

Invasion biology of the gypsy moth (*Lymantria dispar* (L.)) at a northern range boundary  
in Minnesota

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## Thesis Abstract

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidiae), is an invasive defoliator capable of consuming over 300 species of trees. Its North American range now extends north of North Carolina, and west to Wisconsin and Minnesota. Gypsy moth populations in Minnesota have a unique genetic signature, and male moths have been caught for the past 15 years despite low predicted climatic suitability in northern areas of the state. Here, I study the invasion biology of the gypsy moth in Minnesota. I characterize the spatiotemporal patterns in the occurrence of genotypes and assess if there is any evidence of association with putative movement pathways. I also investigate how severe winter conditions may reduce the number male moths captured in the subsequent summer, and test whether egg mass winter mortality is a plausible explanation for such reductions.

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## Thesis Introduction

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae), is a polyphagous insect capable of feeding on over 300 host species, with oak, aspen, and willow being among the highly favored species (Leonard 1981, McFadden and Mcmanus 1991, Liebhold et al. 1995). A forest is susceptible to gypsy moth defoliation when more than 20% of its basal area is preferred species; this metric has been observed on both a stand and a landscape level (Herrick and Gansner 1986, Liebhold et al. 1997). The insect is univoltine with an obligate overwintering diapause in the egg phase (Leonard 1968, Giese and Casagrande 1981). Native to Eurasia, there is little differentiation among European populations, but there is a clear distinction between European and Asian populations. Female European gypsy moth are incapable of flight, whereas females of the Asian variety are capable of flight (Leonard 1981). The Asian variety of gypsy moth pose an enhanced risk to North America due to female flight capability (Schaefer et al. 1984, Reineke and Zebitz 1998, Keena et al. 2001), increased host range (Schaefer et al. 1984, Baranchikov 1989), attraction to light (Schaefer et al. 1984, Wallner et al. 1995)(Schaefer et al. 1984, Wei et al. 2014), and a shorter chilling requirement for diapause completion (Keena 1996, 2015, Wei et al. 2014).

The European gypsy moth was introduced in Medford, MA by Étienne Léopold Trouvelot in 1869 (Forbush and Fernald 1896). The gypsy moth's range in North America now extends north of North Carolina, and west to Wisconsin and Minnesota, (US Code of Federal Regulations, Title 7, Chapter III, Section 301.45), where Northern red oak and quaking aspen are the main susceptible species (Liebhold et al. 1997, Morin et al. 2005). The initial North American population was established from this single

introduction event; thus, this population bottleneck reduced the genetic variability of the North American gypsy moth population (Harrison et al. 1983). As a result North American gypsy moth populations are treated as a unique geographic population within the scientific literature

The United States manages gypsy moth populations through the Slow-the-Spread program (STS). STS involves the annual deployment of pheromone-baited traps to detect and monitor gypsy moth populations. The dataset obtained through this extensive monitoring program is useful to land managers as well as researchers. The spread of gypsy moth can be characterized as stratified dispersal (Hengeveld 1988, Shigesada et al. 1995). Short distance dispersal occurs through the ballooning of first instars, which is generally a short distance phenomenon (1-3 km), but atmospheric conditions can foster greater dispersal distances (7-21 km) (McFadden and Mcmanus 1991, Frank et al. 2013). The anthropogenic movement of juvenile life stages frequently contributes to long distance dispersal. The movement of recreational vehicles and house hold articles from infested areas westward are the largest concern for long distance dispersal (Talerico, 1981; McFadden & Mcmanus, 1991; Liebhold, Halverson, & Elmes, 1992).

As populations invade new areas, low density populations may be susceptible to Allee effects (Johnson et al. 2006, Liebhold and Tobin 2006). Manifested as failure in finding mates, and thus unsuccessful reproduction (Sharov et al. 1995, Tcheslavskaia et al. 2002, Contarini et al. 2009). Allee effects present important management implications, because mating disruption can effectively reduce or eradicate low density populations (Sharov et al. 2002, Tobin and Blackburn 2007, Tobin et al. 2009). Moreover, eradication can be achieved by killing or removing less than 100% of the population (Liebhold and

Bascompte 2003). Because Allee effects are present within low density gypsy moth populations, this facilitates the deployment of trapping grids in successive years to delimit the extent of the population, and maximize the efficiency of eradication efforts through site-specific treatment areas (Liebhold and Tobin 2006).

Along the north shore of Lake Superior in the northeastern region of Minnesota, there have been consistent male moth captures in pheromone-baited traps since 2000, resulting in several population management treatments such as aerial applications of *Bacillus thuringiensis var kurstaki* (BtK) or mating disruption using synthetic pheromones. The presence of spatially autocorrelated trap captures along with evidence of alternate life stages suggests that there is a reproducing gypsy moth population in this region (Tobin 2007). In fact, a gypsy moth quarantine was implemented in Cook and Lake Counties in July 2014. This establishment is perhaps surprising, because the area around Lake Superior has been predicted to exhibit very low to nonexistent climatic suitability (Gray 2004, Régnière et al. 2009). The northern range extent of the gypsy moth has been thought to be limited by climate due to overwintering mortality (Sullivan and Wallace 1972, Smitley et al. 1998, Nealis et al. 1999). The gypsy moth is chill intolerant because mortality occurs at temperatures above the supercooling point of *ca* -27°C, when internal ice formation occurs (Sullivan and Wallace 1972, Madrid and Stewart 1981, Waggoner 1985). Moreover, cooler temperatures may delay development and prevent successful lifecycle completion (Régnière and Nealis 2002, Gray 2004, Régnière et al. 2009). For example, Gray (2004) and Régnière (2002) investigated the climatic suitability for the gypsy moth using a stage specific phenology model, the Gypsy moth Life Stage model (GLS). The GLS uses daily maximum and minimum temperature

records to estimate the probability of a gypsy moth successfully completing its life cycle based on historical temperature records. They predicted that very few moths would be able to successfully complete a lifecycle in a given year in the Arrowhead region of Minnesota (Régnière & Nealis, 2002; D. R. Gray, 2004).

One interesting facet of the invasion of gypsy moth into Minnesota has been the frequency of FS1-Asian alleles, which have consistently been observed at frequencies 2-6x the historic national average. The FS1- Asian allele is found in multiple populations throughout the United States, with a historical mean prevalence of approximately 3.5%. The FS1 locus was identified in 1996 by Garner & Slavicek, who identified this diagnostic region of nuclear DNA using random amplification of polymorphic DNA by polymerase chain reaction (RAPD-PCR). This marker is a single-copy autosomal locus with co-dominant Mendelian inheritance, and is most useful for identifying moths of far eastern origin (Garner and Slavicek 1996). This is important, as the deployment of pheromone-baited traps and mtDNA analysis of captured male gypsy moths has confirmed the presence of Asian genotypes in North America (Bogdanowicz et al. 1993). For example, in 1991 egg masses were discovered on superstructures originating from the Russian Far East in the ports of Vancouver, British Columbia and Seattle, Washington and ballooning larvae were subsequently observed dispersing on shore (Gibbons, 1992; Pfeifer et al, 1995; Hajek & Tobin, 2009). This incident highlighted the need to develop a nuclear molecular marker that could distinguish between Asian and European gypsy moth, which led to the development of the FS1 marker. Reineke (1999) found that the use of the FS1 locus was not suitable for identifying Asian gypsy moth genotypes in central Europe, and concluded that this marker was only effective for distinguishing



between Asian and North American gypsy moth. The occurrence of the FS-1 Asian allele in North America could be the result of its presence within the founding population, or it could be a result of undetected introductions. Due to multiple interceptions of Asian gypsy moth, the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) expanded gypsy moth monitoring activities to include all major US ports. As a result of increased monitoring at ports, a higher than usual occurrence of the FS1 locus can be found in North Carolina, Ohio, Michigan, California, and Washington (Prasher, 1995; Garner & Slavicek, 1996). Due to continued monitoring at ports and other high risk introduction pathways, the list of states in which FS1-Asian allele has been detected continues to grow. Minnesota has two international ports proximate to the Arrowhead region: Duluth, Minnesota and Thunder Bay, Ontario.

The goal of my thesis is to study aspects of the invasion biology of European gypsy moth in Minnesota. Populations of gypsy moth in the Arrowhead region of Minnesota are unique. First, they have a unique genetic signature with FS1-Asian allele frequencies occurring at 2-3x the national average. Second, populations continue to persist despite low predicted climatic suitability. My thesis has two components: 1) examining whether there are spatial or temporal patterns in the occurrence of FS1-Asian alleles and assessing if putative movement pathways are associated the distribution of these alleles, and 2) examining whether winter temperatures affect subsequent summer trap captures of male moths, and if so, determining whether overwintering mortality of egg masses is a plausible explanation for such reductions. It is possible that gypsy moth may persist in “climatically unsuitable” areas if egg masses are insulated by snow in the winter, for example.

The chapters of this thesis were prepared for publication in peer reviewed journals. Although, I am the lead author, the work presented is the combined result of a number of authors. Hence I present these sections in the plural voice. Chapter one is being prepared for submission to *Environmental Entomology*. Chapter two is being prepared for submission to *Diversity and Distributions*. To maintain the integrity of each chapter as a stand-alone unit, there may be a small degree of redundancy between chapters.

## **Chapter I**

Landscape-level patterns of elevated FS1 Asian allele frequencies in populations of European gypsy moth *Lymantria dispar dispar* [L.], (Lepidoptera: Erebidæ) in the Arrowhead region of Minnesota 2013-2014.

## Introduction

The European gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae), is a polyphagous forest insect native to Eurasia. Introduced in Medford, Massachusetts in 1869, its North American range now extends from the Canadian Maritime provinces south to North Carolina and west to Minnesota and Wisconsin. Female European gypsy moths are incapable of flight, attracting flight-capable males through a sex pheromone (Leonard 1981). Long distance dispersal is primarily achieved through the anthropogenic movement of egg masses, typically concealed on outdoor household items, although neonates are capable of short range dispersal through ballooning (Mason and Mcmanus 1981, McFadden and Mcmanus 1991). Larvae have a voracious appetite and feed on more than 300 host plants (Liebhold et al. 1995).

In addition to the European gypsy moth (*Lymantria dispar dispar* (L.)), there are two other subspecies of gypsy moth found in Asia: *L. dispar asiatica* (Vnukovskij) and *L. dispar japonica* (Motschulsky). Gypsy moths originating from Asia are believed to have a greater invasive potential than gypsy moths originating in Europe due to female flight capability (Schaefer et al. 1984, Reineke and Zebitz 1998, Keena et al. 2001), broader host plant range (Schaefer et al. 1984, Baranchikov 1989), and shorter chilling requirements for diapause completion (Keena 1996, 2015, Wei et al. 2014). Moreover, flight-capable female gypsy moths are attracted to illumination in non-forested habitats, such as light standards in urban and industrial areas, which increases the potential of human-mediated movement (Schaefer et al. 1984, Wallner et al. 1995). For regulatory purposes, the United States Department of Agriculture (USDA) classifies any biotype of *Lymantria dispar* (*sensu lato*) possessing female flight capability as “Asian Gypsy Moth”

including: *Lymantria dispar asiatica* (Vnukovskij), *Lymantria dispar japonica* (Motschulsky), *Lymantria umbrosia* (Butler), *Lymantria albescens* (Hori & Umeno), and *Lymantria dispar postalba* (Inoue) (Pogue and Schaefer 2007, USDA-APHIS-PPQ 2010, Kaplan et al. 2014). Here after we refer to this species complex as Asian gypsy moth.

Species and subspecies of gypsy moths are differentiated using morphological characters that can be quantitative, such as forewing length, or qualitative, such as forewing color and patterning (Pogue and Schaefer 2007). However, relying on morphological characters to identify suspect moths is time consuming, difficult, and requires expert knowledge. In addition, crucial morphological characters are often obscured or destroyed if moths are captured within traps containing Tanglefoot®, which are the most commonly used traps in gypsy moth monitoring programs. Moreover, subspecies of *L. dispar* are capable of hybridizing, resulting in offspring with intermediate morphological and behavioral traits (Goldschmidt 1934, Reineke and Zebitz 1998, Keena et al. 2001).

The need for robust molecular markers to identify gypsy moth origins became apparent in 1991 when United States and Canadian inspectors in the ports of Seattle, Washington and Vancouver, British Columbia discovered hundreds of egg masses on shipping structures originating from the Russian Far East. Due to the prohibition of pesticide use in maritime environments, gypsy moth egg masses were entombed using an aerosol formulation of Tanglefoot®. However, gypsy moth larvae were still subsequently observed ballooning onshore from shipping superstructures (Gibbons 1992). In response, pheromone-baited traps were deployed along the ports of Seattle and Vancouver. Molecular analysis of captured moths examined mitochondrial DNA (mtDNA) and

restriction fragment length polymorphisms (RFLPs), revealing the presence of gypsy moths with Asian haplotypes near the Seattle/Vancouver port of entry (Bogdanowicz et al. 1993). Strict eradication programs were consequently enacted in these areas, resulting in the successful eradication of several small gypsy moth populations (Hajek and Tobin 2009). Despite these successes, additional introductions of Asian gypsy moths in North America have continued to occur through global trade routes (Gray 2010, Tobin, Kean, et al. 2014), as well as through the transport of military cargo from Germany (Prasher 1995, Hajek and Tobin 2009). The presence of Asian gypsy moths in Germany and the prevalence of international trade in general have increased the likelihood of future Asian gypsy moth introductions and establishments (Gray 2010, 2015).

Following confirmation that Asian gypsy moths had been introduced into the United States, the need to develop nuclear molecular markers became evident. Mitochondrial markers did not exhibit sufficient variation to distinguish Asian and North American populations, and due to the maternal inheritance, could not detect hybrids. In response, Garner and Slavicek (1996) identified a diagnostic region of nuclear DNA through the use of random amplification of polymorphic DNA by polymerase chain reaction (RAPD-PCR). This diagnostic region can be used fairly robustly to identify the origins of suspect moths through the use of locus-specific primers, and has been designated as the FS1 locus. The FS1 locus exhibits co-dominant Mendelian inheritance of two alleles, the FS1-A, which predominates in Asian populations of gypsy moth, and the FS1-N, which predominates in North American populations of gypsy moth. It is able to diagnose homozygous moths putatively originating from Asia or North America, and heterozygotes (FS1-H) (Garner and Slavicek 1996). Moths can be heterozygous at the

FS1 locus regardless of hybridization between subspecies. Further work by Keena and colleagues (2008) concluded that presence of the FS1-A allele is not necessarily correlated with female flight capability (see Discussion), but surveying for the allele still represents a valuable tool for the early detection of potential invaders that necessitate further scrutiny.

The United States Department of Agriculture, Forest Service, monitors gypsy moth populations along the expanding population front in the eastern and Midwestern United States through the Slow-the-Spread (STS) program. This program uses traps baited with synthetic female mating pheromone to capture male gypsy moths, and estimate the abundance and distribution of their populations (Tobin and Blackburn 2007). State cooperators of the STS program often submit a subsample of captured moths to the USDA-APHIS Center for Plant Health Science and Technology (CPHST) laboratory in Buzzards Bay, MA for subsequent genetic analysis. In addition, the USDA APHIS performs detection surveys at high-risk introduction sites such as ports of entry, selected military bases, and other locations as needed in areas where gypsy moth is not present (USDA-APHIS-PPQ 2010). The FS1-A allele occurs in United States gypsy moth populations at frequencies of approximately 3—6%, but some areas exhibit higher FS1-A frequencies (Prasher 1995, Kaplan et al. 2014). Moths captured in Minnesota, for example, consistently exhibit FS1-A frequencies 2—3x higher than the national average. The reasons for this discrepancy are unclear.

Under eradication programs coordinated through USDA APHIS, Minnesota has been deploying traps in rotating grids throughout the state to detect initial populations of gypsy moth since 1973. Gypsy moth in Minnesota has been managed under the STS

program since 2004 (Leonard 2007). The majority of moths to date have been captured in the northeastern region in St. Louis, Lake, and Cook Counties, in a part of the state known as the Arrowhead region (see Chapter II). The Arrowhead region has several distinct physical, cultural, and economic features. In addition to suitable forest cover types and a thriving wood products industry, this region is home to several international ports, including Duluth, Minnesota and Thunder Bay, Ontario. If the prevalence of the FS1-A allele in this region was the result of one or several recent point introductions, we would expect the distribution of these alleles to exhibit clustered spatial patterns. The anthropogenic movement of gypsy moths mediated through the tourism industry could be another possible source of point introductions. Tourists to the Arrowhead region of Minnesota spend ≈\$1.6 billion annually, approximately half of which is spent during the summer months along the scenic shores of Lake Superior (Davidson-Peterson Associates 2008). Firewood is a high-risk movement pathway associated with the spread of invasive species such as the emerald ash borer (*Agrilus planipennis* Fairmaire) and gypsy moth, for example (Muirhead et al. 2006, Bigsby et al. 2011, Jacobi et al. 2011, USDA-APHIS 2011).

Alternatively, elevated FS1-A frequencies within the Arrowhead region of Minnesota may reflect adaptation to cold climates. Gypsy moth originating from Asia require less cold exposure to complete diapause and develop more rapidly than gypsy moths originating from Europe (Keena 1996, 2015). However, the phenotype requiring less cold exposure for diapause completion is not restricted to *L. d. asiatica* or *L. d. japonica* and it is not fixed in most populations. Less cold exposure for diapause completion would be advantageous in cold climates by allowing eggs to complete post



diapause and to hatch more quickly when temperatures warm (Keena 2015). If FS1-A allele frequencies are elevated at the beginning of the flight period, then this could suggest that this genotype develops at a more rapid rate. However, it is currently unknown if the FS1-A allele is correlated with increased cold hardiness. FS1-A frequencies are not elevated in Wisconsin gypsy moth populations, despite their northern latitude. The lack of elevated FS1-A frequencies in Wisconsin could be a consequence of genetic admixture during the population establishment phase resulting from multiple introductions (see Discussion).

In this paper, we characterized FS1-A allele frequencies in the Arrowhead region of Minnesota over 2 years. We used two approaches. First, we characterized the occurrence of the FS1-A allele in a spatial context. Using spatial regression techniques, we assessed the overall frequency of FS1-A alleles across the landscape and analyzed if there were any spatial patterns, such as clustering or inhibition, in the distribution of FS1-A alleles. Moreover, we assessed if the distribution of FS1-A alleles was associated with any putative movement pathways such as ports, roads, natural areas, mills, campgrounds, or firewood dealers. Second, we analyzed temporal dynamics in the occurrence of the FS1-A allele to assess if the allele was more likely to peak at any particular point during the summer gypsy moth flight period and if these peaks were consistent with potential introduction events.

## Methods

### Sample collection.

Traps to capture adult male gypsy moths were deployed in transects along the north shore of Lake Superior and inland within the Arrowhead region of Minnesota in 2013 and 2014 (Fig. 1.1). The transects spanned Cook, Lake, St. Louis, and Carleton Counties, which exhibited the highest populations of male gypsy moths in previous years (2004-2014). One transect was deployed along the shoreline of Lake Superior ( $n=48$  traps, mean intertrap distance=4.5 km). Another transect, parallel to the first, was deployed approximately 7 km inland ( $n=52$ , mean intertrap distance=4.7 km). In 2014, an additional transect with 50 traps was established in a loop extending north into Lake County (mean intertrap distance=1.9 km) (Fig. 1.1B).

Transects were established 18 July 2013 and 14 July 2014 and monitored weekly through 16 October 2013 and 22 October 2014, respectively, by the Minnesota Department of Agriculture (MDA). Standard milk carton style traps were baited with (+) disparlure (*cis*-7,8-Epoxy-2-methyloctadecane) (Sentry Biologicals Inc., Billings, MT), a synthetic pheromone attractive to flying male gypsy moths (Schwalbe 1981). Each trap contained a 2.2-dichlorovinyl dimethyl phosphate insecticidal strip, 10% by weight (DDVP; Hercon Environmental, Emigsville, PA) to quickly kill the males and preserve their integrity for visual and molecular analysis. Trap captures were annotated with the collection date and location. Contents of traps were mailed weekly to the USDA-APHIS CPHST laboratory in 7.5 in x 10.5 in Kraft paper merchandise bags (Uline, Pleasant Prairie, WI) to screen for the presence of nuclear DNA markers (Garner and Slavicek 1996). In 2013, if the weekly catch for a given trap exceeded 10 moths, CPHST staff took

a random subsample of 10 moths for molecular analysis. In 2014, lower population numbers permitted molecular analysis of all captured moths (see Results).

### **Molecular methods.**

Genomic gypsy moth DNA was extracted using a Proteinase K protocol (Maniatis et al. 1982). An antenna or leg from each moth was placed in a separate 1.2 ml tube containing 500  $\mu$ l of extraction buffer (5% Proteinase K, 0.1% Tergitol, 1X Tris/EDTA buffer) and incubated overnight at 37° C. The extractions were then deactivated by heating at 75° C for 30 min and stored at -20° C until PCR could be performed.

Amplification of the FS1 locus was conducted in polymerase chain reactions (PCR, 10  $\mu$ l total volume) containing 3.5  $\mu$ l molecular grade water, 1  $\mu$ l 10X PCR buffer without MgCl<sub>2</sub> (Sigma Aldrich JumpStart buffer), 1.4  $\mu$ l MgCl<sub>2</sub> (25 mM), 1.6  $\mu$ l dNTP solution (1.25 mM), 0.2  $\mu$ l primers FS1-forward (5'-GGATGGTGGGTGTCGTT-3') and FS1-reverse (5'-GGTTGGTTGATGATTAGATG-3') respectively (10 pmol/ $\mu$ l), and 0.1  $\mu$ l of Sigma Aldrich JumpStart Taq DNA Polymerase (2.5 units/ $\mu$ l). Two microliters of genomic DNA was used as a template. PCR cycling conditions consisted of an initial 1 min denaturation at 94° C followed by 20 cycles of 94° C for 15 s, 58° C for 15 s followed by touchdown to 48.5° C at a rate of -0.5° C per cycle, and 72° C for 20 s. A final 15 cycles at 94° C for 15 s, 48° C for 15 s, and 72° C for 20 s plus 1 sec per cycle and a final hold at 4° C completed the PCR program.

After PCR amplification, 10 $\mu$ l PCR product was loaded onto 1% agarose/ 2% Nusieve gels in 1X Tris/Boric acid/EDTA buffer and run in 1X TBE buffer at 150 volts for 2 hours. Separated bands were stained with Sybr-safe DNA stain and visualized under UV light.

## Statistical Methods.

### *Spatial Analysis.*

Molecular data for each trap were summarized by binomial presence/absence response of the FS1 locus in all males analyzed. Trap response was designated as: (number of FS1-A alleles, number of FS1-N alleles). Because gypsy moths are diploid, each male could have one or two copies of each marker, and a trap with one moth heterozygous at the FS1 locus (A,N) and one moth homozygous for the FS1-N allele (N,N) would have a designated response of (1,3). This binomial variable for each trap, which accounts for differences in sample size, was used for spatial regressions described below. To estimate the mean frequency of FS1-A abundance in the population, this binomial response variable was fitted to a constant intercept model that was back transformed according to the logit link (i.e.,  $P(\text{FS1-A allele occurs}) = \frac{\exp\beta_0}{1+\exp\beta_0}$ ), where  $\beta_0$  is the mean frequency of FS1-A allele occurrence estimated using a constant intercept regression. Ninety-five percent confidence intervals about this estimate were constructed by multiplying the standard error of the intercept by  $\pm 1.96$  (i.e., Z-value for  $\alpha=0.025$ ).

We examined patterns of clustering or inhibition in FS1-A abundance by testing the residuals of this constant intercept model for spatial autocorrelation based upon Moran's I estimated from the spdep package in R (Bivand 2014, R Core Team 2014). Moran's I requires a definition of spatial neighborhood and a weighting scheme. We used a neighborhood radius of 11 km, the maximum intertrap distance that can accurately detect changes in gypsy moth spread rates (Sharov et al. 1997), and an inverse distance weighting scheme to reflect decreasing probability of capturing male gypsy moth in a trap with increasing distance from the trap. This neighborhood structure ensured a

continuous neighborhood throughout the study area while also taking into account the relationship between distance and the attractiveness of the gypsy moth lure (Elkinton and Cardé 1988).

Our implementation of Moran's I captures small-scale spatial variability (Moran 1948). We examined spatial relationships of FS1-A abundance with potential broader-scale spatial trends captured by the traps' latitudes and longitudes, total number of moths captured, and the number of moths that exhibited successful PCR amplification using spatial regression models. These variables are summarized in Table 1.1A. We also analyzed putative movement pathways associated with tourism and wood products industries. Dependent variables in the spatial regressions models included distances to ports, roads, natural areas, mills, campgrounds, and firewood dealers (Table 1.1B). Spatial analyses beyond Moran's I were conducted only on 2013 data due to the low number of moths captured in 2014.

#### *Temporal Analysis.*

Molecular data were summarized by trap and week of moth capture for time series analyses to assess if the frequency of FS1-A alleles differed over time. The frequency of FS1-A allele abundance in the population was considered as a binomial response variable, as previously described. An ANOVA was conducted with a generalized linear model and the binomial family with week of capture as a factor using the R package lme4 (Bates et al. 2014, R Core Team 2014). If the frequency does not vary through time (i.e., FS1-A moths are captured in similar proportions throughout the flight season), we would expect no statistical relationship. Finally, a Durbin-Watson test was conducted using the car package in R (Fox and Weisberg 2011, R Core Team 2014)

to test model residuals for autocorrelation. Similar observations resulting from proximity in time or space can underestimate the true variance and thus bias inferential tests. The Durban-Watson test, commonly implemented in time series analysis, is able to detect autocorrelation in model residuals and is suitable for use in nonlinear models (White 1992).

When preparing data for time series analyses, 40 moths were removed from the data set due to discrepancies in trap capture numbers reported on multiple sampling dates, resulting in a 3.4% reduction in sample size. These moths were retained for the spatial analysis.

## Results

In 2013, a total of 2,479 moths were captured in trapping transects established for FS1-A molecular analyses. The total capture of male gypsy moths in Minnesota's monitoring program for 2013 was 71,258 moths, 99% of which were captured within the Arrowhead region. A subsample of 1,214 moths underwent molecular analyses, with PCR amplification occurring successfully in 980. These moths were captured along the full length of the Lake Superior shore (Fig. 1.2A), and FS1-A alleles appeared to be prevalent throughout (Fig. 1.2C). The FS1-A allele occurred at a mean frequency of 10.2% (95% CI: 8.9%, 11.6%). Fewer male moths were captured in 2014 than in 2013, despite the deployment of 50% more traps across a more extensive area (Fig. 1.2B). Only 58 moths were captured; a reduction of 98%; we note that this population decline occurred following extreme winter conditions as a result of atmospheric blocking of the polar jet stream resulting in a persistent trough of Arctic air masses. This air mass created the below normal temps and above normal snowfall throughout the Midwestern United States during the winter of 2013/14 (Marinero et al. 2015). Molecular analyses were conducted on all captured moths, and PCR amplification was successful in all but one individual. In 2014, the FS1-A allele occurred at a mean frequency of 16.7% (95% CI: 10.9%, 24.7%) (Fig. 1.2D), an increase of 63.7% over 2013.

The greatest contribution of FS1-A alleles was from moths that were heterozygous for the FS1-A allele; however, moths homozygous for the FS1-A allele were also present in both years. In 2013, 171 heterozygous moths and 14 moths that were homozygous for the FS1-A allele were identified, representing 17.5% and 1.4% of the total moth capture, respectively (Fig. 1.3A). In 2014, 15 heterozygous moths and two

moths that were homozygous for the FS1-A allele were identified, representing 26.3% and 3.5% of the total moth capture, respectively (Fig. 1.3B).

### **Spatial Analysis.**

The spatial pattern of FS1-A allele frequencies across the Arrowhead region of Minnesota in 2013 appeared to be random. There was no evidence for clustering in either year (2013: Moran's  $I = 0.002$ ,  $P = 0.84$ ; 2014: Moran's  $I = -0.0085$ ,  $P = 0.97$ ). Moreover, we found no evidence of latitudinal or longitudinal gradients in FS1-A allele frequencies region-wide ( $Z = 1.13$ ,  $P = 0.259$  and  $Z = 0.85$ ,  $P = 0.396$ , respectively; Table 1.2A). These analyses, and those described below, were restricted to 2013 data due to low moth captures in 2014.

The mean frequency of FS1-A alleles was greater among traps with fewer moth captures (Table 1.2A). This relationship, modeled with a decay function, is shown in Figure 1.4. The frequency of FS1-A alleles within individual traps was at most 50%. As sample sizes increased to approximately 100—350 moths per trap in high density areas, FS1-A frequencies decreased and approximated the national mean.

The random pattern of FS1-A on the landscape was supported by lack of association with any putative movement pathways that we studied (Table 1.2B). For example, FS1-A allele frequencies were not correlated with movement pathways typically associated with tourism, such as distance to roads ( $Z = 0.98$ ,  $P = 0.92$ ), distance to natural areas ( $Z = 0.378$ ,  $P = 0.71$ ), or distance to campgrounds ( $Z = 0.74$ ,  $P = 0.46$ ). Likewise, no correlations were found between the occurrence of FS1-A alleles and pathways associated with movement of wood products, such as distances to international



ports ( $Z=1.45$ ,  $P=0.147$ ), distances to mills ( $Z=-0.95$ ,  $P=0.343$ ), and distances to firewood dealers ( $Z=-1.00$ ,  $P=0.317$ ).

### **Temporal Analyses.**

In 2013, the first gypsy moths were recorded from transect traps during the last week of August (Fig. 1.5A). Moth captures peaked in the first week of September, and declined through the next 5 weeks until they ceased on 9 October 2013. Mean FS1-A allele frequency was fairly constant throughout the flight period, and appeared to peak at 12.5% during the first week of October, although trap capture was very low by then. FS1-A alleles were present throughout the 2013 flight period, excluding the final week of moth capture. FS1-A alleles were present in the form of homozygous and heterozygous moths; however, moths that were homozygous for the FS1-A allele were always caught with heterozygous moths or moths that were homozygous for the FS1-N allele (Fig. 1.5C). There was no correlation between the occurrence of FS1-A alleles and study week ( $\chi^2_{(6)} = 8.96$ ,  $P=0.18$ ), indicating that FS1-A allele frequencies are similar throughout the flight season. Weekly samples were independent, with no autocorrelation in the model residuals detected using a Durbin-Watson test ( $D-W=1.84$ ,  $P=0.086$ ).

The 2014 flight period began in mid-August and continued into mid-September, with peak moth capture occurring during the last week of August (Fig. 1.5B). The peak weekly FS1-A allele frequency in 2014 was 21.9%, a 75% increase from 2013, and corresponded with peak trap capture. FS1-A alleles were absent during the first and last week of moth capture. Consistent with 2013 results, moths that were homozygous for the FS1-A allele were always captured with moths that were heterozygous, or homozygous for the FS1-N allele (Fig. 1.5D). During the 2014 peak flight period, weekly FS1-A allele

frequencies were consistently higher than those observed during the 2013 peak flight period. Similar to 2013, there was no correlation between study week and FS1-A allele frequencies ( $\chi^2_{(5)} = 4.15, P=0.529$ ) and no evidence of temporal autocorrelation remaining in the model residuals (D-W=1.95,  $P=0.306$ ). Despite these consistencies, we interpret 2014 results with caution due to the overall trap capture being very low.

## Discussion

The random distribution of FS1-A alleles through time and across space within the Arrowhead region of Minnesota (2013, 2014) do not support the hypotheses that potential Asian gypsy moth genotypes within this region are associated with recent putative introductions, immigration events, or movement pathways. Elevated frequencies of FS1-A alleles do appear to occur in small populations, however (Fig. 1.4). Declining FS1-A frequency with higher trap captures was not an artifact of experimental protocol, as the number of moths amplified in a subsample had no effect on the frequency of occurrence of FS1-A alleles (Table 1.2A).

Elevated frequencies of FS1-A alleles in small populations could simply reflect genetic drift, the effects of which are more pronounced within small populations (Freeland 2005). Genetic drift is a stochastic demographic process that arises from variable reproductive success within a population, and results in variable allele frequencies between generations. As such, rare molecular markers can “surf the wave” of expanding population fronts and reach high frequencies in newly colonized areas following a range expansion, irrespective whether the variants are neutral, beneficial, or deleterious (Travis et al. 2007, Excoffier et al. 2009). Gene surfing occurs more frequently in small populations than in large populations, and is often observed in rapidly expanding populations with limited dispersal capabilities (Klopfstein et al. 2006). As population levels increase, competition becomes important and selection reduces the frequency of deleterious variants (Travis et al. 2007).

Alternative to genetic drift, the FS1-A allele may be linked to physiological traits (e.g., shorter chilling requirements for diapause completion) or behavioral attributes

(oviposition site selection, dispersal capabilities) subjected to selective pressures. The FS1-A allele is likely neutral, as the allele is a 105 base pair region of DNA lacking any long open reading frames that may encode for proteins or peptides (Garner and Slavicek 1996, Keena et al. 2001).

Evidence for FS1 linkage to selected traits comes from the literature and this study. For example, Keena (1996) demonstrated that gypsy moth eggs originating from the Russian Far East, where FS1-A alleles are abundant, required less cold exposure for diapause completion. This phenotypic variation is advantageous in environments with shorter growing seasons, where eggs would be ready to hatch when conditions become favorable. Moreover, regions with cooler spring temperatures, may favor the maintenance of phenotypes with reduced chilling requirements (Keena 2015). The Arrowhead region of Minnesota experiences harsh winters with variable intensities and durations, and reductions in the number of male moths captured have been observed following extreme winters (see Chapter II). Elevated FS1-A frequencies in Minnesota may be due to increased overwintering success in years with harsh winters. Moreover, cool spring temperatures may aid in the maintenance of this advantageous phenotype with reduced diapause requirements.

Potential differences in winter survivorship among moth populations with varying frequencies of the FS1-A allele could also reflect behavioral differences in selection of ovipositional sites. Site selection for oviposition varies among species of *Lymantria* and subspecies of *L. dispar* that are regarded as Asian gypsy moth (Higashiura 1989, Pogue and Schaefer 2007, Jikumaru 2013). Selection of oviposition sites by female gypsy moths presents several potential tradeoffs. Eggs laid higher in a tree canopy, for example, could

facilitate ballooning of first instars, but simultaneously increases the risk of avian predation (Higashiura 1989). Selection of oviposition sites may also vary with mean annual amount of snowfall (Higashiura 1989). Among Japanese populations of *L. d. japonica* in regions with little snowfall, moths consistently oviposited within tree canopies, whereas in regions with large snowfalls, *L. d. japonica* oviposition occurred primarily on tree trunks below the average snow depth, presumably to insulate them. In the Russian Far East, egg masses of *L. d. asiatica* are deposited on the underside of deciduous leaves. This behavior ensures that these egg masses will fall to the ground and be covered by accumulating snow (Pogue and Schaefer 2007, Jikumaru 2013). In Minnesota, overwintering mortality may be reduced in egg masses that are oviposited at low heights and are insulated by snow cover (see Chapter II).

Alternatively, elevated frequencies of the FS1-A allele within the Arrowhead region of Minnesota could be linked to enhanced dispersal abilities of females or first instar larvae, however, evidence is lacking. The presence of female flight capability is not a fixed trait in populations in which it occurs. Moreover, the only way to accurately predict flight capability in female gypsy moths is through the use of female wing morphometrics (Keena et al. 2008, Shi et al. 2015). To our knowledge, potential genetic variation has not been empirically linked to degree of dispersal of ballooning early-instars.

For *L. d. dispar*, the geographic range expands when small isolated populations coalesce with the population front (Liebhold et al. 1992, Sharov and Liebhold 1998, Liebhold and Tobin 2008). Low density populations of the gypsy moth are subject to Allee effects due to mate finding failure (Contarini et al. 2009, Tobin et al. 2013)

however, establishment thresholds vary regionally with the lowest thresholds reported from the northern part of its range (Whitmire and Tobin 2006, Tobin et al. 2007). A lower threshold for successful gypsy moth establishment could be a factor in allowing the FS1-A allele to persist in smaller populations. The invasion of Wisconsin was facilitated by long distance dispersal (Tobin and Blackburn 2008), but these areas may not express elevated FS1-A frequencies due the genetic admixture present when this population was becoming established. The geographical variation in establishment thresholds provides a possible explanation for variation in FS1-A frequencies across the entire range of the gypsy moth.

We conclude that the random distribution of FS1-A alleles in Minnesota favors the hypothesis that this population is comprised of individuals of *L. d. dispar* and not *L. d. asiatica* or other Asian gypsy moth. The elevated FS1-A frequencies in Minnesota populations must be explained another way. They are either due to the population dynamics of small population size (genetic drift, Allee effect) or due to linkage of the FS1-A allele with selected traits (oviposition, overwintering, ballooning). To assess genetic differences within North American populations of gypsy moth, future research should implement the use of microsatellite markers. Microsatellites are commonly used in population genetics due to their high variability, co-dominant inheritance, and increasing availability (Freeland 2005). When the population structure of gypsy moth was assessed, two private microsatellite alleles were found in Michigan and Minnesota, the same states which also exhibit elevated frequencies of Asian mtDNA and FS1-A alleles, respectively (Prasher 1995, Wu et al. 2015). Both Michigan and Minnesota are located along the northern edge of the gypsy moth range. The use of microsatellite data may

elucidate population level processes which contribute to the genetic differentiation that make these northern populations unique.

**Table 1.1:** Explanatory variables used in spatial regression models to study associations with distributions of the FS1-A allele in adult male moths trapped in the Arrowhead region of Minnesota, 2013.

| Variable                    | Description  |
|-----------------------------|--|
| <i>A. Trap variables</i>    |  |
| Latitude                    | Latitude coordinates (decimal degrees); used to examine the distribution of FS1-A allele frequencies in the East/West direction.   |
| Longitude                   | Longitude coordinates (decimal degrees); used to examine the distribution of FS1-A allele frequencies in the North/South direction.  |
| Total catch                 | Total number of male moths captured at each trapping location.   |
| Moths amplified             | The number of moth samples that had successful PCR amplification during FS1 molecular analyses.  |
| <i>B. Pathway variables</i> |  |
| Port distance               | Distance (km) from traps to ports calculated using a GIS. Port layer was obtained from the U.S. Department of Transportation ( <a href="http://www.rita.dot.gov">www.rita.dot.gov</a> ). |
| Mill distance               | Distances (km) from traps to mills were calculated using a GIS. Mill locations were obtained from the Minnesota Department of Agriculture.   |



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| Variable                 | Description  |
|--------------------------|--|
| Road distance            | Distance (m) from traps to roads calculated using a GIS. Road layers were obtained from the Minnesota geospatial commons ( <a href="https://gisdata.mn.gov/">https://gisdata.mn.gov/</a> ).                            |
| Natural areas distance   | Distances (m) from traps to natural areas were calculated using a GIS. Natural area shape files were obtained from the Minnesota geospatial commons ( <a href="https://gisdata.mn.gov/">https://gisdata.mn.gov/</a> ). |
| Campground distance      | Distances (m) from traps to campgrounds were calculated using a GIS. Campground locations were obtained from the Minnesota Department of Agriculture.  |
| Firewood dealer distance | Distances (m) from traps to firewood dealers. Locations of firewood dealers were obtained from the Minnesota Department of Agriculture.  |

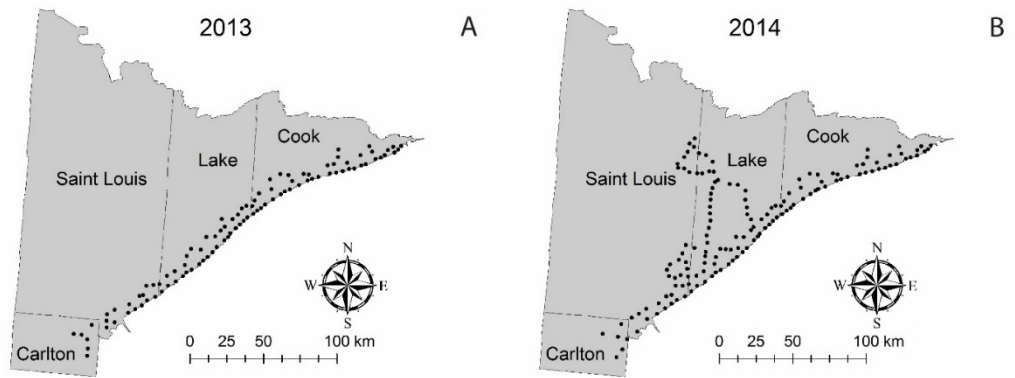
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**Table 1.2:** Association of individual trap location and pathway variables with frequency of the FS1-A allele in male moths sampled in the Arrowhead region of Minnesota, July-October, 2013. Each line is an individual logistic regression, where the response variable is a binomial presence/absence indicator of FS1-A abundance in the moths of the trap analyzed. To obtain the mean proportion of FS1-A among moths in the trap,  $y$  should be back transformed using the logit link function  $P(\text{FS1-A is present}) = \frac{\exp^{\beta_0 + \beta_1 x}}{(1 + \exp^{\beta_0 + \beta_1 x})}$ .

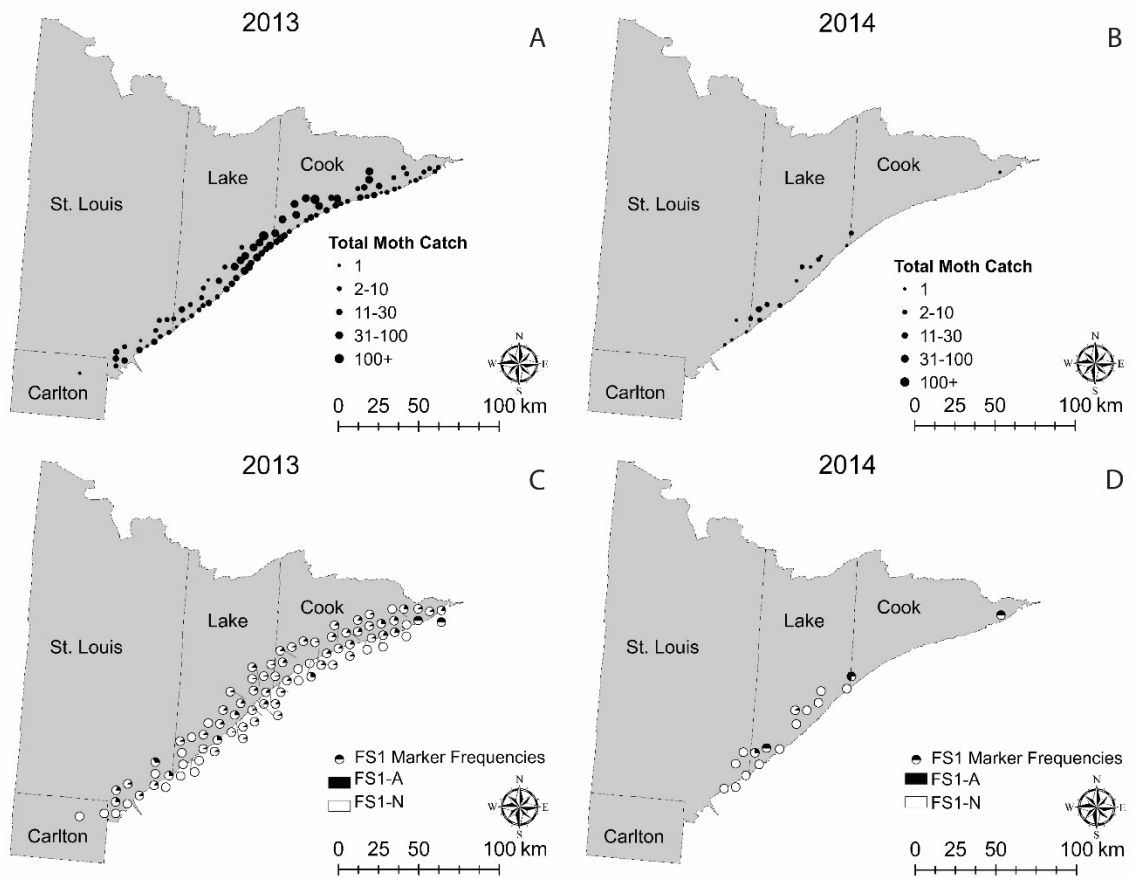
| Variable  | Intercept, ( $\beta_0$ ) |       |             | Explanatory variable, ( $\beta_1$ ) |        |         | Moran's $I^a$ |         |
|---|--------------------------|-------|-------------|-------------------------------------|--------|---------|---------------|---------|
|   | Estimate                 | SE    | P-Value     | Estimate                            | SE     | P-Value | Estimate      | P-value |
| <i>A. Trap variables:</i>                                   |                          |       |             |                                     |        |         |               |         |
| Latitude  | 12.30                    | 12.82 | 0.337       | 0.1592                              | 0.141  | 0.259   | -0.018        | 0.894   |
| Longitude   | -12.93                   | 12.66 | 0.307       | 0.226                               | 0.267  | 0.396   | -0.014        | 0.948   |
| Total catch   | -1.99                    | 0.10  | $P < 0.001$ | -0.0041                             | 0.0017 | 0.013   | 0.002         | 0.832   |
| Moths amplified   | -2.00                    | 0.17  | $P < 0.001$ | -0.011                              | 0.009  | 0.253   | -0.010        | 0.736   |
| <i>B. Pathway variables: Distance from traps to nearest</i> |                          |       |             |                                     |        |         |               |         |
| Port  | -2.31                    | 0.12  | $P < 0.001$ | 0.0054                              | 0.0037 | 0.147   | -0.011        | 0.982   |
| Mill  | -2.07                    | 0.13  | $P < 0.001$ | -0.0042                             | 0.004  | 0.343   | 0.005         | 0.797   |

| Variable        | Intercept, ( $\beta_0$ ) |      |                 | Explanatory variable, ( $\beta_1$ ) |         |         | Moran's I <sup>a</sup> |         |
|-----------------|--------------------------|------|-----------------|-------------------------------------|---------|---------|------------------------|---------|
|                 | Estimate                 | SE   | P-Value         | Estimate                            | SE      | P-Value | Estimate               | P-value |
| Road            | -2.19                    | 0.09 | <i>P</i> <0.001 | 3.04E-4                             | 0.003   | 0.922   | 0.002                  | 0.836   |
| Natural area    | -2.19                    | 0.08 | <i>P</i> <0.001 | 8.23E-6                             | 2.27E-5 | 0.706   | 0.002                  | 0.836   |
| Campground      | -2.28                    | 0.15 | <i>P</i> <0.001 | 1.37E-5                             | 1.85E-5 | 0.461   | 0.010                  | 0.737   |
| Firewood dealer | -2.07                    | 0.13 | <i>P</i> <0.001 | 1.36E-5                             | 1.36E-5 | 0.317   | 0.010                  | 0.724   |

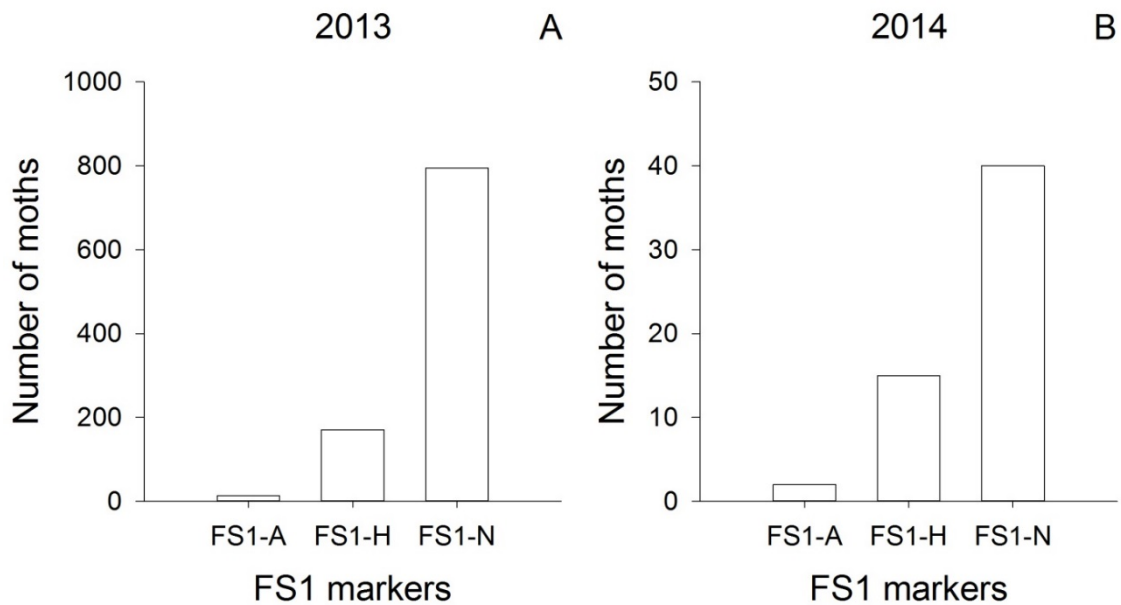
<sup>a</sup> Diagnostic test for spatial autocorrelation remaining in the model residuals. A non-significant test indicates no autocorrelation remaining, providing no evidence that inferential tests associated with the predictor variable of each regression are biased.



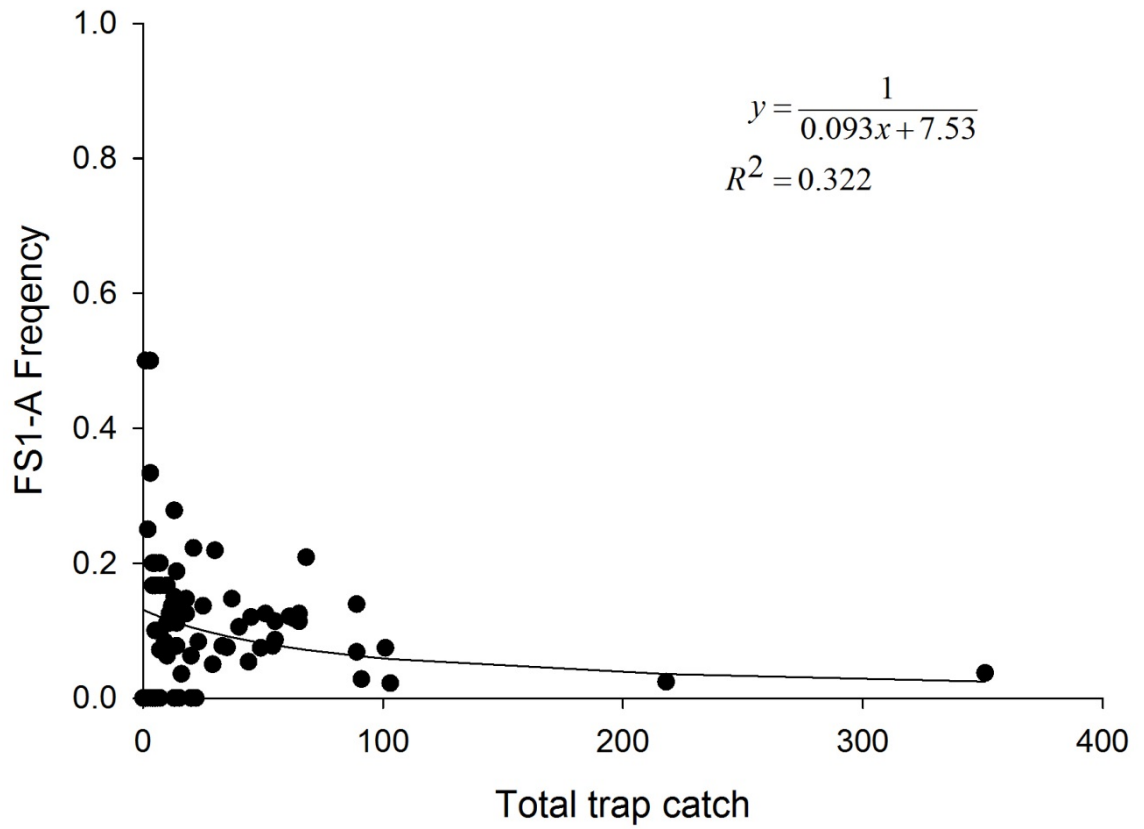
**Figure 1.1:** Placement of USDA APHIS milk carton traps baited with synthetic female sex pheromone. A) 2013; ( $n=100$ ) traps placed 18 July and checked weekly until 16 October. B) 2014; ( $n=150$ ) traps placed 14 July and checked weekly until 22 October.



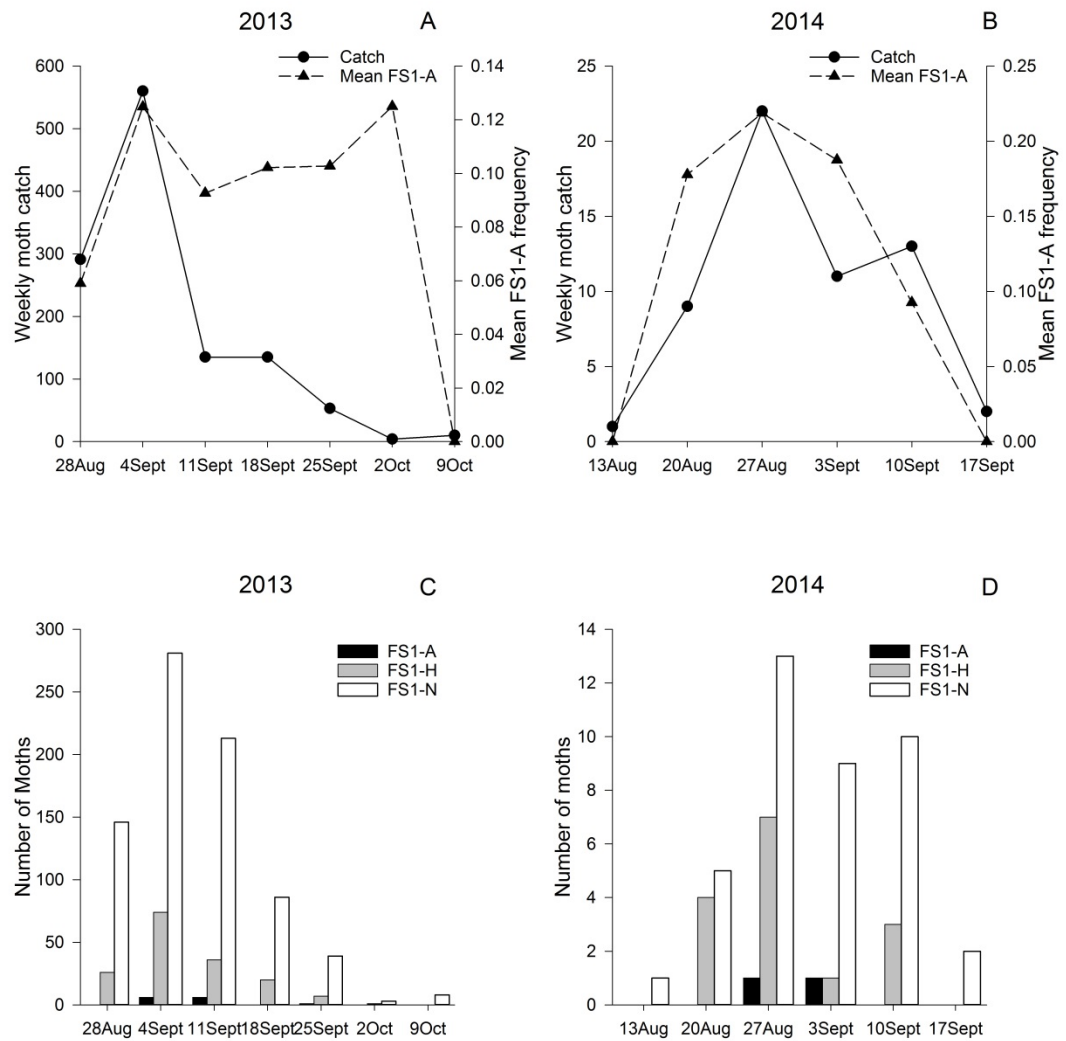
**Figure 1.2:** Spatial distribution of male gypsy moth capture and FS1-A allele frequencies in the Arrowhead region of Minnesota 2013, 2014. Total male gypsy moth capture in traps set for molecular analyses. A) 2013. B) 2014. Pie charts indicate the frequency of FS1 alleles summarized by trap. C) 2013. D) 2014.



**Figure 1.3:** The distribution of FS1 genotypes among moths captured in traps set for molecular analysis in the Arrowhead region of Minnesota. A) 2013. B) 2014. Possible genotypes include FS1-Asian (FS1-A), FS1-Heterozygote (FS1-H), and FS1-North American (FS1-N). Note that the y-axis scales are different between panels.



**Figure 1.4:** Exponential decay relationship between total trap catch of male gypsy moths in the Arrowhead region of Minnesota, 2013, and frequency of FS1-A alleles in captured moths. As populations increase in size, the occurrence of FS1-A alleles decreases.



**Figure 1.5:** Time series analysis of weekly trap catch and weekly mean allele frequencies for moth captures in the Arrowhead region of Minnesota. A) 2013. B) 2014. Weekly distribution of genotypes among moths that underwent FS1 molecular analyses. A) 2013. B) 2014



## **Chapter II**

Range expansion of *Lymantria dispar dispar* (L.) (Lepidoptera: Erebidae) along its northwestern range in North America despite low predicted climatic suitability.

## Introduction

The European gypsy moth (*Lymantria dispar dispar* (L.)) is a forest pest that is capable of defoliating over 300 species of trees and shrubs (McFadden and Mcmanus 1991, Liebhold et al. 1995). Introduced into North America in 1869 at Medford, MA, the current distributional range extends northward to the Canadian Maritime Provinces, southward to North Carolina, and westward to Minnesota and Wisconsin. Gypsy moth generally spreads through stratified dispersal (Hengeveld 1988, Shigesada et al. 1995) in which local population growth and diffusive spread is combined with long-range dispersal (Liebhold and Tobin 2006, Tobin and Blackburn 2008). Female moths are incapable of flight. First instars disperse short distances by ballooning. Long distance dispersal of reproducing populations is achieved through the anthropogenic movement of life stages, such as egg masses (McFadden and Mcmanus 1991, Liebhold et al. 1992, Tobin and Blackburn 2008). As isolated populations grow and coalesce, the population front is capable of advancing large distances (Liebhold and Tobin 2008).

Under the gypsy moth Slow-the-Spread (STS) program, approximately 90,000 pheromone-baited traps are deployed annually along the leading edge of the gypsy moth population front in the United States. Pheromone-baited traps are used to identify isolated colonies and estimate male moth abundance. Through this extensive monitoring program, management practices are prioritized such that the gypsy moth's rate of spread has been greatly reduced (Tobin and Blackburn 2007). Trap catch data from the STS program comprises a highly valuable dataset not only for land managers but also researchers. This dataset has been used to elucidate gypsy moth population dynamics (Johnson et al. 2006),

characterize geographical variation in the strength of Allee effects (Tobin et al. 2007), and describe critical patch sizes induced by Allee effects (Vercken et al. 2011). Moreover, STS data have been used to characterize the range dynamics of the gypsy moth (Tobin, Gray, et al. 2014). The gypsy moth's invasion into the Arrowhead region of Minnesota has been characterized by highly variable moth captures (Fig. 2.1, Fig. 2.2), reflecting a mix of established colonies with random introductions and extinctions (Tobin 2007). Satellite gypsy moth colonies may or may not thrive in new environments (Whitmire and Tobin 2006). Climate can be an important determinant of geographic boundaries of species (Parmesan 1996), particularly for insects because their growth and development are regulated by temperature (Gilbert and Raworth 1996, Bryant et al. 2002). For example, it has long been known that egg mortality can result from exposure to extremely cold temperatures (Summers 1922, Maksimovic 1958). As such, Sullivan and Wallace (1972) predicted that the northern expansion of gypsy moth populations in North America would be limited by winter temperatures, bounded by the  $-30^{\circ}\text{C}$  isoline.

Insects, such as gypsy moth, have several strategies to survive cold temperatures. For example, insects can avoid freezing through the process of supercooling. The supercooling point is the temperature at which ice forms within the insect, and can be interpreted as the minimum temperature at which a freeze intolerant insect can survive (Salt 1961). Historically, overwintering gypsy moth egg masses have been described as freeze intolerant, with a supercooling point of *ca*  $-27^{\circ}\text{C}$  (Madrid and Stewart 1981, Waggoner 1985, Denlinger et al. 1992). However, chill intolerant (Bale 1993) is a more accurate description. Laboratory investigations have demonstrated that mortality occurs

with prolonged exposure to temperatures above the supercooling point, although some degree of chilling is required to develop cold hardiness (Madrid and Stewart 1981, Denlinger et al. 1992). Such laboratory studies are consistent with field observations that have linked egg mass mortality to the number of days that temperatures drop below  $-26^{\circ}\text{C}$  (Smitley et al. 1998).

The northeastern region of Minnesota, known as the Arrowhead region, provides a unique opportunity to investigate the invasion dynamics of the gypsy moth. This area exhibits susceptible forest cover, with greater than 20% of its basal area in preferred host genera such as *Populus* and *Betula* (Liebhold et al. 1995, 1997, Morin et al. 2005). However, this area has also been characterized as climatically inhospitable to gypsy moth (Gray 2004), as winter temperatures consistently drop below  $-30^{\circ}\text{C}$  each year (National Climatic Data Center, National Oceanic Atmospheric Administration). Moreover, phenological modeling has suggested that spring and summer degree day accumulation in this region would allow only a low proportion of the population to successively complete their life cycle each year (Gray 2004). Delayed development due to suboptimal temperatures may also reduce exposure to chilling periods requisite for acquisition of cold hardiness, thus increasing overwintering mortality (Régnière and Nealis 2002, Gray 2004). Yet, despite the very low climatic suitability predicted for the Arrowhead region, male gypsy moths have been caught annually in this region since 2000 (Tobin 2007), while egg masses – generally the most definitive sign of an established reproducing gypsy moth population – were first detected in 2008.

In this study, we seek to correlate annual male gypsy moth trap captures with

measures of preceding winter severity during the range expansion of gypsy moth into the Arrowhead region of Minnesota, USA from 2004-2014. We further test the hypothesis of climatic limitation by examining hatching success rates of 705 egg masses deployed to 12 field sites in Northern Minnesota in the winters of 2014 and 2015. We examine how placement variations in aspect and height may affect overwintering mortality, in an attempt to understand how this insect's establishment and persistence can occur in a putatively climatically-unsuitable location.

## Methods

### 1. Modeling links between winter temperatures and survey trap catch

#### Monitoring Data

Male gypsy moth trap catch data collected annually through STS were obtained from the Minnesota Department of Agriculture for the state of Minnesota, USA, from 2004-2014. Trap catch data for Cook, Lake, and St. Louis Counties, were extracted from the larger dataset because the Arrowhead region had the highest observed male gypsy moth trap captures throughout Minnesota during the study time period. Moreover, this was the most logical region in Minnesota to explore associations between winter conditions and survey trap catch data due to the frequency of severe winter conditions. Thiessen polygons were constructed around 17 weather stations located across the study area (Fig. 2.3). Trap locations were spatially joined to the nearest Thiessen polygon using ArcGIS 10 (ESRI 2010). Male moth capture was quantified for each Thiessen polygon through the summation of male gypsy moths captured from all traps deployed within polygon boundaries (Table 2.1).

The number of traps within each polygon varied annually throughout the study period. The number of traps set annually depended on a variety of factors including moth population densities in previous years, proposed treatment areas, and annual US Forest Service budgets. The action zone in the STS program defines the areas where management strategies are applied against gypsy moth populations, and traps are deployed here on a 2 km grid (Tobin and Blackburn 2007). Trapping within the monitoring zone occurred at densities of 3-8 km, depending on distance to the “action

zone”, as defined by the STS program. The number of traps was summed for each Thiessen polygon for each year. The spatial extent of the STS defined action and monitoring areas varied annually, such that not all Thiessen polygons had at least one trap per year (Table 2.1). As a consequence, the data were not appropriate for analyzing population growth rates. Instead, we focused on absolute numbers of moths captured, standardized by the number of traps deployed (see data analysis).

### **Weather Data**

Daily maximum and minimum temperatures were obtained from the National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center (<http://www.ncdc.noaa.gov/>) and MesoWest (<http://mesowest.utah.edu/>), Department of Atmospheric Sciences, University of Utah for each of the 17 weather stations (Fig. 2.3). Weather data spanned October 2003 through May 2014. For each year, data were analyzed from October of the previous year to May of the trapping year to reflect when overwintering gypsy moth egg masses would be exposed to freezing temperatures. Less than 3% of the daily weather data consisted of missing observations. For a given daily temperature series, if only one observation was missing, the value was interpolated using a first order autoregressive model. If temperature observations were missing for several consecutive days, data were obtained using the single best estimator technique, in which observations from the closest neighboring weather station with the strongest positive correlation to the target station are used (Eischeid et al. 1995).

Measures of winter severity were constructed that quantified the number of cold exposure events as well as total cold exposure (Table 2.2). Nine winter severity variables

were constructed. Cold exposure events were summarized as the number of days that the daily minimums dropped below thresholds of -10°C, -15°C, -20°C, -25°C, and -30°C. Total cold exposure was quantified as mean monthly minimum temperatures for December, January, and February as well as the absolute minimum winter temperature.

### **Treatment Data**

Treatment areas were removed from data analysis during the year in which the treatment was applied to avoid confounding effects of management activities on trap capture. Each year, a number of areas were treated in an effort to eliminate isolated colonies based upon moth abundance in the previous year. Treatments included aerial application of the bacterial insecticide *Bacillus thuringiensis* var *kurstaki* (*Btk*), or mating disruption using a synthetic mating pheromone. In addition, data were also omitted from within a 1.5 km buffer around treatment areas to account for any potential treatment drift.

Past research has shown that mating disruption treatments can persist in the environment in the year following treatment, resulting in trap catch reductions at differing rates due to treatment formulation (PVC flakes or amorphous gel) and weather conditions (Thorpe et al. 2007, Onufrieva et al. 2013). A sensitivity analysis was conducted to determine if the environmental persistence of mating disruption treatments in treatment areas was associated with subsequent reductions in summer male moth captures within Thiessen polygons. In brief, we constructed several artificial datasets where we augmented the numbers of male moths captured in areas where mating disruption treatments had been applied in the preceding year, based on published bias estimates (Onufrieva et al. 2013), and then conducted statistical analyses to see if our



results were robust (i.e. similar to original, empirical dataset). Augmentation schemes were as follows. For mating disruption treatments with Disrupt II® (Hercon Environmental, Emigsville, PA, USA), subsequent summer male moth captures within treatment areas were increased by 70% and 53%, for high and low levels of environmental persistence (Onufrieva et al. 2013). For mating disruption treatments with SPLAT GM™ (ISCA Technologies, Riverside, CA, USA), subsequent summer male moth captures within treatment areas were increased by 44% to account for high levels of environmental persistence, and unchanged for low levels of persistence due to a lack of environmental persistence observed in a warmer climate such as Virginia (Onufrieva et al. 2013).

Sensitivity analyses revealed no differences in our results (i.e. parameter estimates, significance of inferential tests) after accounting for environmental persistence, therefore analyses were conducted with population reduction treatment areas removed only from the year of treatment. The numbers of male moths captured each summer were not adjusted to account for potential environmental persistence of mating disruption treatments.

### **Statistical Analysis**

Linear regressions were used to examine the relationship between summer male moth trap captures and several variables, especially those reflecting various measures of the preceding winter's severity. The unit of replication was the Thiessen polygon (17 in the study area × 11 years). Trap and winter severity variables of interest are defined in Table 2.2 and in section "Weather Data." Trap variables describing the trapping

dynamics within each polygon included: polygon area, the number of traps within each polygon for a given year, number of male moths captured, and the previous year's male moth capture. We first fit variables of interest individually to assess their effect on summer trap captures of male gypsy moths. More male moths were captured when large numbers of moths were captured within polygon areas during the previous flight season (regression coefficient  $t_{152}=9.17$ ,  $P<0.001$ ). Likewise, more moths were captured when more traps were deployed (regression coefficient  $t_{173}=7.73$ ,  $P<0.001$ ). These results confirmed that it was important to account for trapping effort when studying variations in moth catch due to other variables. Hence, we conducted multiple regressions exploring the effects of various measures of winter severity after accounting for variations in moth capture due to trapping effort (i.e. number of traps deployed in a polygon and number of moths captured in the previous year). Trap capture variables were  $\log_2(y+1)$  transformed to satisfy assumptions of homogenous variances and normality of residuals. All data analyses were conducted in R (R Core Team 2014).

We contended with spatial autocorrelation by making the Thiessen polygons of sufficient size to account for any spatial dependence, which for gypsy moth is generally at scales of 10 km (Sharov et al. 1996, 1997). Temporal autocorrelation can also bias inferential tests (Durbin 1970). Temporal autocorrelation in the model residuals was assessed by conducting a Durbin-Watson test using the car package in R (Fox and Weisberg 2011, R Core Team 2014). Inclusion of a  $\log_2(y+1)$  transformed lagged trap capture variable in the multiple regression models successfully eliminated the temporal autocorrelation that was initially present in the model residuals.

## **2. Overwintering mortality within egg masses in Minnesota**

### **Field experiment design**

Laboratory-reared diapausing New Jersey Standard Strain egg masses (NJSS) were obtained from USDA Animal and Plant Health Inspection Service, Center for Plant Health Science and Technology (Otis Air National Guard Base, Buzzards Bay, MA). Egg masses were carefully trimmed from the paper backing on which they were laid, numbered, and measured (length and width). Egg masses were then enclosed in individual mesh pouches to enhance containment and prevent predation. In 2014, mesh pouches were constructed using 20 × 20 mesh Lumite® (Andresen et al. 2001). In 2015, pouches were constructed using 50 × 24 mesh antiviral insect screen (BioQuip Products, Rancho Dominguez, CA) due to product availability.

Egg masses were deployed to 12 field sites on the Grand Portage Reservation in the extreme northeastern portion of Minnesota, located along Lake Superior (Fig. 2.3). Sites were selected to permit the assessment of the impact of temperature buffering due to lake effects. The closest field site to Lake Superior was 18 m, while the farthest field site was 7,493 m. The mean distance to Lake Superior was 3,442 m. Field sites with a species composition primarily consisting of deciduous trees were selected to permit snow accumulation around the boles of experimental trees. Experimental egg masses were deployed in November of each year over two winter field seasons, 2013-2014 and 2014-2015 (subsequently referred to as 2014 and 2015, respectively), and retrieved in April each year. Experimental egg masses were affixed to trees using 14 mm staples (Arrow Fastener Co., Inc., Saddle Brook, NJ). Staples were located above pouch seams to

maintain mesh integrity and to prevent thermal conductance via the metal staple. Each field site consisted of  $n=28$  experimental egg masses, with four experimental egg masses affixed to each tree in a  $2 \times 2$  factorial design (north/south aspect  $\times$  high/low). High placements were located above breast height (1.5m), and low placements were located near ground level ( $\sim 30$  cm). In 2015, 15 “control” egg masses were allowed to overwinter in Syracuse, New York, a climatically suitable location (Gray 2004).

HOBO Pro V2 temperature data loggers (Onset Computer Corporation, Bourne, MA) recording at 30 minute intervals were placed within radiation shields and deployed at each field site to monitor ambient air temperatures at each of the four treatment positions. Radiation shields were constructed using 10.16 cm diameter polyethylene triplewall drain pipes (Advanced Drainage Systems, Buffalo, MN) cut into 30.48 cm sections with 10.16 cm plastic drain grates (NDS, Woodland Hills, CA) affixed at each end. Temperature data loggers were suspended centrally within each radiation shield with a tension wire mechanism utilizing 24 gauge galvanized steel wire. Temperature data loggers with radiation shields were located on a central tree within each field site, and affixed below experimental egg masses to prevent shading.

Egg masses were retrieved each April before eclosion and returned to the lab. Egg masses were removed from their mesh pouches and placed within 29.57 mL portion cups (Dixie®, Atlanta, GA). Experimental egg masses were stored at room temperature ( $\sim 21^\circ\text{C}$ ) and allowed to emerge naturally. Egg masses were maintained at room temperature for 30 days following emergence initiation, to ensure complete emergence. Egg masses were then gently homogenized to separate individual eggs, vacuumed with a

custom mesh attachment to remove hairs and scales, and assessed. Overwintering mortality was quantified by the number of emerged larvae, fertilized un-emerged eggs, and unfertilized eggs. Fertilized eggs are spherical and grey in color, whereas unfertilized eggs are white and may be sunken in appearance. The number of unfertilized eggs was subtracted from the total number of eggs to quantify the total number of viable eggs that overwintered. Overwintering mortality was quantified by dividing the number of un-emerged fertilized eggs by the total number of viable eggs.

### **Temperature data analysis**

From the temperature data loggers, we quantified the number of days that minimum temperatures were less than or equal to thresholds of  $-25^{\circ}\text{C}$ ,  $-27^{\circ}\text{C}$ , and  $-30^{\circ}\text{C}$ . The duration of cold exposure, in hours, was quantified by dividing the number of 30 minute observations at which temperatures were less than or equal to the given thresholds by two. In 2014, one data logger experienced short duration logging failures over the study period. In this case, missing data (0.75% of observations) were interpolated using first or second order autoregressive techniques. In the first year of the study, several additional HOBO temperature loggers were deployed. All temperature observations were averaged for temperature loggers located at the same site and placement. In 2015, one data logger experienced extensive logging failure, such that interpolation of missing data was not possible, so one field site did not record temperature observations for egg masses placed on the southern aspect of a tree.

### **Statistical Analysis**

Overwintering mortality was expressed as a binomial presence/absence response

of the number of fertilized un-emerged eggs relative to the number emerged larvae. This binomial response variable accounted for differences in the number of eggs between egg masses. To estimate the annual mean mortality rate, the mortality binomial response variable was fit to a constant intercept model, that was then back transformed with the logit link function (i.e.  $P(\text{Mortality}) = \frac{\exp\beta_0}{1 + \exp\beta_0}$ ). We explored the effects of height of egg mass placement, aspect, various measures of winter severity, and their interactions on overwintering mortality as a binomial response variable using a generalized linear mixed effects model. These variables are described in Table 2.2. Field site was incorporated as a random effect. The best models describing overwintering mortality exhibited the lowest AIC values. Both years of field data were analyzed independently using the lme4 package (Bates et al. 2014) in R (R Core Team 2014).

## Results

Gypsy moth population levels within the Arrowhead region of Minnesota have exhibited large fluctuations among years since 2004, with numbers generally increasing (Fig. 2.2). Male moth capture peaked in 2013, when 70,133 moths were captured. The greatest number of traps were deployed in 2011 (13,043 traps), and the least were deployed in 2004 (2,077 traps). Summer male moth capture can be high despite relatively few traps being set. For example, in 2009 trap catch increased 257% over 2008, despite 9.9% less traps being set in 2009.

### **Male gypsy moth trap captures**

Winter severity within the Arrowhead region of Minnesota was highly variable between years, although the winter of 2013-14 (October 2013 – May 2014; winter 2014 hereafter) was the coldest according to most measures of winter severity (Fig. 2.4). The mean minimum monthly temperature in 2014 was on average 7°C colder than the mean for the previous decade (December: -23.31°C in 2014 vs. -14.63°C mean 2004-2013, Fig. 2.4A; January: -25.41°C vs. -18.60°C mean 2004-2013, Fig. 2.4B; February: -23.32°C vs. -17.25°C mean 2004-2013, Fig. 2.4C). Winter 2014 had the most days that minimum temperatures reached temperature thresholds of -10°C, -15°C, -20°C, -25°C, and -30°C (Fig. 2.4D-H). In fact, temperatures dropped below -20°C a mean total of 79 times across the 17 weather stations in 2014 compared to a mean total of 38 times in the previous decade (Fig. 2.4F). Surprisingly, 2014 did not exhibit the coldest temperature recorded during the study period. The absolute minimum temperature of -43.3°C in 2014 was not

as cold as the 2005 absolute minimum of  $-47.8^{\circ}\text{C}$ . Both of these temperatures were recorded in Embarrass, Minnesota.

Despite the consistency by which our measures of winter severity described 2014 as the most “severe” winter (i.e., 8 of the 9 metrics in Fig. 2.4), the year with the “mildest” winter temperatures varied depending on the metric used. For example, 2007 had the highest mean minimum December temperature (Fig. 2.4A), whereas 2006 observed the highest mean minimum January temperature (Fig. 2.4B). Overall, 2012 was the mildest winter as described by seven of the nine metrics, such as minimum February temperature (Fig. 2.4C). Despite 2012 being among the milder winters, temperatures potentially lethal gypsy moth egg masses were still observed (Leonard 1972, Sullivan and Wallace 1972, Madrid and Stewart 1981, Waggoner 1985).

In Table 2.3, we present analyses exploring whether various variables describing winter severity can describe variation in male moth capture after accounting for trapping effort and the previous year’s catch. The number of cold exposure events did not affect summer male gypsy moth trap capture at thresholds of  $-10^{\circ}\text{C}$  or  $-15^{\circ}\text{C}$  ( $t_{150}=0.42$ ,  $P=0.66$ , and  $t_{150}=-0.76$ ,  $P=0.45$ , respectively). However, the number of cold exposure events were associated with reduced male moth capture beginning at a threshold of  $-20^{\circ}\text{C}$  ( $t_{150}=-2.143$ ,  $P=0.0338$ ). This pattern remained when examining threshold temperatures of  $-25^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$  as well ( $t_{150}=-2.379$ ,  $P=0.0186$  and  $t_{150}=-2.634$ ,  $P=0.00932$ ). After accounting for the previous year’s capture and the number of traps set, we found that the number of male gypsy moths captured in the subsequent summer were reduced by 50% for every 18 days with minimum temperatures below  $-30^{\circ}\text{C}$  (i.e. 1 divided by the slope



estimate of -0.057 in Table 2.3= 18). Measures of winter severity quantifying total cold exposure, such as absolute minimum temperature and mean minimum January temperature, were not correlated with reduced male moth captures ( $t_{150}=1.49$ ,  $P=0.17$  and  $t_{150}=-0.031$ ,  $P=0.98$ , respectively). However, warmer February temperatures were associated with increased male gypsy moth capture ( $t_{150}=3.039$ ,  $P=0.0028$ ).

### **Overwintering Mortality**

There were marked differences in ambient air temperatures recorded by HOBO data loggers, and thus egg masses placed high or low on the tree (Fig. 2.5). In general, egg masses placed at breast height experienced 2-4× the number of cold days with temperatures less than or equal to  $-25^{\circ}\text{C}$ . We did not observe any differences in temperature between north/south aspects of the tree ( $P>0.05$  for all metrics shown in Table 2.2). Comparing between years, 2014 was a colder winter than 2015, with greater cold exposure measured as the numbers of days and duration that temperatures were below given thresholds. Egg masses generally experienced warmer winter temperatures with increasing distance from Lake Superior in both 2014 and 2015 (duration of temperatures less than or equal to  $-30^{\circ}\text{C}$   $F_{1,46}=5.10$ ,  $P=0.0287$ ; duration of temperatures less than or equal to  $-27^{\circ}\text{C}$   $F_{1,44}=6.33$ ,  $P=0.01567$ , respectively), which may have been related to variability in snow cover across the region.

In 2014, 355 egg masses were deployed to assess overwintering mortality dynamics. Overwintering mortality was high, with only 111 egg masses exhibiting any emergence. The mean mortality rate in 2014 was 96.78% (95% CI: 96.72%, 96.85%). One hundred and eighty egg masses were located high on the tree, and these egg masses

experienced near complete mortality (mean mortality of 99.95% 95% CI: 99.94%, 99.96%). Egg masses located low on the tree had a mean mortality of 93.52% (95% CI: 93.39%, 93.65%). While mortality remained high for egg masses placed low on the tree, the lower placement reduced mortality by 6.43% from egg masses located high on the tree. The maximum eclosion rate was 82%, which occurred in an egg mass placed low on a tree in a site 4.8 km from Lake Superior. Despite this high rate of eclosion, another egg mass with a lower rate of eclosion (40%) had a higher number of first instars that emerged following the winter (638 vs 195). This egg mass was also one that had been placed low on the tree, near ground level. Across all 355 egg masses, 8,997 of 297,683 eggs hatched (3.0%).

In 2015, 366 egg masses were deployed in northern Minnesota to assess overwintering mortality. All 15 “control” egg masses that were sent to overwinter in Syracuse, New York successfully emerged, regardless of high or low placement, with an average eclosion rate of 94.7% (95% CI: 95.08%, 94.29%). Similar to 2014, overwintering mortality of egg masses deployed in northern Minnesota was high, with only 55 egg masses exhibiting any emergences, and an average mortality rate of 98.58% (95% CI: 98.54%, 98.63%). Egg masses that were located high on the tree again experienced near total mortality with an overall mean mortality rate of 99.997% (95% CI: 99.992%, 99.999%), with only one egg mass exhibiting any emergence (only 5% emergence from this one egg mass). Egg masses located low on the tree also exhibited high rates of mortality with a mean mortality rate of 97.1% (95% CI: 97.01%, 97.19%). While egg masses located low on the tree still had high rates of mortality, the lower

placement reduced mortality by 2.9%. The highest eclosion rate in 2015 was 58% for an egg mass placed low on a tree in a site 4.8 km from Lake Superior. Similar to 2014, a separate egg mass, which also overwintered in a position close to the ground within 5 km of Lake Superior, had more first instars emerge (428 vs 414) despite a lower rate of eclosion (36%). Across all 366 egg masses, 3,954 of 279,303 eggs hatched (1.4%).

The rates of successful spring eclosion decreased with cold temperatures experienced during the winter. These relationships are shown in Table 2.4. In 2014, the positive relationship between mortality and the number of days that temperatures reached  $-25^{\circ}\text{C}$ ,  $-27^{\circ}\text{C}$ , or  $-30^{\circ}\text{C}$  became more accentuated at cold temperature thresholds, as evidenced by decreasing AIC values moving through regressions from  $-25^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  (Table 2.4A). In 2015, the strongest relationship between cold temperatures, or their duration, and overwintering mortality was apparent when temperatures reached  $-27^{\circ}\text{C}$  (Table 2.4B). A negative relationship between survival and cold was noted at all temperature thresholds tested for both years, however.

The probability of egg mortality in 2014 in relation to placement (high/low on a tree) and temperature for various durations of temperatures below  $-25^{\circ}\text{C}$  is shown in Figure 2.6. Egg masses located high on the tree consistently experienced near total mortality, regardless of the duration of extreme cold temperatures. Egg masses located low on the tree also experienced high rates of mortality, in general, but mortality exponentially increased with increasing duration of temperatures below  $-25^{\circ}\text{C}$  (Fig. 2.6). We did not observe similar spread in the data to conduct a similar analysis for 2015.

## Discussion

Our findings that cold winter temperatures may reduce the numbers of male gypsy moths captured in the subsequent summer within the Arrowhead region of Minnesota are consistent with high rates of overwintering mortality in exposed egg masses. For this reason, it has been predicted that the Arrowhead region of Minnesota along the expanding range front will be climatically unsuitable (Régnière and Nealis 2002, Gray 2004). In contrast, generally increasing numbers of moths per trap (Fig. 2.2) and repeated observations of egg masses in the field suggest that reproducing populations are present and northward range expansion has occurred. While many range studies use climatic envelopes based on temperature (Sullivan and Wallace 1972, Régnière and Nealis 2002, Gray 2004), our study suggests that thermal insulation provided by snow cover could provide sufficient protection for egg masses placed low on trees (Leonard 1972, Sullivan and Wallace 1972, Madrid and Stewart 1981). That is, even in regions where more than 99.5% mortality may occur due to freezing, enough insects may hatch to sustain the population. In our data, a total of 12,951 eggs eclosed over both years (excluding control egg masses). If 50% of these were female (Campbell 1981), there would have been a total of 6,476 newly-hatched larvae. Anything less than a mortality rate of 90% from early instar to adulthood could thus result in replacement or increase from the 691 egg masses deployed over the two years. Early instar dispersal is a high mortality cost (Campbell 1981), but has not been quantified along the northwestern invasion boundary to date.

While the supercooling point represents a minimum temperature above which pharate larvae may be expected to survive, gypsy moth egg mass mortality can also occur

with prolonged exposure to freezing temperatures above the supercooling point (Sullivan and Wallace 1972, Madrid and Stewart 1981). In this study, winter severity metrics quantifying the duration of cold exposure became statistically significant predictors of male moth trap capture beginning at a threshold of  $-20^{\circ}\text{C}$  (Table 2.3), which is warmer than the  $-27^{\circ}\text{C}$  supercooling point of gypsy moth eggs. This temperature threshold is biologically significant and illustrates the relationship between the length and degree of cold exposure to egg survival. Varying rates of mortality have been observed in laboratory and field studies due to the degree of acquired cold hardiness (Madrid and Stewart 1981, Denlinger et al. 1992) and duration of exposure time (Madrid and Stewart 1981). Cold hardiness increases with prior chilling; eggs that have not been chilled have a mean supercooling point of  $-28^{\circ}\text{C}$  and eggs that have been cold hardened through chilling have a mean supercooling point of  $-30^{\circ}\text{C}$  (Sullivan and Wallace 1972, Madrid and Stewart 1981). Cold hardiness is not inherent to diapause, and increased levels of heat shock proteins in chilled eggs suggests a link between these stress proteins and the acquisition of cold hardiness (Denlinger et al. 1992).

Gypsy moth has an obligate diapause in the egg stage; throughout its native range regional variations occur in chilling requirements for diapause completion. Occasionally, precocious hatching of eggs is observed in fall (Keena 2015). Chilling requirements for diapause completion are not fixed within populations, and may serve as a bet-hedging strategy to ensure that some proportion of egg emergence coincides with spring bud burst. Moreover, the non-diapausing phenotype is not restricted to a single geographical region. This phenotype is inherited via a single recessive gene with no Z-linkage (Keena

2015). A non-diapausing strain of gypsy moth can be selected in one generation (Keena 2015), which suggests that diapause is a highly selectable trait.

The high mortality rates of laboratory generated eggs observed in this work may exceed those occurring under natural conditions in wild populations. The NJSS has been in culture since 1967, and there are several phenotypic differences when compared to wild strains. One such phenotypic difference is that the NJSS egg hatch begins sooner and is more synchronous than wild strains (Keena 1994). Moreover, the NJSS was inadvertently selected to be less cold hardy (Keena 2015). Based on evidence that cold hardiness is a trait that can be under selective pressure, future research on overwintering mortality should seek to use wild collected strains reared in the laboratory for one generation to reduce maternal effects, while maintaining a wild genotype.

High rates of overwintering mortality could also have been a result of experimental egg masses already having met their chilling requirements for diapause completion. Under laboratory rearing protocols, egg masses are allowed to embryonate for 60 days after oviposition, are chilled at 12.8°C for 10-15 days, and then transferred to refrigerators set at 4.4°C for 150 days to satisfy diapause requirements. It is possible that since diapause chilling requirements were already met, post diapause development could have resumed during the experimental period, resulting in less cold hardiness and/or depleting energetic reserves before unfavorable conditions passed.

Reductions in the number of male gypsy moths following severe winters may affect demographic processes that mediate the invasion biology of the gypsy moth. For example, extreme winter temperatures may reduce population densities below critical

thresholds that induce Allee effects, which are manifested through mechanisms of mate-finding failure and unsuccessful reproduction (Sharov et al. 1995, Liebhold and Bascompte 2003, Johnson et al. 2006, Tobin et al. 2009). Gypsy moth Allee thresholds vary regionally, with lower thresholds observed in northern regions (Whitmire and Tobin 2006, Tobin et al. 2007). As such, smaller densities of gypsy moth populations in northern regions, which are more likely to be negatively affected by cold winters than southern regions (e.g., 35°N), may persist due to lower Allee thresholds.

Given that gypsy moth has expanded into areas that have previously been characterized as having an unsuitable climate, and that range retractions have been linked to larval and pupal exposure to supraoptimal temperatures (Tobin, Gray, et al. 2014), climate change may result in range shifts rather than range expansions. Phenological modeling, for example, indicates that the potential southern range of gypsy moth (e.g., Florida) is limited by insufficient chilling periods required to terminate diapause (Gray 2004). However, range expansion, stasis, and retraction has been documented along the current southern distributional range of gypsy moth invasion in Virginia in response to larval and pupal exposure to supraoptimal temperatures (Tobin, Gray, et al. 2014). As such, adverse effects of high temperatures could exceed the beneficial effects on population growth in those regions. To better understand the implications of climate change on gypsy moth biology, research into the prediapause development and overwintering microhabitat dynamics should be conducted. Future research could include manipulative experiments assessing the degree of cold hardiness acquired by diapausing gypsy moth egg masses experiencing prolonged and warmer autumn conditions, for

example.



**Table 2.1:** Number of traps set and male moths captured in each Thiessen polygon within in the Arrowhead region of Minnesota

2004-2014. Weather stations are shown in Fig. 2.3. These data were used to model correlations between measures of winter severity and subsequent summer male gypsy moth capture.

| Weather    | <u>2004</u><br>traps<br>(catch) | <u>2005</u><br>traps<br>(catch) | <u>2006</u><br>traps<br>(catch) | <u>2007</u><br>traps<br>(catch) | <u>2008</u><br>traps<br>(catch) | <u>2009</u><br>traps<br>(catch) | <u>2010</u><br>traps<br>(catch) | <u>2011</u><br>traps<br>(catch) | <u>2012</u><br>traps<br>(catch) | <u>2013</u><br>traps<br>(catch) | <u>2014</u><br>traps<br>(catch) |
|------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Cook       | 9<br>(0)                        | 291<br>(0)                      | 2<br>(0)                        | 2<br>(0)                        | 4<br>(1)                        | 234<br>(125)                    | 418<br>(45)                     | 290<br>(10)                     | 302<br>(9)                      | 262<br>(159)                    | 257<br>(17)                     |
| Crane Lake | NA<br>(NA)                      | 111<br>(0)                      | 2<br>(0)                        | 2<br>(0)                        | 0<br>(NA)                       | 57<br>(140)                     | 117<br>(16)                     | 83<br>(1)                       | 82<br>(1)                       | 91<br>(20)                      | 79<br>(0)                       |
| Duluth     | 517<br>(2)                      | 436<br>(16)                     | 482<br>(3)                      | 617<br>(49)                     | 762<br>(619)                    | 558<br>(625)                    | 1145<br>(847)                   | 479<br>(324)                    | 607<br>(86)                     | 754<br>(3769)                   | 454<br>(81)                     |
| Ely        | 10<br>(0)                       | 305<br>(4)                      | 292<br>(0)                      | 301<br>(1)                      | 311<br>(27)                     | 231<br>(2008)                   | 311<br>(123)                    | 388<br>(271)                    | 397<br>(51)                     | 400<br>(338)                    | 354<br>(16)                     |
| Embarrass  | 32<br>(7)                       | 299<br>(1)                      | 290<br>(0)                      | 313<br>(4)                      | 342<br>(23)                     | 236<br>(714)                    | 577<br>(183)                    | 406<br>(224)                    | 467<br>(44)                     | 181<br>(103)                    | 404<br>(3)                      |
| Eveleth    | NA<br>(NA)                      | 376<br>(2)                      | 181<br>(0)                      | 185<br>(0)                      | 116<br>(14)                     | 283<br>(196)                    | 820<br>(308)                    | 478<br>(117)                    | 467<br>(53)                     | 178<br>(237)                    | 424<br>(23)                     |
| Floodwood  | 25<br>(0)                       | 322<br>(0)                      | 17<br>(0)                       | 6<br>(0)                        | 74<br>(85)                      | 351<br>(1)                      | 294<br>(105)                    | 414<br>(39)                     | 312<br>(4)                      | 379<br>(535)                    | 334<br>(42)                     |

| Weather Station | <u>2004</u><br>traps<br>(catch) | <u>2005</u><br>traps<br>(catch) | <u>2006</u><br>traps<br>(catch) | <u>2007</u><br>traps<br>(catch) | <u>2008</u><br>traps<br>(catch) | <u>2009</u><br>traps<br>(catch) | <u>2010</u><br>traps<br>(catch) | <u>2011</u><br>traps<br>(catch) | <u>2012</u><br>traps<br>(catch) | <u>2013</u><br>traps<br>(catch) | <u>2014</u><br>traps<br>(catch) |
|-----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Grand Marais    | 860<br>(149)                    | 1613<br>(788)                   | 965<br>(160)                    | 1034<br>(2293)                  | 933<br>(1636)                   | 587<br>(1501)                   | 1025<br>(295)                   | 771<br>(729)                    | 282<br>(4509)                   | 159<br>(2966)                   | 166<br>(5)                      |
| Hibbing         | 21<br>(0)                       | 233<br>(0)                      | 9<br>(0)                        | NA<br>(NA)                      | NA<br>(NA)                      | 212<br>(14)                     | 234<br>(27)                     | 265<br>(10)                     | 320<br>(1)                      | 265<br>(352)                    | 317<br>(41)                     |
| Isabella        | 13<br>(1)                       | 375<br>(26)                     | 375<br>(19)                     | 394<br>(14)                     | 384<br>(91)                     | 205<br>(3159)                   | 325<br>(150)                    | 408<br>(801)                    | 207<br>(952)                    | 230<br>(7105)                   | 154<br>(20)                     |
| Kabetogama      | NA<br>(NA)                      | 61<br>(0)                       | NA<br>(NA)                      | NA<br>(NA)                      | NA<br>(NA)                      | NA<br>(NA)                      | 80<br>(6)                       | 56<br>(0)                       | 46<br>(0)                       | 45<br>(7)                       | 78<br>(1)                       |
| Meander         | 5<br>(30)                       | 93<br>(0)                       | 119<br>(0)                      | 62<br>(0)                       | 6<br>(0)                        | 86<br>(584)                     | 73<br>(22)                      | 93<br>(20)                      | 53<br>(25)                      | 40<br>(14)                      | 81<br>(1)                       |
| Orr             | 26<br>(0)                       | 149<br>(0)                      | 8<br>(0)                        | NA<br>(NA)                      | NA<br>(NA)                      | 48<br>(29)                      | 204<br>(16)                     | 136<br>(1)                      | 150<br>(0)                      | 159<br>(68)                     | 213<br>(6)                      |
| Seagull         | 27<br>(2)                       | 138<br>(25)                     | 143<br>(6)                      | 67<br>(9)                       | 71<br>(16)                      | 41<br>(267)                     | 89<br>(24)                      | 80<br>(32)                      | 19<br>(456)                     | 9<br>(181)                      | 12<br>(0)                       |
| Silver Bay      | 77<br>(4)                       | 126<br>(11)                     | 127<br>(8)                      | 164<br>(108)                    | 289<br>(551)                    | 212<br>(1690)                   | 167<br>(219)                    | 178<br>(205)                    | 135<br>(172)                    | 185<br>(3519)                   | 42<br>(22)                      |
| Two Harbors     | 230<br>(3)                      | 366<br>(55)                     | 640<br>(12)                     | 527<br>(52)                     | 577<br>(1448)                   | 386<br>(3506)                   | 664<br>(353)                    | 655<br>(777)                    | 659<br>(253)                    | 486<br>(3484)                   | 178<br>(62)                     |

| Weather Station | <u>2004</u>      | <u>2005</u>      | <u>2006</u>      | <u>2007</u>      | <u>2008</u>      | <u>2009</u>      | <u>2010</u>      | <u>2011</u>      | <u>2012</u>      | <u>2013</u>      | <u>2014</u>      |
|-----------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                 | traps<br>(catch) | traps<br>(catch) | traps<br>(catch) | traps<br>(catch) | traps<br>(catch) | traps<br>(catch) | traps<br>(catch) | traps<br>(catch) | traps<br>(catch) | traps<br>(catch) | traps<br>(catch) |
| Wolf Ridge      | 225<br>(78)      | 775<br>(327)     | 419<br>(41)      | 525<br>(425)     | 646<br>(496)     | 341<br>(3322)    | 348<br>(391)     | 332<br>(1099)    | 288<br>(3734)    | 369<br>(47270)   | 88<br>(13)       |

**Table 2.2:** Variables used in modeling the overwintering dynamics of gypsy moth. A) Trap variables described the attributes of Thiessen polygons used to model the association between winter severity and subsequent moth density in the Arrowhead Region of Minnesota, 2004-2014. B) Egg mass variables described the attributes of each egg mass and its placement during field experiments on the Grand Portage Reservation during the winters of 2014 and 2015. C) Winter severity variables derived from weather station observations from October- May, 2003-2014. D) Winter severity variables derived from data loggers on the Grand Portage Reservation from November to April 2014 and 2015.

| Variable                         | Explanation   |
|----------------------------------|---|
| <i>A) Trap variables</i>         |   |
| Area                             | Area of Thiessen polygon, measured in km <sup>2</sup> .   |
| Traps                            | Number of traps in each Thiessen polygon for a given year. The number of traps set varies annually based on budgets and the previous year's trap catches. |
| Catch                            | Number of male gypsy moths caught in pheromone baited traps during the summer flight period.  |
| $\log_2(\text{Catch}_{(t-1)}+1)$ | Number of male gypsy moths captured the previous year.  |

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| Variable                     | Explanation   |
|------------------------------|---|
| <i>B) Egg mass variables</i> |   |
| Surface area                 | Surface area of experimental egg masses, calculated as the area of an ellipse ( $r_1 * r_2 * \pi$ ) utilizing length and width measurements.  |
| Larvae                       | Number of larvae that successfully eclosed from each experimental egg mass. This variable was used to determine overwintering mortality.  |
| Total eggs                   | Total number of viable eggs in each egg mass, determined by adding the number of emergent larvae and the number of fertile non-emergent eggs. This variable was used to determine the overwintering mortality while accounting for the difference of size between egg masses. |
| Lake Superior distance       | Distance (m) from each field site to Lake Superior. Calculated using a Geographic Information System. This variable was used to detect the presence of lake effects at varying distances to the shore.  |
| Aspect                       | Aspect of experimental egg mass placement. Egg masses were placed on the north and south aspects of trees.  |

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| Variable  | Explanation  |
|---|--|
| Height  | Height of experimental egg mass placement. Egg masses were affixed to trees at high and low heights. |
| <i>C) Weather variables derived from weather station data (trap catch analysis)</i> |  |
| Days below -10°C  | Number of days in which daily minimums were below -10°C.   |
| Days below -15°C  | Number of days in which daily minimums were below -15°C.   |
| Days below -20°C  | Number of days in which daily minimums were below -20°C.   |
| Days below -25°C  | Number of days in which daily minimums were below -25°C.   |
| Days below -30°C  | Number of days in which daily minimums were below -30°C.   |
| Mean December minimum   | Mean observed daily minima in December.  |
| Mean January minimum  | Mean observed daily minima in January.   |
| Mean February minimum   | Mean observed daily minima in February.  |
| Absolute winter minimum   | Lowest observed daily minimum October-May.   |
| <i>D) Weather variables derived from data loggers (egg mass studies)</i>            |  |
| Days below -25°C  | Number of days in which daily minimums were below -25°C.   |

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| Variable             | Explanation  |
|----------------------|--|
| Duration below -25°C | Duration, in hours, in which temperatures were below -25°C.  |
| Days below -27°C     | Number of days in which daily minimums were below -27°C.     |
| Duration below -27°C | Duration, in hours, in which temperatures were below -27° C. |
| Days below -30°C     | Number of days in which daily minimums were below -30°C.     |
| Duration below -30°C | Duration, in hours, in which temperatures were below -30° C. |

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**Table 2.3:** Models constructed to explain the correlation of winter severity with total annual summer trap catch of male gypsy moths after accounting for the previous year's catch and the number of traps set in the Arrowhead region of Minnesota, 2004-2014.

| Winter Severity     | Intercept        |                                   | Explanatory variables            |                                   |                    |                                   |                      |                                   |
|---------------------|------------------|-----------------------------------|----------------------------------|-----------------------------------|--------------------|-----------------------------------|----------------------|-----------------------------------|
|                     | Est<br>(SE)      | $t_{(150)}$<br>( <i>P</i> -value) | $\log_2(\text{Catch}_{(t-1)}+1)$ |                                   | Traps              |                                   | Winter Severity      |                                   |
|                     | Est<br>(SE)      | $t_{(150)}$<br>( <i>P</i> -value) | Est<br>(SE)                      | $t_{(150)}$<br>( <i>P</i> -value) | Est<br>(SE)        | $t_{(150)}$<br>( <i>P</i> -value) | Est<br>(SE)          | $t_{(150)}$<br>( <i>P</i> -value) |
| Days below<br>-10°C | 0.936<br>(1.35)  | 0.69<br>(0.49)                    | 0.469<br>(0.0677)                | 6.92<br>( <i>P</i> <0.0001)       | 0.0045<br>(0.0010) | 4.40<br>( <i>P</i> <0.0001)       | 0.0060<br>(0.014)    | 0.42<br>(0.66)                    |
| Days below<br>-15°C | 2.159<br>(0.964) | 2.24<br>(0.027)                   | 0.484<br>(0.0672)                | 7.19<br>( <i>P</i> <0.0001)       | 0.0042<br>(0.0010) | 4.10<br>( <i>P</i> <0.0001)       | -0.00975<br>(0.0128) | -0.76<br>(0.45)                   |
| Days below<br>-20°C | 2.690<br>(0.698) | 3.85<br>(0.0002)                  | 0.497<br>(0.0662)                | 7.51<br>( <i>P</i> <0.0001)       | 0.0038<br>(0.0010) | 3.71<br>(0.0003)                  | -0.027<br>(0.0126)   | -2.143<br>(0.0338)                |
| Days below<br>-25°C | 2.413<br>(0.571) | 4.23<br>( <i>P</i> <0.0001)       | 0.496<br>(0.0657)                | 7.54<br>( <i>P</i> <0.0001)       | 0.0037<br>(0.0010) | 3.64<br>(0.0004)                  | -0.035<br>(0.0149)   | -2.379<br>(0.0186)                |
| Days below<br>-30°C | 2.192<br>(0.496) | 4.42<br>( <i>P</i> <0.0001)       | 0.485<br>(0.0650)                | 7.46<br>( <i>P</i> <0.0001)       | 0.0037<br>(0.0010) | 3.79<br>(0.0002)                  | -0.057<br>(0.0215)   | -2.634<br>(0.0093)                |
| Mean Dec<br>minimum | 2.758<br>(0.941) | 2.93<br>(0.0039)                  | 0.504<br>(0.0688)                | 7.33<br>( <i>P</i> <0.0001)       | 0.0040<br>(0.0010) | 4.01<br>( <i>P</i> <0.0001)       | 0.084<br>(0.0562)    | 1.49<br>(0.14)                    |
| Mean Jan<br>minimum | 1.473<br>(1.047) | 1.41<br>(0.16)                    | 0.474<br>(0.0676)                | 7.02<br>( <i>P</i> <0.0001)       | 0.0044<br>(0.0010) | 4.34<br>( <i>P</i> <0.0001)       | -0.0016<br>(0.0520)  | -0.031<br>(0.98)                  |

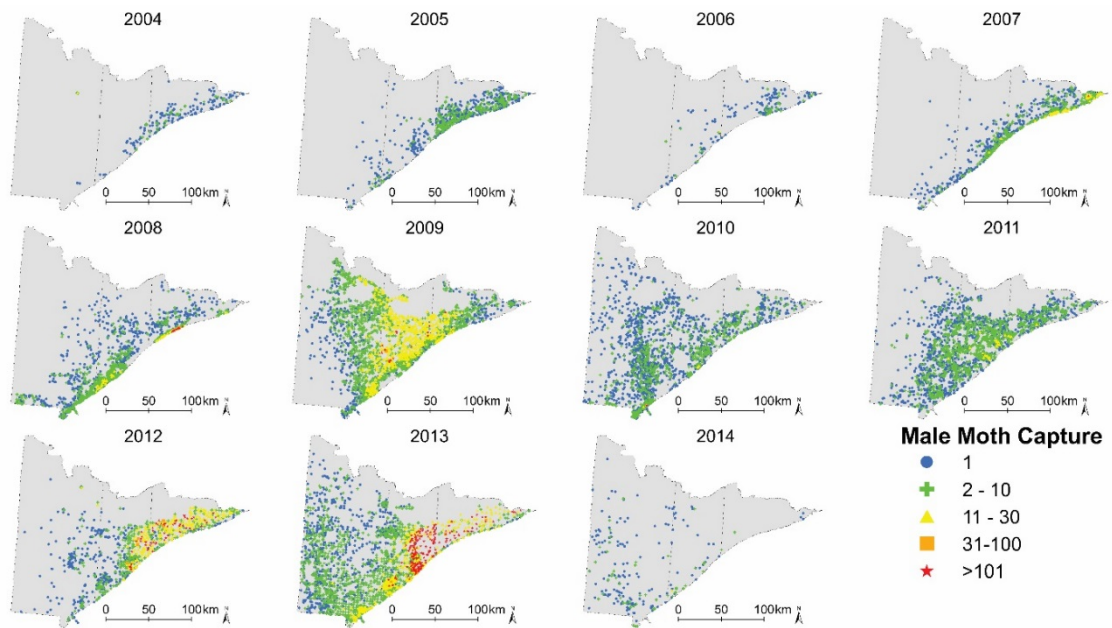


|                  | Intercept        |                                   | Explanatory variables            |                                   |                     |                                   |                    |                                   |
|------------------|------------------|-----------------------------------|----------------------------------|-----------------------------------|---------------------|-----------------------------------|--------------------|-----------------------------------|
|                  | Est<br>(SE)      | $t_{(150)}$<br>( <i>P</i> -value) | $\log_2(\text{Catch}_{(t-1)}+1)$ |                                   | Traps               |                                   | Winter Severity    |                                   |
|                  | Est<br>(SE)      | $t_{(150)}$<br>( <i>P</i> -value) | Est<br>(SE)                      | $t_{(150)}$<br>( <i>P</i> -value) | Est<br>(SE)         | $t_{(150)}$<br>( <i>P</i> -value) | Est<br>(SE)        | $t_{(150)}$<br>( <i>P</i> -value) |
| Winter Severity  | 5.123<br>(1.263) | 4.06<br>( <i>P</i> <0.0001)       | 0.479<br>(0.0644)                | 7.44<br>( <i>P</i> <0.0001)       | 0.0036<br>(0.00099) | 3.64<br>(0.0004)                  | 0.189<br>(0.0624)  | 3.039<br>(0.0028)                 |
| Absolute minimum | 4.710<br>(2.359) | 2.00<br>(0.0477)                  | 0.458<br>(0.0671)                | 6.83<br>( <i>P</i> <0.0001)       | 0.0041<br>(0.001)   | 4.10<br>( <i>P</i> <0.0001)       | 0.0872<br>(0.0631) | 1.38<br>(0.17)                    |

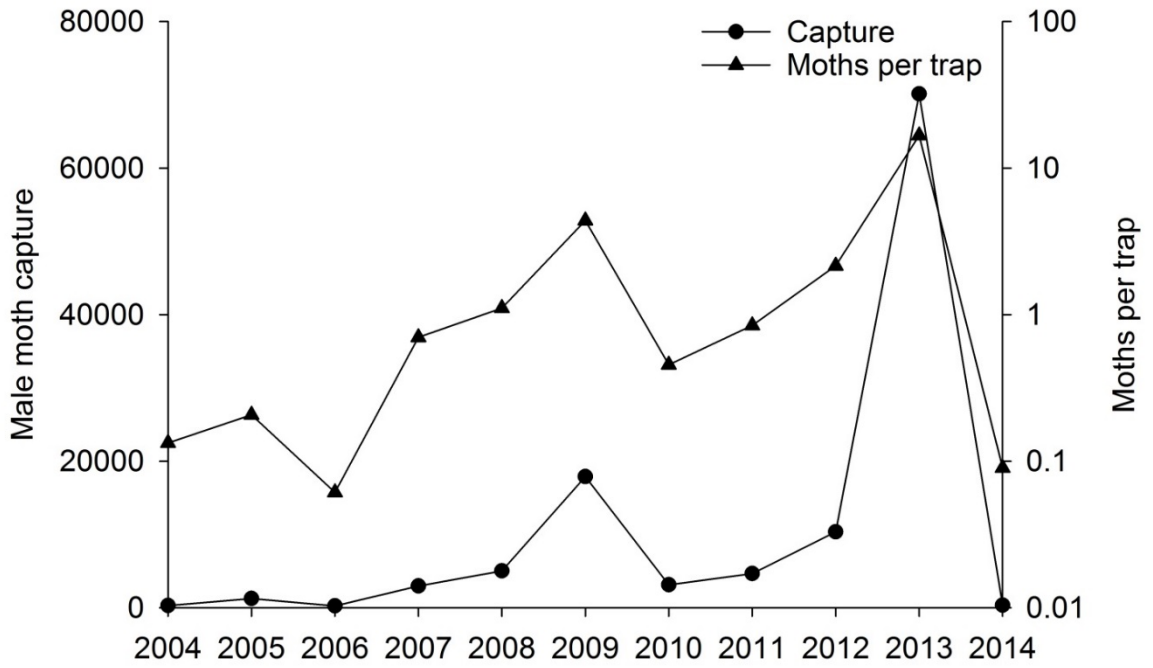
**Table 2.4:** The effect of observed air temperature on overwintering mortality of experimental egg masses allowed to overwinter on the Grand Portage Reservation during the winters of 2014 and 2015. Logistic regressions modeled mortality as a binomial response variable and measures of winter severity as the explanatory variable. The probability of mortality for various measures of winter severity (the number of days that temperatures drop below a given threshold and duration (h) temperatures are below given thresholds, respectively) can be evaluated by back transforming  $y$  using the logit link function  $P(\text{Mortality}) = \frac{\exp(\beta_0 + \beta_1 x)}{1 + \exp(\beta_0 + \beta_1 x)}$ .

|                 | Days below               |           |                         |                 |                 | Duration (h)             |           |                         |                 |                 |
|-----------------|--------------------------|-----------|-------------------------|-----------------|-----------------|--------------------------|-----------|-------------------------|-----------------|-----------------|
|                 | Intercept, ( $\beta_0$ ) |           | Variable, ( $\beta_1$ ) |                 |                 | Intercept, ( $\beta_0$ ) |           | Variable, ( $\beta_1$ ) |                 |                 |
| Temperature Est | Z                        | Est       | Z                       | AIC             | Est             | Z                        | Est       | Z                       | AIC             |                 |
| Variables       | (SE)                     | (P)       | (SE)                    | (P)             | ( $\Delta$ AIC) | (SE)                     | (P)       | (SE)                    | (P)             | ( $\Delta$ AIC) |
| <i>A) 2014</i>  |                          |           |                         |                 |                 |                          |           |                         |                 |                 |
| -25°C           | 3.38                     | 5.83      | 0.12                    | 68.81           | 22021.5         | 3.41                     | 5.58      | 0.016                   | 68.94           | 21859.3         |
|                 | (0.58)                   | (5.66e-9) | (0.0017)                | ( $P < 0.001$ ) | (-)             | (0.61)                   | (2.36e-8) | (0.00023)               | ( $P < 0.001$ ) | (-)             |
| -27°C           | 3.41                     | 5.6       | 0.19                    | 68.15           | 21947.7         | 3.49                     | 5.49      | 0.031                   | 62.4            | 21666.2         |
|                 | (0.61)                   | (2.2e-8)  | (0.0027)                | ( $P < 0.001$ ) | (-73.8)         | (0.64)                   | (4.13e-8) | (0.0005)                | ( $P < 0.001$ ) | (-193.1)        |

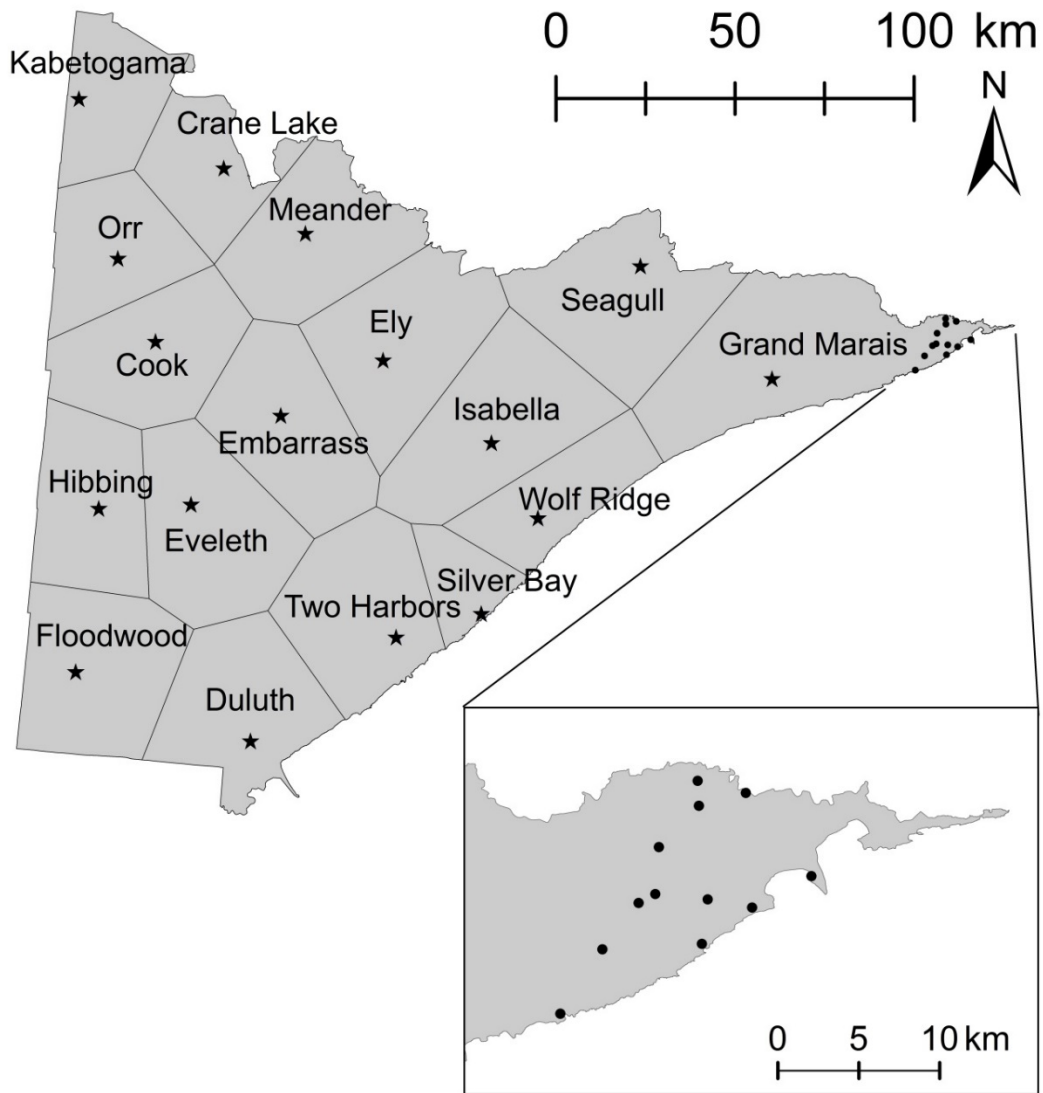
|                 | Days below               |               |                         |               |                 | Duration (h)             |               |                         |               |                 |
|-----------------|--------------------------|---------------|-------------------------|---------------|-----------------|--------------------------|---------------|-------------------------|---------------|-----------------|
|                 | Intercept, ( $\beta_0$ ) |               | Variable, ( $\beta_l$ ) |               |                 | Intercept, ( $\beta_0$ ) |               | Variable, ( $\beta_l$ ) |               |                 |
| Temperature Est | Z                        |               | Est                     | Z             | AIC             | Est                      | Z             | Est                     | Z             | AIC             |
| Variables       | (SE)                     | (P)           | (SE)                    | (P)           | ( $\Delta$ AIC) | (SE)                     | (P)           | (SE)                    | (P)           | ( $\Delta$ AIC) |
| -30°C           | 3.64                     | 15.12         | 0.68                    | 51.62         | 21142.4         | 3.76                     | 5.95          | 0.18                    | 49.78         | 21921.3         |
|                 | (0.24)                   | ( $P<0.001$ ) | (0.013)                 | ( $P<0.001$ ) | (-805.3)        | (0.63)                   | ( $2.76e-9$ ) | (0.0036)                | ( $P<0.001$ ) | (255.1)         |
| <i>B) 2015</i>  |                          |               |                         |               |                 |                          |               |                         |               |                 |
| -25°C           | 4.47                     | 5.54          | 0.15                    | 40.55         | 13611.5         | 4.49                     | 5.20          | 0.026                   | 41.19         | 13256.2         |
|                 | (0.81)                   | ( $3.04e-8$ ) | (0.0038)                | ( $P<0.001$ ) | (-)             | (0.86)                   | ( $2.03e-7$ ) | (0.00063)               | ( $P<0.001$ ) | (-)             |
| -27°C           | 4.37                     | 5.03          | 0.31                    | 39.32         | 12851.4         | 4.65                     | 5.02          | 0.049                   | 39.52         | 13039.9         |
|                 | (0.87)                   | ( $4.88e-7$ ) | (0.0079)                | ( $P<0.001$ ) | (-760.1)        | (0.93)                   | ( $2.25e-7$ ) | (0.0012)                | ( $P<0.001$ ) | (-216.3)        |
| -30°C           | 4.69                     | 5.61          | 0.87                    | 35.74         | 13077.2         | 4.57                     | 60.94         | 0.25                    | 34.97         | 12833.7         |
|                 | (0.84)                   | ( $2.04e-8$ ) | (0.024)                 | ( $P<0.001$ ) | (225.8)         | (0.075)                  | ( $P<0.001$ ) | (0.0072)                | ( $P<0.001$ ) | (-206.2)        |



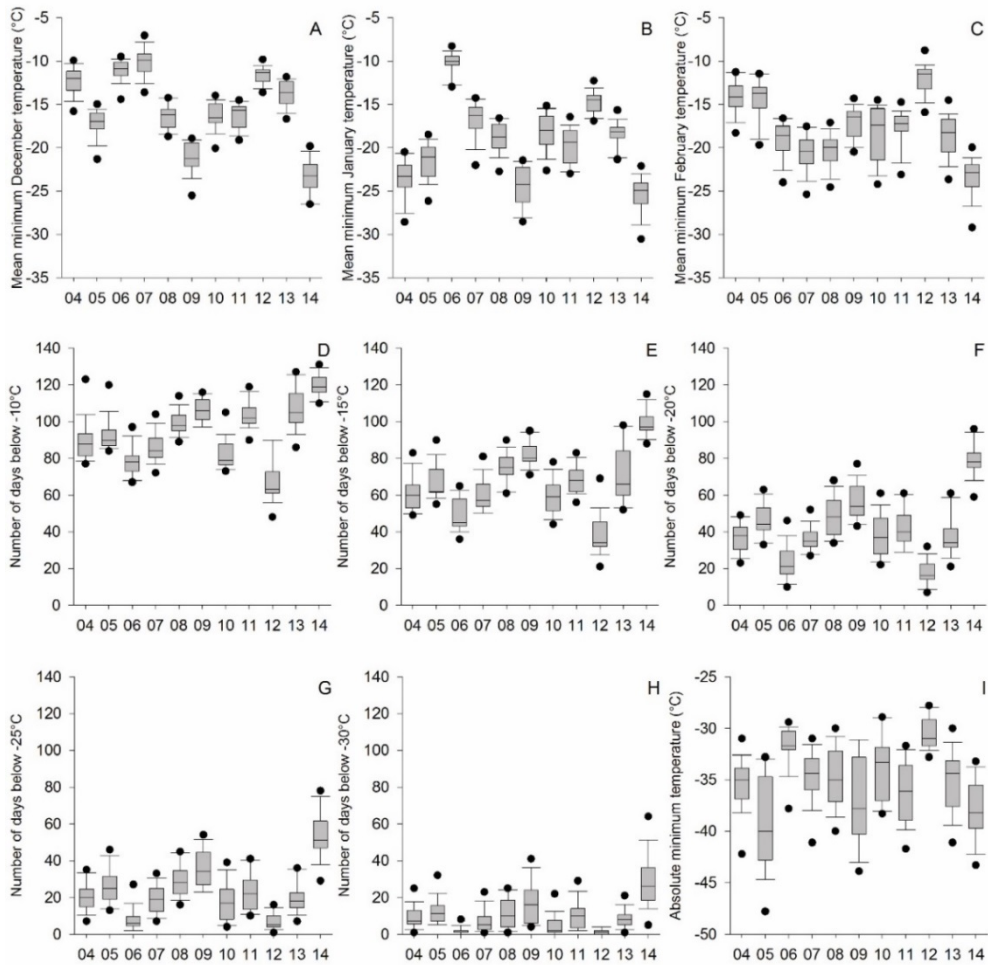
**Figure 2.1:** Distribution of male gypsy moth trap catches within the Arrowhead region of Minnesota, 2004-2014.



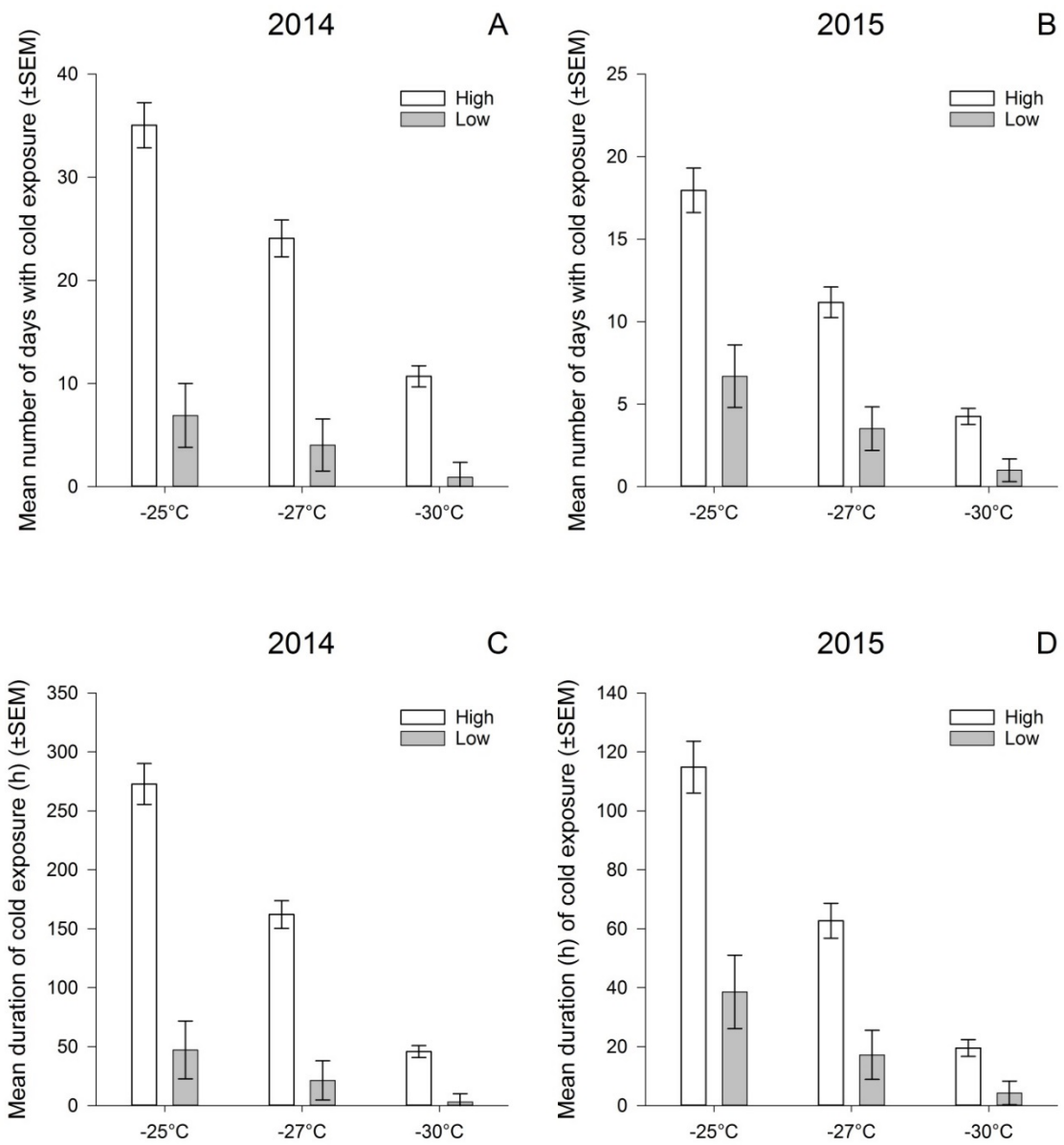
**Figure 2.2:** Annual summer male gypsy moth trap capture and annual number of moths per trap in the Arrowhead region of Minnesota, 2004-2014.



**Figure 2.3:** Map of the Arrowhead of Minnesota with Thiessen polygons drawn around weather stations (denoted by stars) used for modeling association between winter severity and subsequent summer moth capture. Thiessen polygons were used to assign temperature variables to trap catch data for each year, 2004-2014. Inset map shows location of field sites (denoted by circles) within Grand Portage Reservation used to experimentally monitor overwintering mortality during the winters of 2014 and 2015.

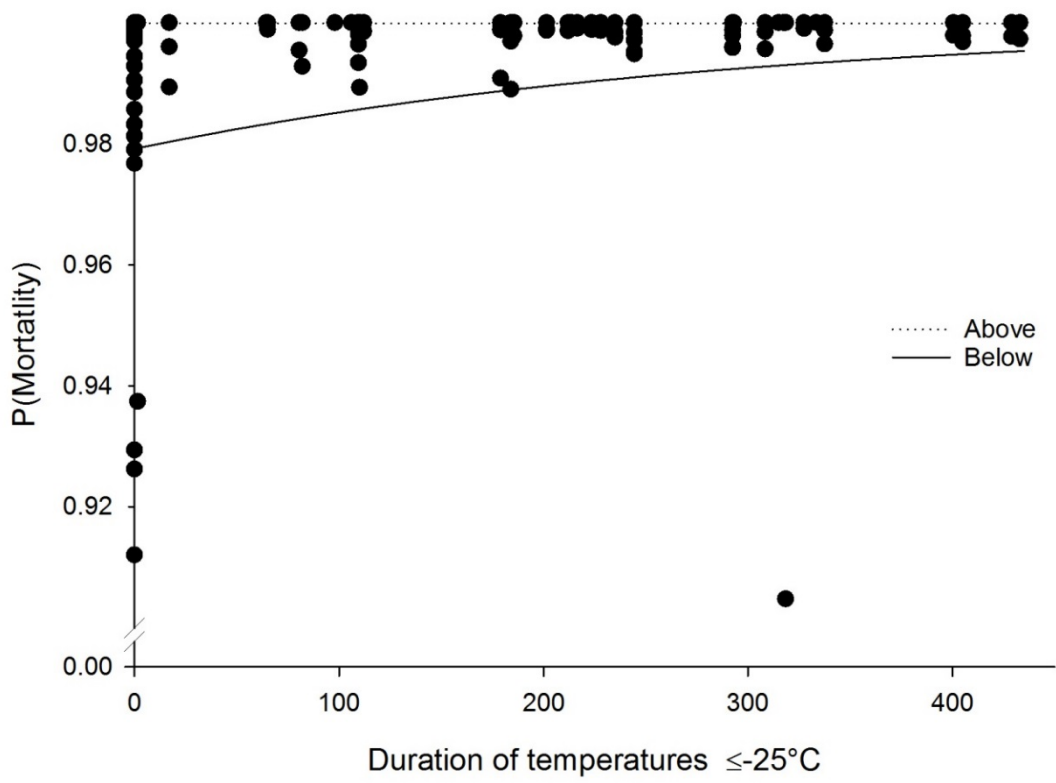


**Figure 2.4:** Box and whisker graphs for various temperature variables characterizing overwintering conditions in the Arrowhead region of Minnesota, 2004-2014: A) mean December minimum temperature, B) mean January minimum temperature, C) mean February minimum temperature, D) number of days below  $-10^{\circ}\text{C}$ , E) number of days below  $-15^{\circ}\text{C}$ , F) number of days below  $-20^{\circ}\text{C}$ , G) number of days below  $-25^{\circ}\text{C}$ , H) number of days below  $-30^{\circ}\text{C}$ , and I) Absolute minimum temperature. The box boundaries indicate the 25<sup>th</sup> and 75<sup>th</sup> percentile. The line within the box indicates the median. The whiskers are error bars, indicating the 90<sup>th</sup> and 10<sup>th</sup> percentiles, and the dots represent outliers beyond the error bars.



**Figure 2.5:** Cold exposure experienced by egg masses at high and low placements at 12 field sites on the Grand Portage Reservation, 2014-2015. Note y axes are on different scales. Number of days ( $\pm$ SEM) that minimum temperatures dropped below various thresholds. A) 2014. B) 2015. Duration, in hours ( $\pm$ SEM), that temperatures were less than or equal to various temperature thresholds. C) 2014. D) 2015.





**Figure 2.6:** Mortality of ( $n= 354$ ) egg mass that overwintered at field sites on the Grand Portage Reservation, MN in 2014. Overwintering mortality was high in 2014, but there were significant differences in the probability of mortality for egg masses with high and low placement. Egg masses ( $n=176$ ) with a low placement had exponentially increasing mortality with increasing winter severity as measured by the duration of temperatures below  $-25^{\circ}\text{C}$ .

## Thesis conclusions

### Chapter I

1. **Gypsy moth populations in northern Minnesota have FS1-A allele frequencies above the national average.** In the two years of this study, FS1-A frequencies varied between 10.2% and 16.7%, compared to a national average of approximately 3.5%.
2. **The distribution of FS1-A alleles in male moths of the Arrowhead region of Minnesota appears to be random in space and time.** I do not find evidence that potential Asian gypsy moth genotypes within this region are associated with recent putative introductions, immigration events, or movement pathways. Interestingly, elevated FS1-A frequencies appear to occur in small populations, although the reasons behind this remain unexplored.

### Chapter II

1. **There is a significant negative correlation between winter temperatures and subsequent male moth trap capture.** We estimated that trap capture was reduced by 50% for every three weeks (cumulatively) that temperatures fall below -25°C.
2. **Overwintering mortality is affected by egg mass placement.** We observed almost 100% egg mass mortality when ambient air temperatures dropped below -27°C, the lower lethal temperature of eggs. However, overwintering success was higher among egg masses placed close to the ground, where snow cover could provide thermal insulation.

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