Assessing Wild Lupine (*Lupinus perennis* L.) Habitat in Ontario, Canada, for the Feasibility of Reintroduction of the Karner Blue Butterfly (*Lycaeides samuelis* Nabokov)

by

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ABSTRACT

Assessing Wild Lupine (*Lupinus perennis* L.) Habitat in Ontario, Canada for the Feasibility of Karner Blue Butterfly (*Lycaeides samuelis* Nabokov) Reintroduction

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Degradation of wild lupine (*Lupinus perennis* L.) habitat led to the extirpation of the Karner blue butterfly (*Lycaeides samuelis* Nabokov) from Ontario, Canada. Lupine habitats in Ontario were evaluated in 2013 during the two estimated flight periods of the Karner blue for their suitability of reintroduction. Specific habitat characteristics were quantified and compared to literature values for these characteristics at extant Karner blue sites in Michigan and New York, USA. While lupine densities, nectar source densities for the first and second broods, shade heterogeneity, ant richness, and climate at some Ontario sites are comparable to Karner blue sites in the USA, the largest individual lupine population in Ontario just exceeds 19,000 stems, far less than the number required in multiple lupine subpopulations to sustain a minimum viable Karner blue population. Extensive lupine planting at and around existing sites is necessary prior to attempting reintroduction of the Karner blue.

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Introduction

The lack of suitable habitat is the single most important factor limiting the success of reintroductions of animals and plants to areas where they once lived but have been extirpated (Kleiman 1989). Land intended for the introduction or reintroduction of a species must be protected from degeneration and exploitation, and must be actively maintained and restored if necessary (Kleiman 1989). Through habitat assessment, shortcomings in a habitat can be identified and targeted with management practices. In the case of reintroductions, the causes of the initial decline of the species also need to be addressed and either controlled or eliminated prior to any attempt to return that species to its former environment (Kleiman 1989).

The management and restoration of wild lupine (*Lupinus perennis* L.; hereafter referred to as "lupine") habitat in Ontario is underway with the intent to recreate suitable habitat for the Karner blue butterfly (*Lycaeides samuelis* Nabokov). Lupine is a xerophytic, herbaceous, perennial Fabaceae (Boyonoski 1992). The decline of the Karner blue butterfly throughout its range over the past 150 years is largely attributed to the loss of lupine (Haack 1993; COSEWIC 2000; Forister et al. 2011). Historically, Ontario contained 5% of the habitat in the global range of the Karner blue, a species that has not been seen in the wild in Canada since 1991 and was listed by COSEWIC (2000) as extirpated in Canada in 1997. Lupine occurs in savannahs, barrens, sand dunes, and similar ecosystems with sandy soils where the tree canopy is incomplete (Boyonoski 1992; Peterson and Reich 2001; Carson 2006; Corry et al. 2008). These are often transition zones between grasslands and woodlands. Lupines are dependent on regular disturbance by fire to maintain early successional stages (Corry et al. 2008). Habitat fragmentation, land clearing for agriculture, construction by humans, and alterations to the fire regime have led to the degeneration of much of North America's savannah ecosystems, including lupine habitat (Peterson and Reich 2001; USFWS 2003).

Historically, the Karner blue could be found in 15 states across the northeastern USA, ranging from Minnesota in the west to Maine in the east as well as southern Ontario (Dirig 1994). It is currently extirpated from nine of the 15 states where it was historically found as well as southern Ontario (Hess 2013). The population in Ohio became extirpated, however it was reintroduced by captive-rearing eggs laid by mated females taken from Michigan, and releasing the resulting adults into managed sites (Candee Elsworth pers. com.). The Ohio population continues to be supplemented regularly through this method. The population in Indiana has recently been reduced to only a few individuals and is a high risk for extirpation (Hess 2013).

The Karner blue overwinters as eggs on the stems of lupine and grasses (COSEWIC 2000). Eggs of the first generation begin to hatch in mid April and larvae feed on cuticle tissue of lupine leaves, leaving behind distinct "feeding windows" in the leaf tissue (COSEWIC 2000; Corry et al. 2008). Larvae progress through five instars over the course of 18-21 days, after which they pupate either within the leaf litter or on lupine (Packer 1990; COSEWIC 2000). Eight days after pupation the adults emerge, beginning in late May (COSEWIC 2000; Corry et al. 2008). Adults nectar at flowers, mate, and females oviposit, generally within three to five days of eclosion (COSWIC 2000; Guiney and Andow 2009). Adults of the second generation begin to eclose in mid to late July and their flight period lasts approximately four weeks (COSEWIC 2000; Corry et al. 2008). Eggs laid by second generation females enter diapause and do not hatch until the following April. The second generation is typically larger than the first, as lupine is more numerous and leaves are larger in June than in early spring, which provides greater larval food and results in higher survival (Schweitzer 1989).

The Karner blue has undergone a number of taxonomic reclassifications since its initial description by Edwards as *Lycaeides scuderri* in 1861. Nabokov reclassified it in 1943 as *Lycaeides melissa samuelis,* a subspecies of the Melissa blue (*Lycaeides melissa* Edwards). It can still be found in the literature under this name, as well as *Plebejus melissa samuelis*. More recently, Forister et al. (2011) compared gene flow between three taxa of butterflies in the genus *Lycaeides*: the Melissa blue, the Karner blue, and the northern blue (*Lycaeides idas* L.). They found similarly low gene flow between (i) the Karner blue and the Melissa blue and (ii) the Melissa blue and the northern blue. Forister et al. (2011) thus concluded the Karner blue should be recognized as a distinct species, which is how I will refer to it: *Lycaeides samuelis* Nabokov.

Eight habitat variables have been identified that affect the suitability of lupine habitat for the Karner blue. The first two variables involve lupine itself. Lupine plants are the sole larval host plant for both the first and second broods of the Karner blue, and lupine population size and density are key factors in the suitability of Ontario sites (Grundel et al. 1998; Chan and Packer 2006). Numerous flowering plant species are used as nectar sources by Karner blue butterflies, and their densities during the two flight periods of the adult broods are the third and fourth variables (Chan 2004). The fifth variable is shade heterogeneity, as measured by overhead tree canopy cover (Lane 1994; Herms 1996). Shade heterogeneity affects oviposition, larval survival, host plant quality and survival, adult behaviour, and mating success (Grundel et al. 1998; Lane 1999;

Pfitsch and Williams 2009). The sixth variable is the presence or absence of larval-tending ant (Hymenoptera; Formicidae) species. Like most members of the family Lycaenidae, Karner blue larvae have mutualistic relationships with ants (Haack 1993; Savignano 1994; Fraser et al. 2001; Pierce et al. 2002). Ants of a number of species increase survival of Karner blue larvae by protecting them from predation and parasitism in exchange for a larval secretion that is high in sugars and amino acids (Haack 1993). The seventh variable is climate. Climatic factors in current and historic Karner blue localities will provide a baseline for evaluating habitat suitability in Ontario. The final variable is the ability of each lupine site in Ontario to sustain a Karner blue metapopulation. The Karner blue, like several other lycaenid species, occurs in multiple subpopulations that collectively comprise metapopulations (Hanski 1998; Fuller 2008). Extinction and recolonization events occur over time in the subpopulations while the metapopulation as a whole remains relatively stable (Carson 2006; Corry et al. 2008; Fuller 2008). This results in a shifting mosaic dynamic with asynchronous fluctuations in populations (Levins 1970; Harrison et al. 1988).

Three main approaches have been developed and applied to lupine habitat assessment over the last several decades: field assessments, modeling, and literature review. Because these approaches are very different, it is unclear what constitutes acceptable Karner blue habitat. The fieldwork approach was used by Herms (1996), Tolson (1997), and Chan and Packer (2006). Herms (1996) conducted lupine habitat assessment at the Allegan State Game Reserve near Allegan, Michigan, where the Karner blue persists today. Tolson (1997) used Herms' (1996) methodology when evaluating lupine habitat near Toledo, Ohio, which eventually led to the reintroduction of the Karner blue butterfly to Kitty Todd Preserve. Lupine habitats in Ontario were evaluated during the summers of 2002 and 2003 by Chan and Packer (2006). Although they determined that none of the five lupine sites evaluated could sustain a reintroduced Karner blue population, they produced minimum standards for habitat quality that were adopted by the United States Fish and Wildlife Service (USFWS 2012). The modeling approach was used by Fuller (2008) to determine the values of habitat qualities needed to maintain a minimum viable population of the Karner blue. The literature review approach was used by Bried (2009), who compiled criteria for habitat qualities from Grundel et al. (1998), Lane and Andow (2003), USFWS (2003), Forrester et al. (2005), Fuller (2008) and several others to create the management plan currently being used to maintain and enhance Karner blue habitat near Saratoga Springs, New York. These various methodologies have produced a range of acceptable and desirable values for habitat variables, complicating the discussion of what constitutes suitable habitat for the Karner blue. In my research I strived to increase the transparency and repeatability of methodologies so future habitat assessments can be more readily compared.

I quantified habitat characteristics at restored and remnant lupine populations in Ontario to determine their suitability for Karner blue butterfly reintroduction. I sought to determine how these habitat variables have changed over the decade since the last assessment was conducted by Chan and Packer (2006). However, in light of some of the more recent research (e.g. Fuller 2008; Bried 2009), it became apparent that I needed to broaden my assessments beyond the minimum ecological requirements produced by Chan and Packer (2006) for the Karner blue. The methodology used by Chan and Packer (2006) had some potential biases: their transects to quantify the density of lupine plants were established in areas of observably high lupine density and the quantification of nectar plant density included unopened flowers (Chan 2004). Consequently, I adopted the methods developed by Herms (1996). Herms' (1996) work provided realistic minimum ecological requirements of the Karner blue to which I compared my results. General comparisons are made to Fuller's (2008) and Bried's (2009) analyses, since our differing methodologies do not allow direct statistical comparisons.

The goal of this project is to determine whether or not the lupine habitats of Ontario are of sufficient quality to warrant the reintroduction of the Karner blue butterfly. In order to accomplish this goal, two objectives must be met:

i. Quantify the habitat characteristics at sites with remnant or restored lupine habitat;
ii. Determine whether sites in Ontario meet the minimum ecological requirements of the Karner blue butterfly based on values determined by previous researchers.

This research has the potential to benefit other species dependent on lupine habitat, as the information generated here will also be used to identify management practices to improve the overall quality of lupine habitat.

Methods

Sites Evaluated

Lupine sites in Ontario occur in oak savannah ecosystems where *Quercus* spp. dominate the overstory. The five primary sites were St. Williams Conservation Reserve (SWCR; latitude 42.700, longitude -80.466), the Karner Blue Sanctuary (KBS; 43.223, -81.887), Pinery Provincial Park (PPP; 43.248, -81.822), Alderville Black Oak Savannah

(ABOS; 43.248, -81.822) and High Park (HP; 43.652, -79.465) (Fig. 1). Chan and Packer (2006) assessed these sites during the summers of 2002 and 2003, and found them all to be unsuitable for various reasons. Each primary site received a full evaluation of the eight habitat variables introduced above. SWCR, KBS, PPP and HP historically supported the Karner blue butterfly (Carson 2006; Chan and Packer 2006). It is likely that savannahs surrounding ABOS historically supported the Karner blue, but the only specimens collected by Bethune (1895), now missing, were mistakenly identified as the northern blue butterfly (Catling and Brownell 2000).

SWCR is in Norfolk County near the northern shore of Lake Erie. The Manestar Tract within SWCR has a population of lupine that was evaluated by Chan and Packer (2006). Norfolk County is a large area with dozens of blocks of land that could be converted to savannah habitat scattered throughout it. Some of these land parcels are owned privately, while some are owned by the Nature Conservancy of Canada, and by the Ontario Ministry of Natural Resources (OMNR). Recent activities in this area have been undertaken to create habitat that may be suitable for the Karner blue including clearing forests and planting lupine and other endemic species.

KBS and PPP are separated by only a few kilometers in Lambton Shores on the southern shore of Lake Huron. These remnant sites were once part of much larger contiguous prairie and savannah habitat that extended southward to the northern shore of Lake Erie, but their area has been drastically reduced by agricultural and industrial development. PPP contains the largest remaining oak savannah in Ontario.

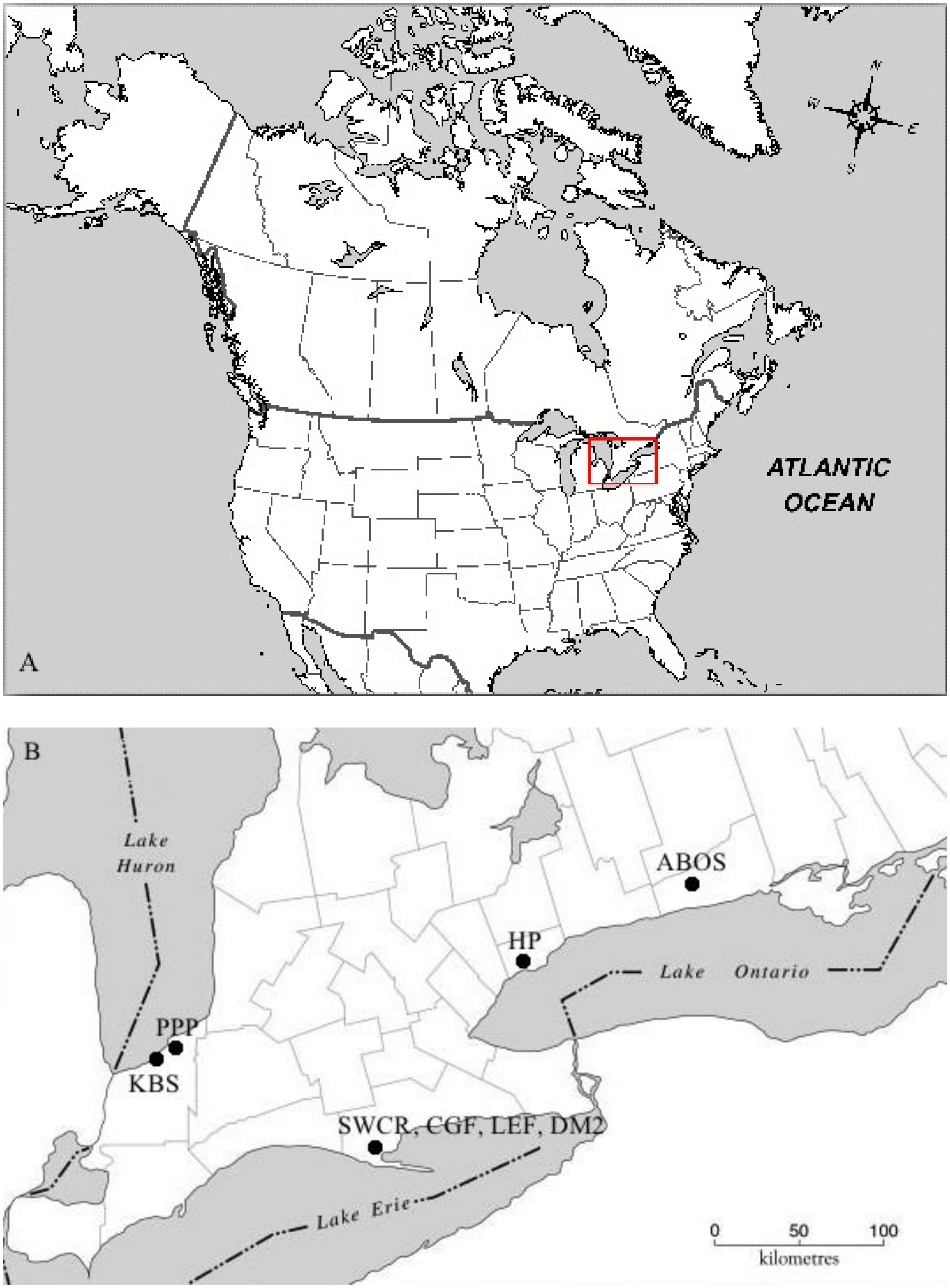

Figure 1. A. Location of the study area in North America. B. Locations of the five primary lupine habitats (SWCR, KBS, PPP, ABOS, and HP) and three secondary habitats (CGF, LEF, and DM2) evaluated in southern Ontario.

ABOS is privately protected land near the southern shore of Rice Lake in Northumberland County. Lupine has been actively planted here since 2001 in an effort to create habitat for the Karner blue butterfly and conserve native savannah species. More recently, the Nature Conservancy of Canada has begun restoring additional blocks of land in the county to increase the amount of savannah habitat.

HP is a heavily trafficked urban park in Toronto on the northern shore of Lake Ontario. Approximately one third of its total area is oak savannah. It is now isolated by the city of Toronto and surrounding urban areas from other prairie and savannah sites in southern Ontario.

I evaluated each site twice, once between 24 May and 13 June 2013, and then again between 29 July and 8 August 2013, to coincide with the known flight periods of the first and second broods of Karner blue adults (Haack 1993; Carson 2006; Pickens 2007). Detailed descriptions of the five primary study sites can be found in Appendix 1. I took high-resolution multi-gigapixel 360° panoramic pictures of each site with a Canon G12 digital camera in conjunction with a GigaPan EPIC 100 mount; internet links to these photographs can also be found in Appendix 1.

Three secondary sites in Norfolk County were also visited: the Carson/Gartshore Farm (GCF; 42.642, -80.575), Lake Erie Farm (LEF; 42.657, -80.573), and DeMaere 2 (DM2; 42.687, -80.466) (Fig. 1). CGF is a privately owned property, while the Nature Conservancy of Canada owns LEF and DM2. I visited these sites on 26 June and 4 July, 2014. These sites are not being managed specifically for lupine, but lupine has been planted on each. Secondary sites received an evaluation of the total lupine population, as it is the most limiting factor affecting habitat suitability for the Karner blue (Corry et al. 2008).

Sites overlay Devonian bedrock in the west and Ordovician bedrock in the east, and are composed mainly of grey-brown luvisol soil (Szeicz and MacDonald 1990; Baldwin et al. 2011). The savannahs across Ontario were formed by the retreat of the Wisconsin glacier between 13,000 and 12,000 years ago (Szeicz and MacDonald 1990; Faber-Langendoen and Maycock 1994). The glacial retreat left behind sediment deposits as outwash and ground moraines of 150-200 m elevations (Faber-Langendoen and Maycock 1994; Baldwin et al. 2011).

These five primary sites and three secondary sites likely represent all extant lupine populations in Ontario. Historically, lupine also occurred at the Niagara Peninsula, the Galt region, London, and Leamington, but these populations are now extirpated (Boyonoski 1992). The entomology collection at Cornell University (Ithaca, New York, USA) contains a specimen of the Karner blue collected by August Schmidt, labeled “Hagersville, Ontario; 12 August 1978”, however there is no recorded lupine population in that area.

Lupinus perennis Abundance and Density

Site visitation order was SWCR, KBS, PPP, ABOS, and HP; the visitation period for each site coincided with the peak flowering time of lupine at that site during the predicted flight period of the first generation of Karner blue adults. I manually counted all lupine stems at each site to determine total population sizes. During the spring site assessment I used transect-quadrat methods to evaluate lupine densities (Bonham 1989).

Transects were established at a density of one per 850 m^2 of lupine habitat, the same density employed by Herms (1996), to standardize sampling effort between sites. All transects were 25 m long and oriented north-south. Six 1 m^2 quadrats were placed along each transect at 0, 5, 10, 15, 20, and 25 m. I employed two different methods of transect placement: the method used by Chan (2004), and the method used by Herms (1996). The Chan (2004) method involved placing transects in areas where I observed relatively high densities of lupine. With the method adapted from Herms (1996), I randomly selected a starting point for the first transect within the discrete area containing lupine, and the remaining transects were placed systematically 10 m away from the starting point of the previous transect. Using the Chan (2004) method provided insight into how the lupine density at each site had changed in the decade since these sites were last evaluated, although it provided an inflated estimate of lupine density over the entire site because the biased method of placement ensured all transects intersected a lupine patch. Using the Herms (1996) method allowed for direct comparisons between habitat qualities at lupine sites in Ontario and habitats with Karner blue populations in Michigan. Regardless of the transect placement method, I counted all lupine stems emerging from the ground within each quadrat along a given transect irrespective of their floral development. Lupine density for each transect was then estimated by averaging the number of lupine stems per six 1 m^2 quadrats per transect (Herms 1996).

Nectar Source Plant Density

During each of the two estimated flight periods of the Karner blue (late May-early June, mid July-mid August), I measured the density of nectar source plant species at each site in the same quadrats used to estimate the density of lupine using the Chan (2004) method of transect placement. Haack (1993) and Chan (2004) have listed known nectar source species for the Karner blue. These lists were used to decide which floral species I encountered should be counted. Within each quadrat I recorded all individuals of those species with open flowers, as well as the total number of flowering plants. Unopened flowers and those that had already senesced were not counted, as they would not provide nectar during the estimated Karner blue flight period. Flowering plant density for each transect was then estimated by averaging the number of known nectar source plants per six 1 m^2 quadrats per transect (Herms 1996).

Shade Heterogeneity/Canopy Cover

During the second visit to each site that corresponded to the estimated flight period for the second brood of Karner blue adults, I quantified the overhead tree canopy cover to determine shade heterogeneity. Variation in tree canopy and thus light infiltration creates variations in microclimate resulting in a heterogeneous habitat, which has been shown to benefit the Karner blue (Lane 1994; Grundel et al. 1998; Chan and Packer 2006). The same quadrats I used to determine density of lupine and nectar source plants using the Chan (2004) method of transect placement were used for quantifying heterogeneity. Within each quadrat I measured the overhead leaf area index (LAI) using a LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, Nebraska, U.S.). LAI is a measurement of the total area of leaf tissue (m^2) produced by the tree canopy divided by the surface area (m^2) of the ground directly below, resulting in a dimensionless quantity. I also took one measurement in a location lacking canopy cover before walking each transect in order to calibrate the LAI-2000 to the current weather conditions. The LAI-2000 internally combined the individual measurements taken from the six quadrats along a transect to yield a single composite value for that transect. These LAI values were converted to percent canopy cover on a per-transect basis (see Statistics and Analyses below).

Larval-Tending Ants

I surveyed the species richness of ants at each of the five sites using a modification of the Ants of the Leaf Litter Protocol from Agosti and Alonso (2000). Through a combination of baited traps, pitfall traps, litter sifting, Winkler-extraction and direct hand-sampling at each site, I endeavored to sample as many ecological niches occupied by different ant species as possible. I established a 50 m transect at each site in an area where lupine was present. At 10 m intervals along the transect, pitfall traps (50 mL polypropylene centrifuge tubes, Fisher Scientific, Hampton) containing 5 mL of 95% ethanol were inserted flush to the ground for a collection period of five hours. Near each pitfall trap (10 cm away on opposite sides) I placed a pair of 14 cm diameter Petri dishes, one containing 28.3 g of canned tuna packed in water and the other a Pecan Sandie™ cookie. I checked baited traps once an hour for five hours and caught representative ants using forceps, keeping those obtained from each trap separate. I also gathered litter from two 1 m^2 areas near lupine patches, sifted it, and collected all ants from the siftate. I collected additional samples of litter from five 0.25 m^2 areas and placed them in Winker extraction mesh bags. The bags were hung for three days, during which time the ants within the litter fell into 355 mL bottles containing 15 mL of ethanol. Finally, my assistant and I spent one hour at each site turning over rocks, breaking fallen tree debris, and collecting any ants found using aspirators and forceps. I transferred all ants caught into labeled 20 mL glass scintillation vials containing 5 mL of 95% ethanol for long-term storage, while maintaining separation based on collection method.

I subsequently sorted ant specimens to genus and morphospecies using Ellison et al. (2012). I then point-mounted and photographed one to six representative specimens of each morphospecies using a Leica M205A microscope in concert with Leica Application Suite (Version 4.3.0, Leica Microsystems Ltd., Switzerland, 2003-2013). The number of ants point-mounted and photographed reflected the quantity of specimens available, and the number of sample wells in a single DNA extraction tray.

In order to verify preliminary identifications, I removed one leg from each point-mounted ant and submitted the samples to the Biodiversity Institute of Ontario where the "DNA barcode" region of the cytochrome *c* oxidase I (COI) mitochondrial DNA gene was sequenced according to Smith et al. (2014). Full results of DNA-barcoding of specimens (taxa and trace files), as well as photographs of each ant can be found in the Barcode of Life Data System (BOLD; www.barcodinglife.org; dx.doi.org/10.5883/DS-ASKBB1). DNA barcoding provided a more accurate species-level identification than manual identification based on morphology alone as well as a permanent database for future comparisons. The sequence of the COI mitochondrial DNA gene was used to embed each ant within a phylogenetic tree of other sequenced ants in BOLD (Ratnasingham and Hebert 2007). The most likely species each specimen belonged to was inferred from genetic distances. A similarity of 98% or higher to existing sequences in BOLD was considered sufficient for species-level identification. An ant identified to species was then assigned the Barcode Index Number (BIN) for that species. BINs cluster operational taxonomic units together so all barcoded members of a species can be grouped with a single identifier (Ratnasingham and Hebert 2007). Species were designated as “larval-tending ants” based on literature syntheses from Haack (1993), Herms (1996), and Lane (1999) of ant species known to larvae of the Karner blue (Appendix 2). I reported the presence or absence of an ant species for each site, as the collection methods I used were not suitable for estimation of species abundances.

Climate Analysis of Sites Known to have Supported the Karner Blue

As I cannot confirm that the potential reintroduction site ABOS in Ontario was a historic home of the Karner blue, I analyzed the climate of ABOS along with other current and historical sites for the Karner blue in Ontario and the USA. Through the use of primary literature, including Wheeler (1991), Dirig (1994), Smallidge et al. (1996) Herms (1997), Tolson (1997), Grundel et al. (2000), Smith et al. (2002), Chan and Packer (2006), Swengel and Swengel (2007), Guiney and Andow (2009), Hess (2013), and Swengel and Swengel (2014), I identified 59 current or former Karner blue sites. When only a location on a map was available, it was located as precisely as possible on Google Maps to determine GPS coordinates with a 13.1 km margin of error. I then determined the values for 19 climatic variables using the WorldClim data set from Hijmans et al. (2005) for all of these localities (Appendix 3) These climatic variables are the means or measures of variability of precipitation or temperature at different times across a year, collected from 1950-2000 at a resolution of 30 arc-seconds (Hijmans et al. 2005).

Statistics and Analyses

General comparisons of the total lupine population at each site were made to the results of Fuller's (2008) model, which outlines habitat characteristics required to maintain a minimum viable population of the Karner blue.

One-sample Wilcoxon tests were used to compare lupine as well as spring and summer nectar source plant densities at each site to values for these same variables obtained from Chan and Packer (2006) and Herms (1996). Nonparametric tests were used as the data violated the normality assumption of parametric tests when a Shapio-Wilk test was performed. The Dunn-Šidák correction for multiple tests was used to account for the increased probability of a type I error resulting from the high number of comparisons made (Ury 1976). An adjusted error rate (α') was determined using the formula from Sokal and Rohlf (2012):

$$\alpha' = 1 - (1 - \alpha)^{1/k}$$

where α is the conventional type I error rate of 0.05, and k is the number of independent tests performed. Five tests were performed for each experimental comparison in this study ($k = 5$).

Lupine densities determined using the Chan (2004) method were compared to the lupine density of that same site as determined by Chan and Packer (2006). Lupine densities determined using the Herms (1996) method were compared to (1) the lowest lupine density reported by Herms (1996) and (2) the lupine density at the site with the highest Karner blue population reported by Herms (1996).

In addition to comparing the density of lupine and nectar source plants to estimates of density from Chan and Packer (2006) and Herms (1996), general comparisons of lupine density in Ontario sites collected using the Herms (1996) method were made to ratings of lupine density assigned by managers at the Saratoga Sandplains, New York, based on Bried (2009). Bried (2009) used quality ratings for lupine density ranging from poor (≤1,801 stems/acre) to very good (>3,603 stems/acre).

Spring and summer nectar source plant densities determined during the first and second Karner blue flight periods were compared to (1) the lowest recorded nectar source density at Michigan Karner blue sites, and (2) the nectar source density at the Michigan site with the highest Karner blue population from the same brood, as reported by Herms (1996). The results of Chan and Packer (2006) could not be compared to my results, as their methodology involved counting plants regardless of floral state, and their surveys did not take place during the estimated flight periods of the Karner blue.

As this study marks the first time that LAI was used to measure the heterogeneity of the habitat at these five sites, no previous data obtained with this method exist for comparison. Consequently, the data were transformed to percentage of canopy cover using the formula from Buckley et al. (1999):

$$\%\ canopy\ cover = \frac{LAI^{1/2} - 0.0841}{0.0196}$$

The canopy cover of a site was considered acceptable if canopy cover values across all transects ranged between 20-60%. Within this range of values, lupine has been experimentally determined to experience high fitness (USFWS 2012). While some shade does provide lupine with protection from moisture loss and reduces early senescence, higher canopy cover levels have detrimental effects on lupine that negatively influence Karner blue fitness (Belsky et al. 1993; Grundel et al. 1998; Grundel and Pavlovic 2007).

The number of ant species at each site and the pairwise differences in ant species richness between those sites are termed α-richness and β-richness respectively. I determined α-richness of captured ants based on DNA-barcode identifications. Comparison of α-richness at sites also reported on by Chan (2004) provides an estimate of change in the total number of ant species at Ontario lupine habitats over the 10 years. β-richness for each combination of paired sites in this study, defined as shared species, enabled comparisons to Chan's (2004) data and inferences regarding how the ant communities have changed over the last decade. Differences in β-richness over the last decade were calculated by subtracting the number of shared species between paired sites in Chan (2004) from the number of shared species at the same paired sites in this study.

In order to determine whether ABOS has the appropriate climate to support a Karner blue population, I used principal components analysis to compare the climate of ABOS to that of 59 known Karner blue localities. I included 19 climatic variables in the analysis, and used verimax rotation to extract the first two principal components (PC1 and PC2). I then plotted the PC1 and PC2 scores for each locality (N = 60), and visually inspected the graph to determine whether the climate of ABOS was similar to that of known Karner blue localities.

All statistical analyses were conducted using JMP (JMP, Version 11.0.0, SAS Inc., 2007) with α=0.05. Figures were produced using SigmaPlot (SigmaPlot for Windows, Version 12.5, Systat Software Inc., 2011).

Results

Lupinus perennis Abundance and Density

The total number of lupine stems at each site varied from 1,826 at DM2 to 19,403 at ABOS (Table 1). Maps showing the locations of patches of lupine and the numbers of stems in each for the primary field sites can be found in Appendix 4 (Maps 1-5).

Using the Chan (2004) method of transect placement lupine densities seem to have increased at SWCR and ABOS since their previous evaluation by Chan and Packer (2006) (Fig. 2; details of statistical results are presented in Table 2). The densities of lupine at KBS, PPP, and HP have not changed in the last decade using this method.

The Herms (1996) method of transect placement generally resulted in much lower lupine densities than those determined by the Chan (2004) method (Fig. 3a). ABOS was the only site to have significantly higher lupine densities when compared to Herms' (1996) minimum value of 0.1 stems/m^2 (Table 2); all other sites did not differ from that minimum density. All sites in Ontario had lower lupine densities than the average lupine density at the Michigan site most populated with Karner blue butterflies in Herms' (1996) study.

Nectar Source Plant Density

SWCR had a relatively high density of nectar source plants potentially available to the first brood of Karner blue butterflies in the spring, while that density at PPP was the lowest (Fig. 3b). These densities were found not to differ from Herms' (1996) lowest reported value, with the exception of SWCR, which had a higher nectar source density

(Fig. 3b; Table 1). None of the Ontario sites exceeded the spring nectar source density at Herms' (1996) site with the highest Karner blue population.

Numerically, ABOS and SWCR had the highest and lowest recorded density of summer nectar sources respectively (Fig. 3c). When the lowest value reported by Herms (1996) was used for comparison, all sites in Ontario except KBS exceeded that density (Table 1). PPP, and ABOS also had higher summer nectar source densities than Herms' (1996) site with the highest Karner blue population, while SWCR, KBS, and HP did not differ from this value (Table 1).

Table 1 Area, area occupied by lupine populations, lupine population size, and planting information for current and potential lupine habitats in Ontario.

Region	Site	Area (ha)	Current Lupine Area (ha)	Number of Lupine	Year of Lupine Planting	Rate of Lupine Planting (kg/ha)
Norfolk County	Manestar Tract (SWCR)	81	0.63	4,867	-	-
	Carson/Gartshore (CGF)	19.7	0.40	11,600	1991	-
	Lake Erie Farms (LEF)	166.6	9.9	2,474	2006	0.014
	DeMaere 2 (DM2)	64.9	18.0	1,826	2010/2011	0.013/0.02
	DeMaere 1	10.0	-	-	2009	0.1
	Anderson	8.9	-	-	2010	0.02
	Soenen 2	6.7	-	-	2011	0.08
	Dekeyser	17.8	-	-	-	-
	Squires	21.6	-	-	-	-
	Weeden	3.6	-	-	-	-
	DeMeyere	20.4	-	-	2011	0.049
	Massecar	12.0	-	-	2012	0.024
	Wiebe	32.4	-	-	2012	0.024
	Hazen	9.3	-	-	2011	0.035
	White	5.3	-	-	2011	0.033
	Rendulich	24.7	-	-	2011	0.035
	Ferguson	80.1	-	-	2011	0.018
	Lightheart	10.1	-	-	2013	0.008
	Lambrecht 50	10.5	-	-	2013	0.008
	Lambrecht 100	26.7	-	-	2013	0.008
	Bergen	12.9	-	-	2013	0.008
	Casier	18.7	-	-	2013	0.012
	Woolley	6.7	-	-	2013	0.012
	DeMaiter	35.1	-	-	2013	0.012
	Saunders	18.0	-	-	2014	0.012

Region	Site	Area (ha)	Current Lupine Area (ha)	Number of Lupine	Year of Lupine Planting	Rate of Lupine Planting (kg/ha)
Norfolk County	Lang	7.5	-	-	2014	0.02
	DeVos-Myke	2.9	-	-	2014	0.02
	Mergl	4.2	-	-	2014	0.029
	TOTAL	**738.3**	**28.93**	**20,767**		
Lambton Shores	Karner Blue Sanctuary (KBS)	15	0.31	2,902	-	-
	Pinery Provincial Park (PPP)	2,532	0.72	5,027	-	-
	TOTAL	**2,547**	**1.03**	**7,929**		
Northumberland County	Alderville (ABOS)	61	0.87	19,403	2000-present	Lupine plugs used
	Hazel Bird	382.2	-	-	-	-
	Webber	131.2	-	-	-	-
	Barr	177.5	-	-	-	-
	TOTAL	**751.9**	**0.87**	**19,403**		
Toronto	High Park (HP)	79	0.64	9,123	2008-present	Lupine plugs used
	TOTAL	**79**	**0.64**	**9,123**		

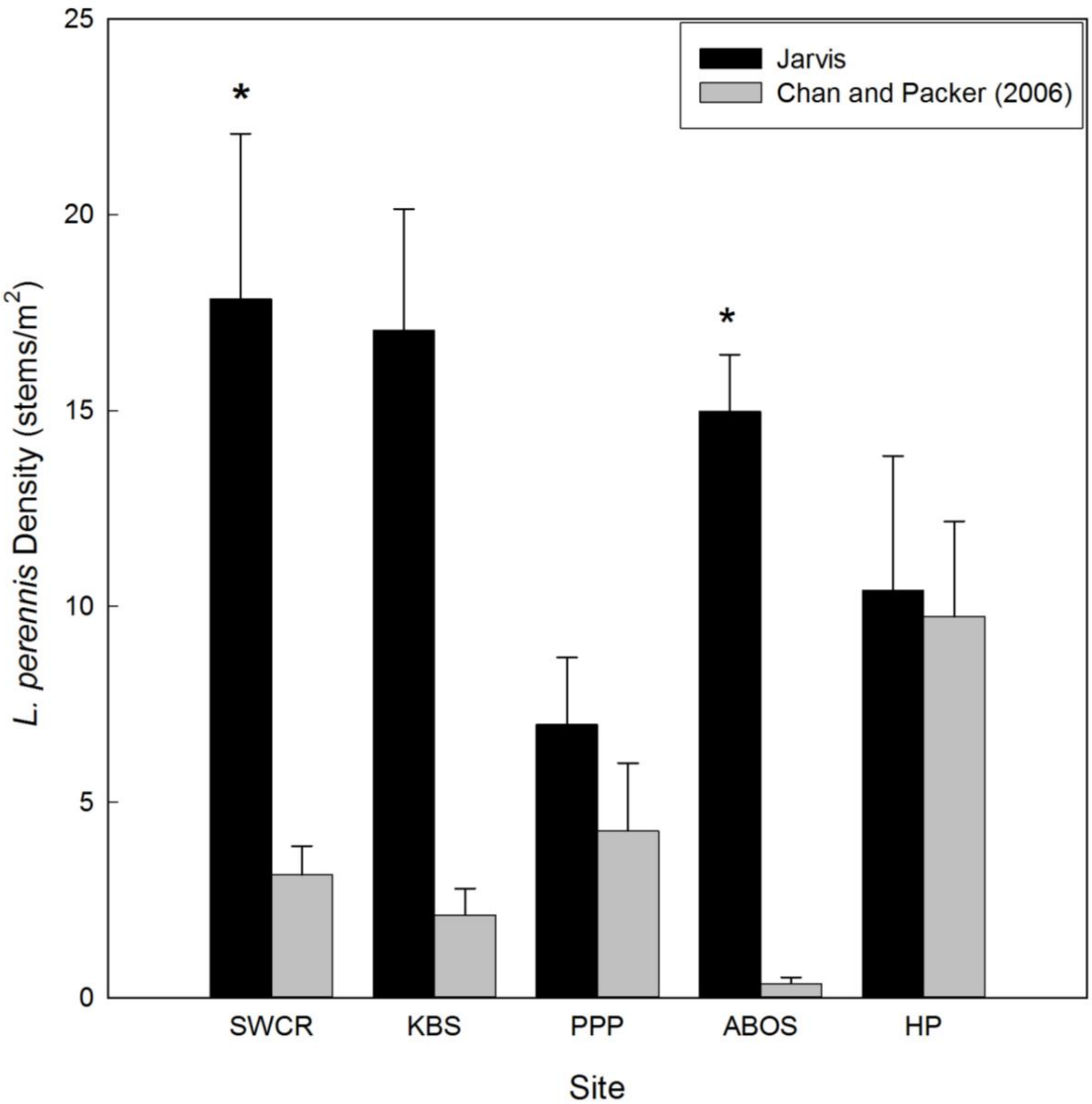

Figure 2. Mean values (± 1 SE) of *Lupinus perennis* density determined using the Chan (2004) method of transect placement in 2013 and the values recorded by P.K Chan in 2003 (Chan and Packer 2006) for each of the five lupine sites in Ontario. Sites denoted with * had significantly different values from the two assessments.

Table 2. Summary of the one-sample Wilcoxon tests performed to compare lupine densities, as well as spring and summer nectar source densities to literature values. P values in bold are significant after applying the Dunn-Šidák correction for multiple tests ($\alpha' = 0.0102$).

One Sample Wilcoxon Test				
Literature Comparison Made	Site	Test Statistic	*df*	p value
Lupine density collected using Herms (1996) transect placement method to Herms' (1996) lowest reported value	SWCR	4.0	6	0.2891
	KBS	3.0	2	0.1250
	PPP	8.0	6	0.1094
	ABOS	18.0	7	**0.0039**
	HP	11.0	6	0.0391
Lupine density collected using Herms (1996) transect placement method to Herms' (1996) best site value	SWCR	-14.0	6	0.9922
	KBS	-3.0	2	0.8750
	PPP	-14.0	6	0.9922
	ABOS	-18.0	7	0.9961
	HP	-13.0	6	0.9844
Lupine density collected using Chan (2004) transect placement method to the density at the same site determined by Chan (2004)	SWCR	13.0	6	**0.0056**
	KBS	3.0	2	0.1250
	PPP	8.0	6	0.1094
	ABOS	18.0	7	**0.0039**
	HP	-2.0	6	0.5938
First brood nectar source plant density to Herms' (1996) lowest reported value	SWCR	14.0	6	**0.0078**
	KBS	0.0	2	0.5000
	PPP	-6.0	6	0.8047
	ABOS	3.0	7	0.3633
	HP	6.0	6	0.1875
First brood nectar source plant density to Herms' (1996) best site value	SWCR	9.5	6	0.0703
	KBS	-2.0	2	0.7500
	PPP	-14.0	6	0.9922
	ABOS	-13.5	7	0.9688
	HP	7.0	6	0.8516
Second brood nectar source plant density to Herms' (1996) lowest reported value	SWCR	13.0	6	**0.0056**
	KBS	3.0	2	0.1250
	PPP	14.0	6	**0.0078**
	ABOS	18.0	7	**0.0039**
	HP	14.0	6	**0.0078**
Second brood nectar source plant density to Herms' (1996) best site value	SWCR	11.0	6	0.0391
	KBS	3.0	2	0.1250
	PPP	14.0	6	**0.0078**
	ABOS	18.0	7	**0.0039**
	HP	13.0	6	0.0156

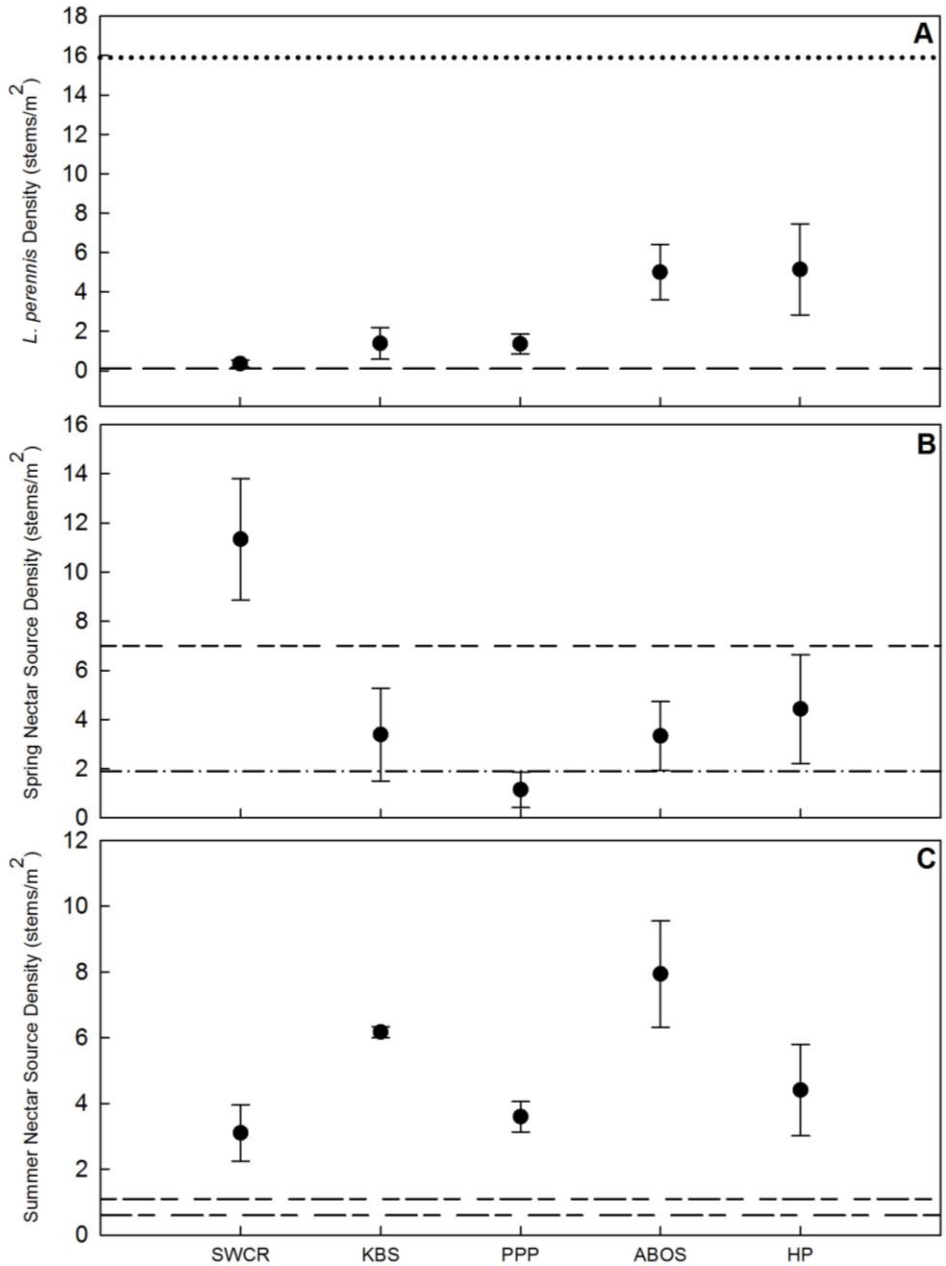

Figure 3. A. Mean values (± 1 SE) of *Lupinus perennis* density at five Ontario sites collected using the Herms (1996) method of transect placement. The upper and lower dashed lines represent *L. perennis* density at the site with the greatest Karner blue population and the lowest *L. perennis* density, respectively, as observed by Herms (1996) near Allegan, MI. B. Mean values (± 1 SE) of spring nectar source plant density. The upper and lower dashed lines represent the spring nectar source plant density at the site with the greatest Karner blue population and the lowest spring nectar source plant density respectively, as observed by Herms (1996). C. Mean values (± 1 SE) of summer nectar source plant density. The upper and lower dashed lines represent the summer nectar source plant density at the site with the greatest Karner blue population and the lowest summer nectar source density respectively, as observed by Herms (1996).

Shade Heterogeneity/Canopy Cover

Ranges for percent canopy cover within each Ontario site were 13.4-64.9% (SWCR), 29.9-35.9% (KBS), 27.2-50.9% (PPP), 27.2-36.8% (ABOS), and 32.5-55.6% (HP) (Fig. 4). Only SWCR failed to score within the 20-60% range recommended by USFWS (2012).

Larval-Tending Ants

DNA-barcoded specimens collected with five different techniques documented 24 different species of ants from lupine habitats (Table 3). Of these 24 species, 11 are known to tend larvae of the Karner blue. Individual sites had 4-8 known tending species. In my analysis of α-richness of ants, I found eight fewer species of ants across lupine habitats in Ontario than Chan (2004) did a decade ago. Chan (2004) found 21 species of ants not found in this study, while I found 13 species that were not present during his evaluation in 2002-2003. Two European species, *Myrmica rubra*, and *Tetramorium caespitum*, were found at HP, which Chan (2004) also found.

Analysis of β-richness showed that HP shares fewer species with the remaining sites than it did a decade ago (Table 4). The number of shared species between ABOS and the remaining sites has increased (Table 4).

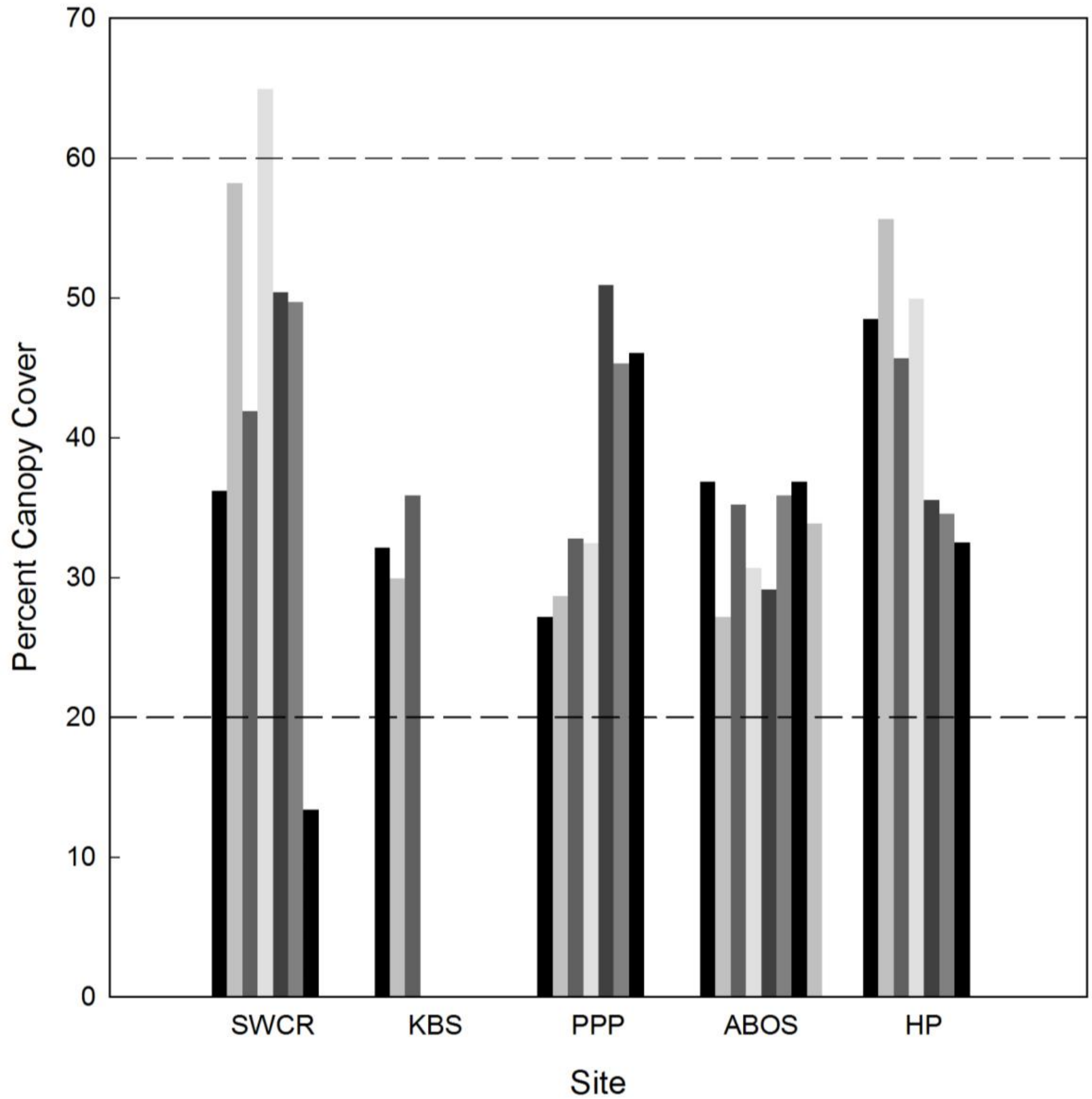

Figure 4. Percent canopy cover of each transect established within five Ontario lupine sites. Data were collected using the Chan (2004) method of transect placement. The dashed lines inclusively represent the range of desirable canopy cover (20-60%) for lupine growth and reproduction to be high (USFWS 2012).

Table 3. Species name, presence (1) or absence (0) of each species from the given lupine site in Ontario, and the Barcode Index Number (BIN) used to identify that species in BOLD. Ant species denoted with * and presence (1) in bold font indicate species that has been observed tending larvae of the Karner blue butterfly.

Species	SWCR	KBS	PPP	ABOS	HP	BIN
Acanthomyops latipes	0	1	0	0	0	BOLD:AAF0797
Aphaenogaster rudis-texana	1	0	0	1	1	BOLD:AAD1927
Aphaenogaster tennesseensis	0	1	1	0	0	BOLD:AAB2794
Aphaenogaster treatae	0	0	0	1	0	BOLD:AAF7307
*Camponotus americanus**	0	0	0	**1**	0	BOLD:AAD4431
*Camponotus pennsylvanicus**	0	**1**	**1**	0	0	BOLD:AAA9461
*Crematogaster lineolata**	**1**	**1**	**1**	**1**	0	BOLD:AAC3275
Dorymyrmex grandulus	0	0	1	0	0	BOLD:AAM6829
Formica glacialis	1	1	1	1	0	BOLD:AAA1468
*Formica schauffusi**	**1**	**1**	**1**	**1**	0	BOLD:AAA1467
*Lasius alienus**	0	0	0	0	**1**	BOLD:AAA9048
Lasius claviger	1	0	0	0	0	BOLD:ABY9254
Lasius sp.	0	1	0	0	0	BOLD:ACE8629
*Lasius neoniger**	**1**	**1**	**1**	**1**	**1**	BOLD:AAB9126
Leptothorax ambiguus	1	0	0	0	0	BOLD:AAG0685
*Monomorium emarginatum**	0	0	0	0	**1**	BOLD:AAO3690
Myrmica AF-smi†	1	1	1	0	0	BOLD:AAA1840
*Mymica americana**	0	0	**1**	0	0	BOLD:AAA1839
*Myrmica punctiventris**	0	0	**1**	0	0	BOLD:AAA1865
Myrmica rubra	0	0	0	0	1	BOLD:AAD0829
Paratrechina longicornis	0	0	1	0	0	BOLD:ACA3963
*Tapinoma sessile**	**1**	**1**	**1**	**1**	**1**	BOLD:AAA3893
Temnothorax sp.	0	0	1	0	0	BOLD:ACM6097
*Tetramorium caespitum**	0	0	**1**	0	**1**	BOLD:AAB8259
Total Tending Species	**4**	**5**	**8**	**5**	**5**	

†*Myrmica* AF-smi is an undescribed morphospecies of *Myrmica* identified by André Francoeur (Ellison et al. 2012).

Table 4. A. Comparisons of β-richness (total number of shared ant species) between lupine sites in Ontario. Above the diagonal represents the species shared between sites in this study; below the diagonal represents the species shared between sites as reported by Chan (2004). B. Differences in β-richness between this study and Chan (2004). The numbers shown are obtained from Table 4A, by subtracting the value from above the diagonal from the value below the diagonal for each pair of sites. Positive numbers indicate there were more shared species found by Chan (2004), negative numbers indicate there were more shared species found in this study.

A.

	SWCR	KBS	PPP	ABOS	HP
SWCR		6	6	6	3
KBS	6		8	5	2
PPP	6	4		5	3
ABOS	2	4	2		3
HP	6	6	6	5	

B.

	SWCR	KBS	PPP	ABOS	HP
SWCR					
KBS	0				
PPP	0	-4			
ABOS	-4	-1	-3		
HP	3	4	3	2	

Climate Analysis of Sites Known to have Supported the Karner Blue

I analyzed climatic variables for the 59 sites known to have supported populations of the Karner blue in the USA and Canada, as well as the restored Ontario site ABOS, with a principal components analysis. The first two principal components (PC1 and PC2) accounted for 51.58% and 19.05% respectively of the variation between the 60 sites analyzed (see Appendix 3 for values used in principal component analysis). Sites with a relatively high positive score for PC1 had low temperature seasonality (BIO4), a high minimum temperature during the coldest month (BIO6), a low annual temperature range (BIO7), a high mean temperature during the coldest quarter (BIO11), high precipitation during the driest month (BIO14), low precipitation seasonality (BIO15), high precipitation during the driest quarter (BIO17), and high precipitation during the coldest quarter (BIO19) (Appendix 3). Sites with a relatively high positive score for PC2 had high precipitation during the wettest month (BIO13), and high precipitation during the wettest quarter (BIO16) (Appendix 3). The principal components analysis placed ABOS centrally within the climate data points representing Karner blue sites (Fig. 5).

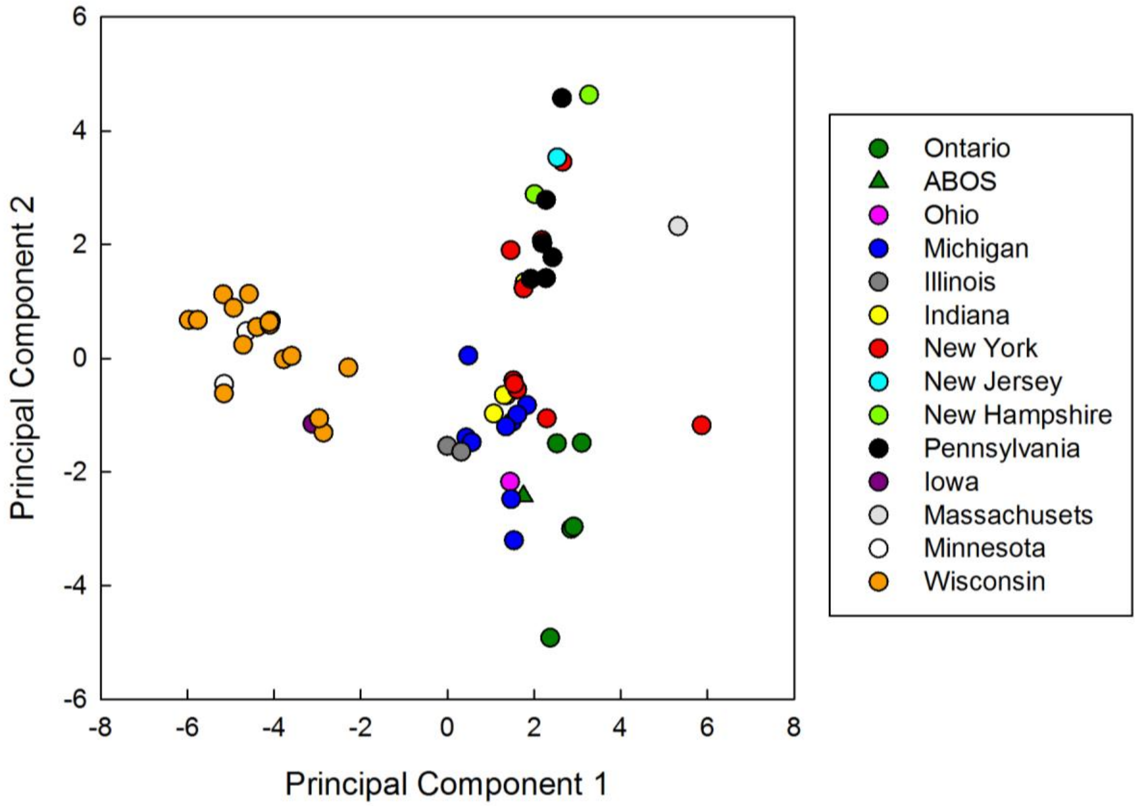

Figure 5. Principal components analysis of 19 climatic variables for 59 current and historic localities of the Karner blue butterfly, in addition to ABOS (indicated with a triangular symbol). Principal components one and two accounted for 70.63% of the variation in the model.

Discussion

Lupinus perennis Abundance and Density

None of the Ontario sites have lupine populations that are large enough to support a Karner blue population. The largest lupine population in Ontario (ABOS) contains ~19,000 stems. In contrast, modeling (Fuller 2008) suggests that the necessary lupine population to sustain a viable population of the Karner blue is at least 128,130 stems in each of the 5-9 subpopulations that make up a greater metapopulation. This indicates that lupine habitat in Ontario is currently not being conserved and created on a sufficiently large scale to sustain the Karner blue. While the lupine density at ABOS is comparable to sites in the USA that support the Karner blue, it represents only a single subpopulation with a few small lupine populations at least 10 km away.

Lupine densities at most Ontario sites were similar to the lowest lupine density seen at the Allegan State Game Reserve, MI (Herms 1996). One Ontario site however, ABOS, had moderate lupine density by the criteria of Herms (1996). Interestingly, ABOS had the lowest lupine density of all sites when evaluated by Chan and Packer (2006) a decade ago. ABOS personnel resolved to create Karner blue butterfly habitat, and over the last decade have planted thousands of lupines on an almost annual basis to supplement their existing population. They have also burned the site at frequent intervals (see management recommendations below). This has resulted in a vast improvement in habitat quality at the site over the past decade.

The lupine density at SWCR was by far the lowest recorded in this study using the Herms (1996) method of transect placement, despite it not being significantly different from the lowest densities in Allegan, MI. This contradicts my result obtained using the

Chan (2004) method of transect placement, highlighting the importance of establishing transects within lupine habitats randomly rather than placing them specifically in lupine patches. While it is important to include a comparison to the lupine densities estimated by Chan and Packer (2006) as they provide the only other existing evaluation of lupine sites in Ontario to date, I was hampered in being able to directly compare to their data because their transects could not be relocated. In reality, the absence of site maintenance over the past decade at the Manestar tract of SWCR should not have led to an increase in lupine density. While the area has been blocked off using felled wood in an attempt to prevent dirt bikes and all-terrain vehicles from damaging the environment, the lack of direct management (e.g. burning or mowing) and proliferation of small trees over the past decade have likely led to the relatively low lupine density quantified using the Herms (1996) method of transect placement. More active management would enhance the quality of Karner blue habitat at SWCR. The OMNR recently has been thinning adjacent forests in an attempt to restore the savannah habitat and may be amenable to increased management of the site (St. Williams Conservation Reserve 2009).

KBS had the lowest total lupine population of the primary field sites, however it was also the smallest site studied. The area of KBS with lupines warranted only three transects, while the medium-sized sites (SWCR, PPP, and HP) were evaluated using seven transects each. Despite the limitations of its size, the KBS had similar lupine densities to PPP.

Nectar Source Plant Density

Nectar plant surveys suggest that nectar sources for Karner blue adults should not be limiting at most Ontario sites. In spring, in contrast to its low lupine density, SWCR had the highest density of nectar source plants. Only PPP had densities of spring-blooming nectar source plants that were so low they could be limiting to the Karner blue. Nectar source densities for the second estimated brood of Karner blue butterflies were excellent at all Ontario lupine sites. Not only did all of the Ontario sites except KBS exceed the minimum summer nectar source density reported in Allegan by Herms (1996), two sites (PPP, and ABOS) had summer nectar source densities that exceeded that at the Allegan site with the highest population of Karner blue butterflies.

Joel Hecht (pers. comm.) questioned the importance of nectar sources to the Karner blue given their very short mean longevity (~5 days) (COSEWIC 2000). Herms (1996) had relatively low densities of summer blooming nectar source plants in Michigan in 1993, however the local population of Karner blue butterflies persisted. While Karner blue butterflies are frequently observed nectaring at flowers (Haack 1993; Grundel et al. 1998; Grundel et al. 2000; Bennett et al. 2013; and others) their need to do so to achieve maximum longevity and fecundity is unknown.

Shade Heterogeneity/Canopy Cover

Lane (1999) argued that sites with a range of canopy cover values from 16-75% provide the benefits to both lupines and the Karner blue of exposure and shade. The canopy cover at all Ontario sites was within this range. At KBS, PPP, ABOS, and HP, values for canopy cover were between 20-60%, which has been recommended as suitable for lupine growth and reproduction (USFWS 2012). Canopy cover plays a complex role in determining habitat suitability for the Karner blue butterfly. Growth and reproduction of lupine are restricted at sites with closed canopies, however shade delays the senescence of lupine plants and increases larval survival rates (Grundel et al. 1998; Lane 1999; Hess 2013; Pfitsch and Williams 2009). Sites with open canopies generally have greater populations of both larval and adult food sources, and females have an increased probability of finding mates, although drought can devastate lupine populations when there is insufficient canopy to mitigate water loss (Lane 1999).

Larval-Tending Ants

All Ontario sites have at least four species of ant known to tend larvae of the Karner blue. While this is fewer than were measured a decade ago, it is likely above an acceptable minimum (Chan and Packer 2006). The β-richness analysis showed that HP had the fewest number of shared species (a species that co-occurs at another site) with any other site in this study. The establishment of the alien European species *Myrmica rubra* and *Tetramorium caespitum*, absent from most other lupine habitats, and the isolation of the site within highly urban surroundings have likely resulted in a unique ant species pool compared to the other sites. *M. rubra*, the European fire ant, is an aggressive species that is known to prey on other ant species (Groden et al. 2005). It is possible the presence of *M. rubra* at HP has affected the invertebrate community and displaced ant species, some of which are known to tend Karner blue larvae. It is unknown whether *M. rubra* and *T. caespitum* would tend Karner blue larvae. In Europe *M. rubra* does tend larvae of the small blue butterfly (*Cupido minumus* Fussly), another lycaenid species of the same subfamily (Fielder 1989). It is possible that the Karner blue could form a mutualistic relationship with *M. rubra* if these species were to co-occur at a site, however it cannot be said with certainty without experimentation.

I found more species from ant genera indicative of forest habitats, such as *Aphaenogaster* and *Lasius*, than were present a decade ago (Chan 2004; Ellison et al. 2012). In contrast, Chan (2004) found many more species of *Formica* that are indicative of more open habitats (Ellison et al. 2012). This suggests that over the last decade, lupine habitats in Ontario have been transitioning from relatively open savannahs to woodlands. However, it is difficult to compare differences in ant richness directly to Chan (2004), because he used one collection method (active sampling) without indicating how much effort was exerted. The ants collected by Chan (2004) were also only identified morphologically by André Francoeur (Canadian expert in many of the groups in question), whereas analysis of the COI mtDNA gene was used here which provides a permanent DNA identification reference for future researchers.

Climate Analysis of Sites Known to have Supported the Karner Blue

My analysis indicates that ABOS, a site where a historical population of the Karner blue cannot be confirmed, is climatically similar to other Karner blue sites. All lupine sites in Ontario fell relatively close to one another in the principal components analysis of climate variables for known Karner blue localities. The climatic suitability of ABOS suggests with continued expansion of the habitat there and in surrounding properties, it may eventually be suitable for Karner blue introduction. The success of ABOS personnel at creating the site with the highest number of lupine stems in Ontario indicates that additional sites outside the historic localities of the Karner blue may be suitable for Karner blue introduction given appropriate conservation efforts.

It is unadvisable to translocate the Karner blue between western and eastern sites for several reasons. First, Karner blue butterflies in the western populations (Wisconsin) are infected with endosymbiotic *Wolbachiaia* bacteria that prevent infected males from successfully fertilizing un-infected females, or females infected with a different *Wolbachia* strain (Nice et al. 2009). These bacteria are absent from the eastern Karner blue populations with the exception of a single individual from the Saratoga Sandplains, NY, found to be infected with a different *Wolbachia* strain (Nice et al. 2009). Introduction of *Wolbachia* into eastern populations could have devastating effects on Karner blue populations. Second, Karner blue butterflies in these two regions differ in mtDNA haplotypes (Gompert et al. 2008). From a conservation standpoint, it is important to maintain those differences. Finally, my principal components analysis of climatic variables appears to separate the eastern and western Karner blue localities into different climatic clusters. This suggests that the butterflies in these two regions experience, and would be adapted to, different climatic conditions.

Eventually Ontario may become one of the only suitable regions for the Karner blue in response to a changing global climate (USFWS 2012). However, a northward shift in distribution of the species, as has been documented for many other butterfly species (e.g. Parmesan et al. 1999) may be impossible in the eastern part of its range without human-assisted migration because of the impeding Adirondack Mountains, Great Lakes, and absence of lupine habitats (Rodenhouse et al. 2009). Even if the Karner blue is translocated to an area where the climate will be more suitable in the future, the expected increase in extreme weather events may have detrimental effects on the species (Fuller 2008). Drought events contributed to the extirpation of the Karner blue in Ontario and Ohio, and over the past few years have led to severe population declines of the Karner blue in Indiana, Ohio, and Michigan (Fuller 2008; USFWS 2012; Maria Albright pers. com.). While this highlights the need for the creation of suitable habitat north of extant Karner blue populations, developing new lupine habitats may not be feasible considering the timescale necessary to develop a landscape mosaic of lupine patches necessary for the persistence of a Karner blue metapopulation.

Metapopulation Structure

Karner blue population persistence requires metapopulations consisting of a number of sub-sites (Fuller 2008). These sub-sites must be sufficiently close for recolonization following localized extinctions (COSEWIC 2000; Carson 2006). Characteristics of sub-sites in New York State have been well documented by Smallidge and Leopold (1997), and it has been concluded that dispersal readily occurs over separation distances of approximately 0.5-2.0 km. The area required to maintain a minimum viable population of the Karner blue is just over 150 ha, distributed among 5-9 sub-sites (USFWS 2003; Fuller 2008). This metapopulation foundation is currently lacking from all Ontario sites where lupine occurs in discrete, isolated sites with few to no lupine patches within 2 km.

The region with the greatest potential to support a Karner blue metapopulation is Norfolk County, encompassing SWCR, adjoining OMNR lands (approximately 11.2 km^2 in total area), numerous properties owned by the Nature Conservancy of Canada, and some significant private land holdings (Map 6, Appendix 4). While the lupine populations at individual sites in this region do not match those of ABOS, 24 properties owned by the NCC have the potential to support lupine populations and already have the connectivity necessary for Karner blue dispersal between them. Currently in addition to SWCR, there are three other properties that already have established lupine populations: the secondary sites CGF, LEF, and DM2. The biggest limitation to developing and maintaining Karner blue habitat here would be that these properties are not currently being burned (with the exception of CGF) or disturbed after their initial rehabilitation by the Nature Conservancy of Canada. The properties owned by the OMNR include SWCR as well as several other plots of land in both the Nursery Tract and Turkey Point Tract of St. William's Conservation Reserve. While the 10-year management plan for this land states a willingness to restore several sites to oak savannah habitat, the Manestar Tract itself (where evaluation took place) is not slated for restoration (St. Williams Conservation Reserve 2009).

ABOS has limited potential to develop a metapopulation structure with other lupine sites in Northumberland County. While lupine was once prevalent across the landscape of the Rice Lake Plains, this is no longer the case (Catling et al. 1992). The Nature Conservancy of Canada is currently in the early stages of restoring five additional sites of degraded lupine habitat in proximity to ABOS. However fewer than 700 lupine seedlings have been planted in these sites to date (Todd Farrell pers. com.). Additionally, these sites are more than 10 km away from ABOS (Map 7, Appendix 4), which far exceeds the recommended distance between subpopulations (USFWS 2003). Additional sites with lupine population are needed as well as connectivity along corridors between these sites and ABOS to facilitate Karner blue dispersal (Fuller 2008). While these sites together with ABOS are still at least a decade away from having a sufficiently large lupine population to support a minimum viable population of the Karner blue, the restoration of these additional sites in conjunction with continued work at ABOS could eventually create a patchwork of sites suitable for Karner blue reintroduction.

The remaining sites in Ontario are unlikely to ever be suitable to support a Karner blue metapopulation. Although PPP and KBS together theoretically are sufficiently large, efforts to enhance lupine numbers and habitat there have been limited; currently lupine populations encompass very small percentages of the potential lupine habitat at these sites. The size and relative isolation of HP within metropolitan Toronto prevent it from being capable of supporting sufficient lupine populations to sustain a Karner blue metapopulation.

General Comparisons to sites in New York State with extant populations of *L. samuelis*

Total lupine populations at sites in Ontario are much smaller than those at the Saratoga Sandplains, and an order of magnitude smaller than the 128,130 stems necessary to support a minimum viable subpopulation of a larger Karner blue metapopulation as estimated by Fuller's (2008) model. Despite this, lupine densities at Ontario sites would likely be considered "good" (0.59-0.88 stems/m^2) to "very good" (>0.88 stems/m^2) under the quality criteria used by management at the Saratoga Sandplains, New York (Bried 2009). The canopy cover levels at all but one site in Ontario would also fall within the “good” (20.1-60%) rating for shade heterogeneity from Bried (2009) (based on Grundel et al. 1998; Lane and Andow 2003). That is presently only a limitation for SWCR.

ABOS is the best individual site in Ontario, however it is only a single site, while 5-9 sub-sites are needed to fulfill the requirements of a metapopulation (Fuller 2008). Additional management units with very small lupine populations in Northumberland County are at least 10 km from ABOS (Todd Farrell pers. com.). In Norfolk County, the Nature Conservancy of Canada and OMNR manage several properties in proximity to SWCR that have the potential to become subpopulations for the Karner blue; the best of these at present are the secondary sites CGF (a private property), LEF, and DM2. The close proximity of KBS and PPP would allow them to function as subpopulations of a metapopulation, however they would not reach the necessary 5-9 subpopulations (Fuller 2008). Currently HP, a large urban park, has no potential to develop a metapopulation structure of lupine habitat.

Limitations

The largest limitation of this study was my inability to match the transects used by Chan and Packer (2006) a decade ago. Chan (2004) described the transects as being placed “to cut through areas with the highest wild lupine densities”. This was impossible to replicate, however I attempted to do so to make comparisons between sites in 2002-2003 and 2013.

Some of the limitations of this study were rooted in the methodology for sampling the different habitat qualities at lupine habitats in Ontario. The multi-purpose use of the same transects for quantification of lupine density, nectar source density, and shade heterogeneity streamlined and simplified the sampling process, allowing all of the sites to be visited during the estimated flight times of the Karner blue. However it did not provide the best representation of spring and summer nectar source densities, as there were multiple transects in which lupine and Karner blue nectar plants did not occur together. This resulted in conservative values for the density of nectar sources because they were present at the site outside of the transects.

Conclusions

As restoration of lupine habitats in Ontario continues it would be worthwhile to look towards other species that have disappeared from these habitats, such as the frosted elfin (*Callophrys irus* Godart). The frosted elfin is another extirpated lupine-dependent butterfly with less demanding requirements for habitat size and population structure than the Karner blue (Pfitsch and Williams 2009; Bried et al. 2012). While the lupine habitats in Ontario are not yet suitable for the Karner blue, they may already be acceptable for the frosted elfin that often persists for years in small populations (Pfitsch and Williams 2009). However, its persistence will ultimately also depend on expansion of the current area of lupine habitat, and continued land management and maintenance.

Improvements have been made to some Ontario lupine habitats since they were last evaluated in 2003. However, no sites in Ontario are currently suitable for Karner blue reintroduction. There are two main issues currently affecting them: individual sites in Ontario have neither the quantity of lupine nor the spatial scale with multiple sub-sites necessary to support a minimum viable metapopulation of the butterfly species. The next step towards a reintroduction of the Karner blue involves the rehabilitation of substantially more lupine habitat in the areas surrounding the sites I evaluated. If more quality lupine habitat can be incorporated into the landscape of Ontario, particularly in Norfolk and/or Northumberland Counties, reintroduction may eventually be feasible, and the iconic Karner blue may inhabit Ontario in the future.

Management Recommendations

The management practices outlined here can benefit all of the lupine habitats in Ontario. Norfolk County, encompassing four current lupine sites (SWCR, CGF, LEF, and DM2) and many additional sites controlled by the Nature Conservancy of Canada and the OMNR, currently has the greatest potential for creating the population structure of lupine that would be suitable for the Karner blue. The multiple lupine populations in Norfolk County as well as the existing connectivity between current and potential lupine sites exceed those at any other Ontario location (Map 6, Appendix 4).

The implementation of the following management practices will immediately benefit lupine habitat and aid in the creation of future habitat for the Karner blue butterfly:

i. Collecting lupine seeds.
ii. Supplementing existing and creating new lupine populations with locally sourced seeds/seedlings.
iii. Planting native floral species and curtailing the development of invasive plant populations.

iv. Burning (or mowing) approximately one third of a management unit at least once every four years, and removing large trees if necessary to maintain desirable shade heterogeneity levels.

v. Focusing restoration efforts away from heavily trafficked areas (roadways, public paths, recreational fields, etc.).

These recommendations were formed through extensive conversation with personnel responsible for habitat management at the Albany Pine Bush and the Saratoga Sandplains, in New York State. Within these two regions are some of the largest remaining populations of Karner blue butterflies. Visits to these sites, as well as the Allegan State Game Area, the Kitty Todd Preserve in Ohio where the Karner blue was successfully reintroduced, and the Rome Sand Plains in New York, which hopes to eventually introduce the Karner blue, were pivotal in understanding what needs to happen to increase the quality of lupine habitat in Ontario.

Enhancing *Lupinus perennis* Populations

Lupine is heavily dependent on disturbance events (Corry et al. 2008). Both burning and mowing have been shown to prevent the development of woody species as well as maintain early successional habitats conducive to the development of lupine populations (Smallidge et al. 1996; Forrester et al. 2005). Both of these management practices are equally effective, and do not affect the quality of host plants for the Karner blue larvae (Pickens and Root 2008). Controlled burns, however, reduce the surface layer of soil organic matter that seems to be important for the establishment of seedlings

(Ernest Williams pers. com.). Low levels of soil organic matter have been associated with lupine populations (USFWS 2012).

The timing, frequency, and spatial distribution of disturbance events will impact lupine establishment and maintenance. The disturbance regime at the Albany Pine Bush in NY, USA, involves burning or mowing one third of a management unit every year (Joel Hecht pers. com.). Disturbance usually takes place either before mid April, or after mid August so the events do not coincide with flight periods of Karner blue adults. However, they now have so much land area to manage that timing of disturbance events has become a relatively unimportant consideration. If sites are mowed, then mower blades are raised to their maximum height to reduce the effects on Karner blue eggs on lupine or grass stems. The disturbance regime is important to maintain because Karner blue have been shown to avoid ovipositing within sites where disturbance events occur less frequently than every four years (Pickens 2009).

In Ontario, the three sites (ABOS, CGF, and HP) that currently have the largest lupine populations are also the only sites employing regular controlled burns as a component of their site management. The other lupine populations in Ontario (SWCR, KBS, PPP, LEF, and DM2) have received little to no management in the last decade. The Manestar Tract of SWCR has been unmanaged – neither burned nor disturbed (with the exception of recreational use) – since its acquisition by the Ontario Ministry of Natural Resources several decades ago. This is evident in the abundance of woody species in areas with lupine patches. The hardwood forest surrounding the tract is slowly encroaching on the property. At the KBS, management previously included controlled burns, but it has been more than a decade since this last took place. The abundance of woody species has created large disconnects between discrete lupine populations, evident in Map 2. Establishment of lupine seedlings there is low to nonexistent. While PPP does employ controlled burns, sometimes a decade or more separates burn events at specific sub-sites within the park (Tanya Berkers pers. com.). The sub-site with the highest lupine density at PPP had been burned only weeks before the evaluation took place, and while the burn did consume the understory ground cover species and allow lupine to regenerate, the established large red and white pine trees remained, resulting in little change to the canopy cover. The effect of the high canopy cover level was seen later in the summer when visibly fewer lupine plants remained. Disturbance events that remove abundant woody species that will shade out both larval and adult food sources must take place for lupines to thrive (Smallidge et al. 1996; Forrester et al. 2005; Pfitsch and Williams 2009). In Norfolk County, two recently restored sites, LEF and DM2, have not experienced any disturbance since their initial plantings with native species by the Nature Conservancy of Canada.

Management at lupine habitats in the USA involves several different methods for planting lupine. At the Rome Sand Plains near Rome, NY, lupine seedlings grown in peat in small pots are transplanted into the site along with 2-3 grains of Soil Moist (JRM Chemical, Cleveland, Ohio, USA), a polymer that aids in water-retention at the roots (Ernest Williams pers. com.). A small amount of wood ash placed in the hole with the plug and polymer grains appears to help in the success of seedlings. When planting needs to occur on a near-industrial scale, lupine seeds can be planted directly into the ground using a seed drill, as is done at the Albany Pine Bush Preserve, Albany, NY, with great success. Because a high proportion of lupine seeds can germinate without stratification, they can be planted as soon as they mature (Peter Carson pers. com.). However stratification will increase the proportion of seeds that successfully germinate (Boyonoski 1992).

Lupine seeds pods can be harvested as soon as they develop a purplish stripe. After removal from the plants, the pods require regular mixing in a protected, dry environment to prevent mold growth as they complete maturation. Once the pods burst, loose seeds can be collected, and a stone mill can be used to remove the remaining seeds from the pods. Lupine seeds will remain viable for three years, which precludes the development of a long-term seed bank containing dormant lupine seeds in the soil (Boyonoski 1992).

Enhancing Nectar Source Populations

Although the necessity of nectar sources for Karner blue adults has been questioned, management at potential Karner blue habitat sites should consider the floral species present. A reduction in ground cover diversity occurred during the original decline of oak savannah sites throughout Ontario as a result of fire suppression and land fragmentation (Abella et al. 2001). The implementation of a disturbance regime can aid in reversing this damage. While disturbance does promote the development of native floral species, invasive species can become a problem. ABOS and PPP have experienced an invasion of spotted knapweed (*Centaurea maculosa* Lam.), a Eurasian member of the Asteraceae family that is widely distributed in eastern Canada (Qaderi et al. 2013). The curtailment of invasive species capable of outcompeting native plants is highly important, as low nectar source diversity has been shown to negatively affect many butterfly population sizes (Schultz and Dlugosch 1999). Dangremond et al. (2010) showed that competition with invasive species could displace native *Lupinus* species, without which the Karner blue could not persist. Supplementing the populations of both lupine and additional native floral species will both provide food for Karner blue larvae and adults, as well as fill environmental niches that may become occupied by invasive species if left empty.

Maintaining Shade Heterogeneity

While shade heterogeneity at KBS, PPP, ABOS, and HP is currently suitable, it is a habitat characterisitc that requires regular management. Burning and, to a lesser extent, mowing will affect understory cover, which increases the openness of the habitat. At most sites (SWCR, HP, PPP), the presence of large woody species is problematic. Large trees can be removed during the winter to reduce the damage to the surrounding environment. The resulting stumps should also be removed to prevent sinkholes that develop as they rot (pers. com. Kathy O'Brien). The thinning of large trees increases the openness of the canopy and benefits lupine populations (Pfitsch and Williams 2009). Burning and mowing mimic the activity of megaherbivores, and have been positively correlated with the presence of both the Karner blue and lupine (Hess et al. 2014).

Maintaining Larval-Tending Ant Richness

The richness of ant species in lupine habitats in Ontario is very different than it was a decade ago when Chan and Packer (2006) evaluated these sites. The most notable differences are the absence of several species within savannah genera and the presence of several species within forest genera. While some Karner-blue-tending ants are forest species, the majority are *Formica* spp. that prefer open habitats (Ellison et al. 2012). Employing disturbance regimes that benefit lupine and nectar sources as well as maintain heterogeneity should create the habitat that should increase the abundance of these species at lupine sites in Ontario.

Recreational Disturbance

Recreational use of Karner blue habitat is a concern. Bennett et al. (2013) used a modeling approach to address the response of the Karner blue to recreational use of its habitat. They found that Karner blue adults react to intruding humans in the same way they react to potential predators, by rapidly flying away from the perceived threat (Bennett et al. 2013). This has negative implications for fecundity and host plant selection, both of which strongly influence population dynamics (Bennett et al. 2013). Human disturbance is a concern at all of the Ontario sites with the exception of ABOS, which experiences limited educational but no recreational activities. Human disturbance is a particular concern at HP, the largest urban park in Toronto that is heavily visited by the public. Map 5 (Appendix 4) illustrates the close proximity of lupine populations to recreational trails, paved roads, and a baseball diamond. Mitigating the disturbance caused by park visitors would involve restricting public access from areas that would be used by the Karner blue, which is not feasible in such a setting. KBS, PPP, and most of the sites in Norfolk County all have the potential to offset the effects of recreational disturbance by increasing conservation efforts further away from trails and publicly used space.

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Appendix 1

Site Descriptions – Primary Sites

St. Williams Conservation Reserve

St. Williams Conservation Reserve was visited between 24 - 28 May, as well as 3 July and 29 July, 2013. The combined tracts of St. Williams Conservation Reserve comprise the largest forest block in the Carolinian Zone of southern Ontario, totaling 1,035 ha. There were several regions of the reserve where prescribed burns have been utilized within the past few years in an attempt to curtail the understory and open the canopy, but the forest in most of these areas was still extremely dense, and the high levels of canopy cover levels precluded the development of lupine populations in most of the reserve. In addition to the reserve itself, there are dozens of properties in the area owned and managed by the Nature Conservancy of Canada, as well as private land holdings that contain savannah. These additional properties represent the best potential for the expansion of lupine habitat in Norfolk County.

The area chosen for survey within the park property was the Manestar Tract, the location of the last known Karner blue adult in 1989 and the site assessed by P.K. Chan (COSEWIC 2000; Chan 2004). The Manestar Tract is located at 42.701, -80.467 in Norfolk County, Ontario, and is accessed via the south side of Concession Road 6. It is an 81 ha tract of land maintained by the OMNR that had previously been privately owned and clearcut, with the intention of farming the land. Little to no restorative effort has taken place there since its acquisition by OMNR. It has since become heavily trafficked by pedestrians, bicycles, and all-terrain vehicles. Despite this level of traffic, a patchy population of lupine has persisted. This tract is the only region of St. Williams

Conservation Reserve where lupine was abundant enough for evaluation. The patches of lupine found there were generally oriented north to south along the sandy trails that run approximately perpendicular to the road (Map 1, Appendix 4).

The soil of the Manestar Tract is sand for a depth of 7-8 m (pers. comm. Peter Carson). The terrain slopes slightly downhill towards the south with some small, interspersed sand hills. White pine (*Pinus strobus* L.) and oak trees (*Quercus* spp.) are the dominant species that comprise the mixed-wood forest that borders the Manestrar tract on the south, east, and west sides. There is also a smaller population of white pine, oak, and staghorn sumac (*Rhus typhina* L.) towards the center of the tract, enclosed by the sandy path on all sides. Within the Manestar Tract a 6,300 m^2 area was established where the majority of the lupine occurs. Seven transects were established within this plot.

The Karner Blue Sanctuary

The Karner Blue Sanctuary was visited between 30 May and 2 June, as well as 4 July and 31 July, 2013. The property is located at 43.223, -81.887 in Port Franks, Ontario, and can be accessed from the corner of Whatman Street and Nipigon Street. It is a 15 ha tract of land that was purchased by Brenda Kulon in 1988 in an attempt to prevent further loss of Karner blue butterfly habitat when housing development began altering the local landscape. Lambton Wildlife Inc., a non-profit organization, has managed the property since its purchase.

The Karner Blue Sanctuary has not been thoroughly burned in a decade, although very small patches are occasionally burned. Some herbicides have been used on the property to kill young oak trees and slow the succession of the savannah into oak woodland. Black oak trees provided the majority of the canopy on the property, but several other species were present, including wild cherry (*Prunus serotina* Michx.), white pine, and sassafras (*Sassafras albidum* Nees). There is currently an effort underway on the property to create more walking trails for public use.

The patches of lupine within the Karner Blue Sanctuary were almost exclusively oriented in an east to west direction, making it difficult to representatively sample with the methodology of north-running transects used in this study (Map 2, Appendix 4). Additionally the lupine tended to be present on south-facing slopes that have little tree canopy overhead. One corridor within the sanctuary measuring 2,200 m^2 was selected for sampling, and two transects were established there. A second bowl-shaped region with a total area of 900 m^2 was surveyed with a single transect.

Pinery Provincial Park

Pinery Provincial Park was visited between 3 - 7 June, as well as 4 July and 31 July, 2013. The property is located at 43.248, -81.822 near Grand Bend, Ontario, and was accessed from Highway 21. The park, located along the southeastern shore of Lake Huron, has a total area of 2,532 ha and is managed by Ontario Parks, a branch of the Ontario Ministry of Natural Resources.

The park contains the largest area of oak savannah in Ontario that is maintained with controlled burns necessary to mimic the ecosystem's natural fire regime. Burns are done in the spring, when a wind from the southeast blows towards Lake Huron, to prevent the resulting smoke and ash from blowing onto nearby residential areas. Some areas of the park relevant to this study were burned as recently as four weeks prior to my field surveys.

There were several discrete populations of lupine scattered throughout the park (Map 3, Appendix 4). They generally occurred in areas where the oak and pine canopy and understory shrubs had been cleared by fire. The first area surveyed near the northeastern corner of the park (latitude 43.251, longitude -81.825) was located behind the Winter Activities Centre. It consisted of a bowl-shaped clearing with a hill at the south edge. It had an area of 2,376 m^2 in which two transects were established.

Not far from the first site and adjacent to a park road was the second area surveyed (43.256, -81.831), in the northeast corner of the park, and south of the Old Ausable Channel that runs through the park. The lupine population led into an oak and white pine forest where flowering plants occurred in the patches where the understory bush had been cleared away. It had an area of 900 m^2 and one transect was established.

The third area surveyed, located at 43.235, -81.860, was also on a roadside in the southwest corner of the park, still south of the Old Ausable Channel. It was a relatively open area with no canopy cover. Some shrubs were present as well as several large snags scattered around the perimeter of the site. There was a large quantity of dead wood on the ground, charred from a previous burn. It had an area of 900 m^2 and one transect was established.

The fourth and final area surveyed, located at 43.235, -81.848, was approximately 150 m north of the shoulder of Highway 21. The area was enclosed with a fence to mark an experimental plot where a controlled fire had taken place four weeks prior to this survey. It had a heavy canopy of oak, as well as white and red pine (*Pinus resinosa* Sol.), and a large understory population of bracken fern (*Pteridium aquilinum* L.). It had an area of 3,069 m^2 and three transects were established. Some smaller patches of lupine were located in the next valley north of this site.

Alderville Black Oak Savannah

The Alderville Black Oak Savannah was visited between 10 - 13 June as well as on 5 July and 1 August, 2013. The property, located at 43.248, -81.822, is near Alderville and south of Rice Lake in Northumberland County, Ontario. The site can be accessed from County Road 18. It is a 61 ha tract of band land owned by the Alderville First Nations. The black oak savannah land was classified as protected in 1998 by a resolution of the local First Nation Chief and Council.

Active conservation of the Alderville Black Oak Savannah (ABOS) began in 2000 through the volunteer work of local community members. The property is the largest tract of oak savannah that remains intact on the Rice Lake Plains. Controlled burns are employed to maintain the integrity of the property as a savannah ecosystem. This savannah is not a traditional home of the Karner blue butterfly, as lupine is not endemic to the property. However, it does occur in the surrounding area. Lupine has been actively planted at ABOS on an almost annual basis. The Alderville Black Oak Savannah joined the Rice Lake Plains Joint Initiative in 2006 with the goal to protect savannah and prairie ecosystems in the entire Rice Lake region. This land is the subject of a long-term restoration and monitoring initiative.

The lupine population on the property was divided into several discrete populations (Map 4, Appendix 4). Lupine planted in some regions of the property has flourished. It has failed to establish in other areas that seemed quite similar to one another at a cursory glance.

The first area surveyed, located at 44.173, -78.089, was a bowl-shaped area with a south-facing slope. Lupine was present continuously across the slope. Black oak (*Quercus velutina* Lamb.) and trembling aspen (*Populus tremuloides* Michx.) trees scattered sparsely on the hill provided some canopy cover. Halfway up the hill on the east side, New Jersey tea (*Ceanothus americanus* L.), red dogwood (*Cornus sericea* L.) and staghorn sumac densely covered the ground. It is a relatively large area, totaling 6,007 m^2, and five transects were established.

The second area surveyed, located at 44.173, -78.091, had a smaller cluster of lupine northwest of the top of the hill in the first area. The lupines were surrounded by several black oaks that formed a circle. Prairie brome (*Bromus kalmia* A. Gray) constituted the predominant ground cover. It had an area of 870 m^2 and a single transect was established.

The third area, located at 44.172, -78.090, contained a small stand of lupine spread across flat prairie in a transitional zone between oak savannah and dense mixed-wood forest where no restoration efforts have been employed beyond the planting of lupine. Lupine was present on both sides of a well-travelled path that intersects the prairie. Big bluestem (*Andropogon gerardi* Vitman) comprised the majority of the ground cover. It had an area of 900 m^2 and one transect was established here.

The fourth and final area sampled at ABOS was a small level clearing bordered by black oak trees to the east and largetooth aspen (*Populus grandidentata* Michaux) trees on the west, located at 44.172, -78.086. It had been burned 4-5 weeks prior to this survey, and the ground was still visibly charred in some places. It had a total area of 900 m^2 and again one transect was established here.

High Park

High Park was visited between 13 - 18 June, as well as 6 July and 1 August, 2013. The property is located within the city of Toronto at 43.652, -79.465. The park contains 79 ha of oak savannah, which is about one third of its total area (Chan and Packer 2004). Created in 1876, it is a heavily trafficked urban park and contains playgrounds, dog walking areas, a pool, a zoo, and a sports field in addition to the natural habitat, trails, and nature center. It lies west of downtown Toronto, near the northern shores of Lake Ontario, and can be accessed from Bloor Street West, Parkside Drive, or The Queensway. Following more than a century of fire suppression, High Park has begun a management strategy with the goal of inhibiting the infiltration of non-native species while enhancing the development of populations of native species, including lupine (Map 5, Appendix 4). To that end, both natural fires and controlled burns occur regularly.

The first area examined, located at 43.649, -79.468, contained scattered lupine populations between several well-travelled dirt paths. The overhead canopy was provided by both black oak and sassafras while juvenile sassafras was present in the understory. The majority of the dense ground cover was comprised of grasses and woodland sunflower (*Helianthus divaricatus* L.). The area had most recently been burned in March 2012. Two transects were established within an area of 1,800 m^2.

The second area, located at 43.648, -79.467, was a clearing with a well-travelled path to the northwest and a baseball diamond to the north. On the north, east, and south sides of the clearing black oak provided the canopy cover, and several immature black oaks were present within the clearing itself. This area was most recently burned in April 2013; as a result the grass had not fully covered the ground and bracken ferns were present. Large pieces of charred debris were present on the ground. The total area was 900 m^2, and one transect was established.

The third area was a patch of meadow between a major dirt path and West Road within the park (located at 43.648, -79.467). The canopy was patchy and created by black oak trees scattered throughout the area as well as a stand of red pines on the north side. The area was burned in April 2013 and the grass has been slow to recover. Bare earth was clearly visible in several areas where ground cover has not become established. There was a large amount of charred litter as well as a few large charred logs on the ground. The area measured 860 m^2, and again one transect was established.

A fourth area was located at between a well-travelled dirt path and a mowed area adjacent to a park road (43.647, -79.467). The very dense population of lupine here provided the majority of the ground cover in concert with some aster species (including *Symphyotrichum ericoides* L. and *S. leave* L.). Immature black oak trees provided canopy cover at the north end, and mature black oaks at the south end. The area sloped gently downwards towards the north and lupine occupied at the top, slope, and bottom of the small hill. Within the total area of 1,049 m^2 one transect was established.

The fifth and final area encompassed a hill just north of the parking lot servicing the Grenadier Café (located at 43.646, -79.466). The south side of the hill had been planted with several plant species including lupine. There is no canopy on the hill itself, but sugar maple (*Acer saccharum* Marshall) were present on the south and west sides.

There was one oak tree on the northeast side, a stand of staghorn sumac on the south side, and basswoods (*Tilia americana* L.) on the east side. The southeast slope was densely covered in sunflower, and the summit of the hill as well as the descending north side is covered in grass. The hill had an area of 1,800 m^2 within which two transects were established.

Site Descriptions – Secondary Sites

Carson/Gartshore Farm (Secondary Site)

The Carson/Gartshore farm was visited on 26 June and 4 July 2014. The property, located at 42.642, -80.575 in Norfolk County, is accessed from the south side of County Road 60. It is a 19.7 ha piece of land privately owned by Peter Carson and Mary Gartshore. Peter and Mary have planted their property extensively with native species since 1991 as a component of a larger effort within the county to conserve native savannah habitat. The property is generally burned every two years. Lupine became established in several large patches throughout the field after a student conducting a research project planted it. The field is bordered on the east and south sides by dense mixedwood forest, and on the west side by agricultural land.

Lake Erie Farms

Lake Erie Farms was visited on 19 and 26 June 2014. The property is in Norfolk County, and can be accessed from the south side of Concession Road 6 (located at 42.657, -80.573). The 166.6 ha property is owned and managed by the Nature Conservancy of Canada. The property was mechanically planted in 2006 with a combination of native wildflowers, grasses including little bluestem (*Schizachyrium scoparium* Michx.) and slender wheatgrass (*Agropyron trachycaulum* Link), and the nuts of local trees. The seed mix was scattered across the property and then rolled to create hard-packed soil. The resulting ground cover community is very dense. The property has not been burned or disturbed with the exception of occasional pedestrian traffic.

All of the lupine on the property occurs on the west side of the trail leading south from a small parking lot on Concession Road 6. An open area that was initially left unplanted as a control zone has since been overtaken by grasses. This is the southern limit of the lupine population at the site. Near the path are scattered young oak trees that occur with increasing frequency towards the western edge of the property. The property is bordered on the west, south, and east sides by dense hardwood forest, and by a population of white pine and staghorn sumac to the north, creating a barrier between the conserved land and the road.

DeMaere 2

I visited DeMaere 2 on 26 June 2014. The property is a 64.9 ha former farm owned by the Nature Conservancy of Canada in Norfolk County, and can be accessed from the south side of Highway 24 (located at 42.687, -80.466). The field was first planted with a seed mix that included lupine in 2010. Most of the field is covered very densely with grass, but the grass thins near the eastern and western margins of the property where it is replaced by native floral species. There is a small population of young white pines near the center of the property.

The lupine population largely occurs in the areas of wildflowers on the east and west sides of the property, although a few scattered clusters can be found closer to the center. The property is bordered on the eastern and western sides by woodlots of hardwood species, and on the south by an agricultural field. Towards the south side of the property the terrain is hillier. There is a small pond in this area, and the vegetative community changes from grasses to shrubs. The Nature Conservancy of Canada has not disturbed the property in any way since it was initially seeded after its purchase.

GigaPan Photograph Links

St. Williams Conservation Reserve
http://gigapan.com/gigapans/130949
http://gigapan.com/gigapans/131033

Karner Blue Sanctuary
http://gigapan.com/gigapans/131811
http://gigapan.com/gigapans/131823

Pinery Provincial Park
http://gigapan.com/gigapans/131937
http://gigapan.com/gigapans/132017
http://gigapan.com/gigapans/132683

Alderville Black Oak Savannah
http://gigapan.com/gigapans/137995

High Park
http://gigapan.com/gigapans/138186

Appendix 2

Tending Ants

Table 5. Ant species reported to tend Karner blue larvae, and location of the observation. (1) Haack (1993), (2) Herms (1996), and (3) Lane (1999).

Ant Species	Location	Reference
Aphaenogaster rudis (Enzmann 1947)	Ontario	1
Camponotus americanus (Mayr 1862)	New York	1
Camponotus ferrugineus (Mayr 1836)	Wisconsin	1
Camponotus noveboracensis (Fitch 1855)	New York	1
Camponotus pennyslvanicus (DeGeer 1773)	Ontario	1
Crematogaster ashmeadi (Mayr 1886)	Wisconsin	1
Crematogaster cerasi (Fitch 1855)	New York	1
Crematogaster lineolata (Say 1836)	Michigan	2
Dolichoderus plagiatus (Mayr 1870)	New York	1
Formica exsectoides (Forel 1886)	Ontario	1
Formica fusca (Linnaeus 1758)	Wisconsin	1
Formica montana (Wheeler 1910)	Wisconsin	1
Formica subsericea (Say 1836)	New York	1
Formica incerta (Buren 1944)	New York	1
Formica schaufussi (Mayr 1866)	New York, Wisconsin	1
Formica lasioides (Emery 1893)	New York	1
Formica neogagates (Viereck 1903)	Michigan	2
Formica obscuriventris (Mayr 1870)	Michigan	2
Lasius alienus (Foerster 1850)	New York	1
Lasius neoniger (Emery 1893)	New York	1
Monomorium emarginatum (DuBois 1986)	New York	1
Myrmica americana (Weber 1939)	New York	1
Myrmica punctiventris (Roger 1863)	Ontario	1
Myrmica AF-scu†	New York	1
Nylanderia parvula (Mayr 1870)	New York	1
Prenolepis impairs (Say 1836)	Wisconsin	3
Tapinoma sessile (Say 1836)	New York, Wisconsin	1
Tetramorium caespitum (Linnaeus 1758)	Wisconsin	1

†*Myrmica* AF-scu is an undescribed morphospecies of *Myrmica* identified by André Francoeur (Ellison et al. 2012).

Appendix 3

Climate Analysis

Table 6. Climatic variables (from Hijmans et al. 2005) used in the principal components analysis to compare the climate of ABOS, current and historic Karner blue butterfly localities.

Code	Description
BIO1	Annual mean temperature
BIO2	Mean diurnal range (mean of monthly (max temp – min temp))
BIO3	Isothermality ((BIO2/BIO7)*100)
BIO4	Temperature seasonality (annual range in temperature)
BIO5	Max temperature of the warmest month
BIO6	Min temperature of the coldest month
BIO7	Temperature annual range (BIO5 – BIO6)
BIO8	Mean temperature of the wettest quarter
BIO9	Mean temperature of the driest quarter
BIO10	Mean temperature of the warmest quarter
BIO11	Mean temperature of the coldest quarter
BIO12	Annual precipitation
BIO13	Precipitation of the wettest month
BIO14	Precipitation of the driest month
BIO15	Precipitation seasonality (annual range in precipitation)
BIO16	Precipitation of the wettest quarter
BIO17	Precipitation of the driest quarter
BIO18	Precipitation of the warmest quarter
BIO19	Precipitation of the coldest quarter

Table 7. Values for the climatic variables (defined in Table 6 above); obtained from Hijmans et al. (2005) pertaining to temperature used in the principal components analysis of ABOS, current Karner blue butterfly localities, and historic Karner blue butterfly localities. All temperature data are in °C x10.

Site	BIO1	BIO2	BIO3	BIO4	BIO5	BIO6	BIO7	BIO8	BIO9	BIO10	BIO11
ABOS	61	101	26	9683	251	-129	380	83	-58	181	-69
ON 1	79	97	27	9209	264	-93	357	151	-34	194	-43
ON 2	79	95	26	9307	262	-90	352	189	-35	194	-44
ON 3	78	95	26	9308	262	-91	353	188	-36	194	-44
ON 4	82	89	24	9454	268	-90	358	194	-34	201	-41
ON 5	74	100	27	9324	262	-99	361	182	-41	190	-49
OH 1	95	112	29	9555	289	-90	379	213	-19	213	-33
MI 1	86	106	29	9237	273	-91	364	192	-26	200	-39
MI 2	78	108	29	9199	270	-95	365	149	-35	192	-44
MI 3	89	107	28	9430	279	-93	372	197	-25	206	-38
MI 4	73	118	30	9573	277	-110	387	145	-43	193	-54
MI 5	82	109	29	9415	276	-95	371	153	-32	199	-43
MI 6	83	116	29	9660	283	-105	388	192	-34	202	-47
MI 7	83	115	29	9612	282	-104	386	192	-33	202	-47
MI 8	93	112	29	9527	287	-91	378	211	-21	211	-35
MI 9	95	106	28	9643	287	-88	375	215	-21	215	-34
IL 1	91	103	26	9842	281	-103	384	213	-41	213	-41
IL 2	97	102	26	9877	287	-96	383	219	-36	219	-36
IN 1	98	107	28	9638	287	-93	380	217	-32	217	-32
IN 2	97	106	28	9623	285	-93	378	216	-17	216	-33
IN 3	110	120	31	9484	302	-83	385	208	-18	227	-18
IN 4	98	115	29	9610	294	-91	385	197	-32	217	-32
NY 1	78	121	30	9677	280	-122	402	131	-39	199	-52
NY 2	84	117	29	9572	283	-111	394	182	-44	204	-44
NY 3	79	106	29	9187	267	-96	363	187	-33	195	-42

Site	BIO1	BIO2	BIO3	BIO4	BIO5	BIO6	BIO7	BIO8	BIO9	BIO10	BIO11
NY 4	70	108	27	9902	265	-129	394	91	-52	193	-63
NY 5	82	116	30	9174	272	-105	377	176	-29	197	-41
NY 6	85	118	30	9552	283	-110	393	204	-43	204	-43
NY 7	74	123	30	9702	277	-129	406	127	-43	195	-57
NY 8	121	85	25	8699	291	-39	330	105	9	233	9
NY 9	76	130	31	9487	280	-127	407	29	-38	195	-52
NJ 1	80	116	31	9027	267	-106	373	172	-41	193	-41
NH 1	68	127	31	9348	267	-130	397	184	-45	184	-58
NH 2	68	119	31	9219	260	-123	383	23	-43	183	-56
PA 1	97	124	33	8946	291	-84	375	188	-23	209	-23
PA 2	91	118	31	8921	280	-89	369	181	-15	203	-28
PA 3	86	117	31	8887	275	-94	369	176	-33	197	-33
PA 4	78	112	31	8820	263	-98	361	168	-28	188	-39
PA 5	71	115	30	9005	258	-113	371	163	-38	183	-49
PA 6	68	115	31	8892	252	-116	368	158	-40	179	-51
IA 1	76	111	26	10886	280	-143	423	208	-74	208	-74
MA 1	91	117	30	9068	284	-95	379	47	207	207	-29
MN 1	70	123	27	11221	286	-164	450	206	-85	206	-85
MN 2	67	114	25	11566	283	-169	452	207	-93	207	-93
WI 1	66	127	28	11092	285	-166	451	188	-88	201	-88
WI 2	64	128	28	10843	281	-163	444	183	-86	195	-86
WI 3	72	117	27	10742	283	-146	429	191	-75	203	-75
WI 4	52	126	26	11687	275	-195	470	192	-112	192	-112
WI 5	50	128	27	11510	273	-194	467	189	-110	189	-110
WI 6	70	116	25	11608	290	-165	455	211	-90	211	-90
WI 7	58	125	27	11175	275	-174	449	193	-96	193	-96
WI 8	66	122	27	11286	283	-168	451	202	-90	202	-90
WI 9	61	126	27	11290	279	-177	456	184	-96	197	-96
WI 10	61	121	27	10880	275	-162	437	181	-88	193	-88
WI 11	71	118	27	10769	282	-149	431	191	-76	203	-76

Site	BIO1	BIO2	BIO3	BIO4	BIO5	BIO6	BIO7	BIO8	BIO9	BIO10	BIO11
WI 12	67	123	28	10835	279	-158	437	186	-82	199	-82
WI 13	67	130	28	10900	286	-163	449	188	-83	200	-83
WI 14	62	132	29	11066	282	-172	454	183	-91	196	-91
WI 15	65	129	28	10883	282	-164	446	184	-85	197	-85
WI 16	78	118	28	10470	283	-135	418	206	-65	206	-65

Table 8. Values for the climatic variables (defined in Table 6 above) obtained from Hijmans et al. (2005) pertaining to precipitation used in the principal components analysis comparing ABOS, current, and historic Karner blue butterfly localities. All precipitation data are in mm.

Site	**BIO12**	**BIO13**	**BIO14**	**BIO15**	**BIO16**	**BIO17**	**BIO18**	**BIO19**
ABOS	832	81	56	10	227	178	212	189
ON 1	971	91	61	12	258	208	251	219
ON 2	887	83	59	10	242	187	241	200
ON 3	892	83	60	9	241	190	240	205
ON 4	782	81	51	12	223	164	218	172
ON 5	944	91	59	12	255	200	253	217
OH 1	850	92	45	21	263	157	263	160
MI 1	942	102	41	20	276	166	265	181
MI 2	857	98	40	22	276	152	237	163
MI 3	956	102	43	20	280	167	270	180
MI 4	880	105	38	23	282	152	246	159
MI 5	873	101	37	22	272	154	239	160
MI 6	861	97	37	25	264	141	259	145
MI 7	866	94	38	24	265	142	263	144
MI 8	836	90	44	20	254	154	254	156
MI 9	822	89	43	20	250	150	250	154
IL 1	897	102	34	29	298	135	298	135
IL 2	919	103	36	28	300	142	300	142
IN 1	960	103	42	24	295	159	295	159
IN 2	983	104	45	22	299	169	299	170
IN 3	1036	115	54	22	328	193	316	193
IN 4	939	101	49	21	294	171	285	171
NY 1	1032	99	64	11	283	219	280	220
NY 2	957	93	58	13	271	196	269	196

Site	BIO12	BIO13	BIO14	BIO15	BIO16	BIO17	BIO18	BIO19
NY 3	934	93	54	15	263	182	261	192
NY 4	940	96	59	14	271	198	236	218
NY 5	1169	118	76	12	331	248	315	252
NY 6	953	93	57	14	272	192	272	192
NY 7	1052	103	65	12	287	221	287	222
NY 8	1119	105	77	9	302	251	294	251
NY 9	1004	102	69	9	276	221	257	230
NJ 1	1162	115	70	13	329	239	320	239
NH 1	1100	102	78	8	296	247	296	255
NH 2	1167	114	87	7	314	271	297	280
PA 1	976	109	57	17	302	190	294	190
PA 2	1031	111	62	17	313	200	302	200
PA 3	979	108	57	19	307	182	295	182
PA 4	1022	108	60	18	313	192	304	192
PA 5	1095	108	70	13	315	223	305	227
PA 6	1184	118	78	12	335	250	322	252
IA 1	814	103	24	42	303	86	303	86
MA 1	1098	113	83	9	302	254	254	271
MN 1	822	113	21	47	320	75	320	75
MN 2	757	113	21	50	315	70	315	70
WI 1	824	106	23	45	315	80	309	80
WI 2	823	107	24	44	310	83	302	83
WI 3	791	99	26	39	285	92	278	92
WI 4	776	108	20	48	314	78	314	78
WI 5	766	105	20	48	308	76	308	76
WI 6	779	111	19	50	316	68	316	68
WI 7	811	112	19	50	325	73	325	73
WI 8	822	106	20	47	318	76	318	76
WI 9	835	108	22	46	320	79	319	79

Site	BIO12	BIO13	BIO14	BIO15	BIO16	BIO17	BIO18	BIO19
WI 10	815	103	25	42	302	88	294	88
WI 11	800	101	27	39	287	92	280	92
WI 12	815	104	25	42	303	85	296	85
WI 13	824	108	24	43	311	84	300	84
WI 14	823	106	23	45	314	80	313	80
WI 15	823	107	24	44	310	83	302	83
WI 16	861	104	29	37	308	104	308	104

Table 9. Climatic variables and their loading values for the first two principal components (PC1 and PC2) resulting from the climate analysis comparing ABOS, current, and historic Karner blue butterfly localities.

Climatic Variable	**PC 1**	**PC 2**
BIO1	0.66134	-0.25431
BIO2	-0.58278	0.62545
BIO3	0.41524	0.66043
BIO4	-0.97045	-0.06606
BIO5	-0.27625	-0.15837
BIO6	0.90848	-0.24357
BIO7	-0.94638	0.18596
BIO8	-0.41287	-0.38239
BIO9	0.75861	0.03362
BIO10	0.05128	-0.38676
BIO11	0.90441	-0.13021
BIO12	0.72908	0.62180
BIO13	-0.28274	0.80497
BIO14	0.89546	0.34869
BIO15	-0.96985	-0.04358
BIO16	-0.41729	0.75963
BIO17	0.92916	0.31106
BIO18	-0.51470	0.61700
BIO19	0.93495	0.27930

Appendix 4

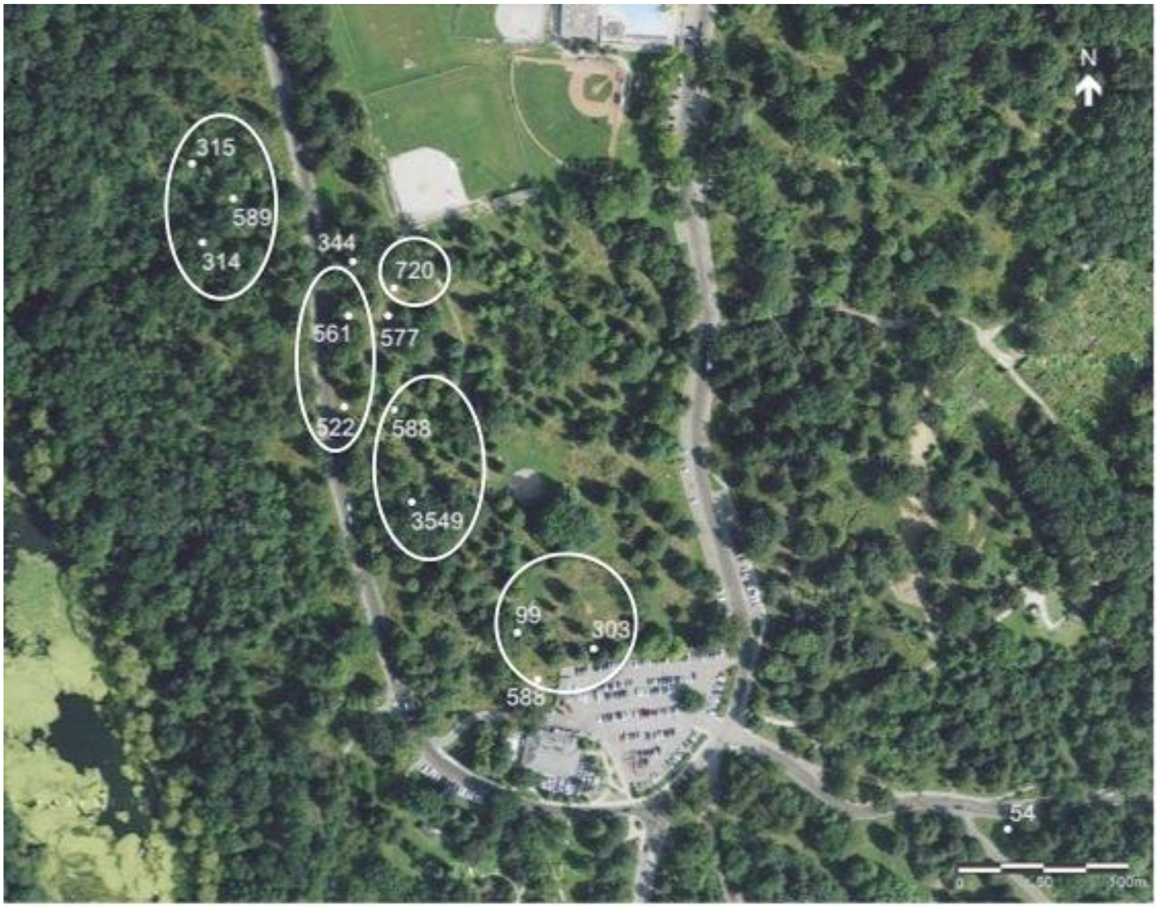
Lupinus perennis Maps

Map 1. Locations of clusters of *Lupinus perennis* and numbers of lupine stems within each cluster (indicated with numbers) at St. Williams Conservation Reserve (SWCR). The circle approximates the area considered as a single lupine population at SWCR.

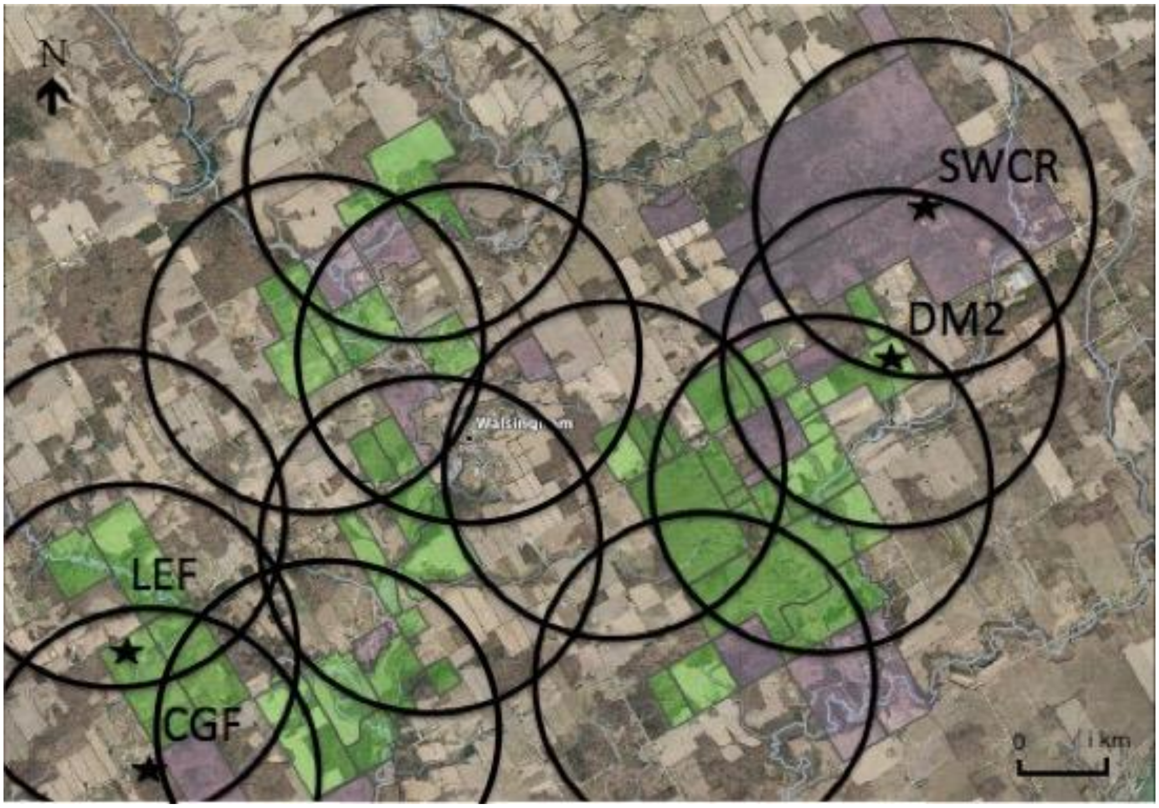
Map 2. Locations of clusters of *Lupinus perennis* and numbers of lupine stems within each cluster (indicated with numbers) at the Karner Blue Sanctuary (KBS). Circles approximate the area considered as two lupine populations at KBS.

Map 3. Locations of clusters of *Lupinus perennis* and numbers of lupine stems within each cluster (indicated with numbers) at Pinery Provincial Park (PPP). Circles approximate the area considered as four lupine populations at PPP.

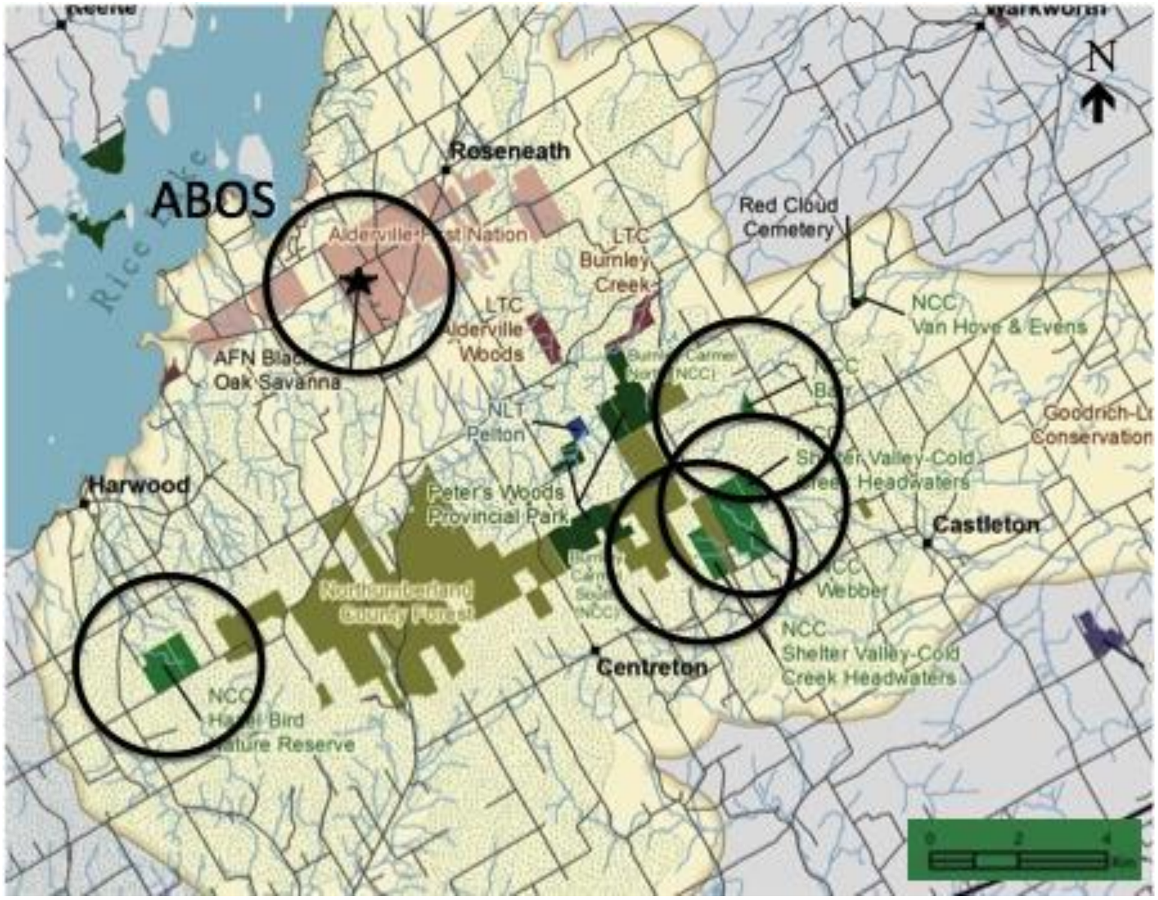
Map 4. Locations of clusters of *Lupinus perennis* and numbers of lupine stems within each cluster (indicated with numbers) at the Alderville Black Oak Savannah. Circles approximate the area considered as four lupine populations at ABOS.



Map 5. Locations of clusters of *Lupinus perennis* and numbers of lupine stems within each cluster (indicated with numbers) at High Park (HP). Circles approximate the area considered as five lupine populations at HP.



Map 6. Locations of St. Williams Conservation Reserve (SWCR), Lake Erie Farm (LEF), Carson/Gartshore Farm (CGF), and DeMaere 2 (DM2) in Norfolk County, Ontario. Circles represent a 2 km radius around each site, the maximum recommended distance between sub-sites in a Karner blue butterfly metapopulation (USFWS 2003). Circles surrounding a site without an identification represent land owned by the Nature Conservancy of Canada where lupine could be planted.



Map 7. Alderville Black Oak Savannah (ABOS) in Northumberland County. Circles represent a 2 km radius around each site, the maximum recommended distance between sub-sites in a Karner blue butterfly metapopulation (USFWS 2003). Circles surrounding a site without an identification represent land owned by the Nature Conservancy of Canada where restoration of degraded lupine populations has begun.