution of genetic variation within and among closely related species (Avise, 2000; Knowles, 2009). Phylogeography typically focuses on haplotypes and genetic lineages within species (Marske *et al*., 2013), and on time scales dating from the Quaternary period, particularly the Pleistocene epoch (Riddle & Hafner, 2007; Marske *et al*., 2013)

To date, plants and mammals are overwhelmingly represented in phylogeography studies. In a review of northwestern North American biota, only two of 126 studies dealt with insects (Shafer *et al*., 2010). In a review of eastern North America, only three of 396 studies looked at insects (Soltis *et al*., 2006).

Phylogeographic studies using North American insects to examine glacial refugia are few and often limited in scope. The study taxa generally have limited distributions (e.g. *Nigronia serricornis* (Megaloptera: Corydalidae) in eastern North America (Heilveil & Berlocher, 2006)) with a strong emphasis on alpine species in the west (e.g., Knowles & Carstens, 2007; Ahern *et al*., 2009; Knowles & Alvarado-Serrado, 2010; Schoville & Roderick, 2010). Maroja *et al*. (2007), however, examined the phylogeography of the spruce beetle, *Dendroctonus rufipennis* (Curculionidae: Coleoptera), a widespread species across North America, from Alaska to Newfoundland. The molecular population structure of this insect showed that they likely spread from three refugia after the last glacial: south of the glacial boundary in the eastern/central United States, south of the glacial boundary but from the Pacific Northwest and/or from farther South in the Rocky Mountains, and Beringia.

Combining different lines of evidence such as distribution and fossil information, in addition to molecular-based methods, can help answer complex questions. Many contemporary arctic-alpine beetles have disjunct distributions; they are found extensively in the arctic tundra, and in limited areas of the Rocky and Appalachian Mountains (Ashworth, 1996; Reiss *et al*., 1999). Yet glacial fossils of these insects have been found in the northwest (Beringia), and extensively along the southern edge of the ice sheets. These arctic beetles are assumed to have become extirpated in the south as a result of climate warming (in 14,500 BP), but survived in alpine areas at higher elevations in the Rockies and Appalachians where climate was suitable.

The model of recolonization hypothesized that after the glaciation, arctic insects dispersed east from their Beringian refugium, while their southern counterparts disappeared, except in alpine refugia (Ashworth, 1996; Reiss *et al*., 1999). Reiss *et al*. (1999) examined this postglacial recolonization model by looking at the molecular patterns of *Amara alpina* (Coleoptera: Carabidae), a beetle with the disjunct distribution shared by many arctic-alpine beetles described above. Their study supported the model of postglacial dispersal, partially. They found that genetic diversity was highest in Beringia and lowest in Hudson Bay. This was congruent with geological evidence that Beringia was unglaciated, whereas Hudson Bay only deglaciated around 6,000 years BP. Additionally, the only haplotype from Hudson Bay was shared with a population currently in the Rocky Mountains (northern British Columbia), likely originally from the southeastern part of Beringia, indicating recolonization from the west to east. However, populations from the Rocky and Appalachian Mountains appeared to be more closely related to the Beringian populations than to each other. Since it was hypothesized that they would be similar to each other if they had been part of a southern refugium, it suggested that during the last ice age southern populations were not continuous. The authors propose a barrier, such as the polar desert in the Great Plains, may have played a role. Nonetheless, this study lends credence to the hypothesis that arctic insects may have populated the North from Beringia, rather than from Beringia and the south (Reiss *et al*., 1999).

The prevalence of insects in the fossil record, their abundance in the arctic, their various distributions appear to make these organisms suitable models for testing hypotheses regarding post-Pleistocene dispersal.

The origin of xeric grassland communities in the Yukon

Xeric grasslands in the Yukon are rare and unique environments, characterised by prairie sage (*Artemesia frigida*), bunch-grasses and forbs. They are isolated communities, associated with arid well-exposed south-facing slopes and with a distinct insect fauna. Located mostly in the south of the Yukon Territory, there are a few outliers on very steep slopes in Old Crow and Firth River (Scudder, 1997a; Boucher & Wheeler, 2001).

It has been suggested that Yukon xeric grasslands are remnants of the late Pleistocene arctic steppe ecosystem (Scudder, 1997a). Regional aridity caused by climatic conditions during the last glaciation in Beringia allowed this steppe-like flora to exist and be widespread. Current

drainage and solar exposure on south-facing slopes has mimicked this aridity which allowed these communities to survive (Zazula *et al*., 2003).

Grasslands with similar plant and insect communities exist elsewhere, also disjunct from the south: the Peace River region (Alberta), interior valleys of British Columbia and northeastern Asia (Scudder, 1997a; Boucher & Wheeler, 2001).

The origin of the Peace River grasslands has been established through multiple lines of evidence: warm and dry conditions during the Hypsithermal caused the current vegetation distribution patterns to be displaced northward. Although this explanation supports disjunct northwestern grasslands, up to a latitude of 54^{o} N (Strong & Hills, 2003, 2005), it has not often been explored to explain the presence of disjunct grasslands in the southern Yukon even though some insect distributions support it.

The current insect evidence is not sufficient to discern between either hypotheses. Some insect distributions suggest a southern origin for the Yukon grasslands such as aculeate wasps (Finnamore, 1997), noctuid moths (Lafontaine & Woods, 1988), and ground beetles (Ball & Currie, 1997).

On the other hand, a faunistic study on the distribution of Diptera in these Yukon xeric grasslands found that the number of endemic flies provides strong evidence that these grasslands represented a refugium during the last ice age. Yet it also showed that there are many species with disjunct northern and southern distributions and concluded that these species could have survived glaciation in both in Beringia and the South or dispersed northward, postglacially. The study could not discount either hypotheses (Boucher, 1998).

A recent review of microlepidopterans in grasslands has suggested that the disjunct distribution of two species in both the Great Plains and Beringia could be explained by a link between the two approximately 11,000 years BP. However, they suggest that the disjunct distributions of some sister-taxa indicate a pre-Pleistocene separation (Schmidt *et al*., 2013).

Hence, while many hypotheses have been postulated as to the origins of South-facing slope grasslands, none have been tested adequately.

Diptera diversity

General patterns

With over 150,000 species worldwide, Diptera is part of the "big four" species-rich orders of insects, surpassed only by Coleoptera. Flies are likely the most ecologically diverse order of insects ranging from blood-feeders to saprophages, passing by leaf miners, predators and pollinators (Grimaldi & Engel, 2005). They are a ubiquitous group, although they are not always abundant in every habitat or region (Grimaldi & Engel, 2005; Pape, 2009).

The number of Diptera species is highest in the Palearctic, with about 45,000 species or 30% of the named world Diptera fauna. However this is thought to be a reflection of the fact that there is a longer taxonomic history in the Palearctic, along with more dipterists than elsewhere (Pape, 2009). In terms of named species, the Neotropical region is second with about 31,000 species, followed, in order, by the Oriental, Nearctic, Afrotropical and Australasian regions (Evenhuis *et al*., 2009).

There are about 21,500 named Diptera species in the Nearctic (Evenhuis *et al*., 2009). Although there have been attempts at estimating the number of still undescribed fauna in the Nearctic, curves displaying the number of species described over time do not show any levelling, making it difficult to speculate. There are relatively few endemic higher taxa in the Nearctic; the northern two-thirds of the fauna (from the arctic and boreal biomes) is mostly Holarctic, and the southern third is shared with the Neotropics (Thompson, 2009).

Canadian Diptera

Diptera is the most diverse and abundant order of insects in Canada, particularly the Arctic. As of 1978, there were approximately 7,000 species known from Canada in 101 families, the largest of which is Chironomidae with 2,000 species. However, Culicidae is considered to be the best known Canadian family (McAlpine *et al*., 1979).

Most Canadian taxa diminish as they go north; for instance, there are more fly species in the subartic than the Arctic. However, the relative abundance of Diptera increases in the Arctic. Danks (1981) recorded 721 species in 39 families from the North American Arctic, although these numbers are an underestimate (T.A. Wheeler, pers. comm.). Calyptrates are best represented in the low arctic, while Nematocera are particularly present in the high arctic.

Chironomidae, Muscidae, Anthomyiidae and Tipulidae are the dominant families present in the north (Danks, 1981).

One striking pattern in arctic Diptera is that there are more species in the west than east, with the fewest species east of Hudson Bay. Even in widely distributed families, this pattern is prevalent. This pattern is likely due to Hudson Bay having acted as a barrier to dispersal from the west during deglaciation (Danks, 1981).

There are severe gaps in our knowledge of insect distributions in Canada, even many years after the publication of Danks (1979), the most complete and still most current publication on Canadian insects. There is no comprehensive work available on the Diptera of the Canadian grasslands. Publications on pests and insects associated with crops are most prevalent (e.g. Gavloski *et al*., 2011; Soroka & Otani, 2011) and summary information can sometimes be found in publications with a specific focus (e.g. sandhill arthropods (Acorn, 2011), arthropods associated with livestock (Lysyk, 2011), insects of the Ojibway Prairie (Paiero *et al*., 2010)), but overall Diptera grassland assemblages remain unstudied. However, herbivory in temperate grasslands is dominated by insects, and Diptera (mainly Cecidomyiidae and Chloropidae) are one of the main groups of stem-boring herbivores in grasslands (Tscharntke & Greiler, 1995). Diptera also represent an important proportion of the soil dwelling-dwelling arthropods, fulfilling an important role in decomposition and distribution of organic matter, but little information on this topic exists (Kirkwood *et al*., 2000).

The Diptera community of the Yukon grasslands is known due to baseline faunistic work (Boucher, 1998). The two dominant families on these grasslands are Chamaemyiidae and Chloropidae, and the most species-rich families are Agromyzidae, Chloropidae and Tachinidae.

Among the Diptera fauna found on these south-facing slopes, two species were flagged as possible Beringian endemic species: *Lasiopogon canus* (Asilidae) and an undescribed species of *Olcella* (Chloropidae). *Lasiopogon canus* is not restricted to grasslands and can occur in many different habitats. *Olcella* sp. appears to be found exclusively on these slopes based on its absence from insect collections. Additionally, a large proportion of the Diptera fauna collected on these slopes (27%) has disjunct distributions with the southwestern United States to southwestern Canada (Boucher, 1998).

The most abundant fly on these grasslands was *Chamaemyia herbarum*, Robineau-Desvoidy (Chamaemyiidae), a species not usually abundant in grasslands. The guild composition

on these grasslands was also unusual: predaceous flies ranked first in terms of abundance, saprophages second and phytophages third. Saprophages usually rank first in most other habitats, though it was suspected that saprophagous flies were less abundant because of the lack of dead organic materials on these grasslands. South-facing slope grasslands are usually very dry and there seems to be little accumulation of organic material (Boucher, 1998).

Diptera phylogeography

The current interest in Diptera for phylogeographic studies lies mostly in understanding the patterns of disease vectors or economically important flies. A Web of Science search, using the key words: Diptera and phylogeograph*, revealed 80 publications on this topic. Although not exhaustive, this search provides a general baseline on the type of phylogeographic work performed with Diptera. Approximately half of the taxa studied were disease vectors (mostly of malaria, but also arboviruses, river blindness, trypanosomiasis and others). The second most common topic involved agricultural pests. Both types of studies mostly aimed at understanding the organism's population structure and genetic diversity, as well as discovering where lineages originated to inform management decisions. Other common Diptera studied can be categorized as parasites (mostly of mammals. including pests of livestock), biting flies, and well-studied laboratory organisms or systems (e.g. *Drosophila* spp.).

Overwhelmingly, a big proportion of the studies were on Culicidae (35%), a family containing many disease vectors. The second most common Diptera family was Tephritidae (16%), which contains many agricultural pests, followed by Drosophilidae (9%) and Simuliidae (7%). Other families on which there has been at least one phylogeographic study were Lauxaniidae, Agromyzidae, Cutebridae, Hippoboscidae, Chloropidae, Ceratopogonidae, Sarcophagidae, Chironomidae, Calliphoridae, Glossinidae, Cecidomyiidae and Neriidae. Most studies examined the patterns of a single species, which at times were several cryptic species. Only about 20% of studies examined more than one species, but about half of those examined only two.

There was no overall pattern regarding the scale of these studies: they ranged from regional to intercontinental and were performed on all continents, except Antarctica. Because of the ecology of disease vectors, many of the studies examined tropical regions. Eighteen studies examined organisms across at least two continents; about half of these examined species of

medical importance and the other half dealt with agricultural pests, models in evolutionary studies and invasive species.

Although North American locations were included in studies performed at intercontinental scales, only four focused specifically on North America. They were performed at a regional scale, rather than continental, two focusing on eastern North America (Brown *et al*., 1996; Noël *et al*., 2005) and the other two on southwestern deserts in the United States and Mexico, (Reed *et al*., 2007; Pfeiler *et al*., 2013).

To elucidate patterns, many different markers were used. As in phylogenetic studies (Gibson *et al*., 2010), there are no markers that are used universally in phylogeographic studies across Diptera. The use of mitochondrial genes was widespread, particularly the gene cytochrome *c* oxidase I (COI). It was used in 51 out of the 80 studies, and in approximately half of those, it was used on its own. The second most frequently used gene was cytochrome *c* oxidase II (COII). Approximately half of the studies used only one gene, and of those, only two examined a nuclear gene exclusively. The most widespread nuclear locus used was the ribosomal internal transcriber spacer 2 (ITS2), but never on its own. Other mitochondrial genes extracted in at least a few Diptera phylogeographic studies are: cytochrome *b* (CytB), 16 S rRNA, and NADH dehydrogenase subunits 1, 2, 4, 5 and 6 (ND1, ND2, ND4, ND5, ND6). Segments of the tRNA-Leu genes were used successfully, along with other gene segments, in only one study, to look at the phylogeny of the olive fly, *Bactrocera oleae* (Tephritidae), (van Asch, 2012) as was the mitochondrial control region (CR) for the phylogeography of the New World screwworm fly, *Cochliomyia hominivorax* (Calliphoridae) (Fresia *et al*., 2013).

Although the use of nuclear genes has become more widespread in phylogeography (Hickerson *et al.* 2010), they are still not widely used (e.g. most studies in Shafer *et al.* 2010 involve either mtDNA and cpDNA) and Diptera studies are no exception. Mitochondrial genes are often more variable and tend to be more appropriate for genus and species-level questions (Gibson *et al*., 2010). However, mitochondrial DNA is also affected by maternal inheritance, haplodiploidy and bias in base composition (Mirabello & Conn, 2008). As such, the use of nuclear genes is becoming more frequent. As mentioned above, the most commonly used nuclear gene in Diptera phylogeography is ITS2. Others represented in at least one Diptera study are: alcohol dehydrogenase (Adh), 28 S rRNA, white eye colour (*white*), white intron 1 (*wint1*),

ribosomal internal transcriber spacer 1 (ITS1), alpha amylase, zinc finger, ribosomal protein S9 (rpS9), elongation factor-1α (EF-1α).

Only two studies failed to recover phylogeographic patterns although this appeared to be due the fact that there was no population structure rather than the nature of the genes or organisms chosen. Both studies were on parasites. In one case, the authors attributed the lack of population structure to high contact between the hosts resulting in high parasite gene flow (Olival *et al*., 2013). In the other, despite two different lineages of host, the parasite did not exhibit population genetic structure (Hoskin & McCallum, 2007). Overall, phylogeographic studies have been effective at extracting patterns in Diptera.

Approach and objectives

Biodiversity patterns can be examined at multiple scales and levels: temporal scales can span millions of years or a few minutes; spatial scales may encompass continents or small patches; and taxonomic levels can incorporate intra-species variation or higher taxa, such as genera or family. Traditionally, different ecological fields have focused only on certain scales or levels. For example, population ecology studies are generally concerned with single species whose distribution is widespread. Community ecology, in contrast, often examines sets of species in specific areas, and can sometimes answer questions using a higher than species-level taxonomic resolution

Advances in molecular biology and ecology have created additional tools for the analysis of ecological patterns and have added a new scale at which biodiversity patterns can be examined - genetic differences (whether inter- or intraspecific). While certain disciplines have incorporated molecular methods in a widespread manner (e.g. systematics), the use of genetic techniques is still not fully widespread. At the other end of the spectrum, fields which have emerged from the advent of molecular techniques have moved away from ecology (Johnson *et al*., 2009; Marske *et al*., 2013).

However, merging different disciplines, particularly combining evolutionary and ecological patterns has allowed new perspectives and insights into old questions, such as the factors dictating community assembly or answering biogeographic questions (Marske *et al*., 2013).

Objectives and expected outcomes

The purpose of this thesis was to examine whether the Diptera on xeric south-facing slopes in the Yukon: (1) survived the last glacial period in Beringia; or (2) have recently expanded there from the south. This was addressed using two different methodological approaches and taxonomic levels:

1- examine the community structure of Chloropidae (Diptera) on xeric south facing slopes in the Yukon and compare it to communities in southern Prairie grasslands, as well as grasslands in the Peace River region (species-level);

2- examine the phylogeographic patterns of higher Diptera in xeric south facing slopes in the Yukon, southern grasslands in the Prairies, as well as disjunct grasslands in the Peace River region (population-level).

With regard to the first objective, if the Yukon grasslands originated during the climate warming which occurred during the Hypsithermal, we expected the chloropid community structure to be similar in all three geographic regions, except that species richness would decrease from the Prairies to the Yukon as species drop out of the assemblages with increasing latitude. If, on the other hand, these grasslands survived the last glaciation in Beringia, they are likely to be relicts of the widespread steppe present in the region during the last ice age. Because these Pleistocene relict grasslands would have been isolated for a long time, we would expect the Yukon Diptera communities to be distinct from southern ones in the Prairies and in the Peace River grasslands.

The second objective built on the first, but allowed further refinement in the analyses. Examining molecular diversity allowed us to look at intraspecific patterns. If the Yukon grasslands had similar origins to the Peace River grasslands (i.e. northward expansion during the Hypsithermal), we expected the nucleotide and haplotype diversity of our target Diptera species to decrease with increasing distance from the Prairies. We also expected shared haplotypes between all regions. On the other hand, if the Yukon grasslands had a Beringian origin, flies from the Yukon would have unique and diverse haplotypes, relative to the southern regions.

Within the context of the second objective, molecular data also permitted us to calculate the divergence time between different populations. We expected divergence time between the Yukon and the two southern sites to correspond either to the time frame for the Wisconsinan

glaciation or other glacial periods if the Yukon Diptera population survived in Beringia, or to the Hypsithermal if the Yukon populations are a relict of a recent southern expansion.

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Table

Table 1.1. Selected ecological categories and associated insects in the East Beringian fossil record (adapted from Kuzmina *et al*., 2008). Some species were reassigned to other categories based on information from Danks & Downes (1997) (see Notes). This list is based on modern habitat preferences, rather than fossil assemblages discussed in the text. Because of conflicting information, this should not be considered a definite list but rather a loose indication of current, simplified habitat associations.

Category	**Examples of associated insects**	**Notes**
Steppe	Coleoptera: Curculionidae -*Coniocleonus* -*Connatichela artemisiae* Hemiptera: Tingidae -*Derephysia foliaceae*	Referred to as steppe-tundra by Kuzmina *et al*. (2008) based on rare insects in modern tundra. Steppe-tundra in this paper is defined as the Pleistocene mammoth steppe. Steppe vegetation assemblages of xerophytes and mesoxerophytes (e.g. tufted grasses, sedges), particularly *Artemisia frigida* are currently found mostly on south-facing slopes in eastern Beringia (Zazula *et al*., 2006).
Dry steppe and tundra	Coleoptera: Curculionidae -*Lepidophorus lineaticollis* -*Lephidophorus thulius* -*Hypera seriata* Coleoptera: Byrrhidae -*Morychus* sp.	These species are now mostly associated with dry steppe and tundra, but some have wider habitat preferences (e.g., *L. lineaticollis* can also be found on river shorelines (Hamilton, 1997)).
Dry tundra	Coleoptera: Curculionidae -*Coniocleonus zherichini* Coleoptera: Carabidae -*Amara alpina* -*Stereocerus haemotopus*	*Sitonina aquilonius* is omitted because it is found in other habitats. *Hypera seriata* was moved to dry steppe and tundra (Scudder, 1997b).

Mesic to wet tundra	Coleoptera: Carabidae -*Pterostichus (Cryobius)* Coleoptera: Staphylinidae -*Tachinus brevipennis* -*Holeoboraphilus nordenskioeldi* Coleoptera: Chrysomelidae -*Chrysolina septentrionalis* -*Phaedon*

CONNECTING STATEMENT 1

There has been much speculation with regards to the origins of grasslands in the Yukon. As described in Chapter 1, paleoecological and ecological studies have postulated different hypotheses to explain these northern grasslands so far out of the range of widespread grasslands. Fossil data suggests that the xeric grasslands in the Yukon are relicts of the ancient mammoth steppe which were widespread grasslands during the last ice age that sustained the large megafauna which survived in Beringia. Disjunct distributions between the Yukon and the southern Prairies of numerous organisms, particularly plants and arthropods, imply a possible recent expansion of the southern Prairies during the Hypsithermal, the warmest period of this interglacial. These grasslands would have become disjunct once the climate cooled. In the following chapter, we examine the plausibility of these two hypotheses to account for the presence of xeric grasslands in a normally boreal zone. We examine the Diptera family Chloropidae, since they are dominant and abundant in grassy, open habitats and verify whether their distribution and community composition is concordant with either a recent southern expansion or survival in a Beringian refugium.

CHAPTER 2. DISTRIBUTION AND COMMUNITY STRUCTURE OF CHLOROPID FLIES (DIPTERA: CHLOROPIDAE) IN NEARCTIC GLACIAL AND POSTGLACIAL GRASSLANDS

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Abstract

1. Two hypotheses have been proposed to explain the existence of xeric grasslands in the Yukon Territory, Canada: (1) they are relicts of the Pleistocene steppe widespread in Beringia during the last ice age; (2) they are recent remnants left behind after early-Holocene Hypsithermal expansion of southern grasslands. To test these hypotheses, we examined Chloropidae (Diptera) assemblages in grasslands from three regions: the Canadian Prairies, the Peace River region of central Alberta, and the southern Yukon.
2. We predicted that if the Yukon grasslands originated during the Hypsithermal, like the Peace region grasslands, chloropid assemblages in all three regions would be similar, except that species richness would decline in the north along a latitudinal gradient. If they are relicts of the Pleistocene steppe, the Yukon chloropid assemblage would be distinct from southern assemblages.
3. Although there was a latitudinal gradient in species richness, Yukon assemblages were distinct from Prairie and Peace region assemblages, based on cluster analysis, non-metric multidimensional scaling and pairwise comparisons of Morisita similarity indices.
4. Community-level analyses suggest that Yukon assemblages have been separated from those in southern grasslands in the Prairies and Peace regions since before the Hypsithermal and are more likely relicts of Beringian insect communities inhabiting Pleistocene steppe than recent expansions from the southern Prairies during the Hypsithermal.

Keywords: Biogeography, Beringia, Pleistocene, Hypsithermal, insects

Introduction

The Yukon Territory represents the Canadian part of Beringia, an unglaciated area which extended through a land bridge from eastern Asia to the Yukon during the Pleistocene ice ages (Harington, 2005). Multiple studies have confirmed the role of Beringia as a refugium over the last ice age (DeChaine, 2008; Shafer *et al.*, 2010) and efforts have been made to understand and explore diversity in the Yukon (e.g. Danks & Downes, 1997; Boucher & Wheeler, 2001; Danby, 2003; Slough & Mennel, 2006; Slough & Jung, 2007; Bowden & Buddle, 2010; Harris & Taylor, 2010; Danby *et al.*, 2011; Klimaszewski *et al.*, 2012).

Located mostly on south-facing slopes, Yukon xeric grasslands are a distinctive ecoregion. These localized, dry grasslands are at the northernmost limit of prairie-type grasslands in North America, and the insect and plant assemblages on these slopes are distinct relative to the surrounding region (Scudder, 1997a; Vetter, 2000).

There are multiple hypotheses about the origins of these isolated grasslands. Paleoecologists tend to note the similarity of the contemporary floral and faunal assemblages on these grasslands to fossil assemblages found throughout Beringia during the last ice age, which is thought to have been indicative of steppe. Among macrofossils identified from the Pleistocene steppe are sage (*Artemisia frigida* Willd. and *Artemisia* sp., Asteraceae), bunch-grasses and other grass-like plants. While the total assemblage of the Pleistocene steppe has no modern analogue, there are similarities between them, such as the presence of *A. frigida*, which suggests that they might be relicts of that steppe (Vetter, 2000; Zazula *et al*., 2003, 2006). In the insects, the Heteroptera community of xeric grasslands is distinct (Scudder, 1997b) and there are endemic species of other insect orders restricted to these slopes, such as *Connatichela artemisiae* Anderson (Curculionidae) (Anderson, 1997) which suggests these grasslands are ancient.

In contrast, some entomologists and botanists have noted the similarity of northern grassland insect and plant communities to those further south (Scudder, 1997a; Vetter, 2000; Strong & Hills, 2003). Finnamore (1997) noted that as many as 32% of aculeate wasp species on these slopes have disjunct distributions between the Prairies and the Yukon, an indication that these insects migrated north recently, likely during the Hypsithermal approximately 9,000 BP, the warmest period of this interglacial. Lausi & Nimis (1991) also suggested recent origins of some of the vegetation in Yukon grasslands, although they suggested the grasslands themselves were ancient.

Some of the species present in the Yukon grasslands are also present in the Peace region of central Alberta, another grassland area disjunct from the southern grasslands. Warm Hypsithermal conditions allowed the southern Prairies to expand northward. Once the climate cooled and boreal forest returned, pockets of grassland vegetation were left behind, mainly in the Peace River valley (Strong & Hills, 2003, 2005; Bromilow & Sperling, 2011). Because grasslands extended at least as far north as 56° N during the Hypsithermal, some have speculated that they could have extended further.

Comparisons of arthropod assemblages in the Yukon and southern grasslands are hampered by a lack of baseline data. Based on a faunistic study of the Diptera from Yukon grasslands (Boucher 1998), we selected the family Chloropidae as a model taxon. Most ecological studies using Chloropidae focus on agricultural pest species (e.g. Nielsen, 1985; Reader & Duce, 2009; Springer & Arnold, 2008; Knodel *et al.*, 2009; El-Wakeil & Volkmar, 2011) or medically important species (e.g. Jiang & Mulla, 2006; Mulla & Chansang, 2007; Chansang *et al*., 2011). Yet in addition to being agriculturally and medically important, Chloropidae are ubiquitous in most habitats and are ecologically diverse, occupying a wide range of trophic roles (Ferrar, 1987; Wheeler, 2010; Nartshuk, 2014). They are also abundant and species rich in various landscapes (Dąbrowska-Prot & Wasiłowska, 2010; Wheeler, 2010), particularly open habitats such as meadows or grasslands (Kozlov & Zvereva, 1997; Beaulieu & Wheeler, 2005). Chloropidae have been used as biodindicators of industrialization, urbanization, agricultural and anthropogenic pressures, and wetland restoration (Dąbrowska-Prot, 1984, 1987; Kozlov & Zvereva, 1997; Keiper *et al.*, 2003; Kubík, 2006; Dąbrowska-Prot & Wasiłowska, 2010, 2012; Krupauerová, *et al.*, 2012; Barrie, 2013). This habitat specificity and dominance in open habitats makes them well-suited for a study of the origin of grassland communities. Although rare chloropid species differ in comparable habitats, many of the dominant species would be expected to be present in all sites within a region (Beaulieu & Wheeler, 2001, 2005), unless other factors, such as history, intervene.

We used chloropid assemblages based on field collections and museum specimens to test two hypotheses on the origins of Yukon xeric grasslands as either relicts of the Pleistocene steppe, or recent remnants left behind following Hypsithermal expansion. To do this, we treated the Peace River grasslands as a reference point on how an assemblage is affected by a recent northward expansion. We hypothesized that if the Yukon grasslands originated recently (i.e.

during the Holocene), the assemblages from the Yukon would be similar to those of Peace River, but with fewer species because of the latitudinal diversity gradient. If the Yukon grasslands originated during the Pleistocene, the Yukon community should be relatively distinct from the Prairie and Peace region assemblages.

Materials and methods

Field collection

We collected Chloropidae in the three focal regions: Prairies, Peace region and southern Yukon (Fig 2.1). Sites were selected based on occurrence of native grassland vegetation and minimal anthropogenic alteration (Appendix 2.1). Prairie collection sites were: Onefour Heritage Land, AB; Cypress Hills Interprovincial Park, SK; and Criddle/Vane Homestead Provincial Park, MB. Peace region collections were made at Dunvegan, AB. Yukon collection sites were: Alaska Highway, 13.1 km west of Takhini River, YT; Klondike Highway, 26.3 km S of Carmacks, YT; Klondike Highway, 8.8 km South of Twin Lakes, Conglomerate Mountain, YT.

Prairies and Yukon samples were collected in July 2012 and Peace region samples in late June and early July 2012. Sampling was done in June and July to capture part of the peak in activity of chloropid flies in temperate (Beaulieu & Wheeler, 2005; Lévesque-Beaudin & Wheeler, 2011; Grégoire Taillefer & Wheeler, 2012) and arctic regions (T.A. Wheeler *et al.*, unpublished data).

Flies were collected by sweeping, which has been used effectively as a sampling technique in many studies involving Chloropidae (e.g. Kozlov & Zvereva, 1997; Dąbrowska-Prot & Wasiłowska, 2010, 2012). Sweep net samples were collected in good weather (i.e. sunny or partly cloudy), at mid-day, for approximately two hours per sampling event. Patches of vegetation were swept randomly, with care not to resample patches. Each site was swept by 2–3 individuals at a time (except Aweme) for 1–3 collection events. Chloropidae were collected into 95% ethanol and subsequently dried and mounted. Flies were identified to species (or morphospecies in the case of undescribed species) and have been deposited in the Lyman Entomological Museum (LEM), McGill University, Ste-Anne-de-Bellevue, Quebec.

Because our research question spans millennial time scales, our goal was to obtain an approximate picture of the species diversity in these grasslands, instead of a time-specific dataset. This allowed us to expand our dataset to include museum specimen data.

Museum data

The LEM Diptera have been databased at the specimen level (http://data.canadensys.net/ipt/resource.do?r=lemq-specimens), facilitating extraction of occurrence records. Identifications of databased Chloropidae were verified and specimens identified only to genus were further identified to species or morphospecies. We extracted data about all LEM Chloropidae collected in previous years from our 2012 collection sites (Aweme, Cypress, Onefour, Dunvegan, Takhini, Conglomerate, Carmacks). We incorporated data from one additional site in the Peace region: Peace River, AB (Peace), to augment our collections from Dunvegan. Three additional Yukon sites were also incorporated, based on sampling in June–July 1997 and 1998: 5 km south of Jakes Corner, Mount White above Little Atlin Lake, YT; 4 km east of Carcross, slope above Nares Lakes, YT; and Klondike Highway, 15 km South of Carmacks, Bushy Mountain, YT (Boucher, 1998) (Appendix 2.1).

Including museum data posed a challenge in that most analytical methods which incorporate abundance require data to be standardized. Consequently, to incorporate abundance data, we used only specimens collected by sweeping during June and July, by T.A. Wheeler and/or his students (to standardize collecting techniques as much as possible). As for 2012 field sampling, the older samples were collected for approximately two hours, mid-day, in good weather and all Chloropidae from those events were processed and pinned. In addition, for abundance-based analyses, we only included data for which there was both older museum data and 2012 field data (the exception was the Peace River site—although we did not collect there in 2012, omitting this site would have left only one site from the Peace region).

Compared to the long-term historical events which affect the composition of modern assemblages, a difference of 10–15 years between collection events was deemed acceptable in building a picture of current community structure. Combining older data with recent data within sites minimized discrepancies based on change that may have occurred in each site due to factors other than glacial and postglacial history.

To include species information from sites for which methods other than sweeping were used and/or details about the collection events were unknown, presence-absence data was used to reduce the impact of non-standardized data collection.

Analyses

Presence-absence data

Non-metric multidimensional scaling (NMDS) ordination and multi-response permutation procedures (MRPP) were performed in PC-ORD v. 5.31 (McCune & Mefford, 2006) using the full data set incorporating all sampling methods. Data were divided by year to establish whether including samples from multiple time points had an impact on overall patterns. Prior to analysis, presence-absence data were transformed through Beals smoothing to reduce noise and even out the pattern of joint occurrences inherent in presence-absence data matrices because of the large number of zeros (McCune & Grace, 2002). Jaccard distance was used for both analyses.

The NMDS ordination was run initially with 6 axes, stepping down in dimensionality until a low stress value was achieved. The test was then rerun with the recommended solution of 2 axes, using the final configuration from the previous run as the starting configuration. Both ordinations were based on 100 runs with real data and 1000 Monte Carlo simulations to determine the validity of the final configuration.

The MRPP for significant difference between *a priori* defined groups tested three configurations (Table 2.1). Two were defined by our original hypothesis and one resulted from the NMDS ordination. The first configuration grouped the Prairies and Peace region sites together, with the Yukon sites as a separate group. The second grouped the Peace region with the Yukon, with the Prairie sites as separate. The final configuration based on the ordination divided the sites into three groups: (1) Aweme and Onefour, (2) Cypress, Dunvegan and Peace, and (3) Yukon sites.

Abundance data

Because sampling effort between sites was uneven, we used rarefaction analysis to analyze abundance data. We used iNEXT (Hsieh *et al.*, 2013) to obtain rarefaction and extrapolated curves using the nonparametric method described in Colwell *et al.* (2012). This program uses unconditional variance estimators which do not converge to zero and as such assumes the data are part of a larger assemblage. This method also allows the species richness curve to be extrapolated and therefore allows comparison of samples beyond the smallest sample collected (Colwell *et al.*, 2012; Gotelli & Chao, 2013).

We used EstimateS v. 9 (Colwell, 2013) to calculate the abundance based coverage estimator (ACE), which calculates total species richness by enlarging the observed number of species based on a correction term, which is dependent on the abundance of the rarest species. We set the upper abundance limit for rare species at 10, as suggested by Colwell (2013). EstimateS was used to calculate the Simpson reciprocal diversity index, a diversity measure weighed toward the most abundant species (Magurran, 2004; Colwell, 2013; Gotelli & Chao, 2013). To calculate these measures and their standard deviations, 100 randomizations and 500 bootstraps were carried out.

The program SPADE (Chao & Shen, 2010) was used to calculate the Morisita similarity indices between communities at all sites. It uses the original Morisita index which is nearly unbiased and influenced mostly by dominant species, which makes it resistant to undersampling (Chao *et al*., 2008). To calculate standard error for each value, the program performs 200 bootstrap replications (Chao & Shen, 2012).

We used cluster analysis, performed in PC-ORD, to group sites based on abundance data. The distance measure used was Sørensen and sites were linked by group average. We also used the same program to test two configurations by MRPP (Table 2.2), using the same distance metric.

Results

Presence-absence data

A total of 11197 individuals, representing 96 species (Appendix 2.2), were used in analyses based on presence-absence data. Species richness was highest in the Prairies, followed by the Peace region and the Yukon (Fig. 2.2). The Prairies also had the highest number of species not found in the two other regions (29), whereas the Peace region and the Yukon both had the same number of unique species (11). More species were shared between the Prairies and the Peace River region than between the Yukon and the two southern regions. Fifteen species were shared between all sites.

The Yukon assemblages clustered closely together in the NMDS ordination of assemblages divided by sites and collection year (Fig. 2.3). Although the Prairie and Peace region sites were more variable, the Peace region sites did not cluster separately from the Prairie sites. Inclusion of the collection year into the analysis did not appear to affect patterns, although

Aweme did have two outlying data points in 1999 and 2000. Although they were not part of the main Prairies-Peace region cluster, these two data points appeared to be more related to the Prairie and Peace region cluster.

The MRPP was congruent with the NMDS analysis (Table 2.1). Although all three tested configurations were significant, grouping the Peace region sites with the Yukon sites had the lowest chance-corrected within-group agreement (A) of all the configurations (A=0.17) making it the least optimal solution. The third configuration based on the NMDS results which divided the sites into three groups and placed the Peace region sites with Cypress while leaving the other regions in separate groups was the best configuration.

Abundance data

A total of 8434 Chloropidae, representing 91 species (Appendix 2.3) was used for abundance-based analyses. We obtained the highest number of individuals at Dunvegan (N=3536). In comparison, the second ranked site, Aweme, contained less than half as many specimens (N=1329). The Yukon sites had the lowest overall number of individuals (Table 2.3).

The contribution of museum and field data used for the analysis differed between regions (Table 2.4). The Yukon data set was based mostly on museum data, except the Carmacks site, in which about a third of the specimens used for the analysis were collected in 2012. The data for the Prairie sites were strongly based on 2012 specimens, except for Onefour where more than half of the specimens were collected in 2000. For the Peace region, Peace River data was solely based on data from 1997. The Dunvegan data set was based evenly on museum and 2012 collection data.

Rarefied ACE measures suggested we recovered on average about 80% of the diversity at each site (Table 2.3). Sampling was most complete at Conglomerate and Carmacks (95% and 88% of species, respectively), although it is likely that for Conglomerate this completeness is an artefact of low sample size. Takhini and Dunvegan had the lowest estimates of completeness with 71% and 73%, respectively.

Rarefaction and extrapolation curves supported ACE results (Fig. 2.4). Curves for Conglomerate and Carmacks levelled off, whereas Takhini and Dunvegan rarefaction curves had the steepest slopes. The two aforementioned Yukon sites also had the lowest species richness, whereas Cypress, a Prairie site, had the highest. The confidence intervals of the species richness

of the other sites overlapped. Although Takhini had the third lowest species richness, its confidence interval overlaps with those of Aweme, Onefour and Dunvegan.

Onefour had the highest Simpson reciprocal diversity index value (6.67), while Peace and Cypress both had values above 5.5. Dunvegan's Simpson index was lower (4.31), but still higher than the Yukon sites. All Yukon sites had a Simpson diversity index below 3.5, with Conglomerate the lowest (1.61). Aweme was similar to the other Yukon sites (Table 2.3).

Pairwise comparison of the original Morisita index showed that the Yukon sites were most similar to one another, and least similar to the Prairie and Peace region sites (Table 2.5). Although the southern sites (Prairie and Peace region) were more similar to one another than to the Yukon, there was much more variation in index values. Aweme was the least similar to the other Prairie and Peace region sites (0.04–0.24). Although these values were low, it was still more similar to those than to the Yukon (~0). The other outlier was Peace, whose Morisita similarity values with other Prairie and Peace sites were lower (~0.5) than pairwise values between Cypress, Onefour and Dunvegan (>0.8). Peace had a relatively high Morisita index when compared with Takhini (~0.4).

Cluster analysis revealed two distinct groups: one containing the Yukon sites and the other the Prairie and Peace region sites (Fig. 2.5). In the Prairie-Peace cluster, Aweme was at the base of the group. The MRPP concurred with these results. Of the configurations tested, only the one that grouped the Peace region sites with the Prairies, and left the Yukon sites separate was significant (Table 2.2).

Discussion

Overall patterns

A decrease in species richness from the tropics to the poles is one of the oldest and best known patterns in ecology (Willig e*t al*., 2003), and our prediction that Chloropidae species richness would decrease with increasing latitude fit this pattern. Although some Diptera families are particularly species rich in the North (e.g. Scathophagidae, Piophilidae) this is not the case for Chloropidae and many other acalyptrate families (McAlpine *et al.,* 1979).

In addition to latitudinal differences, the grassland regions in this study are also separated by different distances and ecozones: the Peace River grasslands are isolated from the Prairies by approximately 300–400 km of aspen parkland and boreal forest (Bromilow & Sperling, 2011),

and the Yukon grasslands are separated from the Peace River grasslands by at least 800 km of boreal forest and mountains (Strong & Hills, 2005; Shorthouse, 2010). Because sites that are closer together tend to be more similar (Legendre, 1993) it is not surprising that the Peace and Prairie region sites were more similar to one another than to the Yukon. But do latitudinal and landscape differences alone account for the patterns seen?

While increasing latitude likely plays a role in decreasing species richness, the role of the landscape separating regions in defining assemblages is less certain. Because Chloropidae are small, with low dispersal ability (T.A. Wheeler, personal observation), it is doubtful that migration between regions is a defining factor in the formation of assemblages. In addition, the Yukon assemblages were distinct from the Prairie and Peace region sites in all analyses, while the latter consistently grouped together. This pattern does not seem strictly consistent with isolation by distance or latitudinal differences. It is thus likely that history has played a large role in forming these communities.

The origins of the Yukon grasslands

The level of similarity in the Chloropidae of the Peace region and Prairie assemblages is most consistent with an expansion of the Prairie grasslands in the Holocene. The two regions would have become linked during the Hypsithermal when prairie vegetation expanded northward. Pockets of prairie vegetation were left behind in areas where the microclimate prevented the establishment of boreal forest and permitted the grasslands to persist. This hypothesis has been supported by modern distributions, pollen stratigraphy, paleoecological evidence and mapping the potential distribution of Hypsithermal vegetation (Strong & Hills, 2003, 2005). This hypothesis was also supported by the phylogeography of grassland butterflies with disjunct and continuous distributions (Bromilow & Sperling, 2011).

The similarities between the Peace River grasslands and the Yukon xeric grassland assemblages have led some to hypothesize that the Yukon grasslands originated in the same way as the Peace River grasslands. Both are present where microclimatic conditions allow them to persist in an otherwise boreal forest landscape. The Peace region grasslands are mainly located in the Peace River valley, on steep south-facing slopes where the microclimate is hot and dry (Moss, 1952; Wilkinson & Johnson, 1983) and the Yukon xeric grasslands are present mainly on south-facing slopes where the soil is well-drained and net radiation is high (Vetter, 2000;

Guthrie, 2001). Both also contain many floral elements that are otherwise present only in the southern Prairies (Vetter, 2000; Strong & Hills, 2003). In our assemblages, for instance, our Peace site shared a relatively high Morisita similarity index (0.4) with Takhini which suggests that both share some dominant species. However, when assemblages present on these slopes are analysed in more depth, it appears that despite similarities, their origins are different.

In a preliminary study, Strong & Hills (2003) suggested that northern grasslands could be the result of the Hypsithermal grassland expansion. However, they later found that southern grasslands likely only extended to 54° N (Strong & Hills, 2005). Using quantitative phytogeographic methods, Lausi & Nimis (1991) found that grasslands in the Yukon were ancient, although some plants likely migrated through the ice free Cordilleran corridor formed during glacial retreat. However their methods subdivided entire grassland communities into types based on their ecology and distribution, rather than examining communities as a whole. This approach downplays the individualistic nature of species niches (Williams & Jackson, 2007) by examining vegetation types as units. Nonetheless, their conclusion regarding the ancient nature of grasslands is supported by the fact that there is a large number of endemic species in these grasslands (Lausi & Nimis, 1991) and areas of high endemism have been linked to refugia (Tribsch & Schönswetter, 2003)

Our results likewise do not support the conclusion that the Yukon grasslands were formed as an extension of the southern grasslands during the Holocene. While some species may have migrated north during the Holocene, it is unlikely that they were grassland specialists. Had the differences between regions been due only to a latitudinal gradient, we likely would have seen species dropping out with latitude, and the Yukon grasslands would be similar to the composition of the Peace River grasslands with lower diversity. Instead, while there is a gradient of decreasing species diversity with increasing latitude, there are distinct differences between southern communities and the Yukon sites. The Yukon sites are more similar to one another than to any of the more southern sites.

Specific patterns

Although analyses revealed two distinct groups: a southern assemblage in the Prairies and Peace region, and a Yukon assemblage, there were still differences between sites. In the southern group, Aweme was an outlier in the NMDS analysis and its Morisita similarity index was low

compared to the other sites. This index is influenced by dominant species and *Apallates coxendix* (Fitch) dominates at Aweme whereas *Incertella incerta* (Becker) dominates in the other Prairie and Peace region sites.

Distance may play a role in explaining these differences; Aweme is almost 800 km east of the cluster of Prairies sites. However, because the more distant Peace region sites do not show this trend, distance is likely not the only cause for this pattern. Another possibility is that the insect community in Aweme has been strongly affected by introduced species. The Aweme sampling sites have been heavily colonized by European Brome grass (*Bromis inermis* Leyss) which is not considered a good habitat for insects (Roughley, 2000). This may explain the lower chloropid species richness at Aweme (Fig. 2.4).

Although they are different species, the dominant flies at Aweme and the other southern sites occupy the same trophic roles. *Apallates coxendix* and *I. incerta* are both generalist feeders on decaying plant matter. The dominant trophic groups in the Yukon are different. One of the dominant species, the undescribed *Olcella* sp. 2, is likely saprophagous on dead and dying insects, based on known habits of other *Olcella* species (T.A. Wheeler, unpublished data). The other abundant Yukon species, *Meromyza pratorum* Meigen, is a stem borer in grasses (Ferrar, 1987).

These differences in the trophic guilds of the dominant flies between the southern Chloropidae and the Yukon ones extend to other Diptera as well. Saprophagous flies which dominate in most habitats (Blades & Marshall, 1994; Beaulieu & Wheeler, 2005; Grégoire Taillefer & Wheeler, 2012) represent a small portion of the total abundance of Yukon grasslands (Boucher, 1998). This may due to the dry, windy nature of the habitat which prevents decaying vegetation from accumulating (Boucher, 1998). Hence, these grasslands appear to be ecologically different from southern ones, reinforcing the notion that they are not recent extensions of the Prairies.

Based on the NMDS of the presence-absence data, Cypress was most closely related to the Peace region sites. Although this pattern was not repeated in the cluster analysis of the abundance data, the high Morisita similarity index between Cypress and Dunvegan supported their similarity. Both sites were the least disturbed by anthropogenic activities out of the southern group. Hence, the Cypress-Dunvegan closeness suggests that in the absence of anthropogenic factors, the Prairie and Peace region would be perhaps even more similar than our patterns show.

The Yukon sites were also not uniform; the Simpson inverse diversity index at Conglomerate was approximately half that of the two other sites and species richness in Takhini was more than double that of the other sites. Our results are similar to those found by Boucher & Wheeler (2001) in a study of Agromyzidae (Diptera) in the same sites. Higher Agromyzidae species richness and abundance was found at Takhini and Carmacks. Chloropid species richness was not as high at Carmacks as at Takhini. Boucher & Wheeler (2001) associated the high diversity of Agromyzidae with higher vegetation diversity at Takhini and Carmacks. It is likely that vegetation diversity is also a factor, either directly or indirectly, in the diversity of Chloropidae.

Conclusion

Our results do not support the hypothesis that the Yukon xeric grasslands are remnants of a Hypsithermal expansion of the southern Prairies. The analysis of Chloropidae assemblages suggests that these grasslands have evolved separately from the south for quite some time, since the assemblages are distinct based on community-level analyses. This makes it more likely that these grasslands are relicts of the Pleistocene steppe. A phylogeographic study using populations of species on these Yukon grasslands would confirm this hypothesis by showing when organisms present on these slopes might have diverged.

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Tables

Table 2.1. MRPP results for presence-absence data divided per site and per year testing three different configurations (groupings). Site codes as in Fig. 2.1.

Groupings	**Avg distance within group**	**Chance-corrected within-group agreement (A)**	**p**
1: AWE, CYP, ONE, DUN, PEA **2:** TAK, ATL, NAR, CON, CAR, BUS	**1:** 0.35 **2:** 0.21	A = 0.31	0.00000014
1: AWE, CYP, ONE **2:** DUN, PEA, TAK, ATL, NAR, CON, CAR, BUS	**1:** 0.36 **2:** 0.32	A = 0.17	0.000089
1: AWE, ONE **2:** CYP, DUN, PEA **3:** TAK, ATL, NAR, CON, CAR, BUS	**1:** 0.34 **2:** 0.29 **3:** 0.21	A = 0.35 **1 vs 2:** 0.10 **1 vs 3:** 0.31 **2 vs 3:** 0.34	0.00000006 **1 vs 2:** 0.00062 **1 vs 3:** 0.0000026 **2 vs 3:** 0.0000022

Table 2.2. MRPP results based on abundance data testing two different configurations (groupings). Site codes as in Fig. 2.1.

Groupings	**Avg distance within group**	**Chance-corrected within-group agreement**	**p**
1: AWE, CYP, ONE **2:** DUN, PEA, TAK,CON, CAR	**1:** 0.79 **2:** 0.74	A = 0.07	0.076
1: AWE, CYP, ONE, DUN, PEA **2:** TAK, CON, CAR	**1:** 0.78 **2:** 0.46	A = 0.19	0.0036

Table 2.3. Number of specimens (N), raw species richness (S_{obs}), rarefaction estimated species richness (S_{est} ± SD) standardized at 348, abundance based coverage based estimator (ACE ± SD), and the Simpson reciprocal diversity index (Simpson).

Site	**N**	**S_{obs}**	**S_{est}**	**ACE**	**Simpson**
Aweme	1329	32	20.96 ± 1.43	25.83 ± 4.73	3.07 ± 0.08
Cypress	777	45	35.99 ± 2.74	47.2 ± 9.01	5.59 ± 0.41
Onefour	432	32	25.36 ± 3.01	34.52 ± 7.47	6.67 ± 0.27
Dunvegan	3536	50	24.14 ± 3.01	30.65 ± 8.7	4.31 ± 0.16
Peace	685	20	16.38 ± 2.26	21.06 ± 6.62	5.62 ± 0.27
Takhini	896	27	17.32 ± 2.41	24.4 ± 5.52	3.36 ± 0.12
Conglomerate	348	14	13 ± 1.02	13.63 ± 0.15	1.61 ± 0
Carmacks	431	17	13.34 ± 1.36	15.11 ± 2.17	3.12 ± 0.1

Table 2.4. Total number of specimens and species per site, per year, per region.

Region	Site	Museum	Field	Total	Species
Prairies	Aweme	437 (1999) 63 (2000)	829 (2012)	1329	28
	Cypress	83 (2000) 71 (2005)	623 (2012)	777	45
	Onefour	247 (2000)	185 (2012)	432	27
	Prairie total			**2538**	**68**
Peace region	Dunvegan	1101 (1997) 639 (2003)	1796 (2012)	3536	49
	Peace River	685 (1997)		685	20
	Peace total			**4221**	**51**
Yukon	Takhini	388 (1997) 472 (1998)	36 (2012)	896	25
	Conglomerate	177 (1997) 138 (1998)	33 (2012)	348	13
	Carmacks	143 (1997) 155 (1998)	133 (2012)	431	14
	Yukon total			**1675**	**29**
All sites				**8434**	**92**

Table 2.5. Pairwise Morisita similarity indices calculated between all sites for abundance data. Standard error was calculated based on 200 bootstrap replications. Site codes as in Fig. 2.1.

Site	CAR	CON	TAK	PEA	DUN	ONE	CYP
AWE	0.005 ± 0.001	0.000 ± 0.000	0.001 ± 0.000	0.038 ± 0.006	0.106 ± 0.010	0.237 ± 0.033	0.096 ± 0.012
CYP	0.109 ± 0.019	0.042 ± 0.011	0.057 ± 0.010	0.471 ± 0.031	0.880 ± 0.013	0.822 ± 0.027	
ONE	0.149 ± 0.022	0.053 ± 0.015	0.152 ± 0.022	0.504 ± 0.034	0.839 ± 0.028		
DUN	0.140 ± 0.020	0.020 ± 0.005	0.204 ± 0.012	0.541 ± 0.028			
PEA	0.203 ± 0.026	0.007 ± 0.004	0.397 ± 0.027				
TAK	0.835 ± 0.022	0.712 ± 0.025					
CON	0.864 ± 0.025						

Figures

Figure 2.1. Map of sampling sites.Site codes: ATL – Little Atlin; AWE – Aweme; BUS – Bushy Mountain; CAR – Carmacks; CON – Conglomerate; CYP – Cypress Hills; DUN – Dunvegan; NAR – Nares Lake; ONE – Onefour; PEA – Peace River; TAK – Takhini. Site descriptions in Appendix 2.1.

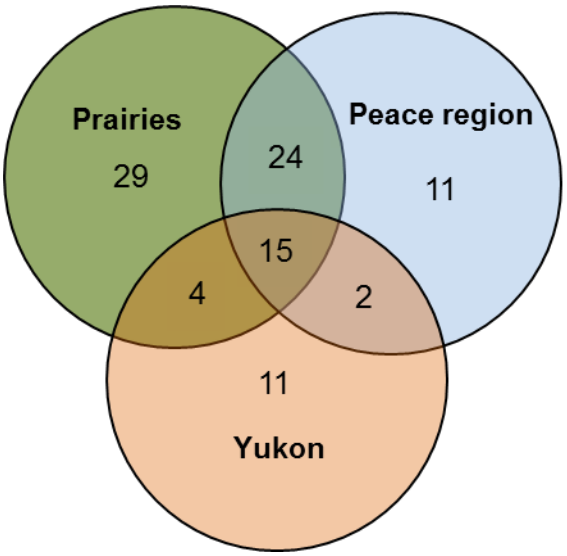

Figure 2.2. Venn diagram showing number of species within and between regions using presence-absence data set.

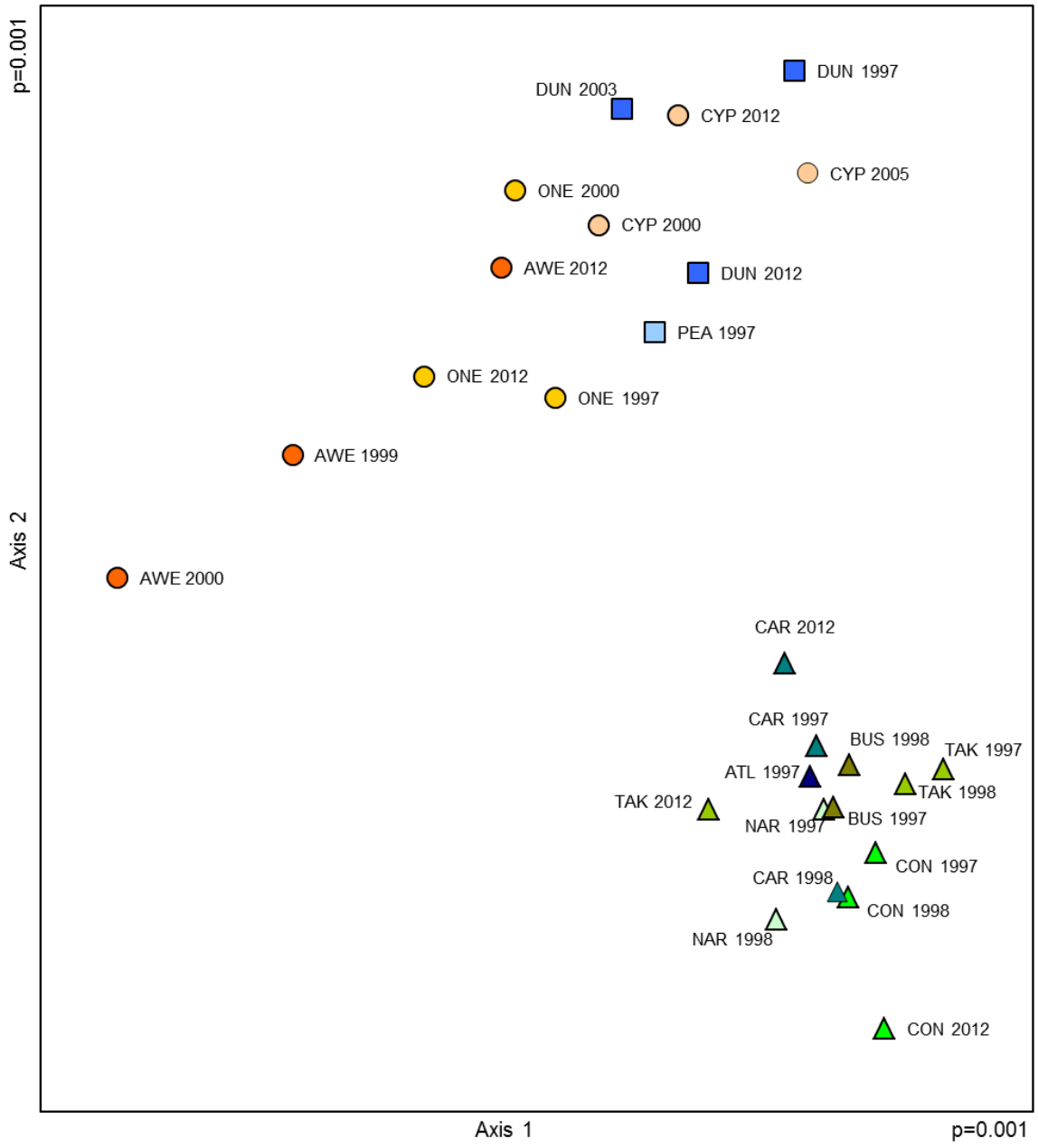

Figure 2.3. Non-metric multidimensional scaling ordination based on presence-absence of species by sites and by year. Ordination differs from randomly derived matrices at $p<0.001$ (Monte-Carlo test, 1000 permutations). Final stress=6.437. Site codes and shapes as in Fig. 2.1.

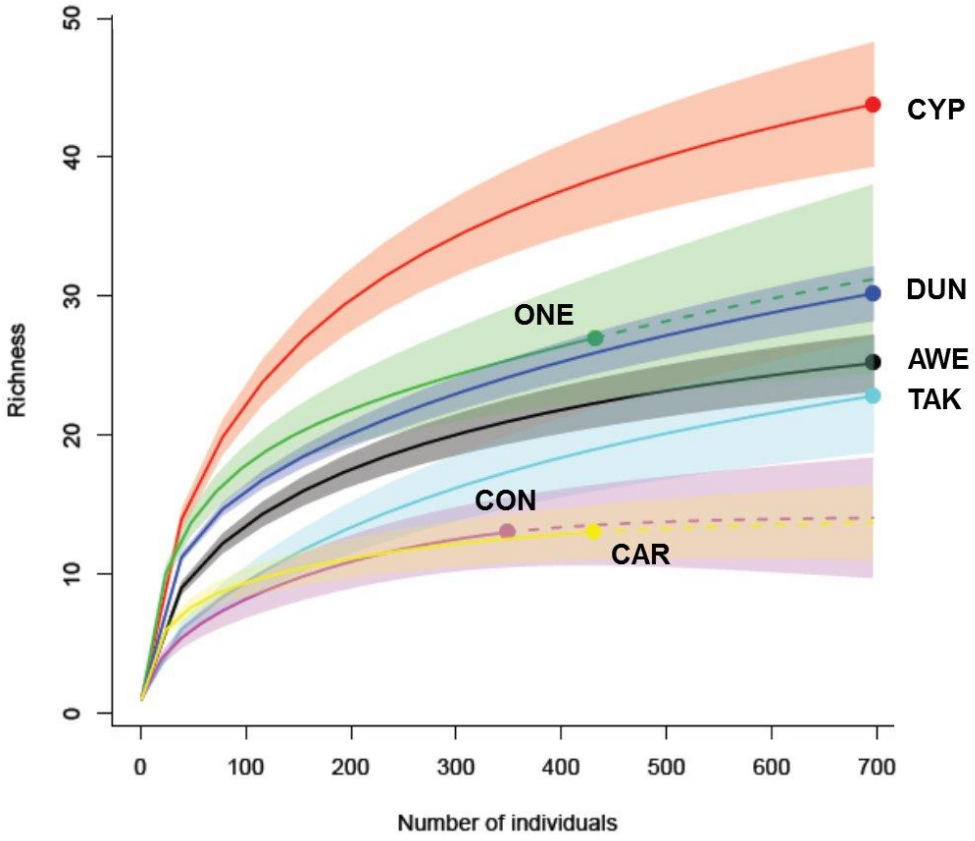

Figure 2.4. Individual-based rarefied and extrapolated estimates of expected species richness plotted against the number of individuals, up to 696 individuals, based on abundance data. The shaded areas represent the unconditional CI based on 500 bootstrap replications and the circle represents the reference sample. Three curves (Onefour (N=432), Carmacks (N=431), Conglomerate (N=348)), have been extrapolated (dashed lines). Site codes as in Fig. 2.1.

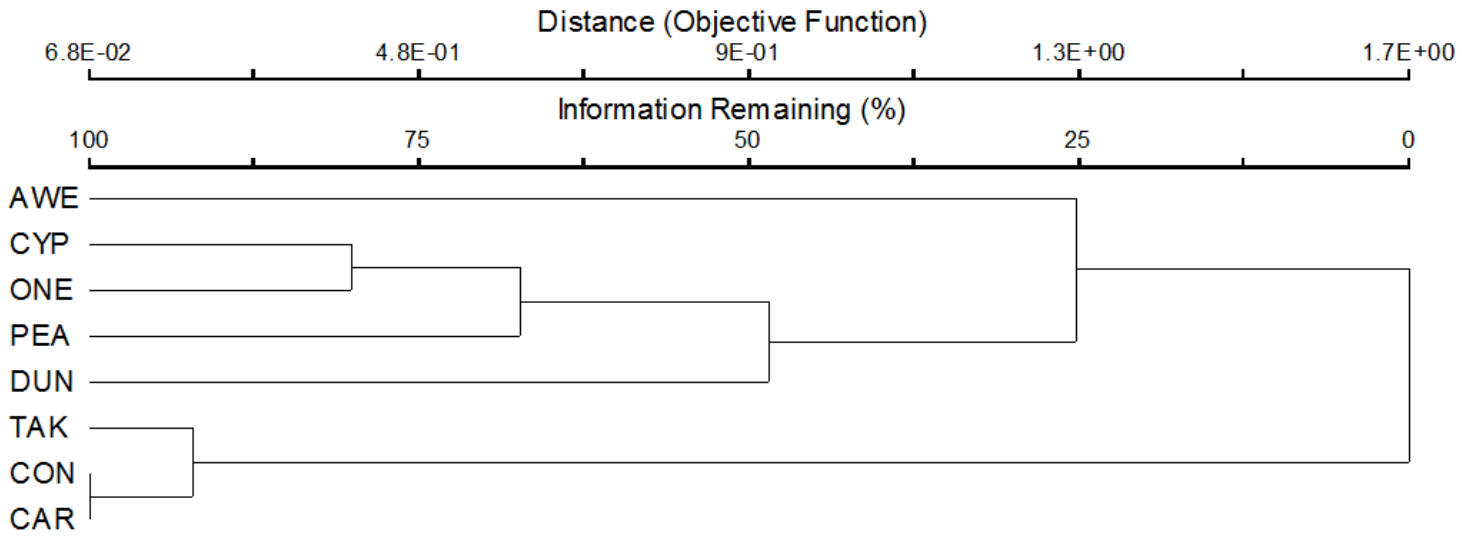

Figure 2.5. Cluster dendrogram based on abundance data set using group average and Sørensen distance. Site codes as in Fig. 2.1.

Appendices

Appendix 2.1. Collection site description and coordinates.

Prairies

Sampling in the Prairies was focused on dry sites in the mixed grassland ecoregion which tend to be dominated by blue grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths), speargrass (*Heterostipa comata* (Trin. & Rupr.) Barkworth), low sedge and pasture sage (*Artemisia frigida* Willd.) (Shorthouse, 2010). Although Aweme is much further east, it is still categorized as mixed grassland with similar vegetation (Roughley, 2000). The Cypress upland ecoregion is characterized by the fact that it is the highest land elevation in the Prairies. In Cypress Hills, we collected in fescue grasslands where the dominant species is rough fescue (*Festuca campestris* Rybd.) (Shorthouse, 2010).

Site	Lat. N	Long. W	Sampling years
AB, Onefour Heritage Rangeland (ONE)	49.15666	-110.2635	1997, 2000, 2012
SK, Cypress Hills Provincial Park (CYP)	49.6718	-109.4615	2000, 2005, 2012
MB, Aweme, Criddle-Vane Homestead Provincial Park (AWE)	49.70853	-99.60275	1999, 2000, 2012

The Peace region

Sampling in the Peace River grasslands was restricted to xeric, steep slopes which tend to have *Hesperostipa spartea–Carex–Artemisia frigida* associations (Schmidt *et al.*, 2013).

Site	Lat. N	Long. W	Sampling years
AB, Dunvegan (DUN)	55.92543	-118.59856	1997, 2003, 2012
AB, Peace River, Grouard Hill (PEA)	56.2325	-117.2770	1997, 2000

Yukon Territory

Sites were dominated by prairie sage (*A. frigida*) and bunch grasses (*Festuca* sp. *Calamagrostis* sp.) and situated on exposed arid south-facing slopes. Detailed descriptions for each site are in Boucher (1998) and Boucher & Wheeler (2001).

Site	Lat. N	Long. W	Sampling years
YT, Alaska Hwy, 13.1 km W of Takhini River (TAK)	60.81407	-135.9706	1997, 1998, 2012
YT, 5 km S of Jakes Corner, Mt. White above Little Atlin Lake (ATL)	60.29667	-133.9833	1997, 1998
YT, 4 km E of Carcross, slope above Nares Lakes (NAR)	60.16833	-134.645	1997, 1998
YT, Klondike Hwy, 26.3 km S of Carmacks (CAR)	61.87667	-134.115	1997, 1998, 2012
YT, Klondike Hwy, 8.8 km S of Twin Lakes, Conglomerate Mtn. (CON)	61.6273	-135.8802	1997, 1998, 2012
YT, Klondike Hwy, 15 km S of Carmacks, Bushy Mountain (BUS)	61.9700	-136.2033	1997, 1998

Appendix 2.2. Presence-absence matrix of species and morphospecies collected at each site, divided by collection year. Site codes as in Fig. 2.1.

	AWE 1999	AWE 2000	AWE 2012	CYP 2000	CYP 2005	CYP 2012	DUN 1997	DUN 2003	DUN 2012	ONE 2000	ONE 1997	ONE 2012	PEA 1997	TAK 1997	TAK 1998	TAK 2012	ATL 1997	NAR 1997	NAR 1998	CON 1997	CON 1998	CON 2012	CAR 1997	CAR 1998	CAR 2012	BUS 1997	BUS 1998
Apallates coxendix (Fitch)	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Apallates neocoxendix (Sabr.)	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Apallates particeps (Becker)	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphanotrigonum scabrum (Aldrich)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Apotropina hirtoides(Sabr.)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Biorbitella frontoorbitalis (Sabr.)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Biorbitella hesperia (Sabr.)	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Biorbitella virgata (Coqu.)	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorops sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Chlorops sp.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0

Chlorops sp.3	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	1	1	1	1	0	1	0	0	0
Chlorops sp.5	1	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorops sp.6	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conioscinella triorbiculata (Sabrosky)	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conioscinella sp.1	0	0	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	0	0	1	1	0	1	0	1	1	1	
Conioscinella n.sp.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	
Conioscinella sp.4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dasyopa sp.1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	0	1	
Dasyopa n.sp.2	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	
Dasyopa sp.3	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dicraeus incongruous Aldrich	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dicraeus ingratus (Loew)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dicraeus tibialis (Macquart)	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplotoxa versicolor (Loew)	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplotoxa sp.A	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplotoxa sp.B	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplotoxa sp.C	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elachiptera costata (Loew)	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Elachiptera decipiens (Loew)	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elachiptera flaviceps Sabrosky	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elachiptera nigriceps (Loew)	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elliponeura diplotoxoides Becker	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epichlorops puncticollis(Zett.)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eribolus longulus (Loew)	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eribolus nana (Zetterstedt)	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fiebrigella oophaga (Sabrosky)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Incertella incerta (Becker)	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	0	0	1	1	1	1	0	
Incertella insularis (Malloch)	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Incertella minor (Adams)	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Incertella ovalis (Adams)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Incertella n.sp.A	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	
Lasiosina sp.A	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Liohippelates bishoppi (Sabrosky)	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liohippelates pallipes (Loew)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malloewia aequa (Becker)	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Malloewia ?diabolus (Becker)	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malloewia neglecta (Becker)	0	0	0	0	1	1	1	1	1	0	0	1	1	1	0	0	1	1	0	0	0	0	1	0	0	1	1	
Malloewia ?nigripalpis (Malloch)	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malloewia n.sp.1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Melanochaeta eunota (Loew)	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meromyza canadensis Fedoseeva	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meromyza columbi Fedoseeva	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	1	1	1	1	1	
Meromyza communis Fedoseeva	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meromyza nigriventris Macquart	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Meromyza pratorum Meigen	1	0	0	1	1	1	1	0	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Meromyza sabroskyi Fedoseeva	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meromyza sp.1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meromyza sp.2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meromyza sp.4	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meromyza sp.5	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neodiplotoxa sp.A	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neodiplotoxa sp.B	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neodiplotoxa sp.C	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Olcella cinerea (Loew)	0	0	0	0	0	1	1	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Olcella parva (Adams)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	1	0
Olcella provocans (Becker)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Olcella pygmaea (Becker)	1	0	1	0	0	1	0	1	1	0	1	0	1	1	0	0	1	0	0	1	0	0	1	0	1	1	1
Olcella n.sp nr *parva*	1	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Olcella sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Olcella sp.2	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	0	1	1	0	1	1
Olcella sp.3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	1	0	0	1	0
Oscinella sp.1	1	0	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscinella sp.2	0	0	1	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Oscinella sp.3	0	0	0	1	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscinella sp.4	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscinella sp.5	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscinella ? sp.7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscinella sp.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudopachychaeta approximatonervis (Zett.)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhopalopterum carbonarium (Loew)	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhopalopterum criddlei (Aldrich)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhopalopterum luteiceps (Sabr.)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhopalopterum painter (Sabrosky)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhopalopterum soror (Macquart)	0	0	0	0	1	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhopalopterum umbrosum (Loew)	0	1	0	0	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhopalopterum sp.1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thaumatomyia glabra (Meigen)	1	0	1	1	0	1	1	1	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0
Thaumatomyia grata (Loew)	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Thaumatomyia pulla (Adams)	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Thaumatomyia pullipes (Coquillet)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Thaumatomyia sp.A	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thaumatomyia sp.B	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricimba brunnicollis (Becker)	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	1	0	1	0	1	1	0	1	1	1
Tricimba cincta (Meigen)	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	1	1	1	1	1	1	1
Tricimba linealla (Fallen)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricimba melancholica (Becker)	1	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	0	1	1	1	1	1	0	1	1

Appendix 2.3. Number of specimens of each species and morphospecies collected at each site. Site codes as in Fig. 2.1.

	AWE	CYP	ONE	DUN	PEA	TAK	CON	CAR
Apallates coxendix (Fitch)	716	12	35	82	1	0	0	0
Apallates neocoxendix (Sabrosky)	13	0	0	0	0	0	0	0
Apallates particeps (Becker)	25	3	0	0	0	0	0	0
Apotropina hirtoides (Sabrosky)	0	0	0	1	0	0	0	0
Biorbitella frontoorbitalis (Sabr.)	0	0	0	2	0	0	0	0
Biorbitella hesperia (Sabrosky)	0	0	0	85	0	0	0	0
Biorbitella virgata (Coquillet)	0	0	1	1	0	0	0	0
Chlorops sp.1	0	0	0	0	0	9	0	0
Chlorops sp.2	0	0	0	0	0	0	0	22
Chlorops sp.3	0	0	0	0	0	5	33	2
Chlorops sp.5	10	4	56	0	2	0	0	0
Chlorops sp.6	0	0	0	2	0	0	0	0
Conioscinella triorbiculata (Sabr.)	5	0	0	9	0	0	0	0
Conioscinella sp.1	0	9	1	252	0	175	13	13
Conioscinella sp.4	2	0	0	0	0	0	0	0
Dasyopa sp.1	16	0	0	0	0	4	0	1
Dasyopa n.sp.2	0	0	0	1	0	1	2	0
Dicraeus incongruus Aldrich	0	10	0	0	0	0	0	0
Dicraeus ingratus (Loew)	0	5	0	0	0	0	0	0
Dicraeus tibialis (Macquart)	0	1	0	2	0	0	0	0
Diplotoxa versicolor (Loew)	4	59	22	66	134	0	0	0
Diplotoxa sp.A	0	0	5	0	0	0	0	0

Diplotoxa sp.B	0	0	3	0	0	0	0	0
Diplotoxa sp.C	0	1	7	0	0	0	0	0
Elachiptera costata (Loew)	2	0	0	2	0	0	0	0
Elachiptera decipiens (Loew)	0	2	0	1	0	0	0	0
Elachiptera flaviceps Sabrosky	0	0	0	1	0	0	0	0
Elachiptera nigriceps (Loew)	0	1	0	0	0	0	0	0
Elliponeura diplotoxoides Becker	0	6	0	0	0	0	0	0
Epichlorops puncticollis (Zett.)	0	0	0	0	0	1	0	0
Eribolus longulus (Loew)	0	1	0	0	0	0	0	0
Eribolus nana (Zetterstedt)	0	2	0	1	0	0	0	0
Fiebrigella oophaga (Sabrosky)	0	0	0	0	0	0	1	0
Incertella incerta (Becker)	39	299	139	1582	101	5	2	17
Incertella insularis (Malloch)	0	1	2	0	0	0	0	0
Incertella minor (Adams)	80	27	0	33	0	0	0	0
Incertella ovalis (Adams)	2	0	0	0	0	0	0	0
Incertella n.sp.A	0	0	0	0	0	3	0	0
Lasiosina sp.A	2	0	0	0	0	0	0	0
Liohippelates bishoppi (Sabrosky)	11	0	0	1	0	0	0	0
Malloewia aequa (Becker)	0	2	0	4	1	1	0	0
Malloewia ?diabolus (Becker)	0	7	0	9	0	0	0	0
Malloewia neglecta (Becker)	0	79	6	39	3	1	0	4
Malloewia ?nigripalpis (Malloch)	13	0	0	124	0	0	0	0
Malloewia n.sp.1	0	0	0	1	0	0	0	0
Melanochaeta eunota (Loew)	0	2	0	0	0	0	0	0
Meromyza canadensis Fedoseeva	0	1	0	0	0	1	0	0

Meromyza columbi Fedoseeva	0	4	31	412	206	276	2	58
Meromyza communis Fedoseeva	0	0	1	8	68	0	0	0
Meromyza nigriventris Macquart	0	1	0	0	0	0	0	0
Meromyza pratorum Meigen	0	14	10	12	0	363	272	222
Meromyza sabroskyi Fedoseeva	0	6	38	0	0	0	0	0
Meromyza sp.1	0	53	0	0	0	0	0	0
Meromyza sp. 2	0	0	1	0	0	0	0	0
Meromyza sp.4	0	1	0	0	0	0	0	0
Meromyza sp.5	0	0	0	2	0	0	0	0
Neodiplotoxa sp.A	90	0	1	0	0	0	0	0
Neodiplotoxa sp.B	1	0	10	0	0	0	0	0
Neodiplotoxa sp.C	0	8	0	1	2	0	0	0
Olcella cinerea (Loew)	0	5	0	226	5	0	0	1
Olcella parva (Adams)	59	22	7	191	53	4	0	0
Olcella provocans (Becker)	0	0	0	0	0	1	0	0
Olcella pygmaea (Becker)	2	2	0	16	15	2	1	75
Olcella n.sp near *parva*	201	2	1	0	0	0	0	0
Olcella sp.2	2	0	0	0	1	23	12	0
Olcella sp.3	0	0	0	0	0	2	0	10
Oscinella sp.1	3	54	1	3	69	0	0	0
Oscinella sp.2	12	16	0	136	8	0	0	0
Oscinella sp.3	0	8	0	16	0	0	0	0
Oscinella sp.4	0	20	0	1	0	0	0	0
Oscinella sp.5	0	1	0	7	0	0	0	0
Oscinella ? sp.7	0	0	0	1	0	0	0	0

Oscinella sp.8	0	0	0	0	0	1	0	0
Pseudopachychaeta approximatonervis (Zett.)	0	0	0	1	0	0	0	0
Rhopalopterum carbonarium (Loew)	0	3	0	1	0	1	0	0
Rhopalopterum criddlei (Aldrich)	4	0	0	0	0	0	0	0
Rhopalopterum luteiceps (Sabrosky)	4	0	0	0	0	0	0	0
Rhopalopterum painteri (Sabrosky)	0	0	8	0	0	0	0	0
Rhopalopterum soror (Macquart)	0	10	1	9	1	0	0	0
Rhopalopterum umbrosum (Loew)	2	3	10	11	8	0	0	0
Rhopalopterum sp.1	0	0	2	7	0	0	0	0
Thaumatomyia glabra (Meigen)	8	5	28	147	1	1	0	1
Thaumatomyia grata (Loew)	0	0	0	8	0	0	0	0
Thaumatomyia pulla (Adams)	0	0	5	0	0	2	0	0
Thaumatomyia pullipes (Coquillet)	0	0	0	0	0	0	1	0
Thaumatomyia sp.A	0	0	0	2	0	0	0	0
Thaumatomyia sp.B	0	3	0	2	0	0	0	0
Tricimba brunnicollis (Becker)	0	0	0	1	1	3	2	3
Tricimba cincta (Meigen)	0	1	0	2	0	5	4	2
Tricimba linealla (Fallen)	1	0	0	0	0	0	0	0
Tricimba melancholica (Becker)	0	1	0	12	5	6	3	0

CONNECTING STATEMENT 2

While Chapter 2 examined the community composition of a family of flies from a biogeographic perspective, the following chapter is a study of intraspecific phylogeographic patterns. Community composition suggested that Diptera communities present on grasslands in the Yukon survived the last glaciation in Beringia, but molecular data will permit examination of the history of species in more detail. The community as a whole may suggest it is more likely that xeric Yukon grasslands are remnants of the mammoth steppe, but particular species may have migrated north recently.

In this chapter, we examine the phylogeographic patterns of three species of higher Diptera. Each species has a different distribution pattern which allows us to examine whether species with widespread distribution survived in different refugia than species with disjunct distribution. Examining molecular diversity and divergence times allows an in depth look into the histories of individual species.

CHAPTER 3. PHYLOGEOGRAPHY OF HIGHER DIPTERA IN GLACIAL AND POSTGLACIAL GRASSLANDS IN WESTERN NORTH AMERICA

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Abstract

Aim The objective of this study was to test, using mitochondrial DNA (mtDNA) from three Diptera species, the two hypotheses on the origin of Yukon's xeric grasslands: they are remnants of (i) the late Pleistocene arctic steppe ecosystem or (ii) southern grasslands which became disjunct because of a change in climate during the Holocene.

Location Grasslands in western North America: the Canadian Prairies (AB, MB), the Peace River (AB) and the southern Yukon grasslands (YT).

Methods We sequenced two mtDNA gene regions (658 bp from cytochrome *c* oxidase subunit I and 510 bp from cytochrome *b*) from three acalyptrate Diptera— one species with a continuous distribution across regions, and two with disjunct populations between regions. We used a Bayesian approach to determine population groupings without *a priori* assumptions and then performed analysis of molecular variance (AMOVA) and exact tests of population differentiation (ETPD) to examine their validity. Molecular dating was carried out to establish divergence time of clades.

Results Two geographically structured populations were found for all species: a southern (including Prairies and Peace River regions) and a northern Yukon population. Although AMOVA did not show significant differentiation between populations, the ETPD did. Divergence time between Yukon and southern populations predated the Holocene for two of the species. The species with ambiguous divergence time results had a high number of haplotypes suggesting survival in a refugium.

Main conclusions Results support the hypothesis that certain fly populations in Yukon grasslands are remnants from the Beringian mammoth steppe of the Pleistocene, rather than the result of a northern expansion during the Holocene.

Keywords: acalyptrate, COI, Cyt *b*, Holocene, Nearctic, Pleistocene, refugium

Introduction

Xeric grasslands in the Yukon Territory and Alaska are rare and unique environments. Characterised by prairie sage (*Artemisia frigida* Willd.), bunch-grasses and forbs, these isolated communities are associated with arid well-exposed south-facing slopes and have a unique insect fauna. While located mostly in the south of the Yukon Territory, there are a few northern outliers on very steep slopes in Old Crow and along the Yukon and Firth Rivers (Scudder, 1997).

To date, only paleoecological and distributional data have been used to infer the origin of these grasslands in the Yukon (e.g. Finnamore, 1997; Schweger, 1997; Boucher & Wheeler, 2001). Fossil evidence suggests that these communities are analogues of the late Pleistocene arctic steppe ecosystem (Schweger, 1997; Zazula *et al*., 2003, 2006). Regional aridity during the Wisconsinan glaciation would have allowed this xeric steppe-like flora to be widespread (Guthrie, 2001; Zazula *et al*., 2003). Subsequent changes in climate have reduced this ecosystem to a few arid, exposed areas. However, some plant and insect distributions suggest that these grasslands may have a southern origin instead (Finnamore, 1997; Boucher & Wheeler, 2001).Warm and dry conditions during the Holocene Hypsithermal warming period could have caused vegetation distributions to be displaced northward. Subsequent cooling would have caused vegetation to recede, leaving behind disjunct populations in sites with warmer microclimates. This explanation has been found to support northwestern grasslands in the Peace River region of Alberta, up to 54° N (Strong & Hills, 2003, 2005), but has not been formally tested as an explanation for the presence of these south-facing grassland slopes in the Yukon.

There are limitations associated with the data currently used to ascertain the origin of these south-facing slopes. Paleoecological data, such as pollen records and fossils, are sometimes unidentifiable to appropriate levels of taxonomic resolution and offer only a snapshot of the assemblage at one time. These data are sparse and the record is incomplete (Shafer *et al*., 2010). Likewise, modern-day species distributions offer only limited insight into origins and past dispersal.

The objective of this study was to evaluate, through a phylogeographic framework, the two hypotheses on the origin of Yukon's xeric grasslands on south-facing slopes: they are remnants of (i) the late Pleistocene arctic steppe ecosystem or (ii) southern grasslands which became disjunct because of a change in climate during the Holocene. To test these hypotheses, we selected three Diptera species. Individual phylogeographic patterns can be difficult to

decipher due to factors such as mutation rates, gene flow within population or variable genetic diversity through time (Bromilow & Sperling, 2011). Multiple species allow examination of congruence in patterns (Riddle & Hafner, 2007). The Diptera species selected for study were small acalyptrate flies which have limited dispersal abilities (pers. obs.) a characteristic that would enhance genetic signals of past separation (Federov & Stenseth, 2002). One of the selected species has a continuous distribution and two have disjunct distributions between the southern grasslands and the Yukon (Fig. 3.1–3.3). By using species with different distribution patterns, we hoped to characterize the isolation which may have been due to range disjunction to ensure patterns were due to historical rather than landscape factors (Bromilow & Sperling, 2011).

Bromilow & Sperling (2011) examined the effect of Hypsithermal prairie expansion on butterflies in the Peace River region and southern prairies, and we expected patterns in Diptera to be similar to their results if the south-facing slopes in the Yukon have a similar origin. While there would be population structure and some isolation, there would be little genetic divergence between all regions. If, on the other hand, the south-facing slopes are remnants of the Pleistocene mammoth-steppe in the Pleistocene Beringian refugium, there would be a history of deep divergence between the south and the Yukon. We would expect higher genetic diversity in the Yukon than in the south and endemic Yukon haplotypes (Beatty & Provan, 2010).

Materials and methods

Sampling sites

Diptera were collected from grasslands in three regions: the Canadian Prairies (AB, MB), the Peace River region (AB), and southern Yukon (YT) (Table 3.1, Fig. 3.4).

Vegetation in the Prairie biome is broadly characterized by grasses (Poaceae), sedges (Cyperaceae), Asteraceae, especially sages (*Artemisia*), and other forbs (Strong & Hills, 2005). Our sampling efforts focused on dry sites in the mixed grassland ecoregion, dominated by blue grama, speargrass, low sedge and *A. frigida* (Shorthouse, 2010). Sites characterized by different vegetation (e.g. tall-grass prairie, Cypress Uplands) were also sampled for widespread Diptera species.

The Peace River grasslands are isolated from the southern Prairies by 300–400 km and are restricted to the Peace River valley and its tributaries (Strong & Hills, 2003). Sampling in the

Peace River grasslands was restricted to xeric, steep slopes which tend to have *Hesperostipa spartea-Carex-A. frigida* associations (Schmidt *et al*., 2013).

Yukon grasslands are characterized by prairie sage, bunch grasses and forbs and generally associated with arid, well-exposed south-facing slopes (Boucher & Wheeler, 2001).

Taxonomic sampling

During collection, emphasis was placed on acalyptrate Diptera, collected into 95% ethanol and dried with hexamethyldisilazane prior to mounting. All individuals were identified to named species or morphospecies to determine those present in all three regions. Geographic distributions were determined using published literature and museum records (see Fig 1–3). Three species were selected for analysis: *Incertella incerta* (Becker) — a widespread generalist species in western Canada, present in habitats between region (Fig. 3.1); *Meromyza columbi* Fedoseeva (Chloropidae) — a phytophagous species restricted to grasslands with a disjunct distribution between the three studied regions (Fig. 3.2); and *Trixoscelis fumipennis* (Melander) (Heleomyzidae) — a species widespread in southern Canada, south of the Peace River region, but disjunct between Peace River and the Yukon (Fig. 3.3). Although the latter species is mostly present at disturbed sites (Foster & Mathis, 2011), in the Yukon, it has been collected primarily on south-facing slopes and some lowland sites with similar plant assemblages.

Molecular techniques

Our DNA extraction, amplification and sequencing protocols follow Gibson *et al*. (2010a). Total genomic DNA was extracted non-destructively using whole specimens. For each species, 20–21 specimens were extracted for each region. Since specimens were mounted on points, water was used to dissolve glue prior to extraction when necessary. The DNA was extracted using a DNeasy Tissue kit (Qiagen Inc., Santa Clara, CA, USA). After extraction, specimens were critical point dried. All specimens were assigned unique identifiers and vouchers are deposited at the Lyman Entomological Museum, McGill University (LEM) (Table 3.2).

Two mitochondrial gene regions were targeted and amplified: (1) the barcoding region, a 658 bp fragment of the cytochrome *c* oxidase subunit I (COI) gene using the forward primer LC01490 (5’-GGTCAACAAATCATAAAGATATTGG-3’) (Folmer *et al.*, 1994) and the reverse primer COI-Dipt-2183R (5’-CCAAAAAATCARAATARRTGYTG-3’) (Gibson *et al*.,

2011); (2) a 510 bp fragment of cytochrome *b* using the forward primer CytB-Dipt-11035F (5'-GGNTTYKCNGTNGAYAAYGC-3') (Gibson *et al.,* 2011) and the reverse primer CytB-Dipt-11545R (5'-ACDGGDCGDGCYCCRATTC- 3') (Gibson *et al*., 2011). Amplifications were carried out in 25 µL reactions: 16.75 µL ddH_2O, 2.5 µL 10X Ex-Taq PCR buffer (containing 20 mM $MgCl_2$), 0.625 µL 25 mM $MgCl_2$, 1 µL of each 10 µM primer, 2 µL 10 µM dNTPs, 0.125 µL ExTaq HS DNA polymerase (Takara Bio USA, Madison, WI, USA), and 1 µL genomic DNA template. Amplification cycles were performed on an Eppendorf ep Gradient S Mastercycler (Eppendorf AG, Hamburg, Germany) as follows: 94˚C for 3 min; 30 amplification cycles of 94˚C for 45 s, 45˚C for 45 s, 72˚C for 1 min; and a final step for 5 min at 72˚C.

Amplification products were visualised on 1% agarose electrophoresis gels and target genes were isolated and purified using the E-Gel® system (Invitrogen ™, Carlsbad, CA, USA) as outlined in Gibson *et al*. (2010b). Sequencing of purified products was performed at the Agriculture & Agri-Food Canada, Eastern Cereal and Oilseed Research Centre Core Sequencing Facility (Ottawa, ON, Canada). The same primers were used as in the PCR reactions to sequence both forward and reverse strands. Sequencing reactions were carried out in a volume of 10 µL and used an ABI BigDye® Terminator v3.1 Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA, USA).

The chromatograms for sequences LEM-0276023–0276140 were edited and visualised using Sequencher 4.7 (Gene Codes Corp., Ann Arbour, MI, USA). Other chromatograms (sequences LEM-0049920, LEM-0049922–0049923, LEM-0276204–0276286) were edited and visualised using ChromasPro (Technelysium, South Brisbane, QLD, Australia).

Sequences were aligned using Clustal X v.2.0 with default parameters (Larkin, *et al*., 2007). Overhangs were removed in BioEdit v.7.2.3 (Hall, 1999). Nucleotide sequences were translated into amino acids using the invertebrate mitochondrial genetic code with ORF Finder (Stothard, 2000) to place sequences in the appropriate reading frame.Genbank numbers for all sequences are in Table 3.2.

Statistical analyses

All analyses were performed on the concatenated dataset and each gene separately. Mitochondrial haplotype diversity (h) and nucleotide diversity (π) were calculated for the entire dataset and for each region using the program DnaSP v.5 (Librado & Rozas, 2009).

Haplotype networks were constructed using statistical parsimony with the program TCS v.1.2.1 (Clement *et al*., 2000). The cut-off value for parsimonious branch connections between haplotypes was set to 95%.

To avoid *a priori* assumptions about the data, the program Geneland v.4.0.3 (Guillot *et al*., 2005a) run in R (2013, http://www.R-project.org/) was used to estimate the number of populations in the total sample. It uses a Bayesian approach that can incorporate both molecular and geographic data to estimate population clusters without prior population definitions (Guillot *et al*., 2005b).

The Geneland analysis was performed under the correlated allele frequencies model. Geographic coordinates (WGS84) were converted to UTM. The uncertainty associated with coordinates was set at 1000 km (approximate distance between regions). Although acalyptrate flies are relatively poor fliers, this setting allowed the possibility of migration and compensated for the fact that many of the individuals had been collected at the same locations. The analysis was executed for five runs, with 1,000,000 Markov chain Monte Carlo (MCMC) iterations each. Thinning was set at 400. The minimum and maximum value of K (number of populations) was set to one and 10, respectively. The burn-in was set to 2000.

Since Bayesian methods can overestimate population structure when there is isolation by distance (Frantz *et al.*, 2009), we examined correlation between geographic and genetic distance using partial Mantel tests executed in Isolation by distance web service v.3.23 (Jensen *et al.*, 2005). The partial Mantel test compares genetic and geographic distances while allowing additional variables to be incorporated into the test and for their effects to be isolated (Manel *et al*., 2003). We removed the effect of pre-existing population structure due to disjunction to verify that patterns were not just due to isolation by distance (Bromilow & Sperling, 2011). To build this indicator matrix, for each pairwise comparison, a value of 0 was given when both individual sequences came from the same population and 1 when they did not. For each species, 10000 randomizations were performed. The genetic distances were calculated using Φ_{ST} and Kimura's Two-Parameter Model (Kimura 2P) which accounts for different rates of transition and transversion (Ngan, 2006).

Population structures defined by Geneland were tested within an Analysis of Molecular Variance (AMOVA) framework and with exact tests of population differentiation (ETPD). Both were implemented in Arlequin v.3.5 (Excoffier *et al*., 2005). The AMOVA was examined at

three hierarchical levels: Φ_{ST}—within region (regions being defined as Prairies, Peace River region or Yukon), Φ_{SC}—within regions among populations and Φ_{CT}—among populations as defined by Geneland. Arlequin was also used to calculate M values (absolute value of migrants between populations per generation) estimated with the formula $M= (1-F_{ST})/2F_{ST}$ (Slatkin, 1991).

Divergence times

Divergence times were calculated with BEAST v.1.7.5 (Drummond *et al*., 2012) and the output examined via Tracer v.1.6 (Rambaut *et al*., 2014). Because no suitable calibration points or mutation rates for the study taxa exist, we used an iterative approach to generate time estimates to root the intraspecific analyses and find mutation rates for each species. We adapted a method combining appropriate demographic priors and deep calibration points (Marino *et al*., 2011).

An initial dated phylogeny of a Diptera subset was produced using fossil evidence to estimate the root of the phylogeny of each study taxon. Two fossil dating points were used: 70 Ma for Schizophora (Nardi *et al*., 2010) and 42 Ma for Chloropidae (Wiegmann *et al*., 2011) (Table 3.3). Sequences of Cyclorrhapha, Schizophora and Acalyptratae taxa for this phylogeny were obtained from GenBank (for accession numbers, see Appendix 3.1). The nucleotide substitution model chosen for sequence evolution was the Hasegawa-Kishino-Yano (HKY) model (Hasegawa *et al*., 1985) with partition of nucleotides into their separate coding positions and rate variation described by a four category discrete distribution. Models which consider the genetic code tend to outperform models that do not, even when fewer parameters are considered (Shapiro *et al*., 2006). The two genes were unlinked to allow separate base frequencies to be estimated for each. A relaxed lognormal clock model was used to allow different rates of evolution for each branch (Drummond *et al*., 2006). The tree prior was set as the Yule process (Gernhard, 2008), a model appropriate for multiple species. MCMC chain length was set to 100 million, with a 10% burn-in to obtain a sufficient Effective Sample Size (ESS > 200). Other parameters were set to the default.

Next, a simplified phylogeny of each study taxon was generated, using only one sequence per haplotype and the other Chloropidae taxa as a root to narrow down estimates. For each subset, the appropriate model of evolution was selected using jModelTest 2 (v.2.1.4) (Darriba *et al*., 2012). The model selected for each species was HKY with invariant sites and is the one used

for the following analyses. Nucleotides were partitioned into their separate coding positions. Posterior distributions from the Diptera tree were used as priors to calibrate the Chloropidae and the study taxa nodes (Table 3.3). The intraspecific aspect of the data was ignored and a Birth-Death prior was used (Marino *et al.*, 2011). The analyses were performed twice under a lognormal relaxed and strict clock for a MCMC chain length of 10 million (10% burnin). Analyses were verified for convergence and data suggested that the use of the strict clock was appropriate. Therefore posteriors generated by the strict clock model were used and this model was chosen for the following analysis.

Demographic analyses were run using all sequences. Tree shape was defined by a Bayesian skyline prior, a variable population size coalescent model (Drummond *et al*., 2005). The time to most recent common ancestor (Tmrca, in Myr) and the mutation rate for each taxa from the previous analysis were used as priors (Table 3.3). Analyses were run for a MCMC length of 30 million (until ESS >200) with a 10% burn-in. Maximum clade credibility trees were then viewed in FigTree v.1.4.0 (Rambaut, 2012).

Results

We successfully obtained 17–21 sequences per gene per region for most species, except *T. fumipennis* in the Prairie region where we obtained 13 COI and 15 Cyt *b* sequences (Appendix 3.2). Because no major differences were found in separate analyses, all results presented are for the concatenated dataset.

Haplotype and nucleotide diversity

Both *I. incerta* and *T. fumipennis* had the same overall number of haplotypes (35), and similar values of haplotype and nucleotide diversity. Although they also had the same number of haplotypes in the Yukon, *I. incerta* had higher nucleotide diversity in the Yukon (Table 3.4), and while *I. incerta* had the same number of haplotypes (14) in both the Prairies and Peace River, there were more *T. fumipennis* haplotypes in the Peace River region (18 compared to 11). The latter, however, could be an artefact stemming from fewer sequences obtained from that region for this species.

Overall, *I. incerta*, nucleotide diversity decreased from north to south, but haplotype diversity remained similar in the two southern regions and decreased in the north. For *T.*

fumipennis, nucleotide and haplotype diversities were highest in the Peace River region and lowest in the Yukon. The overall nucleotide diversity of *M. columbi* was much lower than the other species and nucleotide and haplotype diversity were highest in the north.

Haplotype networks

Only *M. columbi* had a haplotype shared among all three regions (Fig. 3.5). No haplotypes of *I. incerta* and *T. fumipennis* were shared between the two southern regions (Prairies and Peace River) and the Yukon.

There were, however, shared haplotypes for each species between the two southern regions: *I. incerta* had one shared haplotype between the Prairies and Peace River region (Fig. 3.6), *M. columbi* had three shared haplotypes (Fig. 3.5) and *T. fumipennis*, two (Fig. 3.7).

The two species with widespread components, *I. incerta* and *T. fumipennis*, showed similarities in their networks not exhibited by *M. columbi*. Both had fewer, more common clustered Yukon haplotypes: *I. incerta* had two clusters, one of which was separated from other haplotypes by at least six base pair differences; and *T. fumipennis* had one, also separated from other haplotypes by at least five base pair differences.

The haplotype network of *M. columbi* was quite different; all haplotypes were separated by few base pair differences and fewer more common haplotypes. Nonetheless, despite links to haplotypes of other origins and one shared haplotype, the *M. columbi* Yukon haplotypes formed two clusters.

Population structure and migration

Multiple runs in Geneland were consistent for each species (Table 3.5). In both *M. columbi* and *T. fumipennis*, two geographically structured populations were found: one in the Peace River plus Prairie region and one in the Yukon. However, although *I. incerta* individuals grouped into the same geographically structured populations, three populations were found by the program. The third "ghost population", which contained no individuals, was likely an artefact of the Bayesian analysis overestimating the genetic structure due to isolation by distance or data that did not adhere to modelling assumptions (Guillot, 2008). It may also be caused by MCMC that are too short (although increasing the length does not solve the problem) (Guillot, 2008). Guillot (2008) recommends ignoring these ghost populations and Frantz *et al.* (2009) suggest

testing for isolation by distance and performing additional tests, which we did. As expected, the partial Mantel test showed that the pattern exhibited by *I. incerta* did exhibit isolation by distance, but population structure was also correlated with genetic distance when removing the effect of geographic distance (p=0.06, R=0.77), implying that population disjunction also plays a role in forming this pattern. The two other species overall did not show isolation by distance (results not shown).

The AMOVA to assess whether there was any differentiation between the southern population grouping of the Peace River and Prairie regions, and a Yukon population, was not significant for any of the species (p ~3, for all Φ_{CT}) (Table 3.6).

The results of the ETPD were not concordant with the AMOVA; there was a significant difference between the southern population assemblage (Peace River and Prairies) and the Yukon population (Table 3.7). In addition, comparisons among the two southern assemblages were not significant, supporting the population delimitations suggested by Geneland.

The computation of M values showed that migration between the Peace River region and the Prairies was high for all species, especially *T. fumipennis* whose M value was estimated to be infinity (Table 3.8). Although unrealistic, this value stems from an F_{ST} value of 0, meaning that there is no differentiation between the two regions. In contrast, migration between those two regions and the Yukon was low for all species

Bayesian analyses and estimates of divergence time between populations

Bayesian trees recovered groupings found in the haplotype networks, but did not resolve areas of uncertainty. Clusters found in the networks, particularly those from a single region of origin, tended to be recovered in single clusters in the tree (e.g. Yukon 2 in the *I. incerta* tree (Fig. 3.8)). Unsurprisingly, unresolved regions in the haplotype networks were reflected in the Bayesian analyses through low posterior values (e.g. node of the clade Yukon 1, Yukon 2 and PRR in the *Meromyza* tree (Fig. 3.9)).

The iterative method used to find divergence times yielded conservative time intervals for nodes spanning hundreds of thousands and sometimes more than a million years. Furthermore, it is difficult to provide estimates for the divergence of Yukon populations from southern ones. Firstly, Yukon individuals were not always assigned to the same monophyletic grouping (e.g. Yukon 1, 2 and 3 in the *Meromyza columbi* tree (Fig. 3.9)). Secondly, branches at divergence

nodes often had low posterior values (e.g. node of the group containing Yukon 1, Yukon 2, PRR in *Meromyza columbi* tree (Fig. 3.9)). Nonetheless, nodes at the base of groupings (which represent the group's Tmrca) with high posteriors allowed placement of the origin of the group within a time frame. Two of the species (*I. incerta* (Fig. 3.8), *T. fumipennis* (Fig. 3.10)) had nodes at the base of Yukon groups with high posteriors which range from well before the beginning of the last glacial, the Wisconsinan (which began approximately 120 ka) and from before or during the last ice age (which began approximately 1.8 Ma). The highest posterior density (HPD) interval of expansion for the Yukon population of *T. fumipennis* was 0.33–1.2 Ma (mean=0.83). For *I. incerta*, one of the Yukon groupings (Yukon 1) had an HPD interval between 0.16–0.7 Ma (mean=0.67), and the Yukon 2 grouping 0.21–1.29 Ma (mean=0.98).

For *M. columbi*, only one of the nodes of a Yukon grouping had a high posterior (Yukon 3), with an HPD interval between 0–0.54 Ma (mean=0.38) (Fig. 3.9).

Discussion

Survival in a Beringian refugium or Hypsithermal expansion

Overall results support the hypothesis that certain fly populations in xeric grasslands in the Yukon are remnants from the Beringian mammoth steppe of the Pleistocene, rather than the result of a northern expansion during the Holocene, as has been suggested by others. Despite the fact that the AMOVA does not show population differentiation between the Yukon and southern regions for any of the species, other analyses support this population structure, notably, the ETPD and the Geneland analysis. As well, *M. columbi* has its highest haplotype diversity in the Yukon and this pattern is often associated with survival in a refugium (Beatty & Provan, 2010).

An additional piece of evidence which supports this hypothesis is that the Tmrca of the *T. fumipennis* Yukon grouping places it well within the Pleistocene. However, haplotype networks and Bayesian analyses for *M. columbi* and *I. incerta* do not show a clear pattern of population divergence. *Incertella incerta* has the simpler pattern of the two where two Yukon clusters are recovered. The time intervals at the base of these two clusters overlap (0.16–0.7 vs. 0.21–1.29) and it is possible that they originated at the same time and that additional sampling would recover missing intermediate haplotypes. After all, removing one intermediate haplotype because it was missing information from one gene created a network which added steps between both Yukon clusters (Fig. 3.11). However, the low haplotype diversity in the Yukon suggests that this

is unlikely. With the same amount of sampling, the diversity is much lower in the Yukon than in the south. One cluster (Yukon 2) also has many more base pair differences separating its haplotypes from others, accounting for high nucleotide diversity in this region. These numerous base pair differences are likely the result of a longer history of divergence, supported by the longer branch recovered in the dated BEAST phylogeny leading to the node of the Yukon 2 clade.

The branches preceding the Yukon 1 grouping are short and nodes have low posterior values. Although this could be due to conflicting phylogenetic signal, this pattern is seen during rapid diversification events in species trees (He *et al*., 2014). A rapid expansion event in an intraspecific tree would likely show the same signal. It is thus likely that this cluster has a more recent history.

This overall pattern is concordant with the history of Beringia as a refugium during the Pleistocene. While the region remained unglaciated during this last ice age, multiple glacials and interglacials affected the ranges of species creating bottlenecks during glacials and allowing expansion during interglacials. The genetic patterns of *I. incerta* suggest that this species was affected by at least two such events. The few frequent haplotypes in each cluster suggests that both clusters were subjected to bottlenecks, and the branch lengths connected to each grouping suggest that they were affected by bottleneck events at different times.

The haplotype network and dated tree of *M. columbi* are more difficult to interpret. Few nodes have high posteriors and the one node at the base of a Yukon grouping has an HPD interval ranging from 0–0.54. This time frame encompasses the Holocene and the late Pleistocene. The low posterior values of the nodes and short branches preceding groups Yukon 1 and Yukon 2 suggest a period of rapid changes, but it is difficult to speculate beyond this fact. Nonetheless, as mentioned before, the high haplotype diversity in the Yukon, coupled with few shared haplotypes with the South suggest the survival of the species in Beringia (Beatty and Provan 2010).

Comparison of patterns among study taxa

Although results suggest that all three species survived in Beringia during the last ice age, patterns between species differed considerably. This is not surprising given the different distributions and life histories of each species. Some of the species' genetic patterns fit well with

the ecological information that is known about them. The lower overall haplotype and nucleotide diversity for *M. columbi*, which has a disjunct distribution compared to the other species, is consistent with patterns exhibited by butterflies with disjunct versus widespread distributions (Bromilow & Sperling, 2011).

The AMOVA, which tested the population structure found by Geneland, was not significant for any of the species. This contrasted with the ETPD which was significant for this structure. There are a few possible explanations. When examining disjunct and continuous species, Bromilow & Sperling (2011) found that species with continuous distributions tended to lack significant population structures. This would correspond well with the distribution of *I. incerta* and it could be that the two other species' distributions have not been recorded adequately. However, this would contradict results found with the ETPD. The non-significance of the AMOVA could also be due to an artefact of small sample size. While this may be true particularly for *T. fumipennis*, where there was lower sampling in the Prairies, the sampling for *M. columbi* seems to have been sufficient given the higher frequency of many haplotypes. Another possibility is that the non-significance is due to the high variance both among population and within region, particularly for *I. incerta* and *T. fumipennis.* Certain haplotypes, even within populations or regions, are highly differentiated with many base pair differences in both the southern region and the Yukon. The two groupings of *I. incerta* haplotypes from the Yukon could indicate two different glaciation events and it could be more appropriate to treat them separately.

Comparison to patterns of other organisms

Phylogeographic studies have shown that Beringia has been a refugium for multiple species in multiple higher taxa, including plants, mammals and fish (Shafer *et al*., 2010). Some of these studies have found substructure within Beringia (Shafer *et al*., 2010), as we did with *I. incerta*. In the ground squirrel, *Spermophilus parryii* (Richardson), for instance, at least four clades whose divergences can be dated back to glaciation events have persisted through several glacials (Edingaas *et al*., 2004). Another study on an insect species, the spruce beetle, *Dendroctonus rufipennis* (Kirby) (Curculionidae), also found two distinct clades in Beringia separated by a more southern clade which suggested secondary contact between both northern clades following glacial cycles (Maroja *et al*., 2007).

No studies to date have examined the phylogeography of grassland species in the Yukon. The study closest in scope to ours was Bromilow & Sperling (2011) who evaluated the population structure of continuously distributed and disjunct butterfly species in the Peace River grasslands and southern grasslands. Unlike their study, the two southern regions that we studied did not show any significant population structure. We also found more gene flow between both regions than they found in butterflies. The M-values for all three of our species between the Peace River region and the Prairies were considerably higher (>12.5) than those found for any of the continuous species in their study (highest value: 10.24; average: 4.71). This result was unexpected, since acalyptrates are thought to be poor fliers. During field observation, they were seen to hop around from vegetation to vegetation rather than fly. Although none of the species chosen by Bromilow & Sperling (2011) were known to be migratory, they likely have better flying ability than acalyptrate flies. Therefore, the amount of gene flow between individuals from the Peace River region and the Prairies is difficult to explain. One possibility is that because of their small size they can easily be passively dispersed by wind, increasing their dispersal ability. The dispersal capacity, as well as gene flow among populations of acalyptrates is poorly known.

Research on Yukon grasslands has been mostly focused on fossils and current species assemblages (e.g. Boucher, 1998; Vetter, 2000; Berman *et al*., 2011). Yet many questions remain unanswered about these grasslands. In a comparison of species present in Alaska, in the Boreal forest on the northern Great Plains, and the southwest Yukon, Vetter (2000) found that 25% of plant species in each region were restricted to that region alone. These grasslands are not uniform in their composition and could potentially have different origins or at least be good systems to study modern landscape genetics if these differences are recent. These grasslands offer a unique opportunity to study ice age dynamics through extant systems.

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Tables

Table 3.1. Sampling locations and their coordinates divided by designated region.

Location	Abbreviation	N	W
Prairies region			
Onefour Heritage Land, AB	Onefour	49.15666	-110.2635
Cypress Hills Interprovincial Park, AB	Cypress Hills AB	49.6291	-110.2623
Route 41, South of Cypress Hills Interprovincial Park, AB	Cypress Hills AB	49.49087	-110.2547
Route 41, near Cressday, AB	Onefour	49.2444	-110.2525
Midland Provincial Park, McMullen Island, near Dinosaur Trail, AB	Dinosaur	51.46943	-112.771
Cypress Hills Interprovincial Park, SK	Cypress Hills SK	49.6718	-109.4615
Aweme/Criddle Vane Homestead Provincial Park, MB	Aweme	49.70853	-99.60275
Peace River Region			
Dunvegan, AB	Dunvegan	55.92543	-118.59856
Peace River, Grouard Hill	Peace	56.2325	-117.2770
Southern Yukon			
Klondike Highway, Robinson Road House, YT	Robinson	60.44839	-134.84961
Klondike Highway, 8.8 km South of Twin Lakes, Conglomerate Mountain, YT	Conglomerate	61.6273	-135.8802
Klondike Highway, 15 km South of Carmacks, Bushy Mountain, YT	Carmacks	61.9700	-136.2033
Alaska Highway, 13.1 km west of Takhini River, YT	Takhini	60.81407	-135.9706

Table 3.2. Unique identifiers of vouchers deposited at the Lyman Entomological Museum and their associated accession numbers, by species and location. Abbreviations as in Table 3.1.

Location	Unique identifiers	Accession COI	Accession Cyt *b*
Meromyza columbi			
	LEM-0276023	KP037115	KP037303
	LEM-0276024–27	KP037169–172	KP037356–9
	LEM-0276028	-	KP037355
Onefour	LEM-0276029–30	KP037167–8	KP037353–4
	LEM-0276136	KP037146	KP037332
	LEM-0276261–2	KP037118–9	-
	LEM-0276263–4	KP037116–7	KP037304–5
Cypress Hills	LEM-0276047	KP037150	KP037336
AB	LEM-0276255	KP037124	KP037310
Dinosaur	LEM-0276257–60	KP037120–3	KP037306–9
	LEM-0276031–8	KP037159–66	KP037345–52
Dunvegan	LEM-0276204	KP037145	KP037331
	LEM-0276245–54	KP037125–34	KP037311–20
Peace	LEM-0276134–5	KP037147–8	KP037333–4
	LEM-0276040	KP037157	KP037343
Takhini	LEM-0276045–6	KP037151–2	KP037337–8
	LEM-0276133	KP037149	KP037335
	LEM-0276235–44	KP037135–44	KP037321–30
Carmacks	LEM-0276039	KP037158	KP037344
	LEM-0276041–4	KP037153–6	KP037339–42

Incertella incerta			
	LEM-0276048	KP037106	KP037293
	LEM-0276059–60	KP037087–8	KP037273–4
	LEM-0276061	-	KP037275
Onefour	LEM-0276062	KP037089	KP037276
	LEM-0276216	KP037070	KP037256
	LEM-0276220–2	KP037064–6	KP037250–2
	LEM-0276224	KP037062	KP037248
	LEM-0276049	KP037107	KP037294
Cypress Hills	LEM-0276215	KP037071	KP037257
AB	LEM-0276217–9	KP037067–9	KP037253–5
	LEM-0276223	KP037063	KP037249
Cypress Hills	LEM-0276055–7	KP037108–10	KP037295–7
SK	LEM-0276058	KP037082	KP037268
	LEM-0276063–65	KP037092–94	KP037279–81
	LEM-0276067–70	KP037096–99	KP037283–86
	LEM-0276075	KP037104	KP037291
	LEM-0276139–40	KP037083–4	KP037269–70
	LEM-0276225	KP037061	KP037247
Dunvegan	LEM-0276226	-	KP037246
	LEM-0276227–28	KP037059–60	KP037244–5
	LEM-0276229	-	KP037302
	LEM-0276231–34	KP037111–4	KP037298–301
Carmacks	LEM-0276208	KP037078	KP037264
	LEM-0276066	KP037095	KP037282
	LEM-0276071–4	KP037100–3	KP037287–90
	LEM-0276076	KP037105	KP037292
	LEM-0276077–8	KP037090–1	KP037277–8
Robinson	LEM-0276137–8	KP037085–6	KP037271–2
	LEM-0276205–7	KP037079–81	KP037265–67
	LEM-0276209–14	KP037072–7	KP037258–63

Trixoscelis fumipennis			
	LEM-0049920	KP037201	KP037388
Aweme	LEM-0049922–3	-	KP037389–90
	LEM-0276093	KP037215	KP037404
Cypress Hills	LEM-0276094–6	KP037212–4	KP037401–3
AB	LEM-0276099	KP037209	KP037398
	LEM-0276132	KP037178	KP037365
Cypress Hills			
SK	LEM-0276100	KP037208	KP037397
	LEM-0276101–2	KP037206–7	KP037395–6
Onefour	LEM-0276131	KP037177	KP037364
	LEM-0276285–6	KP037198–9	KP037385–6
	LEM-0276054	KP037200	KP037387
	LEM-0276087–92	KP037216–21	KP037405–10
Dunvegan	LEM-0276097–8	KP037210–1	KP037399–400
	LEM-0276127	KP037205	KP037394
	LEM-0276273–4	KP037187–8	KP037374–5
Peace	LEM-0276128	KP037204	KP037393
	LEM-0276265–72	KP037179–86	KP037366–73
	LEM-0276079–82	KP037173–6	KP037360–3
	LEM-0276083–6	KP037222–5	KP037411–14
	LEM-0276129–30	KP037202–3	KP037391–2
Robinson	LEM-0276275–6	KP037189–90	KP037376–7
	LEM-0276277	KP037191	-
	LEM-0276278–83	KP037192–97	KP037378–83
	LEM-0276284	-	KP037384

Table 3.3. Priors for calibrating the different phylogenies in BEAST. Tmrca is the time to most recent common ancestor (in Myr) and μ is the mutation rate (mutations per site per million year).

Analysis	**Taxon**	**Distribution**	**Calibration type**	**Priors**
Diptera phylogeny	Schizophora	Lognormal	Fossil (Wiegmann *et al.*, 2011)	mean=3 st. dev.=0.78 offset=70
	Chloropidae	Lognormal	Fossil (Wiegmann *et al.*, 2011)	mean=3 st. dev.=0.7 offset=42
Simplified Diptera phylogeny	Chloropidae	Normal	Posterior distribution from Diptera phylogeny	mean=59.41 st. dev.=8.9
	Incertella incerta	Normal	Posterior distribution from Diptera phylogeny	mean=31.93 st. dev=11.54
	Meromyza columbi	Normal	Posterior distribution from Diptera phylogeny	mean=23.48 st. dev=10.3
	Trixoscelis fumipennis	Normal	Posterior distribution from Diptera phylogeny	mean=29.3 st. dev=13.14
Demography	*Incertella incerta*	Normal	Tmrca from simplified phylogeny	mean=1.788 st. dev=0.63 μ=0.0042661
	Meromyza columbi	Normal	Tmrca from simplified phylogeny	mean=1.32 st. dev=0.47 μ=0.0023813
	Trixoscelis fumipennis	Normal	Tmrca from simplified phylogeny	mean=2.23 st. dev=0.64 μ=0.0027401

Table 3.4. Nucleotide (π) and haplotype (h) diversity for each species, per region

Species	***I. incertella***	***M. columbi***	***T. fumipennis***
For all regions			
Overall π	0.00501	0.00206	0.00489
Overall h	0.943	0.908	0.954
No. of haplotypes	35	21	35
Prairies			
π	0.00256	0.00172	0.00376
h	0.936	0.867	0.962
No. of haplotypes	14	9	11
Peace River			
π	0.00337	0.00131	0.0049
h	0.956	0.829	0.971
No. of haplotypes	14	7	18
Yukon			
π	0.00449	0.00183	0.00196
h	0.732	0.918	0.745
No. of haplotypes	8	10	8

Table 3.5. Number of populations (K) for each species, as estimated in Geneland. Highest posterior probability (post. prob) out of five runs is included, along with the population assignment of each region. All runs for each species revealed concordant results.

Species	**Estimated K**	**Highest Post. Prob**	**Population assignment**		
			Prairies	**Peace River**	**Yukon**
I. incerta	3	413.266	A	A	B
M. columbi	2	-235.485	A	A	B
T. fumipennis	2	223.818	A	A	B

Table 3.6. Results of AMOVA testing the structure outlined by Geneland. One population contained all Prairie and Peace River individuals, the other all the Yukon individuals. AP—among populations; AR/WP—among region within populations; WR—within region; Φ_{CT}—fixation index of covariance among defined populations.

	Variance components			**Percentage of variation**				
Species	**AP**	**AR/WP**	**WR**	**AP**	**AR/WP**	**WR**	$\mathbf{\Phi_{CT}}$	**p-value**
I. incerta	1.707	0.051	2.00	45.32	1.37	53.32	0.453	0.338
M. columbi	0.475	0.028	0.919	33.40	1.97	64.63	0.334	0.334
T. fumipennis	2.012	-0.027	1.950	51.14	-0.70	49.56	0.511	0.337

Table 3.7. Results from exact test of population differentiation for each species. Differentiation was examined for each pair of regions, as well as for the structure established by Geneland. Values in bold indicate significant p-values (p>0.05). P—Prairies, PR—Peace River region, YK––Yukon, SOUTH—includes the Prairies and Peace River.

	Non differentiation exact p-values			
Species	**P–PR**	**P–YK**	**PR–YK**	**SOUTH–YK**
I. incerta	1.000	**>0.0001**	**>0.0001**	**>0.0001**
M. columbi	0.11246	**>0.0001**	**0.00103**	**>0.0001**
T. fumipennis	1.000	**0.00571**	**0.00323**	**0.00151**

Table 3.8. M values (number of absolute migrants between regions per generation). Abbreviations as in Table 7.

Species	**P–PR**	**P–YK**	**PR–YK**
I. incerta	12.45565	0.51977	0.85066
M. columbi	13.53033	1.09700	0.84576
T. fumipennis	(infinite)	0.39007	0.49111

Figures

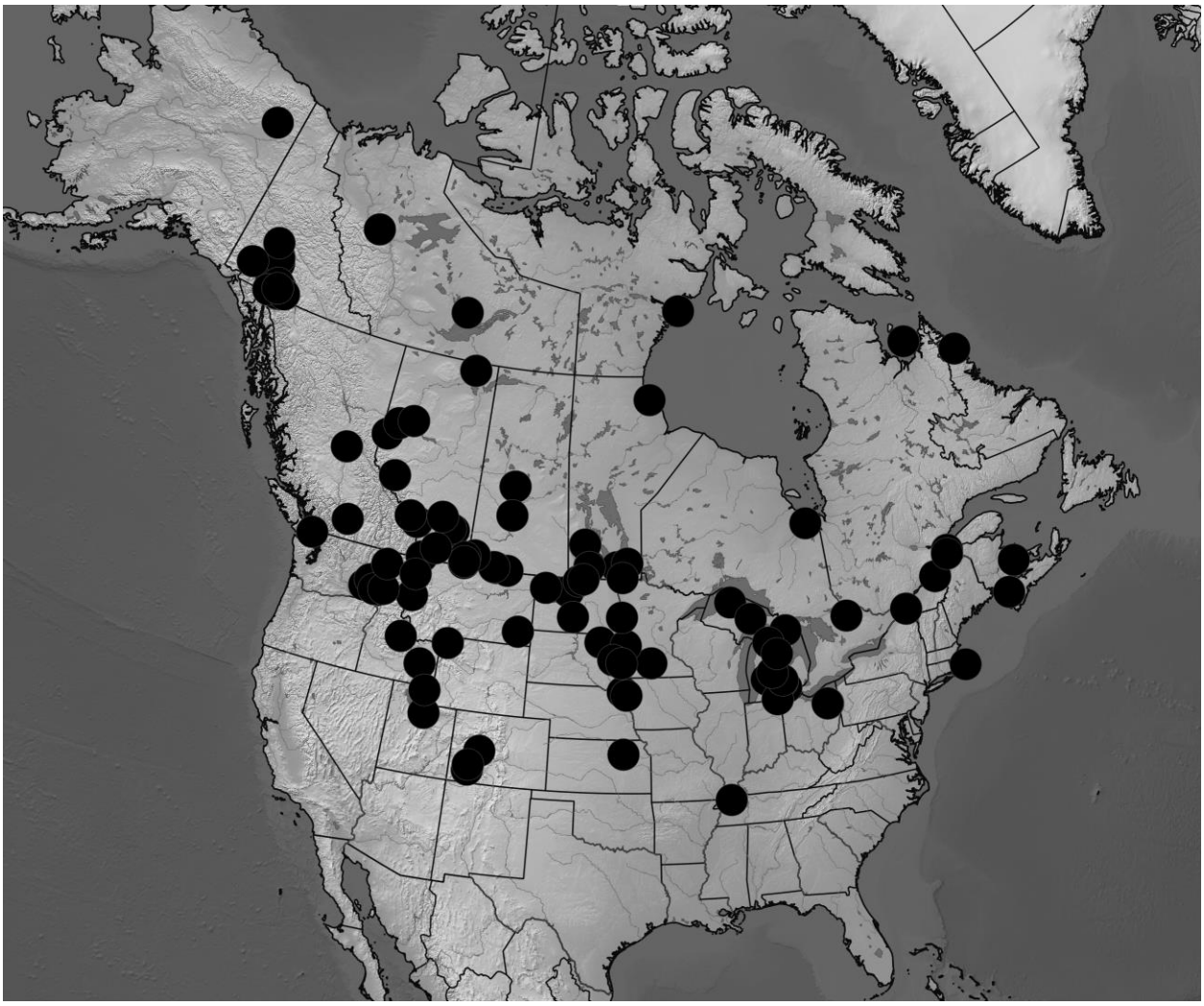

Figure 3.1. Distribution map of *Incertella incerta* (Becker). Each circle represents a record obtained from databases (Canadensys, www.canadensys.net; BOLD, www.boldsystems.org), the Lyman Entomological Museum (specimens not yet databased in Canadensys), the United States National Museum of Natural History and literature (Sabrosky, 1965; Coffee, 1966; Teskey, 1976; Boucher, 1998; Foote, 2004, 2007; MacLoed, 2013). Map projection: Lambert.

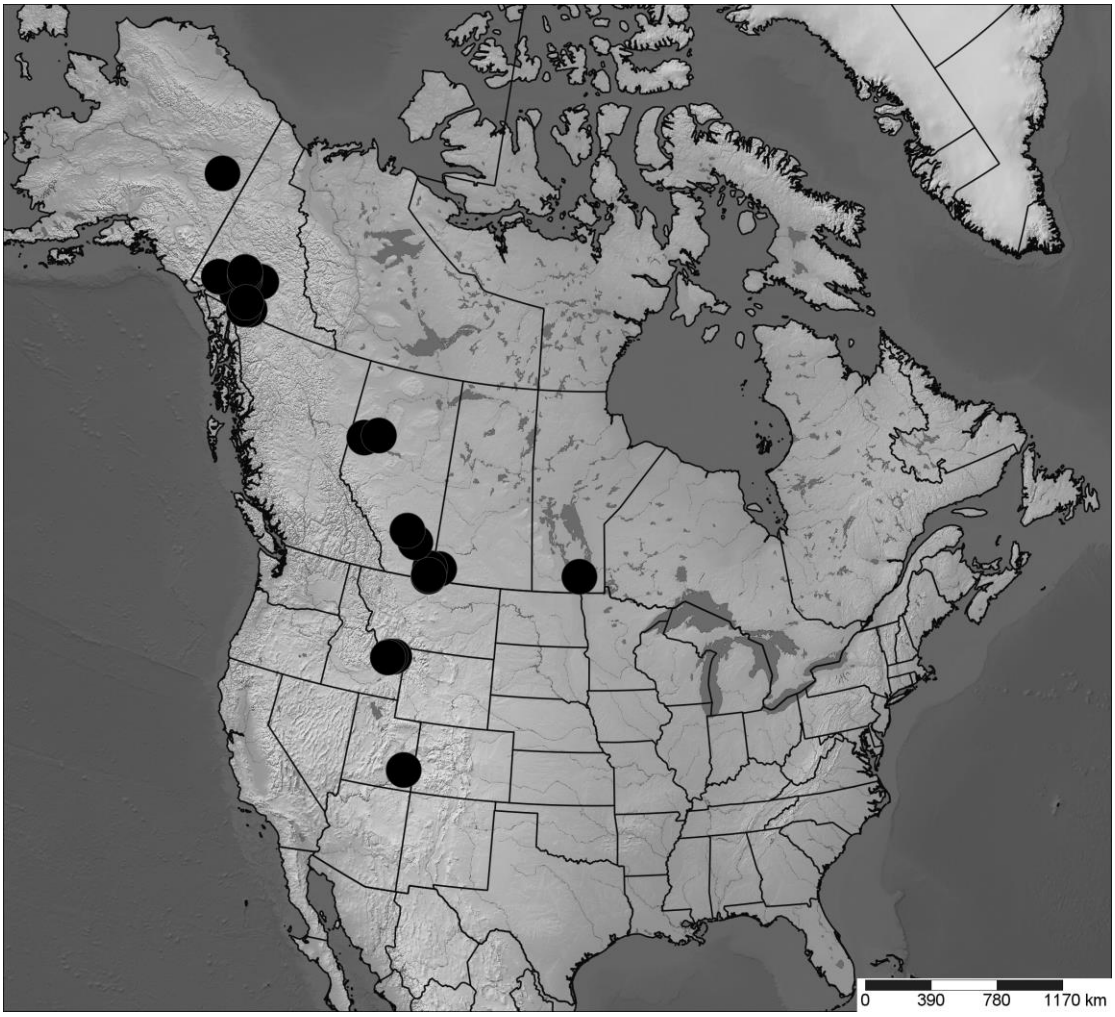

Figure 3.2. Distribution map of *Meromyza columbi* Fedoseeva. Each point represents a record obtained either from Canadensys (www.canadensys.net), BOLD (www.boldsystems.org), Lyman Entomological Museum specimens not yet databased in Canadensys or Fedoseeva (1971). Map projection: Lambert.

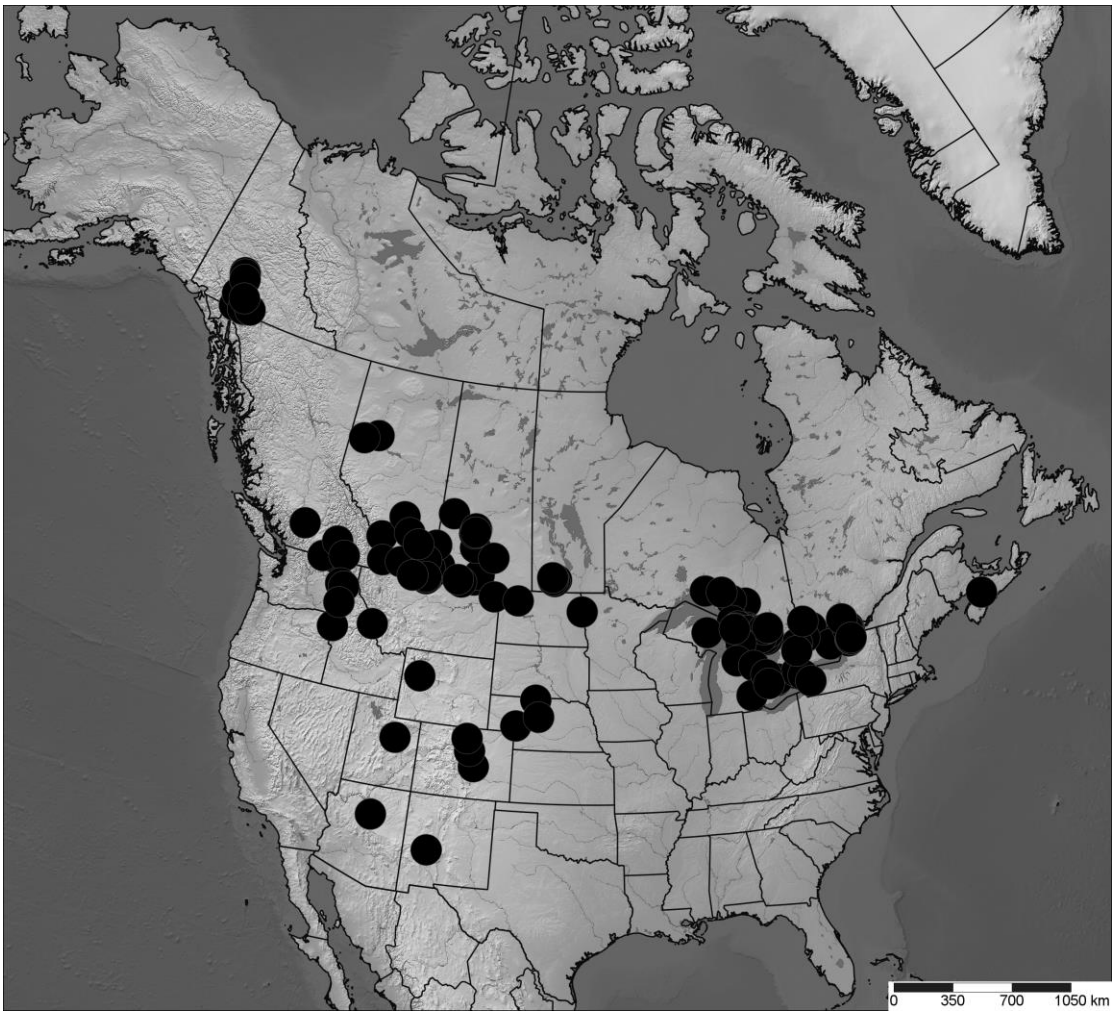

Figure 3.3. Distribution map of *Trixoscelis fumipennis* Melander. Each point represents a record obtained from Canadensys (www.canadensys.net), BOLD (www.boldsystems.org), Lyman Entomological Museum specimens not yet databased in Canadensys or Foster and Mathis (2011). Map projection: Lambert.

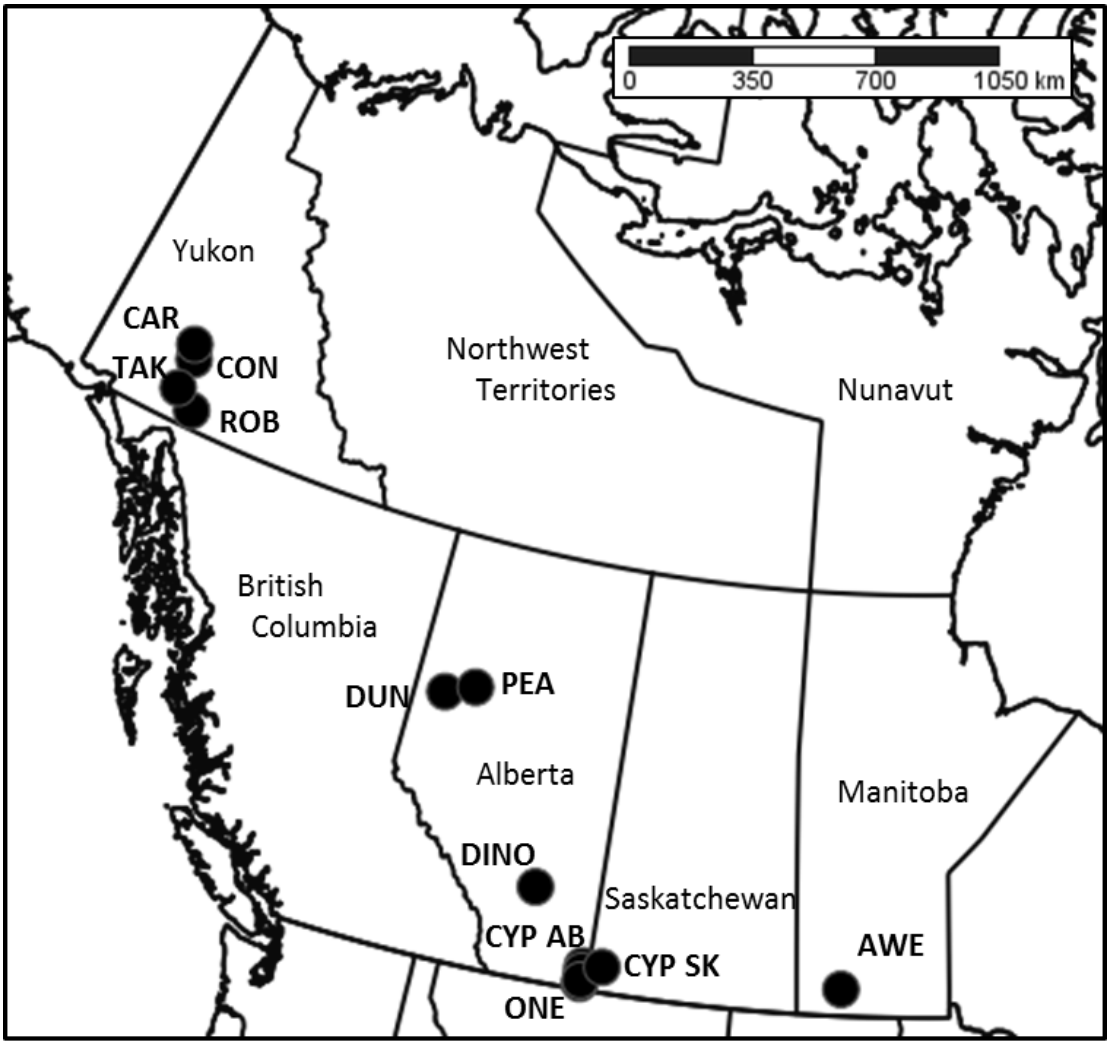

Figure 3.4. Map of sampling localities. Site codes: AWE – Aweme; CAR – Carmacks; CON – Conglomerate; CYP AB – Cypress Hills AB; CYP SK – Cypress Hills SK; DINO – Dinosaur; DUN – Dunvegan; ONE – Onefour; PEA– Peace; ROB – Robinson; TAK – Takhini

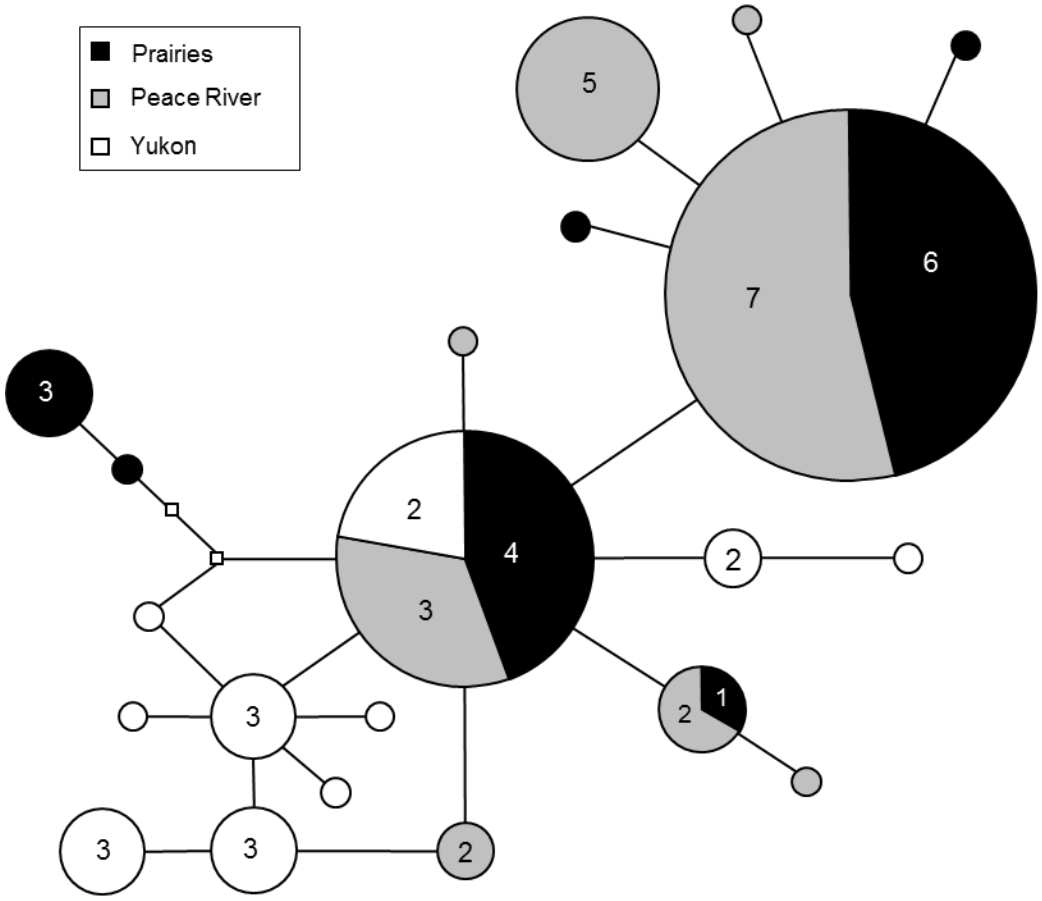

Figure 3.5. Haplotype network for *Meromyza columbi* Fedoseeva based on combined results from COI and Cyt *b***.** Each circle represents a single haplotype and small squares represent theoretical intermediates. Line lengths are arbitrary. Partitioned haplotypes represent haplotypes that are shared between regions. The number within each circle or partition represents the number of individuals with this particular haplotype when n>1. When there is no number within a circle, n=1.

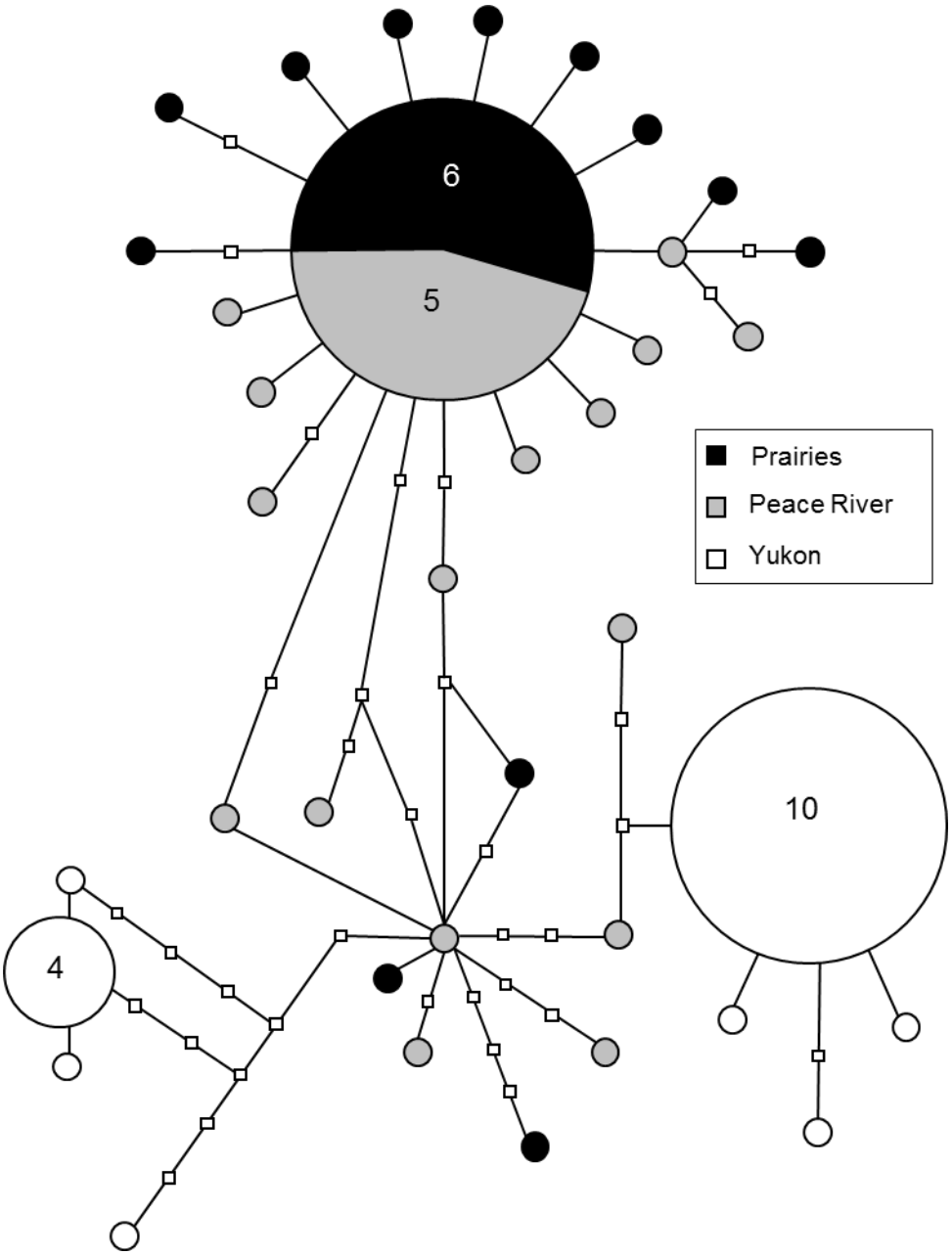

Figure 3.6. Haplotype network for *Incertella incerta* (Becker) based on combined results from COI and Cyt *b*. Explanation of symbols as in Fig. 3.5.

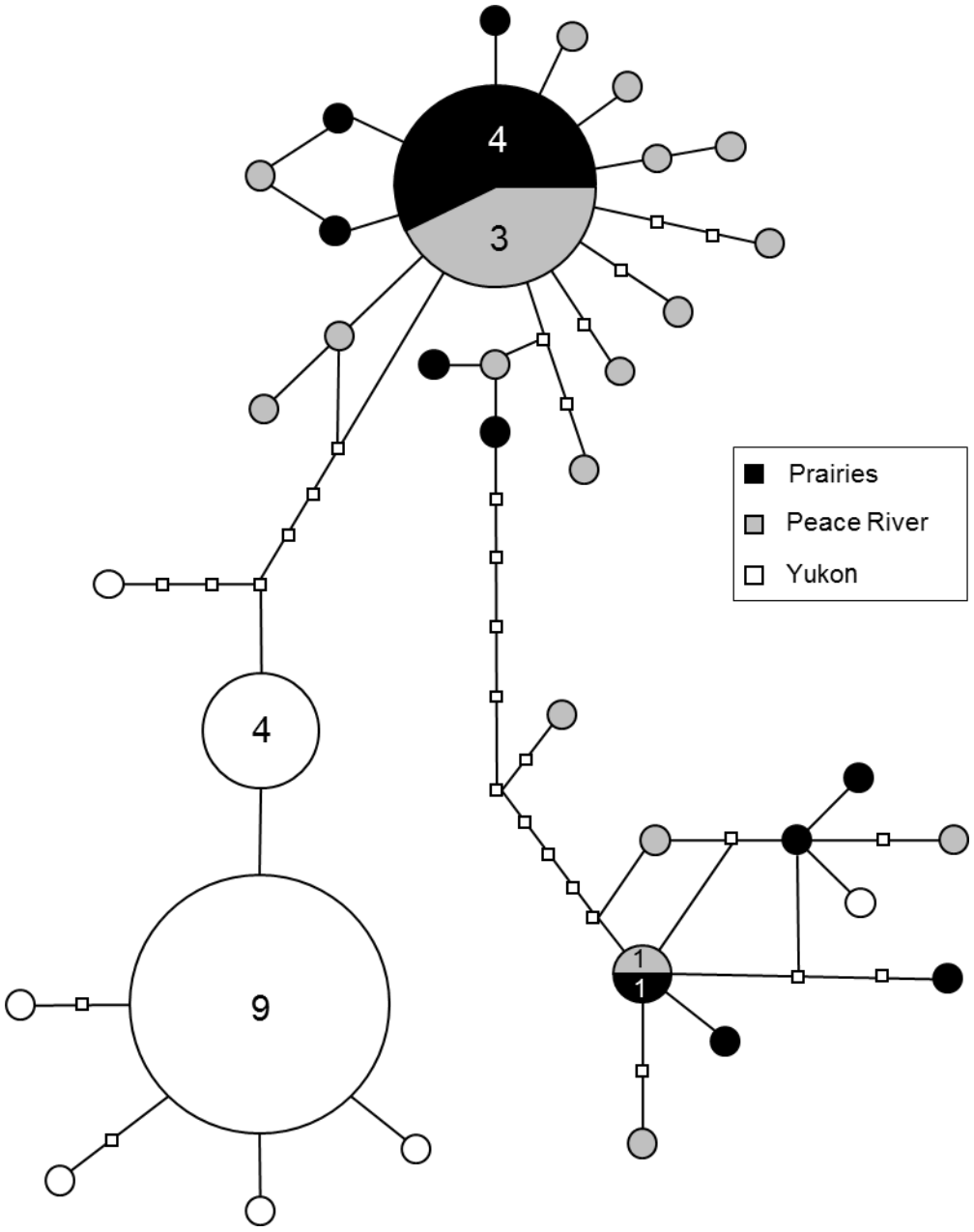

Figure 3.7. Haplotype network for *Trixoscelis fumipennis* Melander based on combined results from COI and Cyt *b*. Explanation of symbols as in Fig. 3.5.

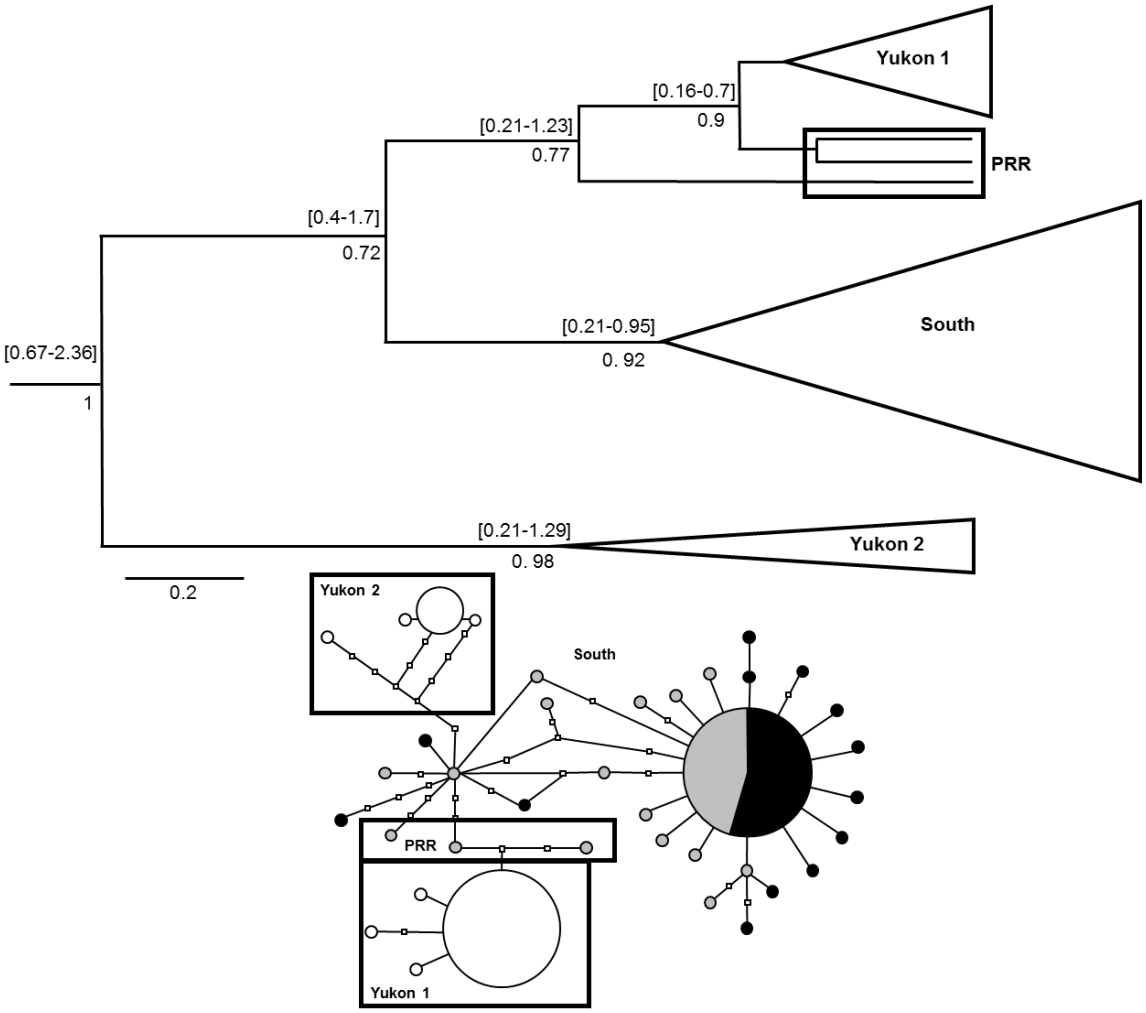

Figure 3.8. Maximum clade credibility tree of *Incertella incerta* (Becker) with posterior values of nodes below the branch connecting to it and 95% highest posterior density (HPD) interval of the age (in Myr) of the node above.

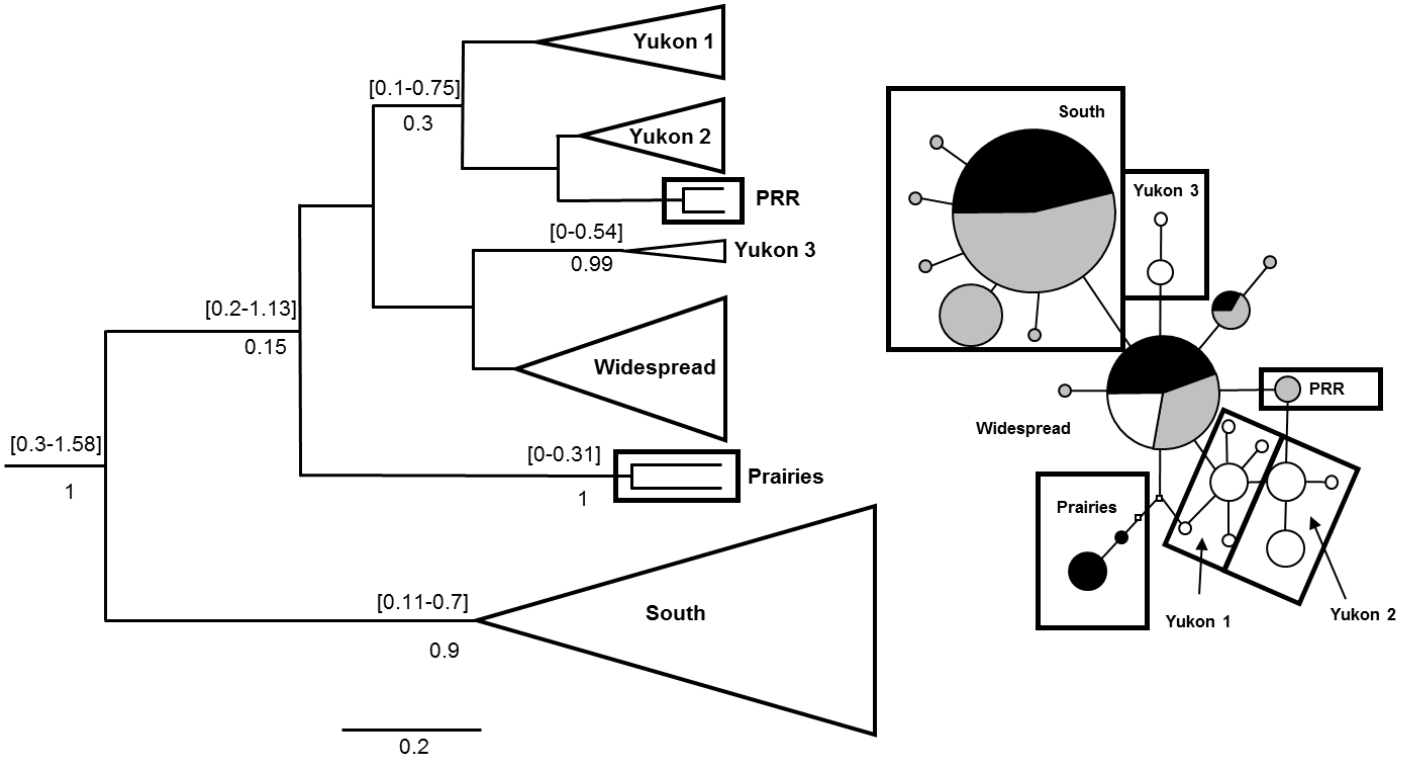

Figure 3.9. Maximum clade credibility tree of *Meromyza columbi* Fedoseeva with posterior values of nodes below the branch connecting to it and 95% highest posterior density (HPD) interval of the age (in Myr) of the node above.

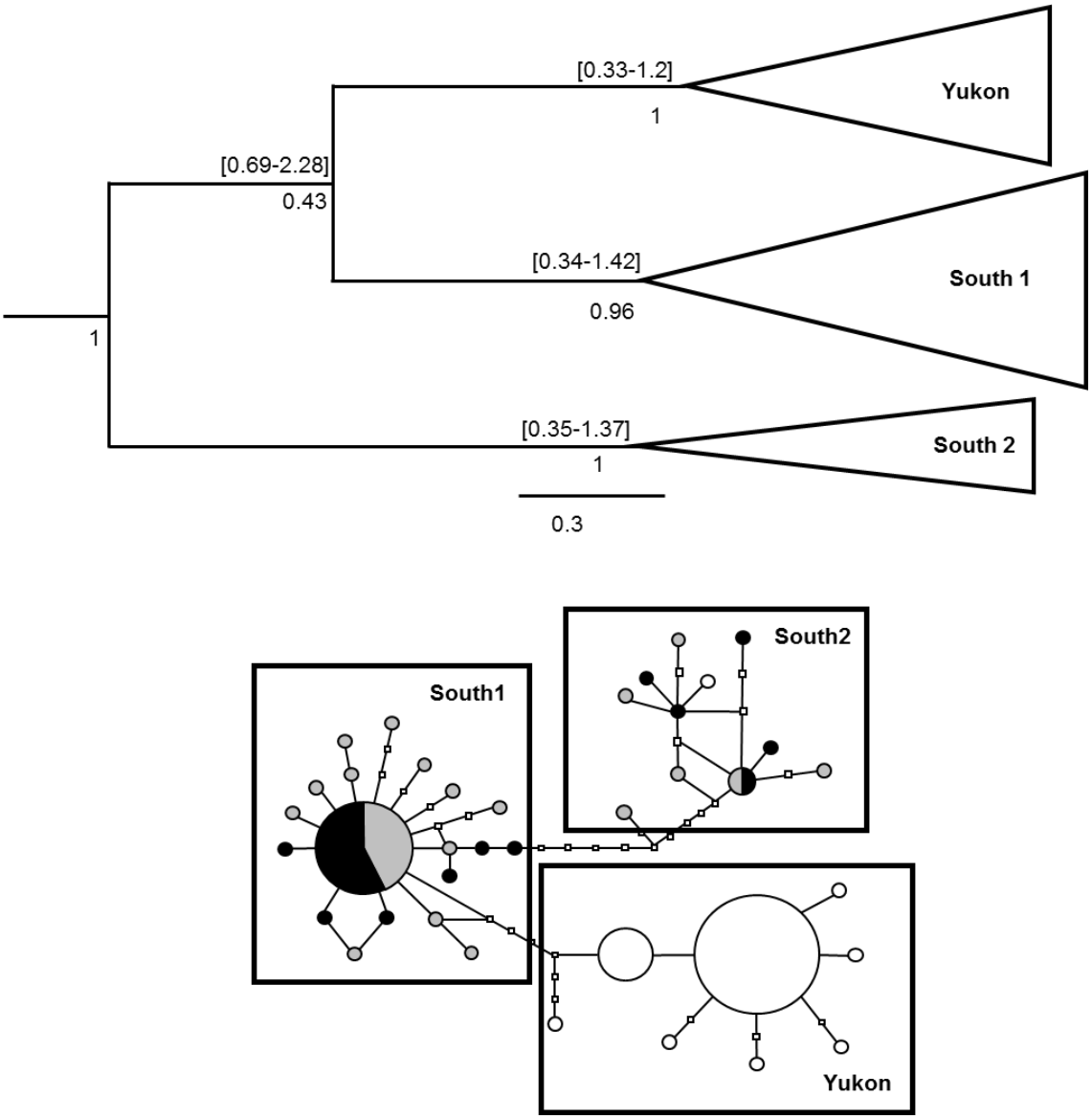

Figure 3.10. Maximum clade credibility tree of *Trixoscelis fumipennis* Melander with posterior values of nodes below the branch connecting to it and 95% highest posterior density (HPD) interval of the age (in Myr) of the node above.

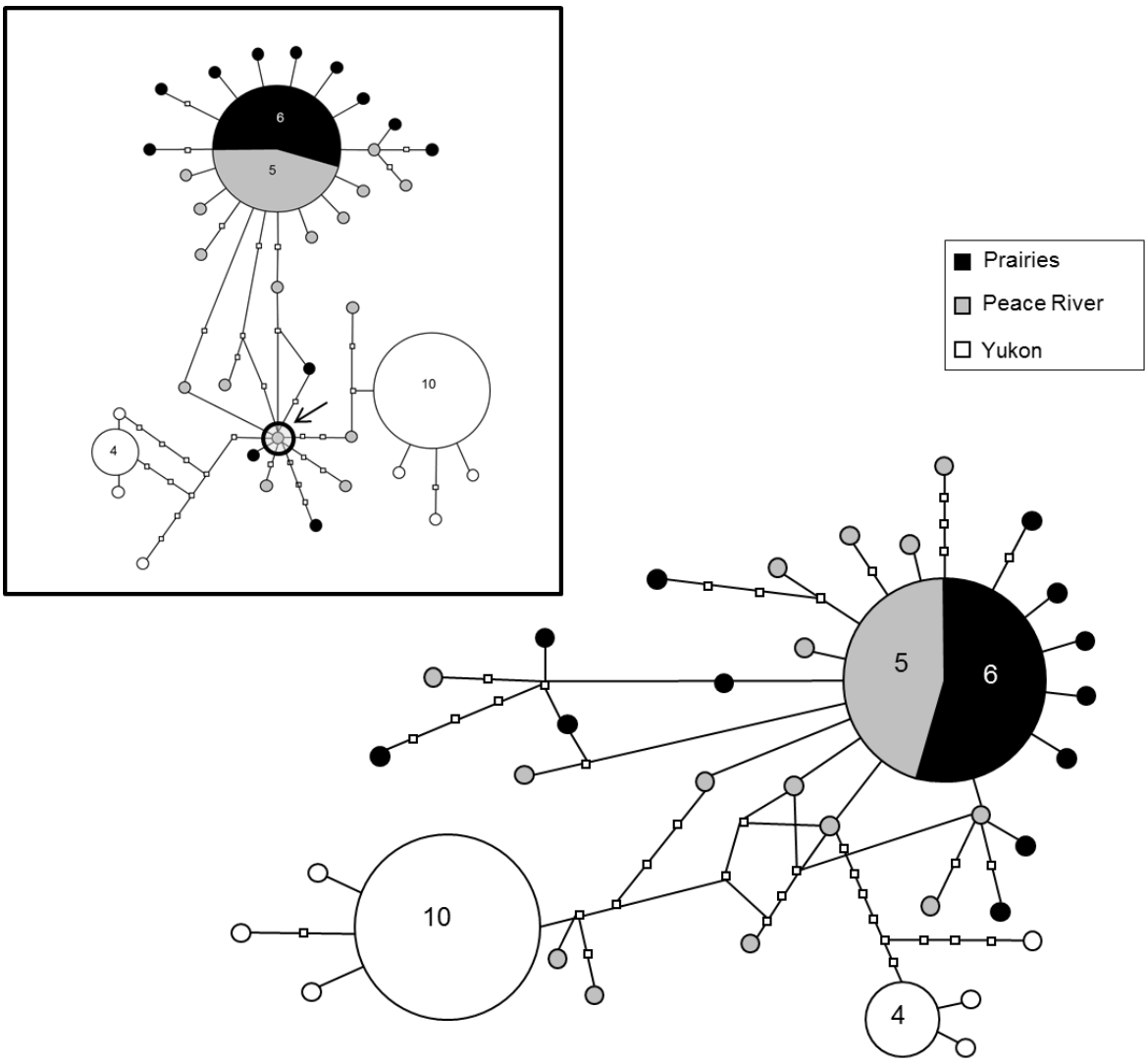

Figure 3.11. Alternate haplotype network for *Incertella incerta* (Becker). Explanation of symbols as in Fig. 3.5. One intermediate haplotype was removed from this network because it only contained information from Cyt *b*. Inset shows the haplotype network containing all information. The removed haplotype is circled and indicated with an arrow.

Appendices

Appendix 3.1. Additional taxa (with GenBank accession numbers) used for calculating divergence times.

Taxon represented	Family	Species	COI accession	Cytb accession
Chloropidae	Chloropidae	*Thaumatomyia notata*	KC192976	KC177599
Acalyptratae	Carnidae	*Hemeromyia anthracina*	FJ025644	FJ025740
Acalyptratae	Sphaeroceridae	*Copromyza* sp. JHK-2012	JX260391	JX887702
Acalyptratae	Sphaeroceridae	*Rachispoda* sp. JFG-2010	HM062544	HM062566
Acalyptratae	Drosophilidae	*Drosophila* sp. JFG-2010	HM062530	HM062555
Acalyptratae	Heleomyzidae	*Epistomyia* sp. JHK-2012	JX260392	JX887703
Cyclorrhapha	Phoridae	*Conicera dauci*	HM062538	HM062562
Cyclorrhapha	Lonchopteridae	*Lonchoptera tristis*	HM062534	HM062558
Cyclorrhapha	Platypezidae	*Platypeza* sp. JFG-2010	HM062540	HM062563
Cyclorrhapha	Syrphidae	*Toxomerus marginatus*	HM062546	HM062568
Schizophora	Muscidae	*Spilogona* sp. JFG-2010	HM062536	HM062560

Appendix 3.2. Number of sequences per species per location. Abbreviations as in Table 3.1.

Species	**Location**	**# sequences**	
		COI	**Cyt *b***
Incertella incerta	**Prairies**		
	Onefour	9	10
	Cypress Hills AB	6	6
	Cypress Hills SK	4	4
	Total	*19*	*20*
	Peace River Region		
	Dunvegan	17	19
	Yukon		
	Robinson	19	19
	Carmacks	1	1
	Total	*20*	*20*
Meromyza columbi	**Prairies**		
	Onefour	12	11
	Cypress Hills AB	2	2
	Dinosaur	4	4
	Total	*18*	*17*
	Peace River Region		
	Dunvegan	19	19
	Peace River	2	2
	Total	*21*	*21*
	Yukon		
	Carmacks	5	5
	Takhini	14	14
	Total	*19*	*19*

Trixoscelis fumipennis	**Prairies**		
	Aweme	2	4
	Cypress Hills AB	6	6
	Onefour	5	5
	Total	*13*	*15*
	Peace River Region		
	Dunvegan	12	12
	Peace River	9	9
	Total	*21*	*21*
	Yukon		
	Robinson	20	20

CONCLUSION

The arthropod communities of natural grasslands in Canada have not been extensively studied. Although the Diptera composition of the Yukon xeric grasslands was previously examined (Boucher, 1998), it was a faunistic study examining only the species present in the Yukon grasslands along with their distributions. Although it provided helpful baseline data, that study was not sufficient to determine the origins of the Diptera community. The endemic species present on south-facing slope grasslands suggested a Beringian origin, while species with disjunct distributions could have signified that these species had moved northward along with their associated flora when the climate warmed during the Hypsithermal. By examining the abundance and diversity of the family Chloropidae in the Yukon and comparing them to southern grasslands, we found that the Chloropidae community present in xeric grasslands in the Yukon is distinctly different from its southern counterparts, suggesting a Beringian origin.

Phylogeographic studies on grassland species in North America are likewise lacking. As mentioned in the previous chapter, there is only one study with a similar scope to ours which examines the phylogeography of butterflies in the Prairies and Peace River grasslands (Bromilow & Sperling, 2011). Our study is the first to look at the phylogeography of Diptera in Yukon grasslands. Intraspecific haplotype patterns showed that the Diptera taxa we examined survived the last glacial in at least two refugia, in the south and in Beringia. There was no suggestion of a recent southern expansion into the north for all species whether they had widespread or disjunct distributions. Calculating the divergence time between southern and northern populations reinforced the notion that the divergence between southern and Yukon groups pre-dated the end of the last glacial.

Much of the phylogeographic work with a Beringian scope has been done on arctic and arctic-alpine organisms (e.g., most organisms studied in Shafer *et al.* 2010). The Arctic is considered to be one of the systems most threatened by climate change. Grasslands in the Yukon are likewise threatened. A recent study of grasslands near Kluane Lake found a reduction in grassland extent due to forest encroachment, particularly on flat terrain and south-facing slopes (Conway & Danby, 2014). As well, some grassland sites which were sampled by Boucher (1998) have now been invaded by roadside weeds (pers. obs.). Although not as extensive as arctic systems, these grasslands represent a unique and important ecosystem. Many insect species, such

as the weevil *Connatichela artemisiae* (Anderson, 1997) and the grass fly *Conioscinella* n. sp. 3 (in Chapter 2) are endemic to eastern Beringian grasslands. Our study has shown that flies present on these south-facing slopes represent unique genetic lineages. The insect fauna of these grasslands may be as distinct and unique as the grasslands themselves.

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