TESTING LOCAL ADAPTATION OF THE FEDERALLY ENDANGERED KARNER BLUE BUTTERFLY (LYCAEIDES MELISSA SAMUELIS) TO ITS SINGLE HOST PLANT THE WILD LUPINE (LUPINUS PERENNIS)

Kevin Handel

A Thesis

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Committee:

M. Gabriela Bidart-Bouzat, Advisor

Juan L. Bouzat

Shannon Pelini
ABSTRACT

M. Gabriela Bidart-Bouzat, Advisor

Local adaptation has been shown to be a key process that drives the evolution of species, influencing both their physical characteristics and ecological interactions. Local adaptation is generally expressed by higher fitness of individuals raised in their native habitats versus in a foreign location. The influence of local adaptation is especially prominent in species that subsist in small and/or highly isolated populations. This study evaluates the degree to which the federally endangered Karner blue butterfly (*Lycaeides melissa samuelis*) is locally adapted to its exclusive larval host plant, wild lupine (*Lupinus perennis*). To test for local adaptation of the Karner blue butterfly, individuals from a laboratory-raised colony were reared on wild lupine plants from populations belonging to either their native or foreign region. Specifically, nine wild lupine populations from three different regions: Indiana (native), Michigan (foreign) and Wisconsin (foreign) were grown in a common garden using growth chambers and one Karner blue larva was placed on each plant. Fitness traits related to growth and development of this butterfly on the different populations were recorded. Survival and days from pupation to eclosion both showed significant differences across wild lupine populations, indicating that wild lupine source can affect some fitness-related traits of Karner blue butterflies. However, this influence was not manifested as higher fitness on native plant populations. Results from this study have implications for programs attempting to reintroduce Karner blue butterfly populations across their historical range.
The apparent absence of local adaptation to wild lupine suggests that at least some individuals of this species could be translocated from native populations to foreign reintroduction sites without experiencing decreased fitness levels. However, because variation was observed for some fitness related traits and our experimental design did not encompass all spatial, temporal, and environmental factors that could influence adaptation, future studies are recommended. Future research should evaluate butterflies from different sources and test plants on a wider spatial scale and between diverse microclimates.
I dedicate this thesis to my parents, who have unwaveringly provided me with the support, encouragement, and independence to pursue my own path.
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INTRODUCTION

Plant-insect coevolutionary associations are known to drive local adaptation of populations across their geographic range (Cogni et al. 2011). Spatial heterogeneity of habitat characteristics and biotic factors can cause selection to favor different traits in different populations (Kalske et al. 2012). Local adaptation is a key mechanism in evolutionary ecology, which results in individuals experiencing increased fitness within their native population compared to foreign populations (Fraser et al. 2011). This fitness variation can lead to lowered genetic diversity of populations by reducing the ability for individuals to disperse between populations; and thus, to inbreeding and outbreeding depression (Jourdan-Pineau et al. 2012). Vayssade et al. (2014) notes that both “inbreeding and inbreeding depression are key processes in small or isolated populations and are therefore central concerns for the management of threatened or (re)introduced organisms.” Likewise, Saccheri et al. (1998) showed that inbreeding in wild populations of the Glanville fritillary butterfly (*Melitaea cinxia*) significantly increased extinction risk by lowering fitness. Given the potential evolutionary and ecological consequences of local adaptation, the study of this topic is particularly relevant for species of conservation concern subsisting in small and fragmented populations (Jourdan-Pineau et al. 2012). The existence of local adaptation has been examined across many systems, including Chinook salmon (*Oncorhynchus tshawtyscha*), fire salamanders (*Salamandra salamandra*), brown trout (*Salmo trutta*), purple sea urchins (*Strongylocentrotus purpuratus*), and the common frog (*Rana temporaria*) (Fraser et al 2011; Manenti & Ficetola 2013; Pespeni et al. 2013; Meier et al. 2014; Muir et al. 2014). In some cases, such as in salmon, it has led to
divergent evolution in unrelated populations, likely as a result of the highly structured spatial organization of this economically valuable species (Fraser et al. 2011). In spite of the substantial number of studies on local adaptation, little information exists related to the potential effects of local adaptation in invertebrate specialist herbivores; especially, on the role this process may have on the outcome of insect reintroduction programs in the wild. In most parasite-host interactions, the parasite is expected to experience a greater degree of local adaptation due to “larger population sizes, shorter generation times, and higher mutation rates” (Cogni et al. 2011).

There is a reason to believe that the federally endangered Karner blue butterfly may experience local adaptation across its spatial distribution. Due to an estimated 99% decrease in wild Karner blue butterfly populations, current populations remain geographically isolated from one another, which in turn may promote adaptation to particular microclimates (Karner blue butterfly recovery team 2003). In a 2003 report, the Karner blue butterfly recovery team estimated the dispersal ability of the Karner blue butterfly to be 100-200 m within suitable habitat and 0.5-2 km between habitats. Additionally, Shultz (1998), estimated that a similar endangered butterfly species endemic to the Willamette Valley of Oregon, *Icaricia icarioides fenderi* (Fender’s blue butterfly) had very limited dispersal abilities (total of 0.75 km in lupine habitat, and up to 2 km in non-lupine habitat) during its total lifetime (approximately 9.5 days). This limited dispersal creates a scenario that could lead to local adaptation when considering the reduced size, abundance, and proximity of wild populations. Compounding the isolation of wild populations of this butterfly is this species’ dependence on *Lupinus perennis* (wild lupine) as their only suitable larval food source. It is known that wild
lupine populations can have diverse alkaloid profiles across their natural populations (Adler & Kittelson 2004). These alkaloids are secondary chemicals, which provide defense against pathogens and herbivores (Vilarino & Ravetta 2008; Ganzera et al. 2010). Therefore, it is likely that the remaining isolated Karner blue butterfly populations may have evolved in response to the specific chemical profiles of the lupine populations that they feed on.

Originally well established in at least 12 Midwestern states and the province of Ontario, Canada, the Karner blue butterfly became a federally listed endangered species in 1992, after experiencing a total population decline of 99%. As with may other species, the primary cause of this decline is habitat loss due to fire suppression, agriculture, and urbanization (Karner blue butterfly recovery team 2003). Karner blue butterfly populations are currently restricted to 0.02% of their original range, making ecological restoration of suitable habitat a top priority for the conservation of this species (Chan & Laurence 2006).

Recently there have been great efforts made to restore savanna and barren ecosystems and wild lupine populations in conjunction with the reintroduction of Karner blue butterflies to their historic range. Currently, there are five active translocation programs, comprised of three reintroductions in the following locations: 1) the Oak Openings Region of northwest Ohio, 2) the Oak Openings Region of southeast Michigan and 3) the Concord Pine Barrens in Concord, New Hampshire. In addition, there are two population augmentation programs at the Indiana Dunes National Lakeshore and the Albany Pine Bush, intended to increase the viability of already established native populations (USFW 2012). The main goal of these programs is to reclassify the species’
conservation status by establishing viable metapopulations across the butterfly’s original range (Karner blue butterfly recovery team 2003). Federal reclassification of the species will be accomplished when 27 metapopulations (comprised of 19 viable populations ≥ 3,000 and 8 large viable populations ≥ 6,000) are established within 13 designated recovery units across the butterfly’s historic range, and delisting will be considered at 29 metapopulations (comprised of 13 viable populations ≥ 3,000 and 16 large viable populations ≥ 6,000; Karner blue butterfly recovery team 2003). However, often times, despite the effort of ecological restoration programs, it is not possible to reconstruct ecosystems to the original level of complexity that would be able to support a wide range of organisms and interactions (Brown et al. 2011).

The success of a metapopulation system depends on a balance between population dynamics and habitat quality (Lane & Androw 2003). Dispersal within a metapopulation is a key concern in wildlife conservation and management (Shultz 1998). Therefore, a tight patch of geographically close (< 2 km) suitable habitats, with relatively large adult butterfly populations would be necessary to allow dispersal, and thus, maintain a feasible metapopulation system for the Karner blue and other endangered butterflies (Karner blue butterfly recovery team 2003; Shultz et al. 1998). In addition to a close proximity to nearby Karner blue butterfly populations, restoration sites must also include a suitable abundance and cohesiveness of wild lupine patches and adequate nectar sources for the adult butterflies. Moreover, Grundel & Pavlovic (2007) proposed that three sets of predictors were associated with Karner blue butterfly patch use at Indiana Dunes National Lakeshore (Indiana) and Fort McCoy (Wisconsin). These predictors included wild lupine availability, characteristics of the matrix surrounding lupine patches, and factors affecting
the thermal environment. Although current reintroduction programs may compare habitat characteristics between translocation sites, they largely fail to acknowledge the extent of coevolutionary interaction between a specific Karner blue butterfly population and its local wild lupine population. The extreme dependence of the Karner blue butterfly on their sole larval host may amplify their adaptation beyond what a simple habitat analysis can quantify.

The hypothesis tested in this study relates to whether Karner blue butterflies are locally adapted to distinct populations of wild lupine. I assessed this hypothesis by testing if this species would experience decreased fitness when reared on wild lupine plants from foreign populations compared to those of their native populations. During this experiment, Karner blue butterfly larvae originating from Indiana were raised on nine different populations of wild lupine (including three native Indiana populations, and six foreign Wisconsin and Michigan populations), and fitness-related measurements were recorded. Results from this study provide valuable information for current reintroduction programs, which usually translocate individuals from native wild populations and captive breeding programs to genetically, geographically, and historically distinct environments. If evidence of local adaptation is found, it may be advisable to evaluate first potential matches or mismatches between the source population of butterflies and the target populations of their host plants present in the reintroduction region.
MATERIALS AND METHODS

Study Organisms

The Karner blue butterfly (*Lycaenides melissa samuelis*) is a federally endangered, specialist herbivore native to the oak savannas and pine barrens of the Midwestern United States. The Karner blue butterfly is a small, blue and grey insect specialist with a wingspan of approximately 2.5 cm. It is a sexually dimorphic species, with larger females that display a dotted orange pattern on the outer margins of the hind wing that is absent in males (Karner blue butterfly recovery team 2003). The Karner blue butterfly has two generations per year, with the first group of adults eclosing in late May to mid-June, and the second group in mid-July to early August (Lane & Andow 2003). As with all butterflies, the Karner blue experiences four distinct life stages including egg, larva, pupa, and adult. As a specialist herbivore, females will only lay eggs on or near their exclusive larval food plant, the wild lupine *Lupinus perennis*. While eggs laid by the first generation hatch during the summer, eggs laid by the second generation overwinter and hatch during the following spring (Karner blue butterfly recovery team 2003). Adult Karner blues are relatively short-lived with an average adult lifespan totaling only 7-10 days.

Since Karner blue butterflies are strict specialist herbivores of the wild lupine during their larval stages, their populations are completely dependent on the distribution of this plant species. Wild lupine is a perennial legume that inhabits the dry, sandy soils commonly found in oak savannas and pine-barrens (Gibbs et al. 2012). Given that these ecosystems can only be maintained through natural or artificial disturbance, the recent
large-scale suppression of these disturbance regimes has reduced the suitable habitat for wild lupine (Forrester et al. 2005). Additionally, because of their sparse appearance, these ecosystems have commonly been targeted for development, due to the relatively small amount of deforestation needed. Not only has the number of oak savannas drastically declined, but also the existing savanna environments have experienced reduced ecosystem functioning leading to a decrease in the abundance of associated species (King 2003). The interdependent relationship between the wild lupine and the Karner blue butterfly allows the use of these rare insects as an indicator species to gauge the health of these unique habitats. In fact, researchers have found that oak barrens sites managed for Karner blue butterflies also benefit bird species (e.g. field sparrow and grasshopper sparrow) associated with these types of habitats, with the biggest influencing factor being the quality and type of adjacent habitat (Wood et al. 2011).

Karner blue butterfly larvae were obtained as eggs from a colony maintained in the laboratory of Dr. Jessica J. Hellmann (University of Notre Dame, Indiana, USA), which individuals originated from a population of Karner blue butterflies at the Indiana Dunes National Lakeshore, Indiana, USA. Plants used in this study originated from nine different populations of wild lupine across three different regions: Indiana, Michigan and Wisconsin (three populations per region). Michigan lupine populations originated from Allegan County (140.4 km from native population), Wisconsin lupine populations originated from Eau Claire County (490.3 km from native population), and Indiana lupine populations originated from Indiana Dunes National Lakeshore (the same location our test butterflies from the University of Notre Dame captive colony were initially collected from) (Table 1; Figure 1). Lupine populations from the same region – Indiana, Michigan
or Wisconsin – were located at least two miles apart. Additionally, lupine seeds were organized based on genotype to further identify potential genetic differences within natural populations. At the time of collection, individual seeds originating from the same plant were grouped together and classified under a single genotype. Each of the nine wild lupine populations used were comprised of 9 to 10 different genotypes.

**Experimental Design**

In order to test for local adaptation in the Karner blue butterfly, a common garden experiment was performed using growth chambers located at Bowling Green State University. A sample of lupine seeds were collected from each of the described wild populations. To facilitate germination, seeds were individually scarified using a razor blade and subjected to a cold treatment for three days. Then, seeds were removed from the cold treatment and inoculated with a commercial inoculum (Prairie moon nursery, Winona, MN, USA). The freshly inoculated seeds were then planted in a commercial soil mix (Fafard 52; Sun Gro Horticulture Canada Ltd., Agawam, MA, USA) and grown under a photoperiod cycle of 16 h light and 8 h dark until large enough for experimental use as a host plant (minimum of 7-8 full expanded leaves).

Karner blue butterfly eggs provided by the lab of Dr. Jessica J. Hellmann (UND) were kept at the same conditions as the lupine populations and monitored for hatching larva twice per day. Once each larva emerged, it was placed on an individual plant randomly selected from one of the nine lupine populations, until there was one larva on each of 30 plants per population (30 plants × 9 populations × 1 larva/plant and population = 270 larvae). Unused eggs were returned to the University of Notre Dame. Each
individual potted lupine plant housing one larva was placed in a growth chamber maintained at 21°C with a photoperiod cycle of 16 h light and 8 h dark. To avoid insect dispersal, each potted plant was covered with a plastic cage, which had holes covered with mesh fabric to allow aeration (Figure 2). Larvae were individually monitored daily for growth and progression through life stages. Following pupation, individuals were moved to 2 oz. plastic containers (Fabri-Kal, Kalamazoo, MI, USA) to easily identify progression through the pupal stage. During the final phase of pupation, individuals were moved to larger plastic containers (16 oz. Fabri-Kal) with a mesh lid for aeration and monitored daily for adult eclosion. After adult eclosion, a generic wooden coffee stirrer was included in the containers to allow perching and individuals were fed a 10% honey solution along with regular misting of distilled water. Several measurements were recorded for each butterfly throughout its life cycle: survival, larval weight, pupal weight, adult weight, developmental time (i.e., days from hatching to pupation and adult eclosion), and gender. After completion of the experiment, each individual adult was transported back to the University of Notre Dame’s captive colony. In order to evaluate wild lupine chemical responses to Karner blue butterfly herbivory, plant material was collected from a sample of individuals from each of the nine plant populations, before and after larval herbivory. These plant samples will be analyzed to detect changes in secondary chemicals resulting from herbivory.

**Statistical Analyses**

The data generated in this experiment was statistically analyzed using SAS (Version 9.1). Dependent variables included: pupal weight (g), adult weight (g), days
from egg hatching to pupation, days from pupation to eclosion, days from egg hatching to adult eclosion, and survival. Pupal weight was measured immediately following pupation, and adult weight was measured immediately following eclosion. Analyses of Variance were conducted for each dependent variable as well as tests for normality and homocedasticity (Table 2). Data was transformed when necessary using inverse and rank transformation. Additionally, survival across population was evaluated using a chi-squared test. Post-hoc multiple comparisons (i.e., Student-Newman-Keuls, SNK) were performed to evaluate specific differences between means of measured variables among regions and populations. Separate comparisons were performed for females and males to account for statistically significant sexual dimorphism since males are smaller and have shorter developmental times than females. Evidence of local adaptation would be supported if Karner blue butterflies experience significantly lowered performance (i.e., decreased weight, survival and longer developmental times) when reared on foreign lupine populations (i.e., Michigan and Wisconsin) versus when reared on their native Indiana populations.
RESULTS

Results were considered to provide evidence of local adaptation if individuals had increased performance (i.e., increased survival, weight, and shorter developmental times) when reared in their native Indiana populations compared to foreign Michigan and Wisconsin populations.

Results of analyses of variance (ANOVA) showed significant differences related to gender and plant genotype, but no significant effects of region or population (within region) on the fitness-related variables measured (Table 2). However, when analyses of variance were performed separately for each gender, there was a significant effect of population on days from pupation to eclosion for male Karner blue butterflies (P=0.013; Figure 3). Furthermore, post-hoc comparisons among populations for this variable using a Student-Newman-Keuls test showed significantly fewer days from pupation to eclosion for males from Indiana population I3, and Michigan population M3, compared to Michigan population M2. ANOVAs testing effects of population (by gender) on all other fitness-related traits were associated with non-significant P-values ranging from 0.321 to 0.964 (Figures 4-12). In addition, there were significant differences in survival of individuals between populations, as shown by a significant Chi-squared test (P=0.011; Figure 13). Highest survival was observed for two Michigan lupine populations (M1 and M3).
DISCUSSION

Significant variation in Karner blue butterflies was based on gender and plant genotype. Differences associated with gender are explained by documented sexual dimorphism in the Karner blue butterfly yielding smaller and faster developing males. Significant results related to genotype suggest the evolution of adaptation in the Karner blue butterfly may be more influenced by wild lupine intra-population genetics than an individual plant’s geographic origin. Significant post-hoc analysis results were associated with days from pupation to eclosion, implying important disparity among tested lupine populations for this important developmental stage in male Karner blue butterflies. However this particular result is inconsistent evidence of local adaptation since one native Indiana population (I3) showed significantly faster development during this stage compared to only one foreign Michigan population (M2), with all other foreign populations statistically equal (Figure 3). Furthermore, no significant difference was shown between any other populations for the same variable. A significant difference in survival between lupine populations was found using a chi-squared test. However, this result is contrary to the expression of local adaptation because the highest survival rates were observed in two Michigan populations (M1 and M3), rather than in the populations considered native to the selected Karner blue butterflies (Figure 13).

Overall, experimental results did not fully indicate the presence of local adaptation in the Karner blue butterfly individuals used in this study. A possible explanation for the potential lack of local adaptation is that this study did not fully capture the spatial scale of the distribution range of the Karner blue butterfly. Currently,
native Karner blue butterfly populations are established across five states – Minnesota, Wisconsin, Indiana, Michigan, New York – while populations in two states – Ohio and New Hampshire – are comprised solely of non-native reintroduced individuals (Karner blue butterfly recovery team 2003). Since the individuals used in this study originated from Indiana Dunes National Lakeshore and were reared on wild lupine populations from Indiana Dunes National Lakeshore (native habitat), Allegan County, MI (~140.4 km to native habitat) and Eau Claire County, WI (~490.3 km to native habitat), our study did not evaluate the entire current native range of the Karner blue butterfly (Table 1; Figure 1). Therefore, it is possible that local adaptation is evident only on a broader scale than our wild lupine populations allowed us to test. It is also possible that an important environmental aspect was not adequately represented amongst the wild lupine collection sites. Local adaptation in the Karner blue butterflies used in this study may be related to an environmental source of variation independent of geographic region. If the butterfly source population is adapted to a particular wild lupine alkaloid profile determined by specific micro-environmental conditions, then, it is possible that our sample lupine populations did not represent consistent variation in this habitat characteristic.

Based on the limitations of our wild lupine samples, it is advisable that future studies of local adaptation in the Karner blue butterfly include lupine populations across a larger span of the butterfly’s native range (e.g. New York to Minnesota) as well as between contrasting environments (e.g., distinct microclimates within a particular native habitat). Hanks and Denno (1994) found evidence of large-scale local adaptation in the armored scale insect *Pseudaulacaspis pentagona*, reinforcing the importance of spatial scale when evaluating local adaptation. The study showed that individuals reared on their
natal host tree exhibited significantly higher survival rates compared to individuals raised on distant trees (≥ 300m from host tree); however, there was no significant difference when raised on neighboring trees (< 5m to host tree) (Hanks & Denno 1994). In a study by Bischoff et al. (2006) strong small-scale local adaptation in grassland plant species was detected and recommended that ecological restorations should use seeds from distant populations with similar habitats opposed to nearby heterogeneous environments.

Experimental results may also have been influenced by the degree of inbreeding that occurs within a captive colony of an endangered species. Individuals used in this study originated from only one population in Indiana, which was propagated in laboratory conditions for several generations. Therefore, this could have led to inbreeding and inbreeding depression among these individuals. In fact, inbreeding has been shown to negatively affect many fitness-related traits in various insect species, including butterflies (Saccheri et al. 1998; Franke & Fischer 2013; Liu et al. 2014; Vayssade et al. 2014). Franke and Fischer (2013) showed that the tropical butterfly Bicyclus anynana experienced reduced fitness even with relatively low levels of inbreeding. Additionally, notable deformities were observed and documented following eclosion in several individual Karner blue butterflies used in this study. Deformities were manifested as malformed adult wings, and suggest some genetic complications within the source colony. Captive-breeding programs focused on the conservation of endangered species are commonly subject to inbreeding as a result of limited wild broodstock and compounded by the number of generations held in captivity (Rollinson et al. 2014). Therefore, the Karner blue butterfly may be particularly susceptible to captive inbreeding stemming from the loss of 99% of the wild population combined with a two-generation
per year life cycle. According to Rollinson et al. (2014), this scenario dictates a management choice between perpetuating genetic homogeneity or introducing new, genetically diverse individuals and increasing the likelihood of outbreeding depression. Furthermore, if the choice is made to insert new individuals into a captive colony, the ability to safely and efficiently collect wild individuals is complicated by the species size, fragility, rarity, and short adult lifespan. Not to mention the possibility that consistent collections from native populations may create negative consequences in an already fragmented and delicate endangered wild population; thus, counteracting the intentions of reintroduction programs (Saccheri et al. 1998; Sletvold et al. 2012; Long et al. 2013; Angeloni et al. 2014; Brzeski et al. 2014; Vayssade et al. 2014).

Maternal effects could have also influenced results of this study. Several details linked to the rearing of previous generations could highly influence fitness-related traits measured in the offspring tested. It has been widely acknowledged that maternal effects can greatly influence phenotypic variation (Bernardo 1996; Mousseau & Dingle 1991; Mousseau & Fox 1998; Roach & Wulff 1987). The most apparent source of maternal effects is based on the wild lupine source used in previous generations of the captive source colony. If previous generations were not reared on wild lupine consistent with the Indiana Dunes National Lakeshore lupine populations, then, it is possible that the individuals used in this study would not express significant adaptation to the plant populations classified as native.

Temporal scale is another factor that determines the degree of local adaptation expressed by a species. Local adaptation is more likely to be exhibited the longer a population remains small and isolated (Angeloni et al. 2014). While the Karner blue
butterfly has experienced a 99% decline over the past 100+ years, it is estimated that 90% of this reduction occurred in the last 20-25 years (USFW 1992). Therefore, it is possible that wild populations have not been isolated for a long enough period to detect local adaptation, at least in the studied populations. However, if populations remain fragmented with inadequate dispersal corridors between heterogeneous environments, local adaptation will increase over time even with the naturally random mating of wild individuals (Angeloni et al. 2014). It is difficult to predict the speed of local adaptation due to extensively varying characteristics influencing evolution across a species range. However, Ledger and Rice (2007) estimated local adaptation occurred in the invasive California poppy (Eschscholzia californica) 110-150 years after introduction to Chile.

Results of this study may have significant implications for current and future reintroduction programs. The data suggests that at least some populations of the Karner blue butterfly could maintain consistent fitness levels if they were geographically translocated to restoration sites. However, this scenario may change when assessing other populations in the studied regions or beyond these regions; or when using butterflies from a different population or region. In fact, another experiment conducted by Dr. Gabriela Bidart-Bouzat at Bowling Green State University (unpublished results) provided evidence of local adaptation in Karner blue butterflies originating from Michigan when raised on some wild lupine populations from Ohio. Therefore, it is always recommended to screen the potential match between a plant population and the butterflies to be reintroduced in an area. To advocate more specific reintroduction guidelines requires further research across an expanded range of populations and regions. In addition, future studies should evaluate different spatial scales while sourcing individuals from different
Karner blue butterfly colonies and lupine populations. Until a more thorough investigation has been completed, it is recommended that conservation efforts focus on maintaining and strengthening native populations currently established combined with expanding the wild lupine habitat surrounding these populations in a manner that would allow adult individuals to easily disperse to new territories. Reintroductions should be viewed as a final approach only after local extinction or the failure of conventional restoration strategies. When translocations are pursued, it is advisable to fully analyze and compare the source habitat with the reintroduction site, in order to ensure uniformity of biotic and abiotic conditions. Furthermore, evidence must indicate strong health and stability within source populations to ensure viability following any necessary collections, along with strict collection guidelines and possible replacement efforts.

Unfortunately, the potential for full recovery and future reclassification of the Karner blue butterfly remains uncertain, with extensive conservation efforts still needed. Despite restoration efforts, population size has remained low since the species was federally listed as endangered with only two additional populations established between 1992 and 2011, resulting in a range wide population increase from 114 to 116 (USFW 2012). Since species relisting standards were set in 2003, only three Karner blue butterfly populations have met criteria to be considered a viable metapopulation (USFW 2012). Furthermore, as habitat loss inevitably increases and intensifies habitat fragmentation, established populations can become increasingly more isolated, thus reducing population resilience. Additionally, impending climate change is predicted to have harmful consequences on remaining populations (Swengel et al. 2011; Hoving et al. 2013; National Park Service 2014). With future conditions predicted to be increasingly
unfavorable, it is likely that reintroductions will become increasingly required, which reinforces the need to examine the extent of local adaptation. Even though no clear evidence of local adaptation was found in this study, there were some differences in performance of the butterfly on different wild lupine populations, in terms of both survival and developmental time. These differences may still be important to consider in potential conservation-related management of this species. In any case, an evaluation of the potential match between donor butterfly populations and receiving wild lupine habitats is essential for a successful re-introduction program.
REFERENCES


important traits of Holstein cattle in Iran. *Journal of Dairy Science*, 93, 3294-3302.


Table 1. Place of origin of the Karner blue butterflies and location of all the nine wild lupine populations used in this study, and their distance to the selected source population of Karner blue butterflies.

<table>
<thead>
<tr>
<th>Native vs. Foreign Karner Blue Butterfly Habitat</th>
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<td></td>
<td>I2</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>I3</td>
</tr>
<tr>
<td>Foreign</td>
<td>Michigan</td>
<td>Allegan</td>
<td>≈ 140.4</td>
<td>M1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M3</td>
</tr>
<tr>
<td></td>
<td>Wisconsin</td>
<td>Eau Claire</td>
<td>≈ 490.3</td>
<td>W1</td>
</tr>
<tr>
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<td></td>
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<td>W2</td>
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<td>W3</td>
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</table>
Table 2. Analyses of variance for several fitness-related variables of the Karner blue butterfly reared on different wild lupine populations originating from three different regions.

<table>
<thead>
<tr>
<th>Source</th>
<th>Pupal Weight (g)</th>
<th>Adult Weight (g)</th>
<th>Hatch to Pupation (days)</th>
<th>Pupation to Eclosion (days)</th>
<th>Hatch to Eclosion (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td>1.04</td>
<td>1.54</td>
<td>0.81</td>
<td>1.98</td>
<td>0.65</td>
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<tr>
<td>Population (Region)</td>
<td>0.76</td>
<td>1.03</td>
<td>1.90</td>
<td>1.61</td>
<td>1.59</td>
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<tr>
<td>Genotype (Population)</td>
<td>1.62*</td>
<td>1.90**</td>
<td>1.70**</td>
<td>1.21</td>
<td>1.96**</td>
</tr>
<tr>
<td>Gender</td>
<td>29.34***</td>
<td>61.22***</td>
<td>11.13**</td>
<td>1.20</td>
<td>15.10***</td>
</tr>
<tr>
<td>Region × Gender</td>
<td>0.19</td>
<td>0.63</td>
<td>0.36</td>
<td>0.52</td>
<td>0.22</td>
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<tr>
<td>Population (Region) × Gender</td>
<td>0.55</td>
<td>2.14</td>
<td>0.97</td>
<td>2.12</td>
<td>2.11</td>
</tr>
<tr>
<td>Genotype (Population) × Gender</td>
<td>1.05</td>
<td>1.07</td>
<td>2.01**</td>
<td>1.35</td>
<td>1.80*</td>
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<td>N</td>
<td>219</td>
<td>214</td>
<td>219</td>
<td>213</td>
<td>214</td>
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</tbody>
</table>

* P<0.05; **P<0.01; ***P<0.001.
Figure 1. Map of the three wild lupine collection regions, each contributing three separate wild lupine populations.
Figure 2. Karner blue butterfly larvae were individually placed on a single wild lupine plant and covered with a plastic and mesh enclosure from hatching to pupation.
Figure 3. Average number of days from pupation to eclosion for male Karner blue butterflies raised on nine different populations of wild lupine.

\[ F_{8,100} = 2.59; P = 0.0132 \]
Figure 4. Average pupal weight of female Karner blue butterflies raised on nine different populations of wild lupine.

$F_{8,100} = 0.30; P = 0.9639$
Figure 5. Average pupal weight of male Karner blue butterflies raised on nine different populations of wild lupine.

$F_{8,95} = 0.39; P = 0.9213$
Figure 6. Average adult weight of female Karner blue butterflies raised on nine different populations of wild lupine.
Figure 7. Average adult weight of male Karner blue butterflies raised on nine different populations of wild lupine.

\[ F_{8,96} = 1.16; P = 0.3290 \]
**Figure 8.** Average number of days from hatch to pupation for female Karner blue butterflies raised on nine different populations of wild lupine.

\[ F_{8,100} = 1.11; \ P = 0.3640 \]
Figure 9. Average number of days from hatch to pupation for male Karner blue butterflies raised on nine different populations of wild lupine.
**Figure 10.** Average number of days from pupation to eclosion for female Karner blue butterflies raised on nine different populations of wild lupine.
Figure 11. Average number of days from hatch to eclosion for female Karner blue butterflies raised on nine different populations of wild lupine

$F_{8,100} = 0.73; P = 0.6670$
Figure 12. Average number of days from hatch to eclosion for male Karner blue butterflies raised on nine different populations of wild lupine.

$F_{8,96} = 0.99; P = 0.4509$
Figure 13. Average percent survival of Karner blue butterflies raised on nine different populations of wild lupine.